



HAL
open science

Development of a simplified clinical bacteriology laboratory for low resource settings

Jean-Baptiste Ronat

► **To cite this version:**

Jean-Baptiste Ronat. Development of a simplified clinical bacteriology laboratory for low resource settings. Bacteriology. Université Paris-Saclay, 2023. English. NNT : 2023UPASQ016 . tel-04531693

HAL Id: tel-04531693

<https://theses.hal.science/tel-04531693>

Submitted on 4 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Development of a simplified clinical bacteriology laboratory for low resource settings

*Développement d'un laboratoire de bactériologie clinique simplifié pour
les zones à faibles ressources*

Thèse de doctorat de l'université Paris-Saclay

École doctorale n°569 Innovation thérapeutique : du fondamentale à l'appliqué (ITFA)
Spécialité de doctorat : Sciences pharmacologique
Graduate school : Santé et médicaments. Référent : Faculté de pharmacie

Thèse préparée dans l'unité de recherche **Team RESIST UMR1184, Immunology
of Viral, Auto-immune, Hematological and Bacterial diseases (IMVA-HB)**
(Université Paris-Saclay, INSERM, CEA), sous la direction de **Dr Thierry NAAS**

Thèse soutenue à Paris-Saclay, le 20 avril 2023, par

Jean-Baptiste RONAT

Composition du Jury

Membres du jury avec voix délibérative

Claire JANOIR Professeur des universités, Université Paris-Saclay, France	Présidente
Abdoul-Salam OUEDRAOGO Professeur des universités, Université Nazi Boni, Burkina-Faso	Rapporteur & Examineur
Elizabeth ASHLEY Professeur des universités, Université d'Oxford, U.K.	Rapporteur & Examinatrice
Bich-Tram HUYNH Professeur des universités, Université de Versailles Saint-Quentin, France	Examinatrice
Jordy VILLA Professeur des universités, Université de Barcelone, Barcelone, Espagne	Examineur
Olivier VANDENBERG Professeur des universités, Université Libre de Bruxelles, Belgique	Examineur

Titre : Développement d'un laboratoire simplifié de bactériologie clinique pour les zones à faibles ressources

Mots clefs : antibiorésistance, laboratoire de bactériologie clinique, simplification, zones à faibles ressources

Résumé: La résistance aux antimicrobiens est un problème de santé publique mondial. Les bactéries résistantes aux antibiotiques peuvent causer des infections difficiles, voire impossibles, à traiter, augmentant le risque de mortalité et de morbidité. L'émergence et la prolifération de bactéries résistantes est alimentée par de nombreux facteurs comme par exemple, la sur utilisation d'antibiotique en agriculture, la pollution de l'environnement par les effluents hospitaliers, les conditions d'hygiène précaires. La diffusion de bactéries résistantes n'est pas limitée à un pays ou une région spécifique, mais se produit à l'échelle mondiale, menaçant la santé publique mondiale. L'accès aux tests de diagnostic de laboratoire est crucial pour la détection précoce et la surveillance des infections bactériennes résistantes aux antibiotiques. Cependant, dans les pays à faible revenu et à revenu intermédiaire, l'accès aux laboratoires de microbiologie de qualité est souvent limité en raison de contraintes financières, de l'insuffisance des ressources humaines et de la capacité limitée des infrastructures de santé. Cette situation est particulièrement préoccupante dans les zones rurales et éloignées où les gens ont moins de chances d'accéder à des soins de santé de qualité. Face à ce défi, Médecins Sans Frontières, organisation humanitaire, a mis en place un projet pour y répondre. Les objectifs de cette thèse résident dans l'identification, l'adaptation et l'évaluation de techniques réalisables et abordables pour la bactériologie dans les milieux à faibles ressources, concentrées sur des packages de laboratoire standardisés de qualité garantie qui peuvent être rapidement installés dans les hôpitaux de district en utilisant une approche multidisciplinaire. Grâce à une approche de développement itérative allant de l'ingénierie de conception de produits, au test de solutions de maquette dans un environnement contrôlé, à l'évaluation de différents prototypes

dans des hôpitaux de MSF, nous avons d'abord démontré que pour transporter tous les équipements, être déployés rapidement et fournir un environnement de travail adéquat et sûr, le format des boîtes paillasse en plastiques roto moulés est adapté au contexte difficile dans lequel évolue MSF. Nous avons démontré que des milieux de culture prêts à l'emploi, thermoscclés, a longue durée de vie, utilisés dans les industries alimentaires, permettent la croissance des bactéries liées aux sepsis dans les pays à revenu faible et intermédiaire sans la nécessité d'incubation sous CO₂ et avec un rendement de croissance similaire aux méthodes standard. Nous avons ensuite montré que les tests de sensibilité aux antibiotiques sous forme micro-dilution en bouillon et adaptés spécifiquement choisis pour répondre aux besoins de terrain de MSF, offrent des performances adéquates pour le traitement et la surveillance dans un contexte difficile. Aussi, nous mettons en évidence que la lecture ne nécessite pas un lecteur automatique mais plutôt un système de guidage assisté pour accompagner l'utilisateur dans les différentes étapes de lecture d'un antibiogramme. Dans l'ensemble, nos expériences en laboratoire à l'évaluation finale dans les milieux hospitaliers subsahariens, a permis d'établir que l'adaptation des technologies existantes pour développer un laboratoire capable de fournir des résultats de qualité et exploitables par les cliniciens entre les mains de techniciens de laboratoire inexpérimentés est faisable mais englobe la nécessité aussi d'inscrire toute la chaîne de valeur d'un laboratoire dans cette simplicité sans compromettre les performances attendues. Les différentes expériences ont également souligné qu'en l'absence d'un microbiologiste de terrain, le développement de systèmes experts informatiques couvrant pour fournir en permanence une interprétation des résultats, est essentiel pour la robustesse du concept d'un laboratoire tout en un, de qualité garantie, déployables et faciles à utiliser, le Mini-Lab..

Title : Development of a simplified clinical bacteriology laboratory for low resources settings

Keywords : antimicrobial resistance, clinical bacteriology laboratory, simplification, low resource settings

Abstract : Antimicrobial resistance is today universally recognised as a global threat, because of the rapid emergence and dissemination of resistant bacteria and genes among humans, animals and the environment on a global scale and represents a heavy burden for healthcare systems especially in Low and Middle Income Countries. Access to Clinical Bacteriology Laboratory in Low resource settings, despite its importance to provide result for treatment guidance and surveillance, have proven to be extremely challenging to install in settings outside of major cities. Médecins Sans Frontières, a leading organisation in the field of humanitarian intervention, facing this challenge established a project to address it.

The objectives of this thesis lie in the identification, adaptation and testing of feasible and affordable techniques for bacteriology in low-resource settings concentrate into standardise quality assured laboratory packages that can be rapidly installed at district level hospitals using multidisciplinary approach.

Through an iterative development approach ranging from product design engineering, test of mock-up solutions in control environment, evaluation of different prototypes on MSF hospital we firstly demonstrated that to transport all equipment, be rapidly deployed and provide adequate and safe work environment, the format of plastic roto-moulding box benches is adapted to the difficult context in which MSF operate. Out of the adapted sample work management for blood culture, we demonstrated that ready to use sealed packed culture media, used in food industries and based on two formulations of Chromogenic and Chocolate agar can growth bacteria that are related to

Bloodstream infection in LMIC without the necessity of CO₂ incubation and with yield of growth like standard methods. We then showed that Antibiotic Susceptibility testing in the form of adapted Microbroth dilution microplates panels specifically chosen to meet MSF field needs, provide adequate performance for treatment and surveillance in difficult context. Also, our finding highlights that reading of this panel does not necessitate an automatic reading but rather of an assisted guiding system to accompany the user in the different steps of reading an antibiogram.

Overall, our experiment from control environment to the final evaluation in sub-Saharan hospital settings, established that adapting existing technologies to develop a CBL able to provide quality, actionable results used by clinicians in the hand of unexperienced laboratory technicians is feasible but encompass the necessity also to adapt all the value chain of a laboratory into this simplicity and around a robust quality management system. Also the results shown that simplification of technologies to LRS constraints does not jeopardise performances of results provided by this CBL. The different experiment also pointed that in the absence of a microbiologist, development of computer expert systems to constantly provide results interpretation, guidance and somehow replace the microbiologist is essential for the robustness of the concept for an all in one, quality assured, deployable easy to use clinical bacteriology laboratories, the Mini-Lab.



*"In nature nothing is created, nothing is lost,
everything changes."*

Antoine-Laurent de Lavoisier, Traité élémentaire de chimie, 1783



*This work is dedicated to my beloved children, Oscar and
Leo, and all future generations whose bright and boundless
potential inspires me to contribute to a better world.*



Acknowledgements

It will be 20 years in March 2023 that I participated in an amazing adventure with Médecins Sans Frontières (MSF) in the field and at headquarters. In 2004, **Laurence Flevaud** and **Laurence Bonte** sent me on the stony roads of the humanitarian aid with my first mission in Bentiu (South Sudan) and I am so grateful to them for having accepted me in the humanitarian family. Along the way, I met my amoeba friends, discovered Plasmodium, felt in love with bacteria and learned with my companions of adventure, logisticians, pharmacists, nurses, doctors, surgeons, administrators, and others the power of our commitment to providing humanitarian assistance and care to vulnerable populations. This Ph.D. project is one of the achievements of this journey. This thesis work was carried out in a joint collaboration between the Mini-Lab team of MSF Operational Centre Paris (France), ReSIST INSERM U1184 teams from the bacteriology laboratory of Bicêtre Teaching Hospital in Kremlin-Bicêtre (France), the Institute of Tropical Medicine (ITM) in Antwerp (Belgium), the University Teaching Laboratory Hospital (LHUB) of Université Libre de Bruxelles (ULB) (Belgium) and many other MSF specialists, organisations from different horizons acknowledge here after.

First of all, I would like to thank the members of the jury, and particularly **Pr. Claire Janoir** who chaired the thesis jury. I would also like to express my deepest thanks to **Pr. Elizabeth Ashley** as well as to **Pr. Bich-Tram Huynh**, for their feedbacks and for the time devoted to the improvement of the manuscript. I also thank **Pr. Jordy Vila** and **Pr. Olivier Vandenberg** for their participation as examiner and as rapporteur of the mid-term Ph.D. committee.

I would like to express my deepest gratitude to my thesis supervisor, **Pr. Thierry Naas**, for his trust during the achievement of all this work. I also thank him for his advices, for sharing his outstanding expertise as well as for his continuous support to the Mini-Lab Scientific Committee. This collaboration forged my spirit and allowed me to acquire an intellectual and human richness.

Colleagues of the Team ReSIST, UMR1184, (Université Paris-Saclay, INSERM, CEA), the national reference centre on antibiotic resistance (CNR) and the bacteriology laboratory of Bicêtre Teaching Hospital in Kremlin-Bicêtre (France).

I was fortunate to be surrounded by remarkable experienced and hardworking colleagues at the research laboratory. First and foremost, to **Dr. Saoussen Oueslati**, I want to deeply thank her for the mental support and friendship in both the good and the difficult times. I am very grateful to have met her and our continued friendship is one of the most significant outcomes of this work. Many thanks to all the members of

the ReSIST UMR1184 unit and the CNR for their welcome and support despite my intermittent presence in the lab: **Delphine Girlich, Dr. Laurent Dortet, Florence Bussy, Dr. Remy Bonin, Sandrine Bernabeu, Elodie Croton, Dr. Cécile Emeraud, Dr. Agnès Jousset, Camille Gonzales and Laura Dabos**. I also warmly thank **Dr Nicolas Fortineau** and all the members of the bacteriology-hygiene laboratory of Bicêtre for their support.

Colleagues from the Mini-Lab Project Team

Without their engagement, what was originally an idea on the paper could never have been developed. We have experienced together so much throughout those years; they will all remain dear to my heart. I am deeply indebted to **Albane Mazoyer** and **Céline Franquesa** for their leadership and support throughout challenging times. I am also grateful to **Bernard Baillet**, for all his expertise, his positive attitude to embark in new adventures, his multi-tasks capacities and adaptation to constraints in Haiti and in Carnot, at the bacteriology-hygiene laboratory of Bicêtre to test the Mini-Lab and complete the adaptation of the procedure. His role has been essential to the project and its success. **Dr. Alessandra Pia Natale's** skills and willingness to create robust science out of the chaos have impressed me, I would like to express my deepest gratitude for the support on my thesis and the friendship that emerged from this journey. I would like as well to warmly express my gratitude to the queen of the Standard Operative Procedures, **Julie Hubert**, her constant happiness and motivation to make this concept understandable to others has been inspiring. Also, I would like to extend my sincere thanks to **Dr. Alice Rochard** for her hard work to negotiate with our partners. I am also thankful to **Thomas Vidal** for installing the Mini-Lab in Carnot and for his motivation even in difficult field context. I would like to express my deepest gratitude to our engineers, **Baptiste Boillot** and **Baptiste Le Corre**, who gradually became the two top best Information Technologies microbiologist. Their motivation to learn, the questions raised about the craziness of microbiology decision pathway and the hours spent to digest the expert rules, really impressed me. Also, without their administrative skills, we would have been lost in the MSF maze, I am really grateful of all our project assistants who efficiently supported all of us: **Delphine Berger, Melchior Atger, Marion Ducasse, Audrey Pâquet, Laura Velez and Marie-Hélène Nougaret**. I would also like to acknowledge all the laboratory staff of Mini-Lab in Haiti and in Carnot, **Madeleine Jean-Baptiste, Léonel Joseph, Majorie Benoit Georges, Guillaume Steve Junior Bero, Guershom Nguelem, Apollinaire Ndeomale**. And I thank them for the quality of their work and friendly welcome. I am also grateful to **Dr. Kerry Dierberg**, Infectious disease and IPC specialist, whom has been able to implement an Antibiotic Stewardship Program and re-enforced IPC measure in Carnot, a difficult context. I would be remiss

in not mentioning **Marie-Pierre Bourguignon, Emilie Viard-Gaudin, Sarah Coviaux** and **Lea Courtier, Virginie Segueineau** for their short but important part of this journey shared together.

Colleagues from the Steering Committee

My deepest gratitude goes to **Dr. Annette Heinzelmann** and **Stephane Roque**, the first chair of the committee and the first general director to actively support the project. I am really grateful for their trust and the initial spark that was lit up. I could not have undertaken this journey without the core group of the committee that gave use constant support and positive challenges, **Thierry Allafort, Remy Obert, Isabelle Mouniaman, Dr. Claire Mills, Dr. Claire Rieux, and Dr. Arlene Chua**. I'd like to acknowledge the other members of the steering committee without whom this project would have not been possible: **Dr. Catherine Van-Overloop, Dr. Northan Hurtado, Dr. Monica Rull, Dr. Mathieu Bichet, Dr. Cara Kosack, Dr. Tammam Aloudat, Dr. Esther Casas, Dr. Carolina Jimenez, Dr. Bern-Thomas Nyang'wa, Melissa Mc Rae, Dr. Bart Janssens, Dr. Rupa Kanapathipillai, Dr. Cécilia Ferreyra, Dr. Jorgen Stas-sijns**. Lastly, I am extremely grateful to MSF and our donors for having sponsored this project.

Colleagues From the Scientific Committee.

First of all, I am very much indebted to **Pr. Jan Jacobs** for sharing this journey through the wonderful world of blood cultures and conventional bacteriology. We had the same goal of simplifying clinical bacteriology laboratory for low-resource settings. His extensive knowledge of clinical bacteriology, both theoretical and practical, his attention to detail and scientific meticulousness have been essential to strengthen the science behind the Mini-Lab. I am as well extremely grateful to **Dr Olivier Vandenberg**, his constant motivation to negotiate with the steering committee, his continuous support and faith in the quality of our work has been stimulating to me. **Dr Céline Langendorf** and **Dr Thomas Kesteman**, the first generation of microbiologists to have installed a bacteriology Laboratory in Kabul. They are a source of inspiration for me, their sense of humour and their high level of expertise have been the best cocktail I ever tasted. Also, words cannot express my gratitude to **Dr. Waël Faroug M. Elamin**, whom we first met in South Sudan in 2003 on the front line, and connected again in 2017, on the frontline of AMR. I would also like to thank all the other members of the scientific committee for their voluntary commitment and precious hours of debate: **Pr. Antoine Andremont, Dr. David Dolinger, Dr. Liselotte Hardy, Dr. Sien Ombelet, Justine Michel, John Stelling, Maurice Page, Elsa Tran, Dr. Timothy Walsh, Dr. Nada Malou, Dr. Jane Cunningham, Dr. Stijn Deborggraeve, Dr. Tjalling Leenstra, Dr. Teri Roberts, Yuan Qiong Hu and Dr. Yves Gilles**.

Colleagues From ITM and LHUB-ULB

This endeavour would not have been possible without my co-Ph.D partner, **Dr Sien Ombelet**. I am really grateful for those moments shared together in Italy, Belgium or France and all the hard work to pursue experiments. I am also thankful to **Kaat Eggermont, Tine Vermoesen, Ellen Corsmit, Anna Bolzan** and **Marta Oller Sala**, for executing all experiments on the blood culture bottles, orientation test and identification tests. I would like as well to thank all other scientifics that I have met at ITM and who are inspiring to me in the daily fight for mankind: **Dr. Barbara Barbé, Dr. Anne Sophie and** all the team working around **Pr. Jacobs**.

Also, I would like to express my deepest gratitude to the personal of the LHUB-ULB in Brussels who opened their laboratory to test the first assembled prototype. A very special thank goes to the laboratory technicians that made this study possible **Johann Griselain, Dr. Emmanuel Busha Tibasima** and **Rodolphe Wery**.

Moral Support and Editing Help

Words cannot express my gratitude to my partner, **Dr. Karine Le Roch**, for her moral support, her constant attention over the years, for caring of our children while I was concentrating on my thesis or travelling the world. Also, her carrier has been inspiring to me and our discussions about her Ph.D. has motivated me to embark into this journey. I also could not have undertaken to properly write this thesis, without the editing help, late-night feedback sessions, and moral support provided by **Dr. David Dolinger**.

Colleagues From MSF

To my friends and colleagues of MSF, they are so many to acknowledge, I would like to express my deepest gratitude to **Claudie Pinosa, Eric Pujol, Isabelle Beauquesne**, and **Mareva Vaiho** for their constant support and intellectual investment to develop all trainings of the Mini-Lab. I would like as well to extend my sincere thanks to all colleagues of MSF at headquarters and in the field, at MSF logistic for the time they dedicated to the Mini-Lab project.

Study Sites Country Authorities And Study Personnel

This endeavour would not have been possible without **Dr. Céline Langendorf** from Epicentre (France), principal investigator of the evaluation in Drouillard and in Carnot, who wrote study protocols, coordinate study sites and analysed the results. I'd like to acknowledge **Dr. Valentina Carnimeo** who replaced Dr Langendorf during her maternity leave. I would also like to thank all the study personnel involved in the two field studies for their implications.

I am also thankful to **Haitian and Central African Republic Ministry of Health, Haitian and Central African Republic National Laboratory of Public Health**, for their authorisation and support to conduct the field evaluation in their countries.

Partners From Academic And The Industries

I am also thankful to our academic partners, **Améziane Aoussat, Fabrice Mantelet, Alexandre Licata, Céline Mateev**, and **Pierre Vedel** from the Laboratoire Conception Produits Innovant (ENSAM, Paris, France) who helped us to conceive the box bench, the layout organisation and the interface of the Mini-Lab. Thanks should also go to **Dr. Barbara Zimmer** from the Clinical Laboratory Standard Institute (USA) and to **Dr. Gunnar Kahlmeter** and **Dr. Erika Matuschek** from the European Committee on Antimicrobial Susceptibility Testing (Sweden) for their vital advises during the course of this project. I would also like to thank our partners from the industries who accepted to take the risk of following us into this journey, **Beckman Coulter** (West Sacramento, USA), **Autobio** (Zheng zhou, China), **Microbiologics** (Saint Cloud, USA), **JP Selecta** (Barcelona, Spain), and **Biomed** (White City, USA).

Last but not least, this endeavour would not have been possible without **all the patients recruited**, who took part in those studies and made this research possible.

Table Of Contents

ACKNOWLEDGEMENTS	1
TABLE OF CONTENTS	6
LIST OF PUBLICATIONS AND PRESENTATIONS	10
LIST OF TABLES AND FIGURES	12
ABBREVIATIONS	18
INTRODUCTION	22
1. ANTIBIOTIC RESISTANCE: A CONCERNING GLOBAL HEALTH ISSUE	22
1.1. UNDERSTANDING ANTIBIOTIC RESISTANCE	22
1.2. A PUBLIC HEALTH ISSUE WITH HEAVY CONSEQUENCES	23
1.3. A RISING CONCERN IN LOW RESOURCE SETTINGS	24
1.4. BLOODSTREAM INFECTION, FROM MALARIA TO BACTERIAL SEPSIS PARADIGM CHANGE	25
2. CLINICAL BACTERIOLOGY IN LOW-RESOURCE SETTINGS: A GAP ANALYSIS.....	29
3. BUILDING THE SPECIFICATIONS TO ADAPT THE CLINICAL BACTERIOLOGY LABORATORY FOR MSF FIELD INTERVENTIONS	32
3.1. MSF CLINICAL BACTERIOLOGY LABORATORY INTERVENTION PRIORITIES	32
3.2. DEVELOPING THE REQUIREMENTS OF THE MINI-LAB	33
SCIENTIFIC OBJECTIVES	43
RESULTS	49
CHAPTER 1- HOW TO DESIGN AND DEVELOP A SMALL-SCALE, STAND-ALONE AND TRANSPORTABLE CLINICAL BACTERIOLOGY LABORATORY?	49
1. INTRODUCTION	49
2. DESCRIPTION OF THE OVERALL DEVELOPMENT PROCESS.....	51
2.1. PROJECT GOVERNANCE AND PROJECT TEAM	51
2.2. DEFINING THE METHODS AND PROPOSED IMPROVEMENT.....	53
2.1. ENGAGEMENT WITH MANUFACTURERS AND SOURCING	55
2.2. PROOF OF PRINCIPLE, EVALUATION IN CONTROLLED ENVIRONMENT	55
2.3. FIELD PERFORMANCE EVALUATION	56
2.4. QUALITY MANAGEMENT SYSTEM, TRAINING MATERIALS	56
2.5. PRODUCTION AND MONITORING.....	57
3. BOX-BENCH PLATFORM TO PROTECT AND TRANSPORT SELECTED EQUIPMENT FOR RAPID SETUP.....	58
3.1. INTRODUCTION	58
3.2. DESCRIPTION OF THE DEVELOPMENT METHODOLOGY	58
3.3. DESCRIPTION OF THE FINAL PRODUCT	61
4. ADAPTATION, SIMPLIFICATION AND VALIDATION OF THE ANALYTICAL PROCESS	68
4.1. ANALYTICAL PROCESS DESCRIPTION	68
4.1.1.1. <i>Blood culture bottles (BCB)</i>	69
4.1.1.2. <i>Sub-culturing system</i>	70
4.1.1.3. <i>Pre-Identification system (pre-ID)</i>	71
4.1.1.4. <i>Identification (ID) system</i>	72
4.1.1.5. <i>Antibiotic Susceptibility Testing (AST)</i>	72
5. QUALITY MANAGEMENT SYSTEM	74
5.1.1. FACILITIES AND SAFETY.....	75
5.1.2. EQUIPMENT	76
5.1.3. PURCHASING AND INVENTORY	77
5.1.4. PROCESS CONTROL—SAMPLE MANAGEMENT	77
5.1.5. PROCESS CONTROL—QUALITY CONTROL / OCCURRENCE MANAGEMENT / PROCESS IMPROVEMENT.....	78
5.1.6. ASSESSMENT—AUDITS, EXTERNAL QUALITY ASSESSMENT, NORMS, AND ACCREDITATION	78
5.1.7. PERSONNEL / ORGANISATIONAL	79

5.1.8.	CUSTOMER SERVICE	80
5.1.9.	DOCUMENTS AND RECORDS	80
5.1.10.	INFORMATION MANAGEMENT	81
6.	DEVELOPMENT OF A MICROBIOLOGY DECISION SUPPORT SYSTEM: FOCUS ON THE PRE-ID EXPERTS SYSTEMS COMPONENT	82
6.1.	INTRODUCTION	82
6.2.	BRIEF DESCRIPTION OF THE DEVELOPMENT METHODOLOGY	83
6.3.	DESCRIPTION OF THE PRE-ID EXPERT SYSTEMS	87
6.3.1.	DESCRIPTION OF THE PRE-ID WORKFLOW	88
6.3.2.	PRE-ID BIOTYPE EXPERT SYSTEM	89
6.3.2.1.	<i>Pre-ID Biotype Knowledge database</i>	89
6.3.2.2.	<i>Inference engine</i>	92
6.3.3.	PRE-ID AND ID EXPERT RULES	98
6.3.3.1.	<i>Pre-ID and ID expert rules database</i>	98
6.3.3.2.	<i>Pre-ID and ID expert rules inference engine</i>	100
CHAPTER 2- ADAPTATION AND VALIDATION OF AN INNOVATIVE TECHNIQUE ADAPTED TO LRS CONSTRAINTS FOR THE SUBCULTURE OF BLOOD CULTURE BOTTLES		103
1.	INTRODUCTION	103
2.	CULTURE MEDIA FOR CLINICAL BACTERIOLOGY IN LOW- AND MIDDLE-INCOME COUNTRIES: CHALLENGES, BEST PRACTICES FOR PREPARATION AND RECOMMENDATIONS FOR IMPROVED ACCESS	105
3.	EVALUATION OF INTRAY CASSETTES DIRECTLY FROM BLOOD CULTURES FOR THE DIAGNOSIS OF SEPSIS IN CLINICAL BACTERIOLOGY LABORATORIES AS AN ALTERNATIVE TO CLASSIC CULTURE MEDIA	114
CHAPTER 3- WHAT ARE THE PERFORMANCES OF THREE NEW TYPES OF INNOVATIVE AST PANEL ADAPTED TO LRS?		129
1.	INTRODUCTION	129
2.	VALIDATION OF THREE MICROSCAN® ANTIBIOTIC SUSCEPTIBILITY TESTING (AST) PLATES DESIGNED FOR LOW RESOURCE SETTINGS	131
3.	EVALUATION OF THE BIOMIC VIDEO READER SYSTEM FOR DETERMINING INTERPRETIVE CATEGORIES OF ISOLATES USING MICROSCAN® ANTIBIOTIC SUSCEPTIBILITY TESTING (AST) MICROPLATE DESIGNED FOR LOW RESOURCE SETTINGS	159
3.1.	INTRODUCTION	159
3.2.	METHODS	161
3.2.1.	BIOMIC AUTOMATED SYSTEMS AND VISUAL READING	161
3.2.2.	EASE OF USE	162
3.2.3.	DATA ANALYSIS	162
3.3.	RESULTS	163
3.3.1.	MSF GRAM-POS PANEL READING METHODS RESULTS	163
3.3.1.	MSF GRAM-NEG PANEL READING METHODS RESULTS	164
3.3.1.	MSF GRAM-FAST PANEL READING METHODS RESULTS	165
3.3.2.	EASE OF USE	165
3.4.	DISCUSSION	166
3.4.1.	INTER-READING AGREEMENT	166
3.4.1.	EASE OF USE	167
3.4.2.	STRENGTHS AND LIMITATIONS OF THE STUDY	167
3.5.	CONCLUSION	168
CHAPTER 4- DOES A SIMPLIFIED CLINICAL BACTERIOLOGY LABORATORY PROVIDE QUALITY AND VALUE? ..		169
1.	INTRODUCTION	169
2.	FIELD EVALUATION OF PERFORMANCES OF THE MINI-LAB – PORT-AU-PRINCE BURN HOSPITAL, HAITI	171
2.1.	INTRODUCTION AND BACKGROUND	171
2.2.	METHODS	174
2.2.1.	STUDY DESIGN AND STUDY SITE	174
2.2.2.	STUDY PARTICIPANTS AND SAMPLES	174
2.2.3.	SPECIMEN COLLECTION AND TRANSFER TO THE MINI-LAB	174
2.2.4.	STORAGE OF BACTERIAL ISOLATES AND STRAIN TRANSFER	175
2.2.5.	DATA COLLECTION	175
2.2.6.	LABORATORY ANALYSIS IN THE REFERENCE LABORATORY	176

2.2.7.	DATA ANALYSIS	176
2.2.8.	ETHICAL CONSIDERATIONS	177
2.3.	RESULTS	178
2.3.1.	SAMPLES PROCESSED IN THE MINI-LAB	178
2.3.2.	EASE OF USE AND USABILITY OF THE MINI-LAB	180
2.3.3.	INTER-OPERATOR AGREEMENT ON BCB VISUAL READING	183
2.3.4.	PRE-ID AGREEMENT AND ACCURACY	183
2.3.5.	AGREEMENT AND ACCURACY OF ORGANISM IDENTIFICATION (ID).....	186
2.3.6.	AGREEMENT AND ACCURACY OF ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)	188
2.3.6.1.	<i>Antibiotics for Clinical Use</i>	188
2.3.6.2.	<i>Antibiotics Used for Surveillance</i>	194
2.4.	DISCUSSION.....	196
2.5.	LIMITS.....	198
2.6.	CONCLUSION	199
3.	EVALUATION OF PERFORMANCE AND INTEGRATION IN CLINICAL PRACTICE OF THE MINI-LAB PROTOTYPE VERSION 2 IN A CARNOT DISTRICT HOSPITAL, CENTRAL AFRICAN REPUBLIC	200
3.1.	INTRODUCTION AND BACKGROUND	200
3.2.	METHODS.....	202
3.2.1.	STUDY DESIGN AND STUDY SITE	202
3.2.2.	STUDY POPULATION.....	203
3.2.3.	DATA COLLECTION	203
3.2.4.	LABORATORY ANALYSES IN THE REFERENCE LABORATORY	204
3.2.5.	DATA ENTRY AND DATA ANALYSIS	205
3.2.6.	ETHICAL CONSIDERATIONS	206
3.3.	RESULTS	207
3.3.1.	DESCRIPTION OF STUDY POPULATION	207
3.3.2.	MINI-LAB KEY PERFORMANCE INDICATORS AND COMPARISON WITH LITERATURE DATA.....	209
3.3.3.	AGREEMENT OF MINI-LAB RESULTS VS REFERENCE METHODS OR COMPARATOR	215
3.3.3.1.	<i>Pre-Identification or Referral Diagnosis (Pre-ID)</i>	215
3.3.3.2.	<i>Identification (ID): Comparison to Reference Method and Comparator</i>	217
3.3.3.3.	<i>Antibiotic Susceptibility Testing</i>	221
3.3.3.4.	<i>Proportion of inconclusive results or difficult result interpretation in the Mini-Lab for pre-ID, ID and AST.</i> 225	225
3.3.4.	EASE OF USE AND USABILITY OF THE MINI-LAB	227
3.3.4.1.	<i>Ease of use</i>	227
3.3.4.2.	<i>Competency assessment</i>	228
3.3.4.3.	<i>Incident reporting</i>	229
3.3.5.	ROBUSTNESS AND USABILITY	230
3.3.6.	ANTIBIOTIC PRESCRIPTION PRACTICE OF CLINICIANS RELATED TO PATIENTS WITH POSITIVE BLOOD CULTURE(S) WITH CLINICALLY SIGNIFICANT PATHOGENS(S).....	230
3.3.7.	MICROBIOLOGICAL INDICATORS IN CARNOT DURING THE COURSE OF THE STUDY	234
3.3.7.1.	<i>Blood Culture Positivity Rate</i>	234
3.3.7.2.	<i>Pathogen Allocation per Age Group</i>	234
3.3.7.3.	<i>Antibiotic Resistance</i>	235
3.4.	DISCUSSION.....	236
3.5.	LIMITATIONS.....	240
3.6.	CONCLUSION	241
DISCUSSION:	242
CONCLUSION:	251
REFERENCES	253
ANNEXES	271
ANNEX 1 : CLINICAL BACTERIOLOGY IN LOW-RESOURCE SETTINGS: TODAY’S SOLUTIONS		271
ANNEX 2 : MINI-LAB EQUIPMENT ATLAS		282
ANNEX 3: BENCH AID DESCRIBING THE SAMPLING KIT		285

ANNEX 4: EVALUATION OF THE MINI-LAB CARNOT USING THE SLIPTA CHECKLIST.....	287
ANNEX 5: QC STRAINS IDENTIFICATION SHEET	288
ANNEX 6: LIST OF BACTERIA IDENTIFIED BY MINI-LAB SYSTEMS:.....	288
ANNEX 7: DETAILS OF THE MSF MICROSCAN AST PANELS:	290
ANNEX 8: SUMMARY OF VALIDATION STUDIES RESULTS, IN CONTROLLED ENVIRONMENT, OF THE MINI-LAB ANALYTICAL COMPONENTS.	291
ANNEX 9: SUMMARY OF THE VALIDATION STUDIES RESULTS OF THE MINI-LAB PRE-ID TEST	292
ANNEX 10: EXTRACT OF THE PRE-ID BIOTYPE DATABASE.....	293
ANNEX 11: EXTRACT OF PLATES READING ID NEG BENCH AIDS	294
ANNEX 12: EXTRACT OF PLATES READING AIDS ID NEG BENCH AIDS	295
ANNEX 13: SUPPLEMENTARY MATERIAL OF THE INTRAY PROOF OF CONCEPT	296
ANNEX 14: SUPPLEMENTARY MATERIALS OF THE INTRAY EVALUATION STUDY	297
ANNEX 15: MSF PRESCRIPTION CRITERIAS FOR BLOOD CULTURE COLLECTION	298
ANNEX 16: PROPOSAL OF GROUPING OF BACTERIAL SPECIES ACCORDING TO CLINICAL AND INFECTION CONTROL RELEVANCE [32]	302
ANNEX 17: GENERAL SCHEMATIC OVERVIEW OF THE MAIN MINI-LAB COMPONENTS DEVELOPED OR ADAPTED	306
ANNEX 18: COMPARISON OF THE MINI-LAB FINAL SPECIFICATIONS WITH THE INITIAL TARGET PRODUCT PROFILE.....	307
FUNDING AND CONFLICT OF INTEREST	318

List Of Publications And Presentations

Citation	Chapter
Sien Ombelet*, Jean-Baptiste Ronat*, Timothy Walsh, Cedric Yansouni, Janneke Cox, Erika Vlieghe, Jan Jacobs on behalf of the Bacteriology In Low Resource Settings Working Group. Published in: Lancet Infectious Diseases 2018;18(8):e248-58	Introduction
S. Ombelet, B. Barbé, D. Affolabi, J-B. Ronat, P. Lompo, O. Lunguya, J. Jacobs, L. Hardy, "Best Practices of Blood Cultures in Low- and Middle-Income Countries," <i>Front. Med.</i> , vol. 6, no. June, 2019, doi: 10.3389/fmed.2019.00131.	1
A. Natale, J-B. Ronat, A. Mazoyer, A. Rochard, B. Boillot, J. Hubert, B. Baillet, M. Ducasse, F. Mantelet, S. Oueslati, S. Ombelet, C. Langendorf, T. Naas, O. Vandenberg, J. Jacobs, "The Mini-Lab: accessible clinical bacteriology for low-resource settings", <i>The Lancet Microbe</i> , 2020 1,2,e56-e58,	1
S. Ombelet, A. Natale, J.-B. Ronat, O. Vandenberg, L. Hardy, and J. Jacobs, "Evaluation of MicroScan Bacterial Identification Panels for Low-Resource Settings," <i>Diagnostics</i> , vol. 11, no. 2, p. 349, Feb. 2021, doi: 10.3390/diagnostics11020349.	1
J-B. Ronat, A. Natale, T. Kesteman, A. Andreumont, W. Elamin, L. Hardy, R. Kanopathipillai, J. Michel, C. Langendorf, O. Vandenberg, T. Naas*, F. Kouassi,*; "AMR in low-resource settings: Médecins Sans Frontières bridges surveillance gaps by developing a turnkey solution, the Mini-Lab," <i>Clin. Microbiol. Infect.</i> , vol. 27, no. 10, pp. 1414–1421, 2021, doi: 10.1016/j.cmi.2021.04.015.	1
S. Ombelet , A. Natale, J-B. Ronat, T. Kesteman, O. Vandenberg, J. Jacobs, L. Hardy, "Biphasic versus monophasic manual blood culture bottles for low-resource settings: an in-vitro study," <i>The Lancet Microbe</i> , vol. 3, no. 2, pp. e124–e132, Feb. 2022, doi: 10.1016/S2666-5247(21)00241-X.	1
S. Ombelet, A. Natale, J. Ronat, O. Vandenberg, J. Jacobs, and L. Hardy, "Considerations in evaluating equipment-free blood culture bottles: A short protocol for use in low-resource settings," <i>PLoS One</i> , vol. 17, no. 4, p. e0267491, Apr. 2022, doi: 10.1371/journal.pone.0267491.	1
J-B. Ronat, "Revival of robust manual solutions for sepsis diagnostic and AMR surveillance in low-resource settings: Case study of Médecins Sans Frontières Mini-Lab" Oral presentation, Med Lab Congress, June 2021, Dubai.	1
J. Orekan, B. Barbé, S. Oeng, J.-B. Ronat, J. Letchford, J. Jacobs, D. Affolabi, L. Hardy, "Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access," <i>Clinical Microbiology and Infection</i> . 2021, doi: 10.1016/j.cmi.2021.05.016.	2
A. Natale, S. Oueslati, A. Rochard, D. Lopez-Baez, S. Ombelet, L. Hardy, J. Cunningham, , O. Vandenberg, C. Franquesa, J.-B. Ronat*, T. Naas*, "Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media," <i>Diagnostics</i> , vol. 13, no. 3, p. 523, Jan. 2023, doi: 10.3390/diagnostics13030523	2
J. B. Ronat, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vandenberg, T. Naas, "Validation of three MicroScan ® Antibiotic Susceptibility Testing microplates designed for Low Resource Settings Principal objective of this study was to verify the accuracy of the MicroScan Conclusions Our results indicate that performance of the 3," in Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse, 2020, poster presentation, CA-SFM.	3
J. B. Ronat, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vandenberg, T. Naas, "Evaluation of the PROMPT inoculation system on the MicroScan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings (LRS)," in Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse, 2020, poster presentation, CA-SFM.	3
J. B. Ronat, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vandenberg, T. Naas, "Validation of Three MicroScan ® Antimicrobial Susceptibility Testing Plates Designed for Low - Resource Settings," <i>Diagnostics</i> , vol. 12, no. 2106, 2022, doi: https://doi.org/10.3390/diagnostics12092106 .	3
J. B. Ronat, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vandenberg, T. Naas, "Evaluation of the BIOMIC Video Reader System for Determining Interpretive Categories of Isolates using Microscan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings)," in European Congress on Clinical Microbiology and Infectious Diseases, 2020, poster presentation, ESCMID	3

A. Natale, J-B. Ronat, A. Mazoyer, A. Rochard, B. Boillot, J. Hubert, B. Baillet, M. Ducasse, F. Mantelet, S. Oue-slati, S. Ombelet, C. Langendorf, T. Naas, O. Vandenberg, J. Jacobs, "The Mini-Lab: accessible clinical bacteriology for low-resource settings", <i>The Lancet Microbe</i> , 2020 1,2,e56-e58,	4
J-B. Ronat, A. Natale, T. Kesteman, A. Andreumont, W. Elamin, L. Hardy, R. Kanapathipillai, J. Michel, C. Langendorf, O. Vandenberg, T. Naas*, F. Kouassi*; "AMR in low-resource settings: Médecins Sans Frontières bridges surveillance gaps by developing a turnkey solution, the Mini-Lab," <i>Clin. Microbiol. Infect.</i> , vol. 27, no. 10, pp. 1414–1421, 2021, doi: 10.1016/j.cmi.2021.04.015.	4
J-B. Ronat, "Development of an all-in-one transportable clinical bacteriology laboratory: feedback from testing the Mini-Lab prototype in Haiti", MSF Scientific day, 16 Aug 2021, Online, Oral presentation	4
J-B. Ronat, « Development of an all-in-one transportable clinical bacteriology laboratory: feedback from testing the Mini-Lab prototype in Haiti", ASLM,24 Nov 2021, Online, Oral presentation	4
J-B. Ronat, "Early results of the Mini-Lab field evaluation, an all-in-one transportable clinical bacteriology laboratory for low-resource settings", ECCMID, 29 April 2022, Portugal, Oral presentations	4

List Of Tables And Figures

Tables

Table 1: Specifications set by the scientific committee of the Mini-Lab as of February 2016	34
Table 2: List of all the market reviews made between March 2016 and March 2017.....	53
Table 3: List of the items classified by the type of methodology used after sourcing or adaptation	54
Table 4: Description of the principal function and functions of the Mini-Lab Box-bench taking into consideration the different phases of the Mini-Lab production life cycle.....	61
Table 5 : Description of the trainings available for different target audiences intervening in the Operational Mini-Lab Life cycle	79
Table 6:Expected results found in the pre-ID knowledge database for Salmonella spp. organism	93
Table 7: Examples of messages triggered at the pre-ID step when a Gram-negative bacilli is observed at the microscope from a positive blood culture bottle	99
Table 8: Results of reading methods for Staphylococcus spp. and Enterococcus spp. using MICPOS1 panel.....	163
Table 9: Results of reading methods for Enterobacterales and Non-fermenting gram-negative bacilli. using MICNEG1 panel	164
Table 10: Results of reading methods for fastidious organisms (Haemophilus spp., Streptococcus spp.) using MICFAST1 panel.....	165
Table 11: Reference techniques in the reference laboratory.....	176
Table 12: Primary and secondary outcome of the study.....	177
Table 13: List of organisms identified in the blood cultures of patients included in the study	179
Table 14: Number of strains used for simulated blood cultures	180
Table 15: List of organisms sent from Bicêtre Laboratory (France) to the Mini-Lab in Haiti in February 2020	180
Table 16: Ease of use score (%) per section of the self-administered questionnaire for 2 laboratory technicians (lab tech 1 and lab tech 2) 4 times during the study period.....	181
Table 17: Analytical SOP compliance score (%) assessed by the microbiologist for 2 laboratory technicians (lab tech 1 and lab tech 2), twice during the study period	182
Table 18: Definition of minor and major errors for the interpretation of the results obtained by methylene blue staining as part of pre-ID analyses	184
Table 19: Inter-operator agreement and agreement with the reference test result for pre-ID testing of the isolated strains of clinical samples or simulated blood cultures	185
Table 20: List of discrepancies between pre-ID and final identification.....	185
Table 21: Inter-operator agreement and agreement with reference methods for the identification of isolated organisms from simulated or patient samples, depending on the type of organism (Neg=Gram-negative; Pos=Gram-positive, except for Streptococcus.....	187
Table 22: Criteria used for the definition of extended spectrum beta-lactamase (ESBL) and Methicillin-resistant Staphylococcus aureus (MRSA) from the minimal inhibitory concentration (MIC) results according to EUCAST and AST Panel manufacturers (Beckman Coulter)	188
Table 23: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-negative AST panel for antibiotics used in treatments	189
Table 24: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-positive AST panel for antibiotics used in treatments	191

Table 25: Inter-operator agreement and agreement with reference methods of the results obtained on fastidious organism AST panel for antibiotics used in treatments.....	193
Table 26: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-negative, Gram-positive and fastidious organism AST panels for antibiotics used for monitoring.....	195
Table 27: Methods in the Mini-Lab and in the reference laboratory	204
Table 28: Primary and secondary outcome of the study	205
Table 29: Participants characteristics, N=835	209
Table 30: Range of pathogens isolated in the Mini-Lab during the study period, n=124	210
Table 31: List of isolated organisms in the Mini-Lab considered as contaminants by physicians during the course of the study, n=176.....	212
Table 32: Proportion of contamination per month, number of bottles, n=150	213
Table 33: Proportion of contamination per prescriber	213
Table 34: Filling volume of blood culture bottles per age range	214
Table 35: Summary of the Mini-Lab Key Performance Indicators	215
Table 36: Percentage of agreement of respective pre-ID tests performed at the Mini-Lab versus reference laboratories utilizing identical techniques and reagents.....	216
Table 37: Comparison of InTray Colorex medium results between the Mini-Lab and Bicêtre	216
Table 38: Details concerning the 11 InTray Colorex result discrepancies between the Mini-Lab and Bicêtre	217
Table 39: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory having a possible impact on clinical significance.....	218
Table 40: Description of cases of contaminant identification discrepancies between the Mini-Lab and the reference laboratory having a possible impact on clinical significance.....	219
Table 41: Genus and species agreement comparing Mini-Lab results to MALDI-TOF and to MSF Neg/Pos ID Panel Type 2 plates (Beckman-Coulter) in the reference laboratory for the identification of organisms depending on the type of organism (Gram-negative; Gram-positive)	220
Table 42: Description of cases of pathogen identification discrepancies between ID plates in the Mini-Lab and ID plates in the reference laboratory (comparator) vs reference method .	220
Table 43: Agreement with reference methods of the results obtained on Gram-negative AST panel for antibiotics used for treatment	221
Table 44: Agreement between the results obtained on the Gram-positive AST panel in the Mini-Lab for Staphylococcus sp. and AST reference methods for antibiotics used for treatments.	222
Table 45: Agreement between the results obtained on the Gram-positive AST panel (Beckman-Coulter) in Bicêtre for Staphylococcus sp. and the reference methods for antibiotics with major errors between the Mini-Lab results and the reference method.....	222
Table 46: Agreement with reference methods of the results obtained on Gram-positive AST panel for Enterococcus sp. for antibiotics used for treatment.....	223
Table 47: Agreement with reference methods of the results obtained on fastidious microorganism AST panel for Streptococcus sp and Haemophilus sp. for antibiotics used for treatment	224
Table 48: . Criteria used for the definition of extended spectrum beta-lactamase (ESBL) from the minimal inhibitory concentration (MIC) results as per EUCAST and AST plate manufacturer (Beckman-Coulter) recommendations.....	224
Table 49: Agreement with reference methods of the results obtained on the various AST panels for antibiotics used for surveillance	225
Table 50: Proportion of repeated/ reread pre-ID tests and reasons.....	225

Table 51: Proportion of repeated ID plates and reasons	226
Table 52: Proportion of repeated AST plates and reasons.....	226
Table 53: Proportion of inconclusive results or difficult result interpretation for pre-ID, ID and AST.....	227
Table 54: Ease of use score (%) per section of the self-administered questionnaire for 3 laboratory technicians 3 times during the study period	228
Table 55: Analytical POS competency assessment score (%) evaluated by the microbiologist for 3 laboratory technicians, three times during the study period.....	228
Table 56: Empirical antibiotic treatment received for 105 participants with pathogen positive blood cultures.....	231
Table 57: Proportion of pathogen positive blood cultures based on age groups and prescribers	234
Table 58: Isolated Pathogen Allocation per Age Group(n=121)	235
Table 59: Cost comparison between the Mini-Lab and MSF conventional clinical bacteriology laboratory as of January 2023, this cost does not include the price of transportation from MSF Logistique in Bordeaux (France) to another country	249
Table 60: table describing the formulation of the different InTray agar evaluated	296
Table 61: table describing the formulation of reference agars used during the different studies	296
Table 62: percentage and number of subcultures done that show growth on InTray and BD chocolate agar, with comparison between CO2 incubator and normal incubator and at different times of incubation.....	297
Table 63: percentage of strains growing consistently on InTray and BD chocolate agar (growth on all replicate subcultures), with comparison between CO2 incubator and normal incubator and at different times of incubation	297
Table 64: Proposal of grouping for Gram negative bacteria: enteric bacteria.....	302
Table 65: Proposal of grouping for Gram negative bacteria: others.....	303
Table 66: Proposal of grouping for Gram positive bacteria: streptococci	304
Table 67: Proposal of grouping for Gram positive bacteria: others.....	305

Figures

Figure 1 : Schematic of the development, spread, drivers and tools for the mitigation of AMR. (Zellweger et al. 2017).....	23
Figure 2: Courtesy of Valérie D’Acremont, Swiss TPH on the result from the study done in Tanzania among patients attending OPD with Severe febrile illness. The figure presents the distribution of illnesses by pathogen type (bacterial, viral, or parasitic) found among 227 children tested (D’Acremont, et al., 2014, NEJM.	25
Figure 3: Schematic representation of the cascade from entry site of the micro-organisms toward bloodstream infection and sepsis, adapted from Delano et al. 2016, Fujishima et al. 2016 and Codina et al. 2022.	27
Figure 4: Percentage of all deaths related to sepsis, age-standardised for both sexes, in 2017 (B), extracted from the article of Rudd et al., 2020, The Lancet [49]	28
Figure 5: Figure presenting the development life cycle of the Mini-Lab project with overall phases.....	51
Figure 6: Description of the important phases of the Mini-Lab design and development, MSF	52
Figure 7: BSL3 laboratory in a maritime 41-foot container, IMEBIO (France).....	58
Figure 8 : Standard methodology pathway description used by the LCPI for product development.....	59

Figure 9: artist's view on the capacity to transport the Mini-Lab component on the standard Toyota 4x4, Land Cruiser pick-up, STRATE College (France).....	59
Figure 10: Creativity session at ENSAM laboratory with members of the scientific committee to tailor the laboratory workflow per modules, Mini-Lab (France).....	60
Figure 11: Test of the wooden mock up box bench at MSF logistique in Bordeaux (France) with logisticians, Mini-Lab (France)	60
Figure 12: Life cycle of the Mini-Lab from a production point of view, LCPI (France).....	61
Figure 13: Highlight of the scenario of usage regarding the sample processing part of the laboratory workflow, Mini-Lab (France).....	63
Figure 14: Poster developed for the MSF General Assembly, 2022, highlighting the results of the Mini-Lab box-bench development, Mini-Lab (France).....	64
Figure 15: 3D modelling of the (A) Box-bench in close position and (B) in open position ready to provide adequate space for laboratory workers, Mini-Lab (France)	65
Figure 16: Photo of the Box-bench roto moulding pre-series test, Mini-Lab (France).....	65
Figure 17: Schema of the Mini-Lab modules identifications and functions, Mini-Lab (France)	66
Figure 18: Photo of the different modules when installed. Modules are presented from module 1 to module 6 from top left to right, Mini-Lab (France).....	66
Figure 19: Configuration layout extracted from the document DOC-2.1-AMENAG, Mini-Lab (France).....	67
Figure 20 : Scheme of the analytical workflow in the Mini-Lab. Description of analytical components and overview of performance, Mini-Lab (France)	69
Figure 21: Biphasic blood culture bottle, Autobio diagnostic, China, Mini-Lab (France).....	70
Figure 22: Artist drawing of InTray Chocolate agar being inoculated with loop on top, photo of a closed InTray cassette on bottom, Mini-Lab (France)	70
Figure 23: Artist drawing of the pre-ID testing steps (Gram straining, aminopeptidase, catalase, oxidase), Mini-Lab (France)	71
Figure 24: MSF Neg/Pos ID Panel type 2 and Pastorex latex kit, Mini-Lab (France).....	72
Figure 25: MSF Microscan AST panels (see further description in Chapter 3), Mini-Lab (France).....	73
Figure 26: Snapshot of the QMS Mini-Lab Tool Box structure as available, snapshot taken on the 03 rd February 2022, Mini-Lab (France)	74
Figure 27: Operational Life cycle of the Mini-Lab installation on MSF fields. This life cycle does not take into account the production cycle, Mini-Lab (France).	75
Figure 28: Laboratory technician in Carnot MSF performing routine testing under supervision of a trainer microbiologist, October 2021, Mini-Lab (France).....	77
Figure 29: Schematic view of the Mini-Lab concept, a deployable Clinical Bacteriology Laboratory developed to provide access to blood culture capacities in difficult to reach settings, Mini-Lab (France).....	81
Figure 30: Schematic representation of an expert system. According to Rhoads et al. « An expert system is a form of artificial intelligence that allows a user to use software rules (inference engine) together with a knowledge database to make a conclusion (output) about an input » [114].....	82
Figure 31: Diagram of the Mini-Lab blood culture workflow, extract of the document DOC-9.1.1-HEMLOGI V1, 16/11/2021, page 1, Mini-Lab (France)	84
Figure 32: Mini-Lab pre-ID decision tree for bacilli, this algorithm does not take into consideration growth information obtained from the BCB. Extracted from the document DOC-10.10-PIDADEC, V1.1, 10/08/2022, page1, Mini-Lab (France)	86
Figure 33: Diagram describing the different layers of the expert systems (red rectangle indicated with numbers 1 to 8) assembled as a unique microbiology decision support system (all diagrams	

in the light red rectangle) and embed into the Mini-LIMS (all diagrams in the blue rectangle, Mini-Lab (France).....	88
Figure 34: Snapshot of the mock-up database for Pre-ID tests are fixed, but this can be modified, added to or items removed by the Mini-LIMS administrator, Mini-Lab (France).	90
Figure 35: Snapshot of the pre-ID mock-up database, the top snapshot presents the part of (i) morphotypes class and bottom snapshot presents the (ii) specific morphotypes, Mini-Lab (France).....	91
Figure 36: Results provided by the inference engine for the example used for a gram negative, aminopeptidase positive, oxidase positive bacilli, Mini-Lab (France).	92
Figure 37: Cascade calculation results done by the inference engine for gram negative bacilli, aminopeptidase positive, oxidase positive test results. Extract from the actual Mini-LIMS interface, Mini-Lab (France).....	96
Figure 38: Cascade calculation results done by the inference engine for a gram-negative bacillus, aminopeptidase positive, oxidase positive and colour of colonies white on Colorex, test results entered. Extract from the mock-up interface, Mini-Lab (France).....	98
Figure 39: Extract of the report form provided to clinicians with the different sections were the sentences are automatically filled by the expert system, Mini-Lab (France)	101
Figure 40: Schematic overview (A) and different perspectives (B-D) of the cell phone based ELISA colorimetric reader. Sample images (E), and sample plate(F). Rows and columns are labelled in (E) to correspond with the plate in (F), UCLA (USA).	160
Figure 41: Bliomic Video Reader System, Giles Scientific (USA).....	160
Figure 42: Photo of the MICNEG1 panel reading by: (a) picture extract taken by Biomic V3 picture in the internal cabinet; (b) microplate viewer box by JP Selecta, used for visual reading with interchangeable background (black to white) [26], Mini-Lab (France)	166
Figure 43: Photo taken during the MVP testing in February 2019. From top to down, all modules in closed position, opening of a module, Tetris like assembly in a module, module assembled, laboratory technician working on a module, Mini-Lab (France).....	171
Figure 44: Photo of the emergency entrance of the MSF Burn centre, Drouillard, Haiti; © Lunos Saint Brave / MSF.....	174
Figure 45: Deployment of the Mini-Lab V1 by the logistician team, Mini-Lab (France).....	178
Figure 46 : Training of the laboratory technicians at the Mini-Lab facility, Mini-Lab (France)	178
Figure 47: Competency testing final score assessed by the microbiologist for 2 laboratory technicians 4 times during the study period.....	182
Figure 48: Photo of the microplate viewer box and the attached camera to take the adequate picture and transfer it into the Mini-LIMS, Mini-Lab (France).....	201
Figure 49: Snapshot of the Mini-LIMS interface to calibrate the reading of ID plate on top and for the AST plates down, Mini-Lab (France)	201
Figure 50: Top to down, from transportation, installation of the Mini-Lab, training of laboratory technicians and treatment of the patient at Carnot Hospital, Central African Republic, Mini-Lab (France).....	202
Figure 51: Study flow chart of the study population	208
Figure 52: List of pathogens isolated in the Mini-Lab during the study period, N=124	211
Figure 53: List of isolated contaminants by the Mini-Lab during the study period, N=176. 213	
Figure 54: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory.....	218
Figure 55: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory.....	219
Figure 56: Final score (%) of the ease of use by a technician 3 times during the study period	228

Figure 57: Final score (%) of competency assessment per technician 3 times during the study period	229
Figure 58: Change of antibiotic treatments depending on the bacteriological results	232
Figure 59: Result of PPS survey as per MSF protocol among 97 patients chart reviewed between November and December 2021 for empirical antibiotic therapy.....	233
Figure 60: Result of PPS survey as per MSF protocol among 97 patients chart reviewed between November and December 2021 for patient therapeutic evaluation at 48h and 72 hours of hospitalisation	233
Figure 61: Box-Bench ready to be assembled in MSF Logistic warehouse, Bordeaux, France	314
Figure 62: Module 6, waste management installed in Carnot Hospital, Central African Republic	315

Abbreviations

°C	Degree Celsius
AA	Antimicrobial Agent
AMC	Amoxicillin + Clavulanic acid
AMI	Amikacin
AMIES W/C	AMIES transport medium with coal
AMNP	Aminopeptidase test strips
AMP	Ampicillin
ARS	Assisted Reading System
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Clone Colony
AWB	Air Waybill
BA	Bench Aid
BCB	Blood Culture Bottle
CAT	Catalase
CCV	Ceftazidime + Clavulanic Acid
CE-IVD	European Union directive on in-vitro diagnostic medical devices
CFU	Colony-Forming Unit
CfxS	Cefoxitin screen
CHL	Chloramphenicol
CIP	Ciprofloxacin
CLI	Clindamycin
CLSI	Clinical and Laboratory Standards Institute
CNS	Coagulase-negative <i>Staphylococcus</i>
COAG	Coagulase test
COL	Colistin
CR	Clinical Report
CRAB	Carbapenem-resistant <i>Acinetobacter baumannii</i>
CRE	Carbapenem Resistant Enterobacteria
CRO	Ceftriaxone
CTA	Cefotaxime
CTXAC	Cefotaxime + Clavulanic Acid
CTZ	Ceftazidime
D0	The first day of blood culture bottle (BCB) incubation.

D1	The day after the first overnight incubation at 35°C.
DAP	Daptomycin
DGR	Dangerous Goods Regulations shipper declaration
Dp	Day of positivity
EQC	External Quality Control
ERT	Ertapenem
ERY	Erythromycin
ESBL	Extended Spectrum Beta-Lactamase
ETA	Estimated Time of Arrival
ETD	Estimated Time of Departure
EUCAST	European Committee for Antimicrobial Susceptibility Testing
FDA	U.S. Food and Drug Administration
FEFO	First Expired, First Out
FOS	Fosfomycin
GCP	Good Clinical Practice
GEN	Gentamicin
GLP	Good Laboratory Practice
GmS	Gentamicin (high level)
H-IND	Indole reagent for HNID use
HAS	Hydroalcoholic solution
HNID	Identification plate for Haemophilus and Neisseria
HNID-B	HNID broth
HR	Human Resources
HSE	Hygiene, Safety, Environment
HTM	<i>Haemophilus</i> Test Medium (Broth)
IATA	International Air Transport Association
Icd	Inducible clindamycin resistance
ID	Identification
ID No.	Identification Number
IFU	Instructions For Use
IMI	Imipenem
IND	Indole reagent
IQC	Internal Quality Control
IRPA	Imipenem and ceftazidime resistant <i>Pseudomonas aeruginosa</i>
ISO	International Organization for Standardization
IVD	In Vitro Diagnostic
LEV	Levofloxacin

LHB	LHB broth
LIMS	Laboratory Information Management System
LIN	Linezolid
MAJ	Major Error
MCQ	Multiple Choice Questionnaire
MDRO	Multi-Drug Resistant Organisms
MEM	Meropenem
MF	Mac Farland
MIC	Minimum Inhibitory Concentration
MIC FAST	Antibiotic susceptibility testing plate for fastidious organisms
MIC NEG	Antibiotic susceptibility testing plate for Gram negative bacteria
MIC POS	Antibiotic susceptibility testing plate for Gram positive bacteria
MIN	Minor error
Mini-LIMS	Mini-Lab Laboratory information system
MIO	Mobile Implementation Officer
mn(s)	Minutes(s)
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSDS	Material Safety Data Sheet
MSF	Médecins Sans Frontières
MTA	Material Transfer Agreement
NA	Non Applicable
NC	Negative Control
NC	Non-compliance
NEG/POS ID	Identification plate for Gram positive and Gram negative bacteria
NIT1	Nitrite 1 reagent
NIT2	Nitrite 2 reagent
OX	Oxidase
OXA	Oxacillin
PARC	<i>Pseudomonas aeruginosa</i> Resistant to Ceftazidime
PEN	Penicillin
PEP	Peptidase reagent
PEP	Post Exposure Prophylaxis
PI	Packing Instructions
PIT	Piperacillin + Tazobactam
PPE	Personal Protective Equipment
Pre-ID	Pre-identification tests
PROMPT	Prompt inoculation system

QA	Quality Assurance
QC	Quality control
QUD	Quinupristin + Dalfopristin
RENOK	MICROSCAN RENOK REHYDRATOR
RT	Room Temperature
RUO	Research use only
SC	Subculture
SOP	Standard Operating Procedure
TD	Template document
TDA	Tryptophan Deaminase Reagent
TEI	Teicoplanin
TET	Tetracycline
TFG	Thymidine-free growth well
TIG	Tigecycline
TLB	Tool Box
TMR	Task, Maintenance, Recording
TRS	Trimethoprim + Sulfamethoxazole
UN	United Nations
VAN	Vancomycin
VMJ	Very Major Error
VP1	Voges-Proskauer 1 reagent
VP2	Voges-Proskauer 2 reagent
VRE	Vancomycin Resistant Enterococci
VRSA	Vancomycin Resistant <i>Staphylococcus aureus</i>

Introduction

1. Antibiotic Resistance: a concerning global health issue

1.1. Understanding Antibiotic Resistance

Antibiotics were a large medical innovation of the past century. First identified was penicillin, in 1928 by Alexander Fleming[1]. Since then, multiple other antibiotics have been discovered and developed, which has improved medicines significantly[2]. Anti-microbial resistance among bacteria is a natural and evolutionary process where bacteria develop mechanisms to resist the effects of antimicrobial drugs. This can happen through genetic mutations or the acquisition of resistance genes from other bacteria through horizontal gene transfer. [3], [4].

Since the human discovery of antibiotics, civilisation has disrupted the delicate balance between organisms producing antibiotics and microorganisms, promoting the selection of resistance [5]. In humans, the drivers of antibiotic resistance are complex and multi-factorial, especially in Lower- and Middle-income countries (LMICs), but are also not absent in High Income Countries (HICs)[6]. These drivers and factors include: (i) inappropriate socio-ecological behaviours, (ii) poverty; overcrowding, (iii) lack of surveillance systems, (iv) food supply chain safety and control issues, (v) highly contaminated waste effluents and poor hygiene, (vi) loose rules and regulations on antibiotics importations and distributions, (vii) irregular access to antibiotics, and (viii) inappropriate prescriptions of antimicrobials [7]. Use of antibiotics in food and companion animals, fish, and the environmental resistance gene pool, are also pointed out as key players in to the ever growing burden of AMR[8]. Veterinary hospitals dedicated to pets have cropped up, especially in urban areas of LMICs where use of antimicrobials has also been increased substantially[9]. Small-scale backyard farming systems are being replaced by industrial food animal production (IFAP), due to the rising demand for animal protein in LMICs[9]. The lack of stringent regulations and monitoring and increases in the non-therapeutic use of antimicrobials in industrial farms has led to the persistence of AMR microorganisms. Associated with multiple other factors, such as co-resistance, cross-resistance, bacterial fitness, mixing of new and old animals, and vectors or reservoirs of bacterial infection is causing a significant change the global dysbiosis of eco-epidemiology[9], [10].

AMR is an “ecosystem related” problem, which threatens the interplay of human, animal and environmental health (“One Health”). Resistant bacteria arising in one geographical area can spread via cross-reservoir transmission to other areas worldwide either by human-to human transmission, and /or exposure through the food chain and/or environmental contamination[11] (Figure 1).

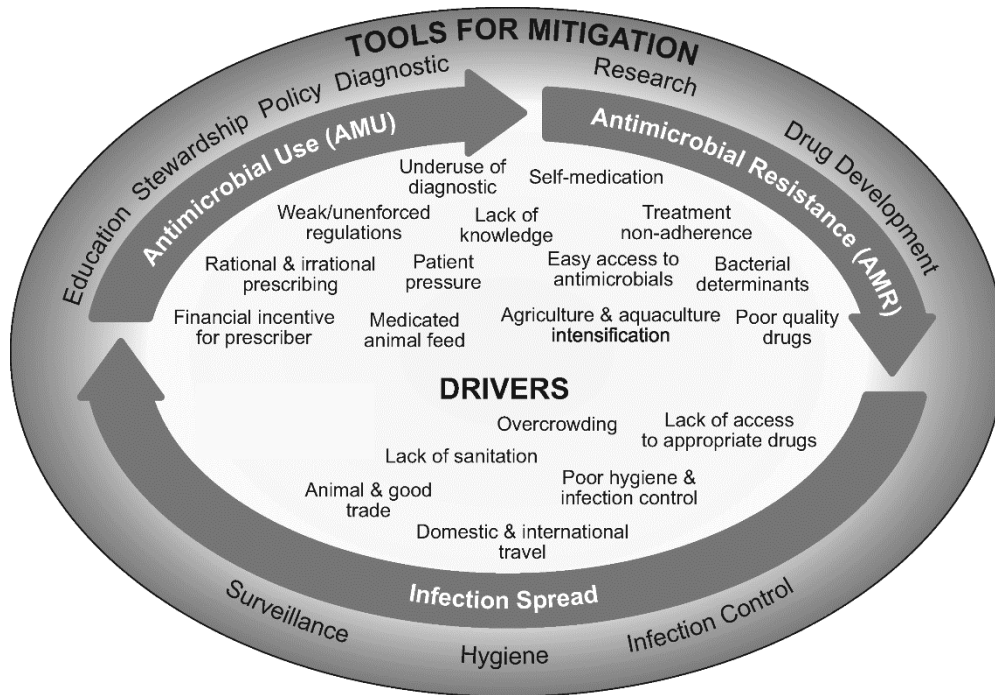


Figure 1 : Schematic of the development, spread, drivers and tools for the mitigation of AMR. (Zellweger et al. 2017)

1.2.A Public Health Issue with Heavy Consequences

Antimicrobial resistance (AMR) is today universally recognised as a global health threat, because of the rapid emergence and dissemination of resistant bacteria and genes among humans, animals and the environment on a global scale and represents a heavy burden for healthcare systems all over the world [12]–[14]. This basically means that standard antibiotic treatment may no longer work, and the subsequent control of infections will be harder if not impossible. As some infections no longer respond to treatments, there is a bigger risk of dying from infections, especially for those who are immunocompromised but not only[14]–[16].

From an economic point of view, antibiotic resistance leads to longer stays in hospital and longer bouts of illness and, consequentially, higher costs for healthcare providers[17]. The increase in AMR is widely considered a public health emergency, as so-called “superbugs” are emerging that can no longer be treated effectively with currently available antibiotics. This has led to warnings by the World Health Organization (WHO)

of the arrival of a “post-antibiotic era”, in which a simple wound infection or pneumonia could become fatal[18].

According to WHO, this situation threatens to set back the achievements of modern medicine. A post-antibiotic era, in which common infections and minor injuries could kill, can be possible[18]. In 2015, antibiotic resistance was estimated to be responsible for approximately 700 000 deaths with a dire prediction of up to 10 million AMR associated deaths by 2050[19]. In a recent systematic study published in the Lancet, Murray, et al., estimated that there were 4.95 million (95% CI 3.62–6.57) deaths associated with bacterial AMR in 2019. This includes 1.27 million (95% CI 0.911–1.71) deaths attributable directly to bacterial AMR[13]. At the regional level, it has been estimated that the all-age death rate attributable to AMR is the highest in western sub-Saharan Africa.[13]

1.3. A Rising Concern in Low Resource Settings

The impact of AMR on morbidity and mortality has been described being higher in LMICs [20]–[24]. The recent study performed by Murray et al. as part of the Global Research on Antimicrobial Resistance Project (GRAM) project, mentioned that AMR all-age death rates were highest in LMICs versus HIC, making AMR not only a major health problem globally but a particularly serious problem for some of the poorest countries in the world[13]. Also, the WHO report from the global antimicrobial resistance and use surveillance system (GLASS), showed that in LMICs, 58.3 % of the *Escherichia coli* are resistant to third-generation cephalosporin as compared to 17.5% in HICs. For *Staphylococcus aureus*, 33% were methicillin resistant in LMICs as compared to 15% in HICs[24].

The situation in LMICs is further aggravated by a lack of accurate information from rural areas and the over emphasis on urban collected data concerning the extent of AMR, especially in sub-Saharan Africa[25], [26]. Uncontrolled use of antibiotics, substandard dosing, substandard quality of antimicrobials, higher incidence of healthcare-associated infections, population displacement, lack of veterinary oversight in antimicrobial use in agriculture, insufficient access to safe drinking water and sanitation facilities, and limited diagnostic facilities are factors likely contributing to higher rates of AMR in LMICs [11], [12], [20], [27]–[30]. Despite impressive economic growth in Asia and even in many African countries, most LMICs on these continents constitute so-called low-resource settings (LRS); these are settings with limited clinical laboratories and other healthcare resources[31]. Most of these settings are in low-income countries, but also in remote or rural areas in middle-income countries. Not all laboratories in LMICs constitute a low-resource setting: some tertiary hospitals in capital cities may be

well equipped and financed. Thus, for the rest of our presentation, the use of LRS will refer to secondary care or district hospitals in LMICs which have limited diagnostic facilities and capabilities[32]. Also, in some countries we do acknowledge that some tertiary care or regional hospital might fall under the definition of LRS, because of lack of functioning microbiology laboratories.

1.4. Bloodstream infection, from malaria to bacterial sepsis paradigm change

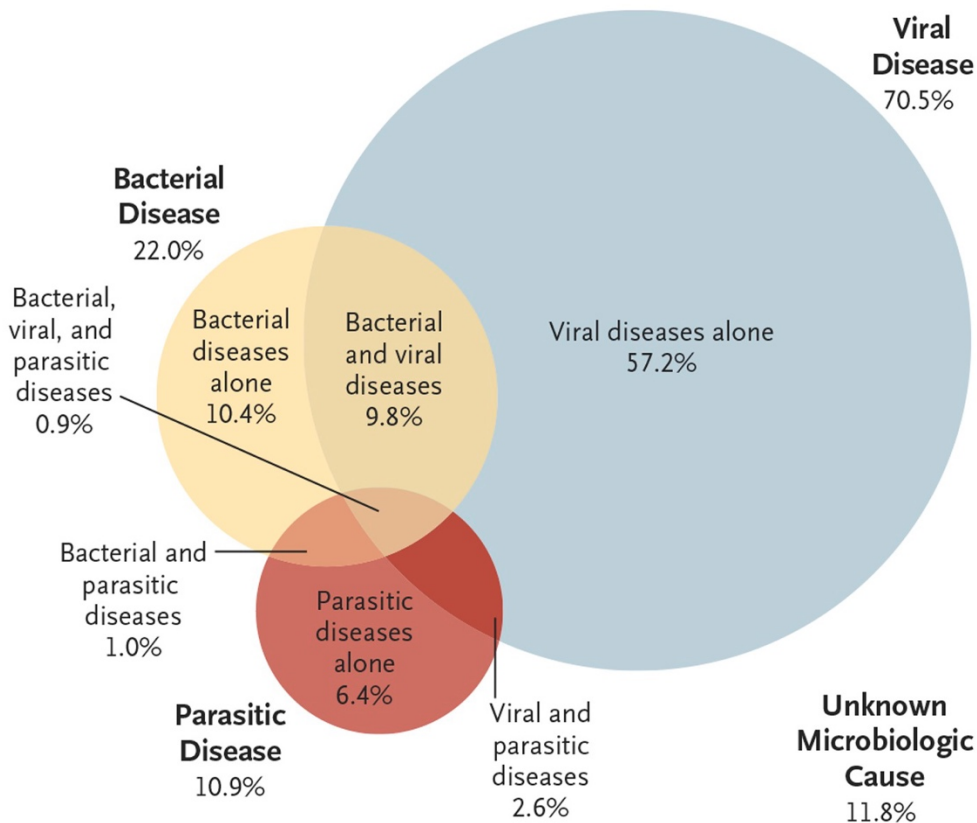


Figure 2: Courtesy of Valérie D'Acromont, Swiss TPH on the result from the study done in Tanzania among patients attending OPD with Severe febrile illness. The figure presents the distribution of illnesses by pathogen type (bacterial, viral, or parasitic) found among 227 children tested (D'Acromont, et al., 2014, NEJM).

With the amendment, in 2010, of the WHO acute fever case management algorithm, from presumptive antimalarial treatment to parasite-based diagnosis for all populations in endemic areas and the introduction of affordable malaria antigen-based rapid diagnostic tests (RDTs)[33] a paradigm shift in the management of febrile illnesses in malaria-endemic countries has occurred. Historically, malaria was considered, by default, the principal cause of fever, and presumptive empiric antimalarial therapy was a widespread policy and practice[33]. A new paradigm has emerged based upon a study carried out by Valerie D'Acromont in Tanzania in 2011. In the study samples were

collected from children attending the Outpatient Department (OPD) with febrile illnesses. The researchers showed that most fevers were related to viral infections and only 10% were due to parasitic infections. Surprisingly, it was shown that 22% were due to bacterial infections of the bloodstream[34].

While challenges remain for malaria RDT implementation, the availability of relatively simple, reliable, and accessible point-of-care tests means that it has become more straightforward to rule malaria out of a differential diagnosis. Since the clinical presentations of febrile episodes are often non-specific, definitive diagnosis requires an array of laboratory tests, many unavailable at the point of care[35]. Nevertheless, when and where testing is available, a large proportion of individuals with fever remain undiagnosed. In many African countries, diagnostic facilities are limited, and surveillance networks are often clustered around research institutions, leaving wide geographic swathes with no data on the aetiologies of febrile illnesses[36]. In the absence of reliable data, health care providers often resort to the prescription of empiric antimicrobial therapies, potentially promoting the emergence and spread of AMR[36]–[38]. This has propelled the improvement of fever case management into the limelight as a global health priority, with a recent proliferation of articles describing aetiologies of non-malarial febrile illnesses (NMFI) in LMICs[35]–[38]

A proportion of the febrile illnesses become severe febrile episodes of which invasive bacterial infections, namely bloodstream infections, severe pneumonia, and meningitis, continue to be leading causes of morbidity and mortality[26], [39]–[42]. Severe localised or systemic infections can cause micro-organisms to enter the bloodstream via the lymphatic system. This presence of bacteria in the bloodstream is called “bacteraemia”. Most of the time, these bacteria are cleared quickly by the immune system. In the case of overwhelming infections or intravascular focus of infection, the immune system may be unable to clear the bacteria from the blood, resulting in a bloodstream infection (BSI) [43] which can lead to sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to bloodstream infection and to death[44]–[47]. Infections that result in excess mortality are the most natural initial direction to focus on in LRS for Médecins Sans Frontières (MSF) related interventions.

In the first WHO report on the global epidemiology and burden of sepsis, published in 2020 [48], it is mentioned that sepsis mortality, a final common pathway to death for severe infectious diseases, but which can also arise as a complication of injuries and non-communicable diseases, is responsible for an estimated 20% of all-cause global deaths [48]. Sepsis disproportionately affects neonates, pregnant or recently pregnant women, and people living in low-resource settings. It was highlighted that the current

understanding of the epidemiology of sepsis is limited, especially where the burden is highest, and is also hampered by poor data quality, especially in LRS[48].

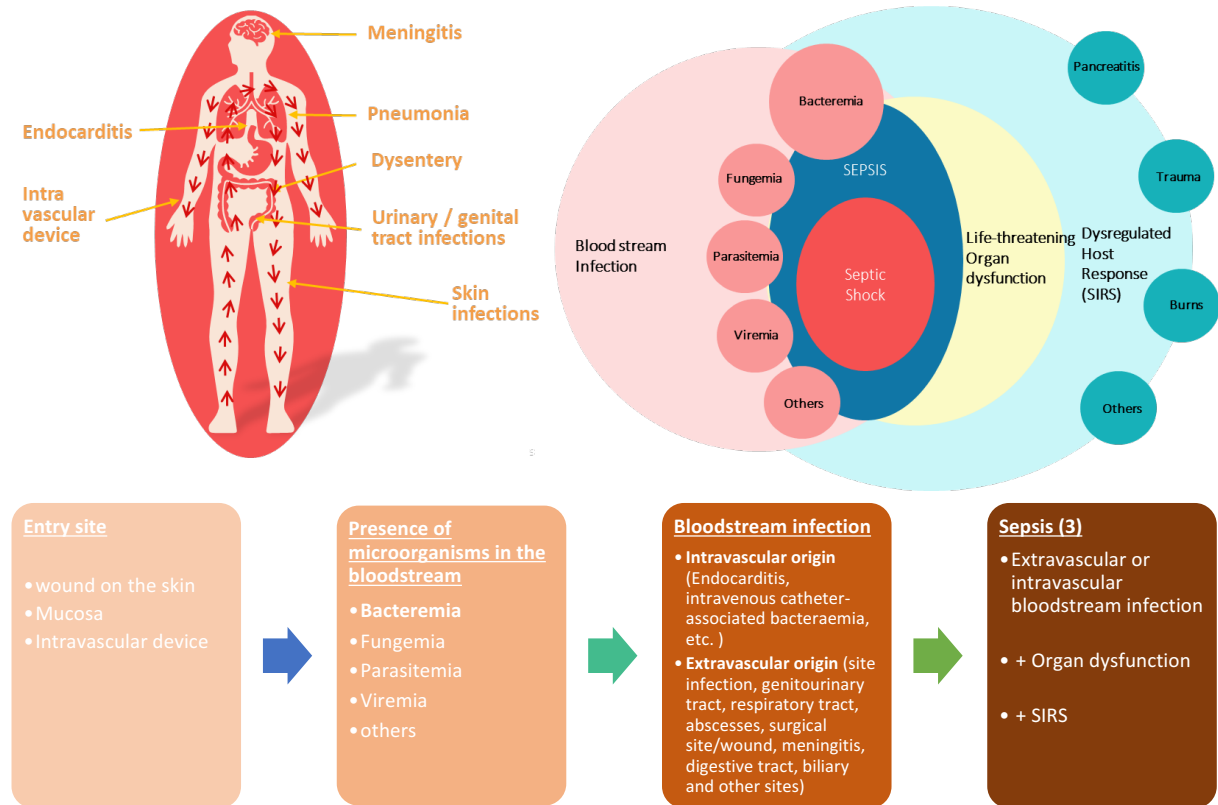
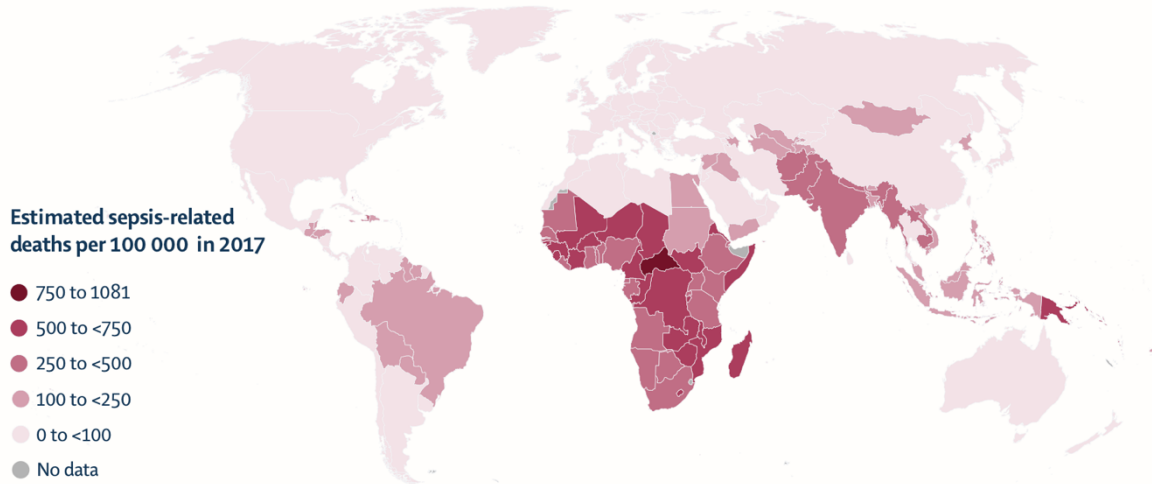


Figure 3: Schematic representation of the cascade from entry site of the micro-organisms toward bloodstream infection and sepsis, adapted from Delano et al. 2016, Fujishima et al. 2016 and Codina et al. 2022.

WHO emphasised that antimicrobial resistance further complicates the management of sepsis across all settings, particularly in high-risk populations, such as neonates and individuals in intensive care units (ICUs)[48]. A recent publication on the global burden of sepsis across all populations and based on death records' analysis by the Global Burden of Disease (GBD) study, estimated that in 2017, sepsis had affected upwards of 49 million individuals and was related to approximately 11 million potentially avoidable deaths worldwide. In addition, sepsis mortality is often related to suboptimal quality of care, inadequate health infrastructure, poor infection prevention measures, late diagnosis, and inappropriate clinical management[49].

In this type of study, robust data are scarce, and the robustness of the data can be affected as the case proportion of sepsis suspected to be due to nosocomial infections is unknown. Individuals admitted to hospital for non-infectious conditions are exposed to infection risk either from invasive devices such as central venous or urinary catheters or through inadequate handwashing practices among healthcare workers and authors emphasise the necessity for further studies[49]. The WHO report, in addition, mentioned that a comprehensive summary of published evidence on the epidemiology of

Health Associated (HA)-sepsis found that 1 in 4 cases of sepsis was acquired in the hospital, increasing to 1 in 2 in ICUs for sepsis with organ dysfunction[50]. Also, the same report mentioned also 25% of sepsis is tracked to Urinary Tract Infections (UTIs) either not, poorly or under treated[50].



Kristina E Rudd, Sarah Charlotte Johnson, Kareha M Agesa, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *The Lancet*; January 2020

Editorial note: The Lancet Group takes a neutral position with respect to territorial claims in published maps, tables, and institutional affiliations

Figure 4: Percentage of all deaths related to sepsis, age-standardised for both sexes, in 2017 (B), extracted from the article of Rudd et al., 2020, The Lancet [49]

Médecins Sans Frontières (MSF), an international medical humanitarian organization, supports health care facilities in over 72 countries, mainly in LMICs. The causes and extent of bloodstream infections remains mainly unknown in MSF hospitals, and they are underdiagnosed due to a lack of access to proper diagnostics. Also, increasing evidence of co-infection of malaria with non-typhoidal *Salmonella* [40], [51], spectrum and frequency ranking of bacteria causing BSIs in LMICs difference from those recorded in HICs further stress the need for another paradigm change. Pathogens like *Salmonella enterica* or *Burkholderia pseudomallei* are uncommon in HICs but account for a large proportion of pathogens in Africa and South-East Asia [52]–[56]. Vaccine preventable pathogens, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, are also more common in LMICs [53], [54].

Also, the use of antibiotics on a large scale on such diseases, with limited guidance from diagnostics testing, is a fundamental part of MSF’s medical response, including in paediatrics / malnutrition programs, In-Patient Department (IPD) and Out-Patient Department (OPD) programs, epidemic responses, surgery, and medical responses to natural disasters. However, globally the increasing risk of antibiotic-resistant bacterial infections, both among those hospitalised as well as individuals in the community, has

shown that all health care actors, including MSF, should begin to make more deliberate efforts to improve pathogen detection, identification and susceptibility testing to facilitate appropriate treatment, prevention of (bacterial) infections and the propagation of AMR.

A measured response is possible. This is possible with the implementation of: (i) better antibiotic stewardship, (ii) better infection and prevention practices, (iii) implementation of clinical bacteriology laboratory, (iv) support of better direct care and (v) increased surveillance capacity. These actions will allow for aggregation of data from areas where the scientific community could not otherwise obtain data. This then allows for the development and dissemination of fit-for-purpose standard treatment guidelines, where in data driven cases empirical antibiotics can be used where the prevalent community-based causes of invasive bacterial infections is understood. However, to make this feasible, existing solutions in clinical bacteriology must be simplified and standardized.

2. Clinical Bacteriology in Low-Resource Settings: A Gap Analysis

WHO has long recognised the need for an improved and coordinated global effort to contain AMR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance provided a framework of interventions focused on slowing the emergence and reducing the spread of antimicrobial-resistant microorganisms[57] In 2012, WHO published “The Evolving Threat of Antimicrobial Resistance – Options for Action”[14] where it was proposed that a combination of interventions that include strengthening health systems and surveillance; improving use of antimicrobials in hospitals and in the community; infection prevention and control; encouraging the development of appropriate new drugs and vaccines; and political commitment was the approach which would lead to affective change.

Following the indication of a primary role for surveillance, in April 2014, WHO published the first global report on surveillance of AMR collecting experiences from national and international surveillance networks and NGOs such as MSF[23] and in 2017 the WHO provided a prioritized list of pathogens for surveillance and research and development of new antibiotics[58]. Recently the WHO published a list of prioritized fungal pathogens to guide research, development and public health action[59].

In the global action plan published in 2015, WHO identified five strategic objectives[18]. The main goal being to treat and prevent infectious diseases with effective and safe treatment choices. Among the strategic objectives, diagnostic tools are mentioned several times as an effective means to reach such an objective. Diagnostics are,

in fact, a strong contributing factor in reaching at least two of the five objectives mentioned by the WHO:

- Strengthen the knowledge and evidence base through surveillance and research, and
- Optimise the use of antimicrobial drugs in human and animal health.

The implementation of relevant diagnostic solutions, permitting rapid identification of pathogens and their subsequent susceptibility and resistance to selected antibiotics, has the ability to improve surveillance and therefore the knowledge and prevention of AMR. However, tools are ineffective unless they can be part of a solution. Development of the Dx tools should always be part of a holistic approach to diagnosis, treatment and monitoring.

Such knowledge will help improve the optimal use of antibiotics with an important consequences on treatment outcomes. This innovation is thus seen as a lever to counter the rise of AMR. Research and development programmes in syndromic driven diagnostic solutions, better understanding of AMR (phenotypic and genotypic), better understanding of the pathogen and human interaction and focused efforts in the development of new antibiotics and new classes of antibiotics are highly needed.

WHO in their global action plan, recognises implementation of Clinical Bacteriology Laboratories (CBL), allowing for the diagnosis of bacterial infections as well as guidance for appropriate treatment, as one of the key solutions for addressing the AMR issue. However, today, hardly any CBLs exist in LRS due to the difficulties of implementation, including high costs, requirements of highly skilled staff and close supervision[60]. In addition, the implementation of CBLs is complicated due to the settings and inappropriateness of many diagnostic tools and methods. Conventional methods for bacteriological culture were not developed for nor have they been adapted to low-resource facilities (e.g. cold chain requirements, logistical constraints, complexity, etc.) and the resulting outputs, if not properly interpreted, are sometime complex to be understood by clinicians[32], [61].

Even today, the strengthening of clinical bacteriology and use of culture based phenotypic methods in LRS is pivotal to diagnose BSIs and improve individual treatment strategies while controlling the spread of AMR[62]. Efforts have been invested in the strengthening of laboratory systems with successful outcomes for severe infectious diseases such as malaria, tuberculosis and HIV[18], [63]. However, compared to these "single pathogen" disease silos, clinical bacteriology covers a wider spectrum of pathogens and clinical presentations, and is less prone to being captured in simple and straightforward diagnostic and therapeutic algorithms[31].

In recent reviews it was stated that available LMIC AMR data lack standardisation data collection and laboratory practises and are often not representative of what is occurring throughout a country or large community[22], [64]. Data is also often collected inconsistently, leading to systematic inaccuracies and underreporting[21]. Data quality and coverage are complicated by a lack of infrastructure and expertise[65], especially in laboratory facilities where quality assurance procedures, skilled personnel, laboratory supplies, and adequate and functioning equipment are all in short supply[32], [66]–[69]. Robust data management is also often lacking. As a result, LMIC clinicians frequently distrust and underutilise laboratory services and ignore reported results (such as failing to de-escalate or discontinue an individual’s antibiotic even when it is indicated by the lab)[22]. Despite a general consensus concerning the need for improved bacteriological diagnostics in LMICs, there is consistent underfunding and underdevelopment[22].

Moreover, microbiology laboratories in LMICs are usually found only in reference hospitals in large cities. AMR data is thus biased towards community-acquired, urban infections and HAIs which are seen at an advanced level of care. Physicians without access to local bacteriology services are deprived of direct diagnostic support and must rely on aggregated referral centre data or imprecise international data to inform antibiotic guidelines and empiric treatment[41]. While trying to predict AMR prevalence more local surveillance can resolve these challenges and have a major impact on individual care management[70].

Furthermore, the rolling-out of CBL in LRS raises numerous challenges, including procurement constraints, product stability and availability of qualified personnel[32]. Automated systems are restrictive due to their high costs and high maintenance requirements, molecular-based methods for identification (ID) and antibiotic susceptibility testing (AST) are also too costly and not yet ready to replace phenotypic methods, and whole genome sequencing (WGS) is not yet applicable in the diagnostic setting as sensitivity of WGS is not on par with phenotypic testing in the case of some antibiotics or it does not take in consideration interaction of multiple drugs [71].

Rapid, affordable, and effective point-of-care (POC) diagnostics, especially those that distinguish between viral and bacterial infections, identify pathogens, and provide antibiotic susceptibility testing (AST) are much needed. Yet, these breakthrough products have not yet materialised, mostly because of market failures and barriers to use and a lack of understanding of the use cases by the manufacturers[65].

In collaboration with a working group of clinical microbiologists and infectious disease physicians from both HICs and LMICs, an extended gap analysis was performed

and has been published as an opinion paper on the topic and can be found in Annex 1:

Sien Ombelet*, **Jean-Baptiste Ronat***, Timothy Walsh, Cedric Yansouni, Janneke Cox, Erika Vlieghe, Jan Jacobs on behalf of the Bacteriology In Low Resource Settings Working Group. Clinical bacteriology in low-resource settings: today's solutions, Published in: **Lancet Infectious Diseases** 2018;18(8):e248-58

** Both authors contributed equally to the manuscript*

3. Building the specifications to adapt the clinical bacteriology laboratory for MSF field interventions

Médecins Sans Frontières (MSF), as a major international medical humanitarian organization supporting over 170 health facilities globally, faced and witness the challenges posed by AMR in LMIC[72]–[80]. Since 2008, MSF has established clinical bacteriology laboratories (CBLs) in some low-resource settings (Mali, Jordan, Liberia, Central African Republic, Yemen) and partner with fourteen private or public CBLs, mainly in urban accessible hospital settings.

As MSF is increasingly confronted with antibiotic resistance in many of its projects, several initiatives were created to tackle antibiotic resistance such as: (i) installation of the first Clinical bacteriology laboratories for diagnostic and research purpose in 2008 (Niger), (ii) the creation of the Antibiotic Task Force in 2014, (iii) the medical conference on AMR in Jordan in 2014 with several Ministries of Health representatives from neighbouring countries, (iv) a partnership with the Global Antibiotics Research and Development (GARDP) created in 2016, (v) development of an electronic Decision Support System (eDSS) for paediatric primary care (MSF eCare) in 2018, (vi) development of a mobile application for Antibiotic Susceptibility Testing measurement and interpretation (Antibiogo) in 2020 and (vii) much more. These initiatives demonstrate how much MSF needs to address the issue of AMR and how within the organisation it is felt and how strong the commitment is to find new strategies to tackle the AMR problem.

3.1. MSF Clinical bacteriology laboratory intervention priorities

While waiting for breakthrough solutions able to diagnose AMR at the bedside and tailored to LMIC requirements, facing the challenge to rapidly install CBL in rural and more remote settings (outside of a capital or main cities), at the end of 2015 MSF decided with key partners to support the creation of an initiative to develop its own solutions to tackle AMR diagnostic and surveillance. Financial support was provided to

implement the project and a governance was created consisting of a steering and a scientific committee.

It was also recognised that this project could potentially have an enormous transformational impact by revealing the distribution of AMR within the MSF field projects and by supporting the challenges faced by MSF in adopting therapeutic guidelines to local data.

Based on the challenges described above and the strategic plans of all Operational Centres (OCs), MSF supported the implementation of a road map that included 3 pillars: infection prevention and control (IPC), antibiotic stewardship and diagnostic/surveillance. Implementation of the Simplified Clinical Bacteriology Laboratory (Mini-Lab) throughout several programs was and is an essential part of this road map.

MSF medical directors, together with experts of the MSF laboratory working group set the objectives to which the Mini-Lab should reach:

- Support the reduction of morbidity and mortality from invasive life-threatening bacterial infection including antibiotic-resistant infections in hospitalised patients.
- Provide detection of invasive bacterial bloodstream infections with blood culture samples
- Priority would be given to those hospitalised:
 - o children with key risk factors for bloodstream infection (i.e. malnutrition, neonates, malaria co-infection, signs of severity),
 - o children with failure to respond to first-line treatment or deterioration while hospitalised.
 - o adults and children with suspected burn-based sepsis
 - o adults and children with HIV infection admitted with fever.
- Strengthen MSF guidelines/recommendations based on current data sets, inform national / international (WHO) stakeholders and sharpen advocacy towards field-relevant diagnostics for bacterial infections based on recent data.

3.2. Developing the requirements of the Mini-Lab

In early 2016, the scientific committee, consisting of renowned academic persons (e.g. clinical microbiologists, AMR experts, IVD developers, biomedical engineers, etc.) together with the project team started to develop the requirements for what was to become the Mini-Lab.

- The Mini-Lab should consist of a small-scale, quality-assured, low-cost, stand-alone and transportable clinical bacteriology laboratory allowing for the diagnosis, identification of causative agent and informed antibiotic treatment decision making for cases of sepsis using manual techniques, but harmonised and simplified to clinical relevance, and performed by trained but non-expert users.
- The type of development process should be done by reviewing, reviving and adapting existing growth-based methods, focusing on minimal bacterial identification and susceptibility testing needs tailored to the available antibiotics in the field with the support of knowledgeable and volunteer academic and non-academic experts. This development process is referring to the existing concept of "**reverse innovation**", a process whereby goods are developed as inexpensive models to meet the needs of developing nations¹.

Table 1: Specifications set by the scientific committee of the Mini-Lab as of February 2016

KEY FEATURES	DESIRED	ACCEPTABLE
INTENDED USE		
Target diseases	Bloodstream infection, osteomyelitis ²	Bloodstream infection
Technical and strategy purpose	<ul style="list-style-type: none"> • It aims to perform bacterial identification and antibiotic susceptibility testing focusing on a minimal but clinically relevant level of identification and susceptibility testing tailored to the required and available antibiotics in the field. • This concept must follow a modular approach in order to permit usage flexibility by field workers /team targeting three different strategies such as for patient care, surveillance and for operational field studies: <ul style="list-style-type: none"> • Patient care: Support the diagnostic of above-mentioned disease and help target / adapt antibiotic therapy • Surveillance: Support clinical based surveillance of antibiotic resistance patterns on the field to capture relevant data to permit the update of empirical treatment guidelines. • Operational studies: Support field studies on diagnostic performance and on infection prevention strategies 	

¹ "GE's Immelt Says 'Reverse Innovation' Needed for Global Growth (Bloomberg)". Retrieved 21 October 2009.

² Any bacterial infections, outside of extrapulmonary Tuberculosis, that could be diagnosed through collection of sterile body fluids might be done using Mini-lab as well.

KEY FEATURES	DESIRED	ACCEPTABLE
Target population	<ul style="list-style-type: none"> Any hospitalised patient with suspected sepsis, immunocompromised or not. Hospitalised patients with infections related to war, trauma or prior surgery. 	Hospitalised patients: Malnourished children, neonates, malaria co-infected children, severely ill children, patients with burn wounds with suspected sepsis, patients living with HIV and admitted with fever.
Target use setting	MSF supported second level of care structure with or without laboratory facilities on site	
Identification target pathogens	<ul style="list-style-type: none"> Minimum and most clinically relevant bacteria causing bloodstream infection and osteomyelitis in LRS with minimal identification to the genus or the family if of clinical relevance and growing in standard broth (to exclude <i>Bartonella</i>, <i>Leptospira</i>, etc). Possibility to have a sub-list of pathogens depending on the geographical zone/site (core pathogens + site depending pathogens). The system should permit the storage and conservation of strains as well for a minimum period of 6 month and safe shipment to referral laboratory in the country or overseas to perform further investigation, confirmation, or quality control. 	
Antibiotic List to be tested	<ul style="list-style-type: none"> Reflect the MSF-WHO essential drug list including and adapted to local spectrum of pathogens and antibiotic resistance patterns list with "must have" and "nice to have" including last resource ATB. Proxy indicator of other resistance. List must be adapted according to the above strategy (patient care, surveillance, etc.). The system should permit the storage and conservation of strains for a minimum period of 6 months and safe shipment to referral laboratories in the country or overseas to perform further investigation, confirmation, or quality control 	
Diagnostic Performance	<ul style="list-style-type: none"> Yield of pathogens must be comparable with current state of the art, "blood culture "system (50 to 60% Se if enough blood volume sampled and culture)][81] System to allow good quality indicators (lower than 3 % contamination rate and between 10 – 15% positivity (pathogen) rate, > 80% appropriate blood filling rate of blood cultures bottles. Antimicrobial susceptibility test (AST) devices that are used to determine minimum inhibitory concentrations (MIC) and/or interpretive category determinations of susceptible, intermediate and resistant should note permit 	

KEY FEATURES	DESIRED	ACCEPTABLE
	"very major" errors to occur [82] and should follow FDA guidance on discrepancies agreements[82] or ISO 20776-2:2007[83].	
Clinical Specificity	Same as conventional culture	
Type of Analysis	Analysis based on existing growth-based methods and non-culture-based tests available on the market, with adaptations and improvements when appropriate and feasible. Manual system is preferred – but "objective" growth indicator needed (agar paddle, agar slant). Screw cap and wide mouth preferable	
Reading system	Visual & semi-automated, with dedicated equipment	Visual
Throughput	30 new tests/day; pick loads of 200 max per 5 days.	10 new tests / days; pick loads of 75 max per 5 days.
Sample type	Blood and bone / Tissue / Pus-aspirate	Blood
TEST PROCEDURE		
Number of steps	< 6	<10
Biosafety	No need for biosafety cabinet at any step of performing the laboratory tests. No Bunsen burner, no gas required	If a biosafety cabinet or other measures are needed, the materials need to fit the below described specifications.
Time to result	<ul style="list-style-type: none"> Total incubation time not longer than 5 days. 85% must turn "growth positive" < 48 h and AST results should be available 24 hours after "growth positive" 	
Volume and type of samples required	<ul style="list-style-type: none"> Should be adequate volume regarding field, patient, and technical constraints in a manner that it does not affect much the diagnostic performance. From 1ml to 10 ml of whole blood sampled by phlebotomy. Disinfection and sampling procedures / materials should allow sampling of blood in a way to limit skin contamination 	<ul style="list-style-type: none"> < 10 ml of whole blood Disinfection and sampling procedures / materials should allow sampling of blood in a way to limit skin contamination of samples and to facilitate the collection of the required volume of blood.

KEY FEATURES	DESIRED	ACCEPTABLE
	<p>of samples and to facilitate the collection of the required volume of blood. Should permit sample collection of patients under antibiotic treatment.</p> <ul style="list-style-type: none"> • Sample kit should include all materials for sample taking (antiseptic pads, needles, gloves, sterile pads, etc.) 	
Sample transport and sample preparation	<ul style="list-style-type: none"> • Blood culture system needs to withstand environmental temperature and delay pending incubation at 35°C. Should withstand a delay of 4 hours to incubation. • No Preparation Requirement 	<ul style="list-style-type: none"> • Blood culture system needs to withstand environmental temperature and delay pending incubation at 35°C. Target lower than 4 hours at a temperature between 20°C and 35°C • No preparation requirement
Internal Control	<ul style="list-style-type: none"> • All necessary internal quality control strains/material should be available within the kit. Its usage, storage and interpretation should be made as easy as possible for non-expert but trained personnel. • The minimum of control strains should be aimed and validated. 	
OPERATIONAL CHARACTERISTICS		
Operating conditions	<ul style="list-style-type: none"> • 15 – 40°C • 25-90% RH 	<ul style="list-style-type: none"> • 15 – 35°C • 25-80% RH
Shelve life (stability)	<ul style="list-style-type: none"> • Upon arrival on the site in the country the product should be stable for 18 months • Reagents / consumables should withstand environmental temperatures between -10 °C and 40°C for a period of at least 48 hours. 	<ul style="list-style-type: none"> • Upon arrival on site in the country, the product should be stable for 12 months • Reagents / consumables should withstand environmental temperatures between -10 °C and 40°C for a period of at least 24 hours.
Storage conditions	Can be stored between 2°C and 40°C. A maximum of 45 L (Net storage capacity) volume of	Can be stored between 2°C to 30°C. A maximum of 108 L (Net storage capacity) volume of

KEY FEATURES	DESIRED	ACCEPTABLE
	reagents/consumables for one week of activity may require 2 - 8°C storage.	reagents/consumables for one week of activity may require 2 - 8°C storage.
In use stability (under tropical conditions)	Minimum of 4 weeks below 40 °C if pouch sealed (ziplock) after use.	Minimum of 4 weeks at 2 to 8 °C if pouch sealed (ziplock) after use.
Reagents reconstitution	<ul style="list-style-type: none"> All reagents and media ready for use except for lyophilised / dehydrated reagents/consumables (in that case appropriate volumes and easy transfer required). This step should not have biosafety requirements such as a biosafety cabinet or hood. 	<ul style="list-style-type: none"> Reconstitution acceptable if very simple to do. All reconstitution fluids (including water) already provided in the kit.
End user profile	Trained laboratory technician in health care facilities: <ul style="list-style-type: none"> Personnel using the Mini-Lab should have at least diploma in laboratory technician science and should be able to read and understand the technical language of the Mini-Lab either English/French/Arabic 	
Biosafety requirement	Biosafety requirement not higher than level 2: <ul style="list-style-type: none"> Biosafety requirements should take in consideration till risk 3 class pathogens. All material, reagent should comprise safely practice or should mitigate the risk of contamination to the technician. Appropriate containment levels (anti splash practices, personal protective equipment) to be provided in case of (suspected) pathogens such as <i>Salmonella Typhi</i>, <i>Brucella spp.</i>, <i>Shigella dysenteriae</i> and <i>Burkholderia pseudomallei</i>. 	
Training / Communication	<ul style="list-style-type: none"> All training materials must be provided to allow training by an experienced staff (laboratory technician / microbiologist/ MD) as well as by self-study. Training materials should consist of easily understandable standard operating procedures, video, bench aids/job aids and should be easily accessible on soft and hard version. Laboratory personnel training aim: Being able to use properly the Mini-Lab and provide results according to specifications set <ul style="list-style-type: none"> Laboratory personnel should have a minimum of Laboratory certificate diploma level after high school (1-2 year diploma). 	

KEY FEATURES	DESIRED	ACCEPTABLE
	<ul style="list-style-type: none"> • No experience in bacteriology required. • Minimum training should consist of 5 days of theoretical training and 5 days of practical exercise. • Mentorship of one month minimum from an experienced laboratory staff with minimum supervision visit every 6-9 months. <p>Prescriber training aim /Diagnostic Stewardship: Practitioners should be able to understand the added value of the use of Mini-Lab, when to ask for analysis and how to interpret the results and best use of them for patient treatment.</p> <ul style="list-style-type: none"> • Medical Doctor or Medical officer with General Practitioner level. • No experience in clinical bacteriology laboratory required. • Minimum training should consist of 2.5 days of theoretical training and 2.5 days of practical exercise. • Mentorship of one month minimum from an experienced MD with minimum supervision visit every 6-9 months. <p>Nurses' training aim: Nurses should be able to collect samples properly in order to mitigate the risk of contamination and provide the best possible quality and quantity of samples accordingly.</p> <ul style="list-style-type: none"> • Certified nurse or nurse assistants. • Experienced in standard phlebotomy • Minimum training should consist of 0.5 days of theoretical training and 1 days of practical exercise. • Mentorship of one week minimum from an experienced Nurse with minimum close supervision first 2 months and visit every 6 month <p>A standard certificate will be provided to all mentees/trainees that have been going through the process of Mini-Lab and a specific register should be available to keep a record of trained staff.</p>	
Equipment	<ul style="list-style-type: none"> • To be carried by hand without need for a specific lifting machine • All equipment of the Mini-Lab will need to be easily transportable from the supply centre to the end- user and from one project site to another without a need for a big truck. All the boxes of the Mini-Lab should be fit to be transported in a standard Toyota pick-up (2m length x 0.98m width x 1.5m height) or van. 	

KEY FEATURES	DESIRED	ACCEPTABLE
	<ul style="list-style-type: none"> • Total weight < 800 kg; maximum weight per case should be 100 kg. • Volume at transport < 4 m³ • List of potential minimum equipment: • Requiring electricity: Charger/ inverter/ UPS, Incubator, computer, light, safety cabinet Class 2 • Not requiring electricity: Should incorporate bench tops and all the material for administrative work, stools, fire extinguishers, eye wash station, first aid kit, fire blanket, etc. 	
Power Requirements	<ul style="list-style-type: none"> • Material requiring electricity should permit a flexible and robust connection to available energy supply and should be able to last up to 8 hours without external power supply (rechargeable batteries). The Mini-Lab should be able to be connected to fluctuant city supply / generators or solar energy • Electrical Requirements: 100-240V 50/60Hz • Wattage: Min 150 W, max 500 W • Consumption per 24 H: min 1000 Wh, max 5000 Wh 	
Waste management	Can require a specific waste management material using easy and accessible system fitting in MSF waste management scheme.	Should not require any specific waste management outside of the existing one present in MSF hospital.
Reporting	<ul style="list-style-type: none"> • The system must be compatible with the existing MSF HIS system requirements with the possibility to capture bacteriology data, provide expert system-based information, provide result to practitioners and support bacteriology data analysis for the trend of resistance • "Expert" system guiding interpretation and directing the report to the clinician (e.g. AST selective reporting). The software should be a decision tool and data entry allowing working without access to the internet but with an automatic connection to the Internet when it is possible in order to upload information on a data server in a cloud accessible by specific persons. The system should be able to work on PC (windows) environment as well as on the Android system. 	

KEY FEATURES	DESIRED	ACCEPTABLE
Need for additional equipment in addition	None, all equipment, consumables, etc. included	Simple equipment acceptable
Need for maintenance/spare parts	<ul style="list-style-type: none"> • Only preventive maintenance required and within the competence of a trained laboratory technician (no requirement for a bio-engineer technician). • All spare parts needed for maintenance and basic repairs (fuse, sockets, etc.) as much harmonised as possible, available on site, well coded, easy to order and deliverable in a short delay (to be defined according to equipment). 	
Specimen collection and transfer device	<p>Regular phlebotomy materials, complemented by specimen collection containers (wide mouth, screw caps), transport media/materials and transfer devices (loops, transfer pipettes, etc.). All materials complying with standard biosafety precautions and in case anti splash practices (e.g. disposable loops rather than metal wired loops and Bunsen burner).</p> <p>Personal protective equipment including gloves, masks, eye protection and gowns.</p>	<p>Use of dedicated / specific non-MSF phlebotomy material specifically fitting with blood cultures collection devices, complemented by specimen collection containers (wide mouth, screw caps), transport media/materials and transfer devices (loops, transfer pipettes, etc.). All materials complying with standard biosafety precautions and in case anti splash practices (e.g. disposable loops rather than metal wired loops and Bunsen burner).</p> <p>Personal protective equipment including gloves, masks, eye protection and gowns.</p>
Development / adaptability capabilities	The Mini-Lab will be able to accept over time new methods that will increase its effectiveness.	
Infrastructure requirement	<ul style="list-style-type: none"> • The Mini-Lab must be able to be set up in non-laboratory specific facility environments. Should just have access to clean water, storage facilities in compliance with the above specification and separate administration/staff room. Examples: set up under a tent or in an existing building with no specific requirements except the one found below. • Space requirement of a minimum of 10 m² and maximum 20m² 	

KEY FEATURES	DESIRED	ACCEPTABLE
	<ul style="list-style-type: none"> • Clean dust-proof room with concrete or washable floor and walls • Access to clean and chlorinated water (10 Litres per day) • Electricity connection (see above specifications) • Internet connection through Wireless, LAN or USB 3G/4G key (Upload: min 128 Kbs; download: min 256Kbs) 	
COST		
Cost per consumables (e.g. cartridges, strips,) (for procurement)	Cost expectation regarding the response scenario for patient care: Fewer than 7 €/specimens (on positive sample), average of 5 euros (including negative if 20% of positive). Other cost scenarios cost should be as minimal as possible	Cost expectation regarding the response scenario for patient care: Fewer than 10 €/specimens (on positive sample), average of 7 euros (including negative if 20% of positive). Other cost scenario cost should be as minimal as possible
Cost per equipment (for procurement)	Less than 10000 € Maintenance cost of < 50 € per trimester, should include preventive weekly, monthly and bi yearly grand service	< 15 000 € Maintenance cost of < 200 € per trimester

Scientific Objectives

This Ph.D. thesis originated from observations after fifteen years of field interventions in LMICs. The central idea of the thesis is the identification and adaptation of feasible and affordable techniques for bacteriology in LRS concentrated into standardised quality assured laboratory packages that can be rapidly installed at district level hospitals as a door opener, improving access to clinical bacteriology, providing local and individual-centric and actionable results for clinicians, thereby improving AMR surveillance and stewardship in LRSs.

At the start of this Ph.D. research, a “gap analysis” of clinical bacteriology in HICs versus LMICs was performed to assess the reasons behind the relative absence of microbiology facilities in LMICs and to summarise the interventions required to turn the situation around and to change the paradigm (section 2 of the bibliography and Annex 1). For the Mini-Lab to bridge existing bacteriology phenotypic methods to be accessible, affordable, supply management friendly, robust, easy to use, addressing LRS constraints highlighted in the gap analysis, a transdisciplinary research approach was needed. The Mini-Lab concept does not consist of only analytical methods but includes critical laboratory peripherals such as (i) quality management and control, (ii) biosafety measures, (iii) training, (iv) transport, (v) equipment maintenance, (vi) deployment, (vii) implementation, (viii) activity follow-up and much more. This study will therefore vary in focus and will include technology scouting methods; microbiology analytical methods, biosafety measures, biomedical engineering and bioinformatic methods, instructional design engineering, identification, adaptation, development and testing, iteration with user centre-based approaches and public health approaches.

The work presented in this thesis is the result of a collaboration between the Mini-Lab team of MSF Operational Centre Paris (France), ReSIST INSERM U1184 team from the bacteriology laboratory of Bicêtre Teaching Hospital in Kremlin-Bicêtre (France), the Institute of Tropical Medicine (ITM) in Antwerp (Belgium), University Teaching Laboratory Hospital (LHUB-ULB) of Université Libre de Bruxelles (ULB) (Belgium) and many other specialists, organisations from different horizons, and all listed in the acknowledgments. The amount of work to develop the Mini-Lab is consequently result of a strong team work and collaborations with numerous actors, therefore it would not be possible to entirely describe all types of processes and the epistemology of decision to achieve this concept. Two Ph.D. students have worked on the project, Dr Siem Ombelet ITM, as part of her Ph.D. thesis work with ITM and myself as part of my day to day work as project chief scientific officer for MSF and Ph.D student with ReSIST INSERM U1184 team.

Specific research questions and objectives of this Ph.D. thesis are the following:

Question 1: How can we design and develop a small-scale, stand-alone and transportable clinical bacteriology laboratory adapted to LRS constraints?

The chapter is divided into sections addressing different objectives to answer the question:

- **Objective 1: Development of an iterative project process to manage the development of a complex concept.** The first chapter will include a deep dive into the overall processes of the Mini-Lab development describing first the overall process based on the gap identification and literature reviews based upon technical questions, followed by the development process from technology scouting to selection, engagement with partners and suppliers and laboratory-based evaluation of some of the analytical components.
- **Objective 2: Development of a platform to protect and transport selected equipment for rapid setup.** Describes iterative product development approach, laboratory workflow analysis used to identify the pathway to incorporate CBL complex workflows, biosafety measures into a deployable platform. The development and evaluation of the different platform iterations from wooden box mock up to final roto moulding plastic developed are presented, incorporating the risk mitigation measures highlighted by a biosafety risk-based analysis and evaluate them for usability and robustness in control environment by expert users.
- **Objective 3: Adaption, simplification, and validations for analytical process of blood culture.** Processing blood culture bottles to provide actionable results to clinicians encompass the use of several systems in the downstream processes, the blood culture system (BCB), a subculturing process (SC), a pre-identification process (pre-ID), identification (ID) process and antibiotic susceptibility tests (AST) process. While the SC process and AST process selection, adaptation and evaluation are presented in Chapter 2 and 3, adaptation and evaluation of the BCB, and ID processes have been done by Dr Sien Ombelet ITM, as part of her Ph.D. thesis work, in this section a narrative review on best practices for performing blood culture in low-resource settings, an *in vitro* comparison of two types of manual blood culture bottles against automated blood culture are presented. The *in vitro* evaluation of a bacterial identification process, its subsequent adaption for use in the Mini-Lab and other low-resource settings, the MicroScan ID (Beckman Coulter, Brea, USA) Dried Overnight Gram-negative

and Gram-positive panels are then presented. Both BCB and the ID processes were evaluated with the constraints of low-resource settings in mind.

- **Objective 4: Development of an all-in-one Quality Management System.** A Quality Management System (QMS) is the cornerstone for quality, valid and acceptable results. The Mini-Lab has been built around the objective of ensuring that the whole process and components meet quality requirements to provide accurate results in the absence of an on-site expert microbiologist. An integrated QMS was designed following important and relevant recommendations of WHO Stepwise Laboratory Improvement Process Towards Accreditation criteria (SLIPTA) and ISO 15189. The method used and result of the adapted didactic documents, pictograms, user manual, bench aids onsite and online training to adequately perform, control, maintain all types of process ranging from installation, waste management, biosafety, pre-analytical to post-analytical are then briefly presented.
- **Objective 5: Development of an expert system to accompany the user through results interpretation and results control.** The process from the culture of bacteria to AST results, in bacteriology, require highly skilled personnel. Firstly to orient selection of downstream testing in a dichotomous approach which are often error prone and secondly the interpretation of results and providing understandable results to clinicians. One of the most complex parts of the dichotomous approach is the pre-identification or orientation testing, which allows for an orientation to an organism's group level, due to morphology (cocci and rods), staining characteristics (Gram staining), and ability to metabolise defined substrates. A probability- based algorithm constructed using the probabilities of test results of validation studies done on selected pre-ID test and a weighted combination of them for each pre-defined group of organisms, to give a final most likely orientation is then described. The process of developing a microbiology decision support system, embed it into a developed-for-purpose Laboratory information system, that incorporates expert rules to orient, warn the user and interpret results all along the analytical process is then described.

Research question 2: how can we validate a subculture system, initially developed for veterinary and food industries bacteriology, for human blood culture?

- **Objectives 6: adaptation and validation of an innovative technique adapted to LRS constraints for the subculture of blood culture bottles.**

In Chapter 2, we focus on the adaptation and evaluation of the culture media system to be incorporated into the Mini-Lab process. Culture media are fundamental to any clinical bacteriology laboratory. Conventional culturing methods in clinical laboratories generally consist of using agar poured plates or commercially available pre-poured ready-to-use agar plates or in-house preparation of agar plates from dehydrated culture media. All methods have advantage and disadvantages, (e.g. shelf life, cold chain requirement, price, additional materials and substrates, quality control, etc.). In this chapter, the culture media selection and processing in LMIC is reviewed, best practices for in-house preparation are compiled, ways to improve access to quality-assured products are discussed and research questions to improve these practices are formulated. In the second part of the chapter the selection process is explored, from the market analysis of ready-to-use culture systems (InTray, Biomed diagnostic, USA) originating from veterinary and food safety microbiology and the performance of several proof of principle studies to explore usage as subculturing systems, Blood Cultures Bottles (BCB). The analytical performance evaluation of this system on a larger scale to confirm the findings of the proof of principles studies is then discussed.

Research question 3: How can AST methods be adapted to LRS constraints?

- **Objective 7: Adaption and verification of the accuracy of the MicroScan MSF Dried Overnight MIC microplate and automated reader**

Determination of the antimicrobial susceptibility patterns of significant bacterial isolates is among the primary responsibilities of the clinical microbiology laboratory. In the context in which the Mini-Lab is operated, robust, easy to use, results reproducibility across laboratories and an AST process in the hands of the “operator” is key. This AST process should be easy to read manually or with the use of a simple reading apparatus for accurate and reproducible results. There are many different methodologies available for detecting organism resistance to antimicrobials, ranging from disk diffusion (Kirby-Bauer), broth microdilution (both manual and automated), agar dilution, and antibiotic gradient methods, all with advantages and disadvantages depending on if they are used in high-resourced or in low-resourced settings. In Chapter 3, the selection, adaptation, and analytical performance verification of three panels of MIC micro broth dilutions (Microscan, Beckman Coulter) developed together with Beckman Coulter and the PROMPT system that allows in a simple way to systematize the inoculum density, using ISO 20776-2:2007 is described. The selection and in laboratory evaluation of a reading apparatus for the determination of automatic versus manual reading performance is then discussed.

Research question 4: is the simplified clinical bacteriology laboratory easy to use by non-experts and does it provide quality and valuable results to clinicians?

- **Objective 8: Field implementation and evaluation of the ease of use, performance of the different Mini-Lab prototype iterations**

Processes, technologies, equipment, etc. developed and tested in control environments and described in previous chapters were integrated into different iterations of the Mini-Lab assembly. To answer the objective of evaluating the usability of the first Mini-Lab prototype (V1) on the field, a first pilot study was implemented in an MSF burns centre in Haiti where the usability of all components associated together was evaluated, using qualitative or semi-quantitative methods to evaluate user experience with the material, process, and test through the use of a questionnaire and regular interviews. Results were then used to further adapt components and develop a second iteration of the prototype (V2). In the second iteration, that incorporated a more mature, and revised process and near to final specifications, the performance was evaluated in a prospective study, as was usability and utility of results by clinicians, by implementing Mini-Lab V2 at Carnot District Hospital, an MSF supported hospital in Central African Republic, a hospital without prior access to a microbiology laboratory. Hospitalized patients who had a blood culture on admission or during hospitalization, and who consented to participate in the study were included. Clinical and therapeutic data were collected by standardised questionnaires using patient records. Microbiological data were extracted from the pseudonymised Mini-Lab database. Bacteria isolated in the Mini-Lab were stored and sent to a reference laboratory in France, for confirmation by reference methods (MALDI-TOF and reference AST testing). Laboratory technicians participated in a regular evaluation of the usability of the Mini-Lab and to determine their technical competencies.

Finally, in the general discussion the finale specifications of the Mini-Lab are compared to the original requirements. The main findings and implications for integration of adapted clinical bacteriology into clinical practice in LMICs are discussed, and strengths and limitations from the development to embedment into clinical care are identified and discussed. Finally, as to the different open questions, perspectives are provided and new research gap unanswered by this work are highlighted.

To help the reader better understand my involvement in this thesis work, I would like to provide some background information. The original idea for this project emerged in 2014 and was further developed in collaboration with Pf Jan Jacobs during a GLASS WHO meeting. I was fortunate enough to secure MSF's sponsorship to initiate

the project in 2016, and for the first two years, I took on the role of project coordinator and scientific lead. During this time, I created the team, identified experts for the consortium, found partners, established project management methodologies, and coordinated the team's efforts to write specifications, perform market reviews, some of which I conducted myself, conceptualise product improvement, laboratory performance protocol.

After recruiting a project manager, I shifted my focus to serving as the Science lead expert, working with partners and a team to evaluate different components of the project and coordinate field evaluations. Throughout this process, I guided the work, reviewed documents, provided scientific and methodological support, and oversaw laboratory evaluations of strains from Haiti or Carnot field evaluations. I was also doing onsite visit in Haiti and Carnot to supervise the studies and provide support to the study coordinators.

However, I would like to emphasize that I start my PhD in 2019 and for the AST part of the project, I was at the forefront from the beginning to the end. Specifically, I was responsible for developing and testing the AST microplate, protocol for evaluation at Bicêtre laboratory Hospital with laboratory bench level work. Also, i was leading all laboratory evaluations of strains from Haiti or Carnot coming back at the reference laboratory and doing the work at bench level too.

Results

Chapter 1- How To Design And Develop A Small-Scale, Stand-Alone And Transportable Clinical Bacteriology Laboratory?

1. Introduction

The original concept of the Mini-Lab propose to adapt all the components of a standard clinical bacteriology laboratory with the target specifications set in the previous chapter. As previously mentioned, a laboratory is not only composed of tests, reagents and equipment, it is composed of much more. The concept built around a strong Quality Management System (QMS) combine a variety of innovative approaches in the field of training, Laboratory Information System (LIS), etc., with the objective of ensuring that the whole process and components meet quality requirements to provide accurate results in the absence of an on-site expert microbiologist.

In this chapter, section 2 will describe the overall processes of the Mini-Lab governance, development from technology scouting to selection, engagement with partners and suppliers and laboratory-based evaluation of some of the analytical components, training development and bring all those elements together to form the Mini-Lab.

Section 3, will explore the outcome of risk mitigation of damage during transport, provide a description of the laboratory bench/shipping container and other safety measures, following an iterative work process with a product and industrial designers.

Section 4 will give a complete overview of the Mini-Lab, the final sample management process developed around the different adapted analytical components.

Section 5 will highlight the work done concerning the QMS for documentation training and facilitating the work of all personnel involved in the operational life cycle of the Mini-Lab.

Section 6 will be a description of the main pillar of clinical bacteriology simplification, the description of one of the components of the Microbiology Decision Support System (MDSS) which is embeded in the Mini-Lab LIMS. This is a major component for supporting the interpretation of results and for the laboratory providing actionable results to the clinician. This component is a complex part of the MDSS, the pre-ID expert system will help for a better understanding of the expert system development.

The work done in the experimentation and/or development process has been described in the following peer reviewed articles and conference abstracts:

- S. Ombelet, B. Barbé, D. Affolabi, **J-B. Ronat**, P. Lompo, O. Lunguya, J. Jacobs, L. Hardy, "Best Practices of Blood Cultures in Low- and Middle-Income Countries," **Front. Med.**, vol. 6, no. June, 2019, doi: 10.3389/fmed.2019.00131.
- A. Natale, **J-B. Ronat**, A. Mazoyer, A. Rochard, B. Boillot, J. Hubert, B. Baillet, M. Ducasse, F. Mantelet, S. Oueslati, S. Ombelet, C. Langendorf, T. Naas, O. Vandenberg, J. Jacobs, "The Mini-Lab: accessible clinical bacteriology for low-resource settings", **The Lancet Microbe**, 2020 1,2,e56-e58,
- S. Ombelet, A. Natale, **J.-B. Ronat**, O. Vandenberg, L. Hardy, and J. Jacobs, "Evaluation of MicroScan Bacterial Identification Panels for Low-Resource Settings," **Diagnostics**, vol. 11, no. 2, p. 349, Feb. 2021, doi: 10.3390/diagnostics11020349.
- **J-B. Ronat**, A. Natale, T. Kesteman, A. Andremont, W. Elamin, L. Hardy, R. Kanapathipillai, J. Michel, C. Langendorf, O. Vandenberg, T. Naas,* , F. Kouassi,*; "AMR in low-resource settings: Médecins Sans Frontières bridges surveillance gaps by developing a turnkey solution, the Mini-Lab," **Clin. Microbiol. Infect.**, vol. 27, no. 10, pp. 1414–1421, 2021, doi: 10.1016/j.cmi.2021.04.015.
- S. Ombelet , A. Natale, **J-B. Ronat**, T. Kesteman, O. Vandenberg, J. Jacobs, L. Hardy, "Biphasic versus monophasic manual blood culture bottles for low-resource settings: an in-vitro study," **The Lancet Microbe**, vol. 3, no. 2, pp. e124–e132, Feb. 2022, doi: 10.1016/S2666-5247(21)00241-X.
- S. Ombelet, A. Natale, **J-B. Ronat**, O. Vandenberg, J. Jacobs, and L. Hardy, "Considerations in evaluating equipment-free blood culture bottles: A short protocol for use in low-resource settings," **PLoS One**, vol. 17, no. 4, p. e0267491, Apr. 2022, doi: 10.1371/journal.pone.0267491.
- **J-B. Ronat**, "Revival of robust manual solutions for sepsis diagnostic and AMR surveillance in low-resource settings: Case study of Médecins Sans Frontières Mini-Lab" Oral presentation, **Med Lab Congress**, June 2021, Dubai.
- **J-B. Ronat**. "AMR Diagnostic stewardship in resource-limited laboratories: today's solutions ,» Oral presentation, **European Congress on Clinical Microbiology and Infectious Diseases**, June 2021, online.

2. Description of the overall development process

2.1. Project Governance and Project Team

The project team was composed of one project manager, assigned to manage the project with the Scientific Leader. A variety of human resources capacities have been utilized during the development process from Médecins Sans Frontières (MSF) as well as, from other partners, employed staff on specific topics, according to the development phases requirements (microbiologist, project assistant, engineer, instructional designer, etc.). A total number of 20 persons over a period of 6 years have worked as part of the project team; they are all acknowledged in the acknowledgement section. This work would have not been possible without the internal technical resources of MSF such as the MSF Logistic supply centre, legal department, fund-raising department, IT department and more.

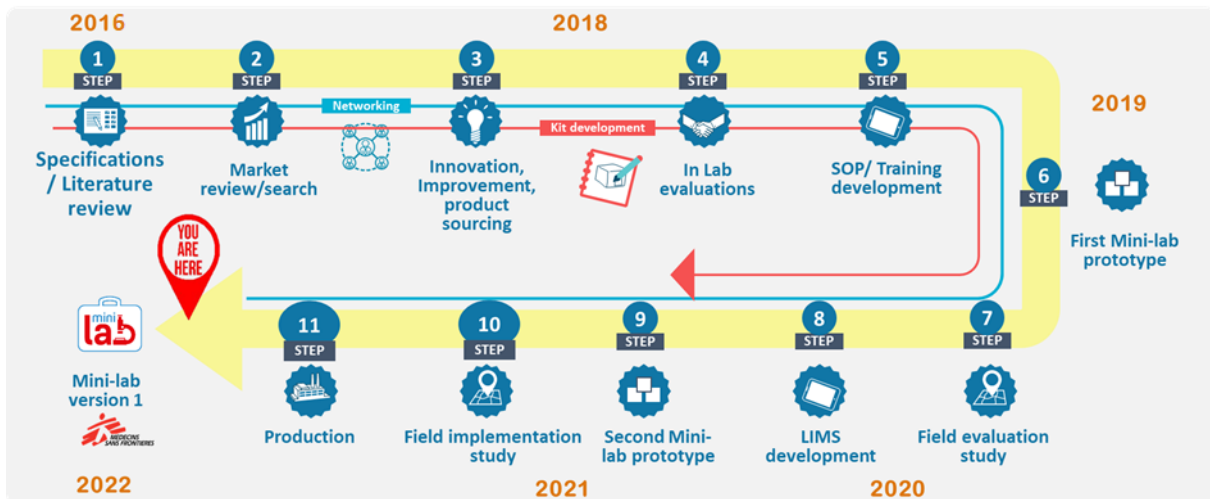


Figure 5: Figure presenting the development life cycle of the Mini-Lab project with overall phases

A Steering Committee was set as a governing body of the project to provide strategic leadership and governance oversight. With delegated authority of the medical directors (Dir Med) platform to make decisions that are in accordance with the objectives, approach and scope of the project as set out in the project charter. A chairperson was appointed to ensure that Committee functioned properly, there was full participation during meetings, all relevant subjects were discussed, and effective discussions were made and carried out. For the past 6 years, a total number of 25 people participated to the Steering Committee and represented all MSF sections. Participants came from different backgrounds in the medical sectors (e.g. infectious diseases practitioners, medical directors, nurses, Infection Control specialist, etc.).

Also, a multi-stakeholder Scientific Committee was set up as an independent external body whose function was to provide scientific oversight on behalf of the Steering Committee in the design, development, scientific rigour, execution, uptake and pro-

motion of the Mini-Lab project. It has acted in an advisory capacity to both the project management team and the Steering Committee at specified intervals to ensure the scientific validity of the program.

This committee consisted of renowned academic or non-academic experts in Microbiology, AMR surveillance, IVD development, IVD regulations, biomedical engineers, etc. A chairperson was assigned to lead the group and maintain consistency. A total number of 22 persons participated in this committee over the last 6 years.

The complete list of persons involved in the Steering and the Scientific Committees can be found in the acknowledgement section and we are deeply thankful for their involvement on a voluntary basis.

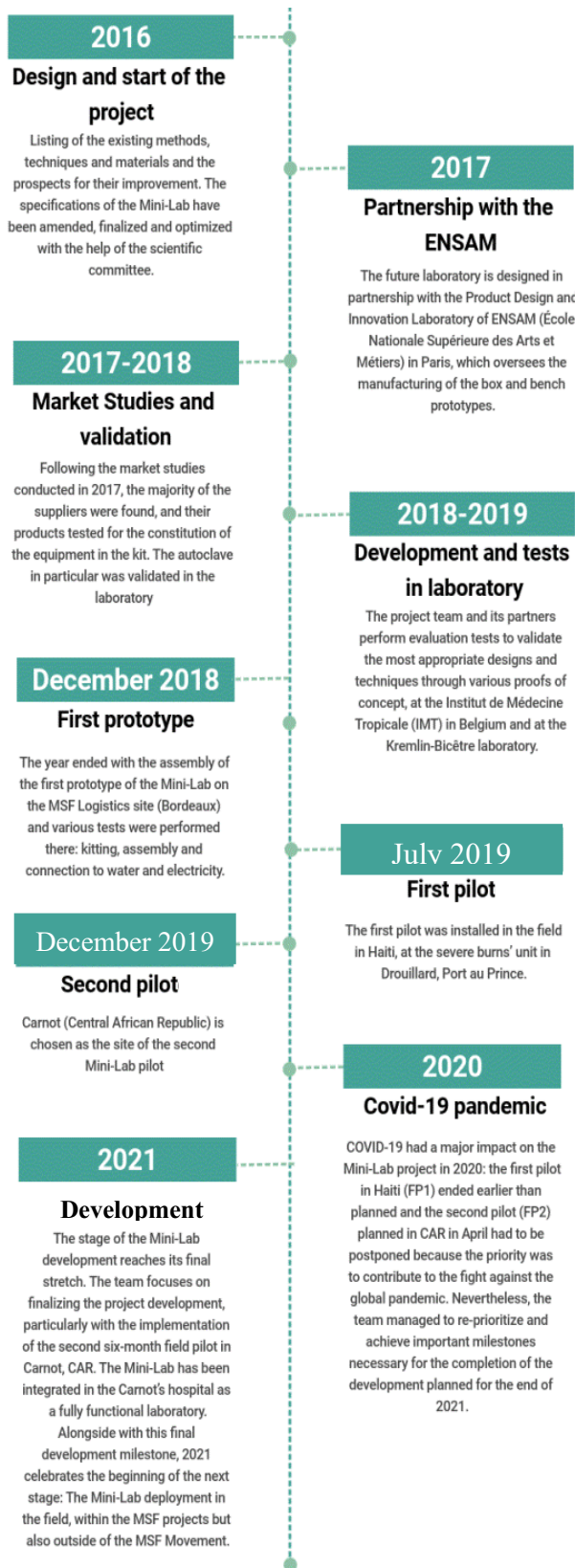


Figure 6: Description of the important phases of the Mini-Lab design and development, MSF

2.2. Defining the methods and proposed improvement

At the initiation of the project an internal market size analysis was done to, understand/target quantity of Mini-Lab to be produced for MSF and in order to negotiate

Table 2: List of all the market reviews made between March 2016 and March 2017

PNA FISH stain
Pyrrolidinyl Arylamidase (PYR)
Ortho-nitrophenyl- β -galactosidase (ONPG)
Indole
Voges-Proskauer (VP)
Blood culture automated system
Blood culture bottle reader
Identification systems
Blood Culture Bottles
Autoclave
Incubators
Coagulase
Oxidase test
AST system
Catalase
Aminopeptidase test (AMNP)
Beta-glucuronidase (PGUA)
Sub-culturing system
Quality control strains
Densitometer
Vortex
Fridges
ID and AST reader
Multi volumes sample transfer system
Strain storage media
Temperature regulated Transport box
Single-use disinfection system
Blood culture collection kit
Personal Protective Equipment
Small consumable for sample transfer
Zipped sample transport
Biohazard Spillage kit
Gram staining

adapted prices with manufacturers. Using the target product profile attributes and requirement ranges, and fully understanding the type of structure in which the Mini-Lab will be utilized and for which patient population (diagnoses of bloodstream infections; BSI, MSF documents, databases, activity reports), were then searched. This was done to estimate the burden of bloodstream infection (BI) among patients seen at In Patient Department (IPD) level. Few projects among MSF operational centres (OCs) had the capacity to record or detect the burden of BSI, therefore calculations are based upon the literature. It was estimated that there are several causes of bloodstream-related co-infection indicators that could support the understanding of the total burden of this disease among MSF treated individuals. Different indicators found in the literature such as the rate of BSI co-infections among malnourished children, among severe malaria cases, neonatal sepsis incidence, etc. [84] [80] [55] [85] [86] from the sub-Saharan region were applied. It was estimated that between 30 to 50 Mini-Lab modules would need to be deployed and between 12000 and 20000 blood culture bottles (BCB) processed per year, if implemented in all specialised In Patient Departments (IPD) with the assumption of a full deployment scenario on all intended use

cases.

Table 3: List of the items classified by the type of methodology used after sourcing or adaptation

Item	Type of methodology
Blood culture bottle reader	adaptation and evaluation
AST system	adaptation and evaluation
Identification system	adaptation and evaluation
Quality control strains	adaptation and evaluation
ID and AST reader	adaptation and evaluation
Blood culture collection kit	adaptation and evaluation
Biohazard Spillage kit	adaptation and evaluation
PNA FISH stain	drop down
Blood culture automated system	drop down
Pyrolydinyl Arylamidase (PYR)	sourcing and evaluation
Ortho-nitrophenyl- β -galactosidase (ONPG)	sourcing and evaluation
Indole	sourcing and evaluation
Voges-Proskauer (VP)	sourcing and evaluation
Blood Culture Bottles	sourcing and evaluation
Autoclave	sourcing and evaluation
Incubators	sourcing and evaluation
Coagulase	sourcing and evaluation
Oxidase test	sourcing and evaluation
Catalase	sourcing and evaluation
Aminopeptidase test (AMNP)	sourcing and evaluation
Beta-glucuronidase (PGUA)	sourcing and evaluation
Sub-culturing system	sourcing and evaluation
Densitometer	sourcing and evaluation
Vortex	sourcing and evaluation
Fridges	sourcing and evaluation
Multi volumes sample transfer system	sourcing and evaluation
Strain storage media	sourcing and evaluation
Temperature regulated Transport box	sourcing and evaluation
Single-use disinfection system	sourcing and evaluation
Personal Protective Equipment	sourcing and evaluation
Small consumable for sample transfer	sourcing and evaluation
Zipped sample transport	sourcing and evaluation
Gram staining	sourcing and evaluation

A list of priority reagents, tests, consumables, equipment necessary to perform blood culture in a standard clinical bacteriology laboratory[87] and set specifications to perform for each of them a market review was then defined. A total of 34 market reviews (Table 2) were done with the support of MSF logistic technical advisors between early 2017 and end of 2018. After each market review, results were presented to the Scientific Committee and brainstorming was done to propose improvements and/or innovations to the pre-selected candidate in each category. Short but comprehensive feasibility studies were then done on the innovations and improvements to be propose to the manufacturer. Each innovation and improvement was ranked in terms of investments and development risk. Only ideas with a lower-risk investment and quick return were chosen to be implemented in the Mini-Lab. Those criteria for selections of improvement followed recommendations made

by K. Culbreath and A. Petti in May 2015[88]. As mentioned earlier, the Mini-Lab concept consists of improving existing techniques but also providing easy means for transport and supply chain management (further described in section 3 of this chapter). Providing high quality results by a laboratory can be difficult to achieve without the

presence of Laboratory Information and Management System (LIMS), in section 6 the development of one of the main elements of the LIMS that will help non-expert users in their daily work, the microbiology decision support system is described.

2.1. Engagement with Manufacturers and Sourcing

Out of the 34-market review done, not all necessitated asking the manufacturer for product adaptations. Only 7 out of 34 required improvements from the manufacturers. The others were sourced using the standard protocol per MSF logistic supply centre. For reasons of independence and to reduce potential conflict of interests, a call for proposals was launched publicly for 3 out of 6 items where more than one manufacturer was using the same initial technology (AST system, ID system, Quality Control) with stringent selection criteria applied and then used in negotiations. For two out of six remaining products with proposed adaptations, it was decided to assemble them internally within MSF, using different sources of items to be included in a kit form (i.e. biohazard spillage kit, blood culture collection kit). For one, the blood culture bottle reader, no option was found on the market and only one manufacturer had a solution approaching our specifications. The manufacturer was contacted directly. For all sourced items, specific attention was provided on the legal engagement and contract negotiation with the support of MSF legal department. For quality and quantitative production requirements, the manufacturer was closely worked with and manufacturer site audits, with the support of an independent auditor, were also performed.

2.2. Proof of principle, evaluation in controlled environment

The development of a diagnostic test usually follows a series of phase gates from identification of the diagnostic target, optimisation of test reagents, to the development of a test prototype that then undergoes a series of evaluations. In addition, previously CE IVD marking was known to not be as stringent as they should be. This principle was applied to the most important items and for some, were the subject of studies performed by Sien Ombelet, Ph.D. Specifically Dr. Ombelet performed studies evaluating the blood culture system, the identification system (ID) and Pre-identification system (Pre-ID). Herein, Chapter 2 describes the development and validation of the sub-culturing system and Chapter 3 describes the development and validation of the AST system.

The laboratory evaluations on adapted items were done using known bacterial strains of LMICs origin for testing of reagents as well as the testing of the ability for use by non-expert users. For material and biomedical items, testing consisted of

making sure that machine turned on or worked when setup and results were consistent with expectations when run in a controlled environment mimicking field conditions (electrical interruption, temperatures, etc.). Final products are briefly described in section 4 of this chapter.

A total of 14 experiments were done between early 2018 and late 2019 on the different types of reagents by the ReSIST team, INSERM U1184 team and the bacteriology laboratory of Bicêtre Teaching Hospital in Kremlin-Bicêtre (France), the Institute of Tropical Medicine (ITM) in Antwerp (Belgium), and the University Teaching Laboratory Hospital (LHUB-ULB) of Université Libre de Bruxelles (ULB) (Belgium). A complete list of laboratory performance evaluations can be found in Annex 8. Most of the equipment was tested in February 2019 at LHUB-ULB during a mock exercise where all pieces of equipment, reagents, etc. were assembled for the first time to test for usability. The process of these evaluations are not fully described in this document. The complete list of reagents, equipment and tests can be found in Annex 2.

2.3. Field performance evaluation

Testing in the field of the assembled prototype in LMICs against a gold standard in order to study the performance characteristics under real conditions and to evaluate the ease of use by non-experts and its clinical impact was critical. To independently evaluate the first and second prototypes assembled with the different components, at different stages of iteration or development, Epicentre research centre (Paris, France) was selected to support the conceptualisation of the study protocols and implementation, considering state-of-the-art field studies recognised guidance. All studies were approved by an Ethical Review Board (ERB) following standards as set for any MSF study. Two evaluations were done, one in Haiti MSF Burn Centre where the usability of the prototype V1 was evaluated between June 2019 and May 2020. A second evaluation was done in Carnot MSF district hospital in Central African Republic for performance and usability between September 2021 and May 2022. This work is presented and described in Chapter 4 of these documents.

2.4. Quality Management System, Training materials

Improving existing techniques, providing an easy means for transport and supply chain management, providing an adapted LIMS with integrated decision support system does not make a clinical bacteriology laboratory solution easier to use by non-expert technicians. Technicians need to have access to the best possible adapted training and teaching methods to facilitate the implementation of the Mini-Lab. As well,

having access to understandable, easy to use and comprehensive Standard Operating Procedures (SOPs) and designed for purposed bench aids within a comprehensive and adapted Quality Management System (QMS) was an essential part of this work. This work has been done alongside the development and evaluation of the different elements constituting the Mini-Lab and its different consolidated V1 and V2 assembled prototypes. This work has first started by doing an extensive landscape analysis of existing training materials and development strategy was then structured to ensure that pedagogy objectives set in the Mini-Lab specifications were answered. Different specialists provided support in the development of documents, training objectives and content, such as instructional designers, QMS specialists, etc. This work reflects the collection of multiple experiences and expertise acquired by the Mini-Lab team.

Results of the development of documents, training, didactic material, etc. synthesized are, presented in section 5 of this Chapter. The documents presented are not intended to be exhaustive but are to provide a description of the development process not the end content. The end content may be accessed by requesting access to the Mini-Lab QMS toolbox and Tembo MSF platform to follow the e-learning training. In Annex 17, schematic figures are presented around the final version of the analytical workflow, and the main elements of equipment that now composed the Mini-Lab.

2.5. Production and monitoring.

One phase often forgotten by manufacturers is monitoring or surveillance after introduction of a product into the market, especially when it comes to knowing the real cost benefit of the product and the usage practices by the end users. This will not be described in depth in this document but a set of Key Performance Indicators (KPIs) (4.5.2 Indicators follow-up Mini-Lab-TLB-V1.2) were defined to support MSF in monitoring the cost and impact of the Mini-Lab. Also, the final assembly process has not been addressed in this document and has been subcontracted to MSF Logistic supply centre in Bordeaux, France.

3. Box-Bench platform to protect and transport selected equipment for rapid setup

3.1. Introduction

The necessity to rapidly deploy a laboratory able to provide microbiology support in a humanitarian context has always been present. For example, in response to the earthquake that struck Haiti on January 12, 2010, the Israel Defence Forces dispatched a rapid-response team intended to establish a 72-bed mobile hospital with bacteriology capacity onsite[89]. Deployable field microbiology laboratories have gained attention with the 2014 Ebola outbreak and the necessity to rapidly deploy specialised laboratory units able to track the highly contagious virus[90] and more recently with the SARS-CoV-2 pandemic[91], [92]. The development of portable or transportable laboratories has so far been done either by integrating existing off the shelf laboratory equipment, test devices and methods, into shipping containers that have been converted into modern laboratories[91]–[93], placed on the back of a truck[94] or for more rapid response within flight cases[90], [95]. This type of service is provided by several companies around the world, ranging from BSL3 or BSL2 type laboratories. Unfortunately, this approach is costly (between 150 000 euro to 350 000 euros) and rarely integrates adequate materials, and equipment. From MSF experiences, container laboratories take time to ship and install and the cost is an issue. Also, deployable laboratories in flight cases have been designed by academic teams and generally only address viral detection with the use of PCR. During the Ebola 2014 outbreak, among the 4 laboratories deployed and visited by MSF, it was noticed that laboratory benches, one of the key elements to provide safe work environments, were replaced by unstable dining tables.



Figure 7: BSL3 laboratory in a maritime 41-foot container, IMEBIO (France)

3.2. Description of the development methodology

Developing a concept to transport all equipment and to be used as laboratory benches within a given space, addressing multiple challenges: (i) the design itself (ii) the material and production method, (iii) the number and types of modules necessary to provide a safe and adequate working environment and (iv) the space and workflow organisation within the laboratory when it has been deployed and is running.

1- Need identification and translation.

- Need definition + Benchmarking
- Job analysis
- Ergonomic analysis
- Aesthetic study
- External Functional Analysis
- Creativity Session

2 – Product definition.

- Research for product architecture and technical solutions
- Internal Functional Analysis
- CAD Design / Sizing and components selection
- Technical and financial Analysis

3 – Product validation.

- Functional model design
- Laboratory test
- Usability Test
- Validation
- Design of two prototypes
- On site test
- Validation and product folder formalisation

Figure 8 : Standard methodology pathway description used by the LCPI for product development.

With the support of the Innovative Product Conception Laboratory (LCPI) from the school of engineering ENSAM (Paris), a standard methodology for product development was applied (Figure 8.) This started with defining the life cycle of the product, an external functional analysis and requirements for all key functions and attributes.

During the internal functional analysis process, the usage scenario of the laboratory



Figure 9: artist's view on the capacity to transport the Mini-Lab component on the standard Toyota 4x4, Land Cruiser pick-up, STRATE College (France)

was defined by observing the different steps in an existing clinical bacteriology laboratory at Bicêtre Hospital and in an MSF laboratory in the Ivory Coast. The outcome of these observations was discussed with a focus group of the scientific committee and along the development of the analytical

component, specific usage scenarios were tailored for the Mini-Lab.

After defining core functions and requirements for the concept and usage scenarios, several iterative back and forth sessions were done with future users, with the team of

ENSAM proposing several concepts and collecting feedback to fine-tune the final concept.

Mock up wooden box benches were then constructed and installed into a room to simulate the deployed laboratory (Figure 10 and 11). After several iterative meetings with the scientific committee and future users, feedback was integrated to adapt the concept, the module organisation, workspace and much more.

The mock-up phase then progressed to a prototype phase where the engineers of ENSAM and Mini-Lab, with support of several

small size manufacturer, developed iterative prototypes composed of different materials (plastic sheet boiler making assembly, phenolic plywood, fiber glass with resin). Each of the prototypes was installed and tested in the field (described in Chapter 4) and user feedback was collected for continuous improvements. All types of material used did not comply with the criteria set in the external function analysis. With the support of a specialist in roto moulding technology (Philippe Vigouroux, ID Roto Solution, France) the concept was further adapted for the complexity of a roto moulding process. After an open request for quotation, a final selection and engagement into a production workflow was made (Rototec, Saint-Malo, France). This workflow started, (i) by selecting the manufacturer able to create the adequate aluminium mould able to address all constraints, (ii) test industrial pre-series to adjust all parameters of the roto moulding machinery, (iii) produce the first batch, and (iv) incorporate the box-bench production mechanism into the final assembly procedures with MSF logistic.



Figure 10: Creativity session at ENSAM laboratory with members of the scientific committee to tailor the laboratory workflow per modules, Mini-Lab (France)



Figure 11: Test of the wooden mock up box bench at MSF logistique in Bordeaux (France) with logisticians, Mini-Lab (France)

3.3. Description of the final product

Figure 12 represents the Mini-Lab life cycle from a design, development, and production perspective with seven main phases. It provides the different important phases

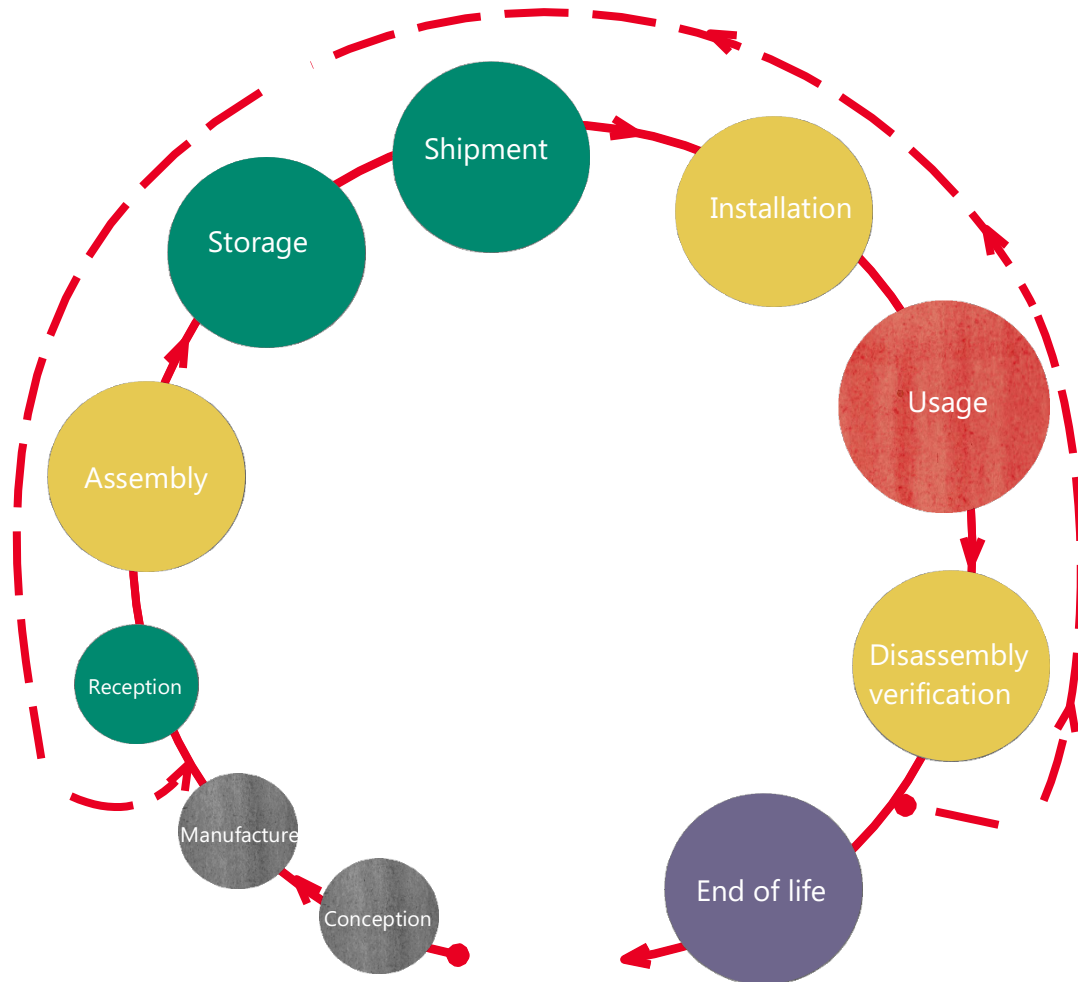


Figure 12: Life cycle of the Mini-Lab from a production point of view, LCPI (France).

that were used to initiate the description of the external function or attributes present in Table 5. In total 35 key functions or attributes were set to develop the Mini-Lab box benches.

Table 4: Description of the principal function and functions of the Mini-Lab Box-bench taking into consideration the different phases of the Mini-Lab production life cycle

Principal Functions	Functions
Allow operators to assemble the kit	Must be packaged with standard tools and machines at MSF logistics
	Be easily located in stock and routed to the place of assembly
	Meet packaging standards
	Be easy to condition, in the right order (comprehension / ergo / readability)
	Protect Mini-Lab subassemblies
	Be stored alongside/above/underneath other products, without damage

Protect Mini-Lab elements	Do not hinder the movement of handlers, allow them to identify the products inside the Box-bench
	Do not exceed a floor area corresponding to the size of a pallet
	Be stored on the ground
	Resist external climatic aggression and physical aggression
	Contain all Mini-Lab elements
	Be easily locatable at the place of storage
Be transported from the place of arrival	Be easily locatable and identifiable by handlers
	Be easy to load, unload by 4 people maximum
	Can be transported by handling machines, have an external volume corresponding to a transport pallet
	Fit at least into an MSF 4x4, not be too heavy
	Resist climatic aggression and physical aggression
	Be stable once charged, not be too heavy, have a well-distributed mass
	Comply with transport standards, air, ground
	Be easy locatable from start to finish
Allow users to install the Mini-Lab in its environment	Be able to handle with standard handling machines and tools, use as few tools as possible during assembly
	Integrate into the environment (inhabitants - existing structures, etc.) - Have a suitable environment
	Connect to existing networks, clean water, dirty water, electricity, internet
	Resist climatic aggression and physical aggression
	Make installation and assembly instinctive for users, be intelligible by any type of user regardless of language
	Provide safe and intuitive work environment
Allow users to process samples	Meet the operating standards of a laboratory
	Be transformed into a standard, flat, robust laboratory bench / workstation with adequate lighting
	Be easily cleanable, be easily maintainable, removable
	Integrate electricity circuit protection
	Enable proper waste sorting and management
	Protect samples from possible airborne contamination
	Resisting climatic and physical aggression
	Facilitate the movement of users, facilitate use
	Do not damage samples
Allow users to disassemble and check the working status of Mini-Lab elements	Be able to handle with standard handling machines and tools, use as few tools as possible when dismantling
	Restoring a healthy environment
	Resist climatic aggression and physical aggression
	Make disassembly and verification instinctive for users, be intelligible by any type of user regardless of language, traceability, check list check
	Contain items that have been cleaned, decontaminated and ready for reuse
	Respect the environment
	Allow easy and intuitive disassembly with as few tools as possible
	Not be dangerous to the operator

Allow the user to dismantle the different parts of the Mini-Lab	Be able to reuse all or part of the different elements of the minilab
	Planning for good waste management

In this part of the document, the final product as of December 2022 is described. The different iterations are presented in Chapter 4 as part of the prototype evolution description for the first pilot study in Haiti and the second pilot in the Central African Republic. Complete scenarios of usage, especially one related to sample management and processing is synthetised in the figure 13 and described in this chapter as part of the analytical workflow description. A complete sample management workflow as diagram is presented in Figure 31. Other scenarios of usage related to installation, waste management, etc. are not described in this document but can be found in the QMS Mini-Lab toolbox.

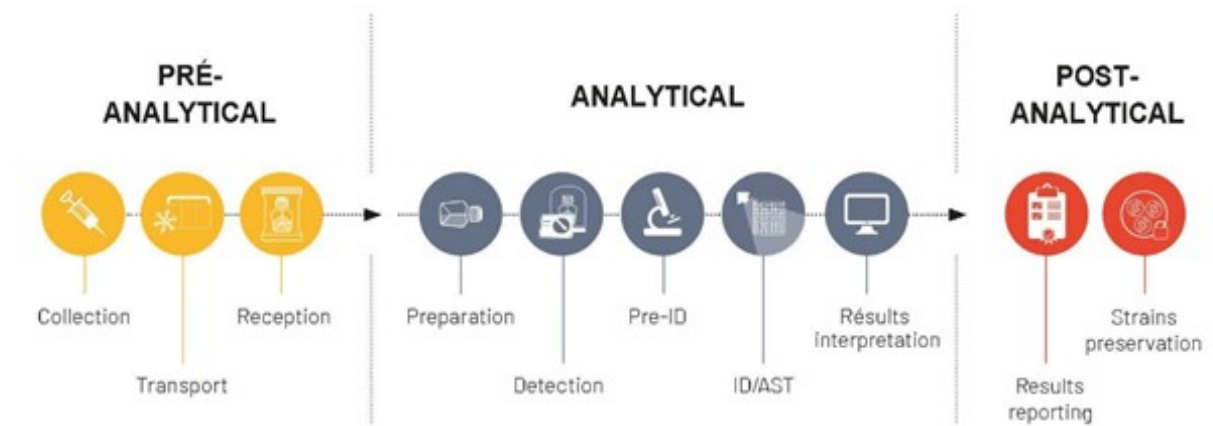


Figure 13: Highlight of the scenario of usage regarding the sample processing part of the laboratory workflow, Mini-Lab (France)



Modular and adaptable box bench



1st PRODUCT DEVELOPED WITHIN MSF, DESIGNED FOR HUMANITARIAN FIELDS





DEVELOPED BY



→ MAY BE USED FOR
OTHER APPLICATIONS





Box-bench platform to transport, protect and rapidly deploy

Transportable by 2 to 4 persons

Can be attached in a container or in a truck or in plane



Sturdy transport boxes ~ 50 kg empty

Impact resistant

Made in plastic (rotomoulding)

Suitable with standard pallet



→ Material can be packed inside the boxes

Transformable into standalone bench / table

-  • Corrosion, disinfection and heat resistant
- Washable surfaces (ISO Standard)
- Integrated lighting 
- Intensity adjustment



Depth 800 mm

Length 1200 mm

- Movable furniture equipped with leveling feature
- Vast work area surface
- Integrated shelve or spout box configuration
- Plug and play modules
- Power circuit protected 
- IP 64 water protection

Height 700 mm

Adjustable: 740 to 920 mm (normal height)
45 mm steps

Figure 14: Poster developed for the MSF General Assembly, 2022, highlighting the results of the Mini-Lab box-bench development, Mini-Lab (France)

The final box-bench design, and a few features are described in Figure 14 as a poster. A detailed 3D modelling is presented in Figure 15 for the box-bench in open and

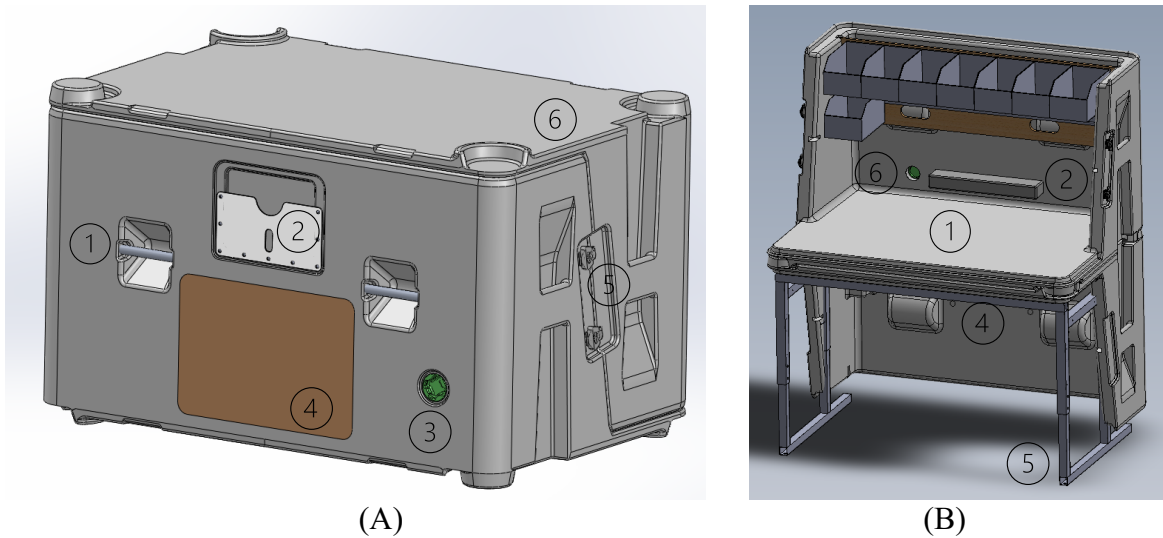


Figure 15: 3D modelling of the (A) Box-bench in close position and (B) in open position ready to provide adequate space for laboratory workers, Mini-Lab (France)

closed positions. The outer size of the box is 75 cm in length, 120 cm in width and 70 cm in height. It provides a total usable volume of stock of 350L. When the box-bench is closed (Figure 15 A), the box is composed of, (1) several built in handles on the four sides to allow easy lifting, (2) on two sides specific built in bulges allow for the insertion of instructions for use (IFUs) or administrative documents for customs clearance, (3) specific connection sockets for electricity, (4) specific flat surface size to accommodate identification stickers, (5) built in butterfly locks to maintain box closure, and (6) each box can be mounted on another one and maintain stability due to specific design forms. (B) When the box-bench is open and in a bench format (Figures 15 B and 16), it is composed of, (1) a flat bench surface of 100 cm length, 65 cm width, (2) an integrated electrical wall multi-socket and two adjustable spot lights, (3) on the upper shelf two sets of mounting rails to accommodate storage boxes or to be transformed into standard shelf, (4) shelves to accommodate further storage capacity, (5) adjustable and stable feet, and (6) circuit protector box.



Figure 16: Photo of the Box-bench roto moulding pre-series test, Mini-Lab (France)



Figure 17: Schema of the Mini-Lab modules identifications and functions, Mini-Lab (France)

The Mini-Lab is composed of 6 Box-bench modules with each having a specific purpose (Figure 17). The Administrative Module (1) allows for the reception of specimens and samples, check conformity, records, print identification and barcode labels, filling of medical and specimen records, quality controls log and all other administrative functions. Hygiene and Security Module (2) allows the technician to perform the Gram stain, provides security elements (eye shower, extinguishers, etc.), preparation of benchtop cleaning solutions and of sampling kits. The Sample Processing Module (3) provides adequate workspace to process and test specimens and samples. The Incubation Module (4) allows for the incubation of blood cultures bottles (BCB), reading of the BCB, incubation of subculture plates, storing of the ID and AST systems and provides a space to store samples for retention. Reading and Interpretation Module (5) allows for the reading of Gram-stained slides under the microscope, utilize reagents for ID and read and record results of the ID and AST systems. Waste Management Module (6) provides a workspace for waste management and disposal and preparation of distilled water.



Figure 18: Photo of the different modules when installed. Modules are presented from module 1 to module 6 from top left to right, Mini-Lab (France).

Regarding deployment of the modules and space organisation, during the past 4 years adjustments of modules within the space of the 20 m² room we were offered to deploy the Mini-Lab have been tried. The concept was proven to be versatile but only under the following constraints to meet safety requirements. Whether the room is square, rectangular, or L-shaped, it is important in the arrangement of the modules with respect to the following rules:

- Module 1 must always be close to the door where the specimen or samples and staff will enter.
- Modules 2 and 6 must be close to each other and near the sink. If this is not possible, the sink must be placed at the entrance of the technical part of the laboratory.
- Modules 3,4,5 must be close to each other.
- If the passage space between the modules is less than 2 m, it is important to avoid having face-to-face modules where laboratory technicians sit and spend most of their time (modules 1, 3, 5).
- Module 6: If the laboratory has two doors, the module for waste management must be placed close to a door that gives direct access to the outside and where personnel and samples will not pass, except hygienists to recover waste. If the room consists of only one door, it is advisable to place module 6 after administrative module 1 and before having access to the technical area consisting of modules 3, 4 and 5.

A complete description of the final product features, specification can be found in Annex 18. The table provides a comparison to the original target product profile of the Mini-Lab and the proposed layout for deployment is shown as an example in Figure 19.

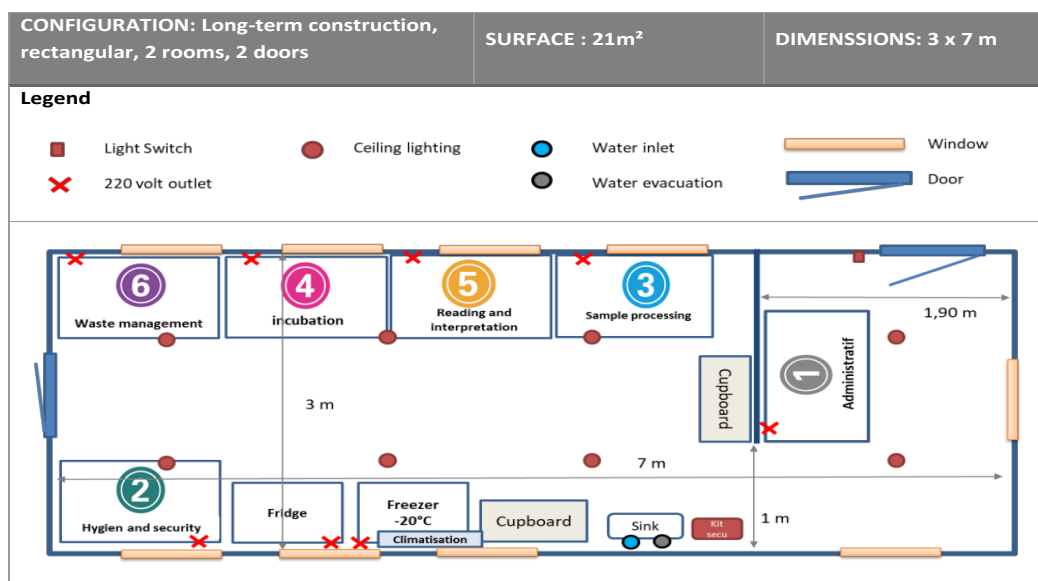


Figure 19: Configuration layout extracted from the document DOC-2.1-AMENAG, Mini-Lab (France)

4. Adaptation, Simplification and Validation of the Analytical Process

4.1. Analytical process description

A simplified description of the analytical workflow is presented in Figure 20 and briefly described below. The main components of this analytical workflow will then be described followed by a synthesis of laboratory validation study's results. A summary of all validation studies can be found in Annex 8.

The Mini-Lab specimen/sample workup is developed around five main analytical components: (i) the blood culture bottle (BCB), (ii) a subculturing system (SCS), (iii) a pre-identification system (pre-ID), (iv) Identification (ID) system and (v) antibiotic susceptibility tests (AST). Individually all these components have received international certifications (CE-IVD or USA Food and Drug Agency, FDA clearance), however further analytical validation in collaborating centres in Europe[96]–[101], and evaluation in clinical settings were performed (see Chapter 4).

The process is initiated when specimens/samples are received in the laboratory (D0), specimens/samples are checked for nonconformity, registered on the LIMS and a unique identifier is provided to the specimen/sample which is then prepared for incubation by inoculating a blood culture bottle, the agar side is then placed in a down position, to flood the solid phase with broth for 15 minutes. Bottles are incubated at 35°C +/-1°C for seven days and visually inspected, using the BCB light box, which is performed twice daily, on days D1 and D2 and once a day after. The second day (D1) blind subcultures are performed on chocolate agar as per procedure on negative BCB. For positive BCB subcultures are performed on both chocolate and Colorex agar as well as a Gram stain. If the Gram staining yields Gram-positive cocci as a result, a coagulase test is performed on the culture broth and then on the day after on colonies to confirm positivity for *S. aureus*. The Mini-LIMS will automatically orient the user on the type of testing to be performed. The same is repeated, on the following days as BCBs become positive. Negative BCBs are re-incubated for a maximum of 6 days, subcultures for max 48h).

On the second day (D2), growth on agar, provides colonies to rapidly orient the clinician in their treatment decisions, a subset of the culture is then used (Gram stain, aminopeptidase, catalase, coagulase, oxidase, indole) to determine phenotypic characteristics of the bacteria. Results of these tests, plus additional information on the type of growth provided by the bacteria on agar (chromogenic, colony type, presence of gas in BCB, type of growth in BCB, etc.) is entered in the Mini-LIMS which includes a probabilistic based algorithm that will interpret the result and provide bacterial groups,

family, genus orientation to the practitioner and for the technician downstream identification (ID) and Antibiotic Susceptibility Testing (AST) procedures.

On the third day (D3), ID and AST panels will be read using the Assisted Reading System, composed of a camera and a viewer box, connected to the Mini-LIMS. This system guides the user in defining the positivity or negativity of reactions on the ID panel wells and determining the Minimal Inhibitory Concentration from the AST panel. The information collected is then interpreted with biotype database of the manufacturer for identification and EUCAST or CLSI AST clinical breakpoints (R/I/S). Identification and AST results are then confirmed and the interpretation provided to the clinician. In addition, the Mini-LIMS will warn the technician in case of errors and provide alerts for IPC in case of important resistance. At each stage, the results are communicated to the clinicians (indicated as a report in the Figure 20). Samples are retained for seven days prior to destruction and bacterial isolates are kept for 6 months at -20°C in brain-heart glycerol broth for quality control or to be referred to National Public Health Laboratory for second line testing or surveillance purpose.

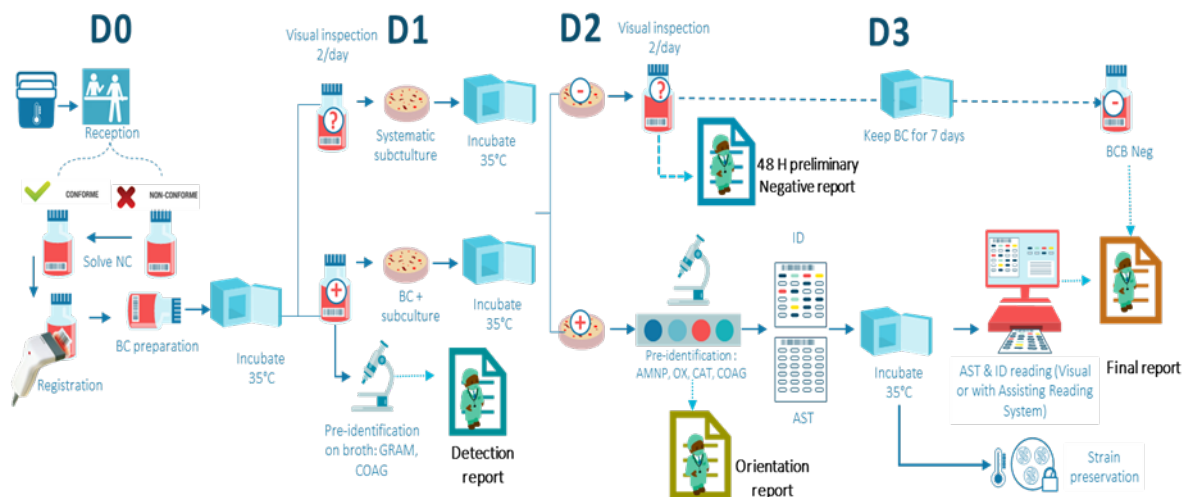


Figure 20 : Scheme of the analytical workflow in the Mini-Lab. Description of analytical components and overview of performance, Mini-Lab (France)

4.1.1.1. Blood culture bottles (BCB)

The blood culture system of the Mini-Lab is composed of a biphasic bottle (Autobio Diagnostics, China) containing both liquid and solid culture media to allow the growth of aerobic bacteria (Figure 21). The bottle is incubated at 35°C in a static incubator and bacterial growth is detected visually with the help of a modified Rhesuscope light box (The Rhesuscope is a device used for the reading of rhesus factors and is composed of an opaline reading plates), increasing the visibility of growth in the BCB.

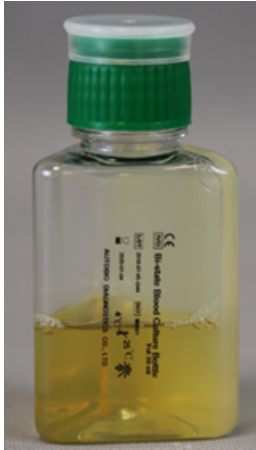


Figure 21: Biphasic blood culture bottle, Autobio diagnostic, China, Mini-Lab (France)

The Autobio blood culture bottle was tested in a comparative evaluation study against the BacT/ALERT (bioMérieux, France) BCB with the BacT/ALERT in both automated and manual mode. In the study, BCBs were inoculated with human blood and spiked with clinical bacterial isolates from LRS[97]. The Autobio bottle showed a recovery rate (yield) of 95.9% of all inoculated BCBs, compared to 95.5% of manual BacT/ALERT, and 96.1% of automated BacT/ALERT[97].

The study also showed that 90.7% of all the recovered Autobio bottles were positive at Day1 (24h after incubation) and 100% at Day2, compared to 75.0% at Day1 and 97.6% at Day2 for the BacT/ALERT manual bottles, showing a media performance of Autobio comparable to that of the BacT/ALERT. In addition, it was also easier to visualize growth in the Autobio BCBs[97].

4.1.1.2. Sub-culturing system

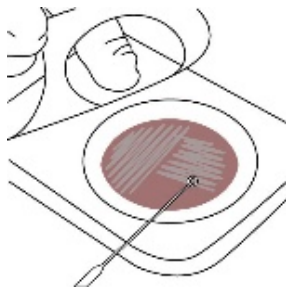


Figure 22: Artist drawing of InTray Chocolate agar being inoculated with loop on top, photo of a closed InTray cassette on bottom, Mini-Lab (France)

The system chosen for subculturing (described in Chapter 2), is a ready-to-use agar plate in a miniaturised sealed cassette (6 cm diameter), for the culture of aerobic organisms transferred directly from the BCBs: the InTray® cassettes (Biomed Diagnostics Inc, OR, United States).

Different InTray media formulations were tested with BCBs samples. Based upon the data generated MH chocolate agar and a chromogenic media (Colorex Screen) were chosen for routine use in the Mini-Lab (Figure 22)[99]. Within the Mini-Lab a colour specific atlas of different types of bacterial growth is available through the LIMS. Pictures can be selected by the technicians and compared with the picture taken from the camera attached to the microscope.

These two agar formulations were then further tested in parallel with standard agar media (PolyViteX, bioMérieux and UriSelect™4, Bio-Rad, respectively), using positive blood culture bottles directly from clinical routine (BacT/ALERT, bioMérieux), and simulated multi-microbial infections prepared using frozen strains from low-resource settings and inoculated into bi-phasic BCBs.

Out of 70 positive clinical BCBs sub-cultured (n=65 adults and n=5 paediatrics), In-Tray MH chocolate agar showed growth in 97% (n=68) with 100% agreement with standard media. Time to detection (TTD) was <20h in 97% of positives. Detection of multi-microbial cultures was 94.4% on Colorex™ Screen and 88.9% of Müller-Hinton (MH) chocolate agar, out of a total of 99 simulated mixed cultures[99].

4.1.1.3. Pre-Identification system (pre-ID)

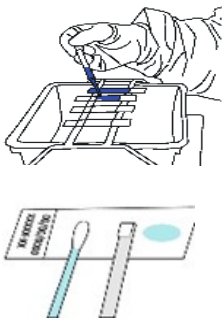


Figure 23: Artist drawing of the pre-ID testing steps (Gram staining, aminopeptidase, catalase, oxidase), Mini-Lab (France)

The pre-identification system allows for an initial identification of an organism's group level. Due to the morphology (cocci and rods) of bacteria, staining characteristics (Gram reaction), and the reaction to L-alanine aminopeptidase, oxidase and catalase tests (Figure 23), microorganisms can be grouped into Gram positives or Gram negatives and further characterised to group level with the support of a probability-based algorithm.

The algorithm developed was constructed using the probabilities of test results from validation studies and a weighted combination for each pre-defined group of organisms, to give a final most likely grouped based probability. In the Mini-Lab, the Pre-ID system is composed of 5 tests: Gram staining (Gram-hucker stain, RAL, France), aminopeptidase (Gram Test Stick, Liofilchem, Italy), coagulase (Coagulase Plasma Lyophilised, Oxoid, UK), oxidase (Oxidase Test Swab, Hardy Diagnostics, USA), and catalase (Catalase reagent dropper, Liofilchem, Italy).

The evaluation study was performed in parallel with the blood culture evaluation study, however, results were not published, where single test results were evaluated against expected results of the known pathogens inoculated. Results showed 86% accuracy for morphology recognition and 96.8% for correct Gram classification when using Gram staining. Aminopeptidase showed 85% overall accuracy in Gram classification with a higher performance for some groups of organisms (e.g.. Enterobacterales, 100% vs Gram stain 95%, Gram-positive rod 100% vs 66.7%).

The oxidase and catalase tests showed overall, 93% and 84% accuracy, respectively. The coagulase test, for rapid distinction of *Staphylococcus aureus* versus coagulase-negative *Staphylococcus* species, did not undergo a full validation but was tested during the first field pilot in Haiti, showing 45% sensitivity and 100% specificity when used directly on blood culture broth, and 92% sensitivity and 90% specificity on cultures on solid media. Final characterisation of the isolated organism at the group level by the

combination of the Pre-ID tests was evaluated in the field pilot 1, showing a 98% agreement with final identification performed by a reference laboratory.

4.1.1.4. Identification (ID) system



Figure 24: MSF Neg/Pos ID Panel type 2 and *Pastorex latex* kit, Mini-Lab (France)

The ID system allows identification of organisms at the species level to provide more accurate information for treatment and surveillance. A customised panel for MSF, called “MSF Neg/Pos ID Panel Type 2” (Beckman Coulter, US, ref C38213) was specially designed using MicroScan technology as the foundation. It consisted of a dried formulation of reagents for longer shelf life (12 months) and storage at room temperature. The panel (also called “dual”) combines the commercially available identification panels for Gram-negative and Gram-positive organisms on one single microplate (Figure 24) and is further described by Ombelet et al. in a recent article[100]. The ID panel was further tested in a validation study using clinical isolates from LRS.

The final identification obtained was evaluated against identification by standard methods (MALDI-TOF), at genera and species level[100].

The results of this study showed excellent performance of the Gram-negative panel, with 94.9% of isolates correctly identified to the species level, in line with several previous studies evaluating the MicroScan panels[100]. The Gram-positive panel had a lower performance with 85.9% of tested isolates correctly identified to species level. This was again in line with other published studies for *Staphylococcus* and *Enterococcus* species. For a more detailed description of this study and the results, refer to the published article [100]. Another limitation of this Dual ID panel is the incapacity to identify *Neisseria.spp* and *Haemophilus influenzae*, agglutination test Pastorex (Biorad, UK) was then incorporated in the ID algorithm the identification of *Neisseria meningitidis* (group A,B,C, Y/W135), *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *Streptococcus* group B. This is a test already well evaluated by MSF and colleagues in the field[102]–[104].

4.1.1.5. Antibiotic Susceptibility Testing (AST)

The AST system, described in Chapter 3, is an essential and innovative component of the Mini-Lab, developed in collaboration with Beckman Coulter® (West Sacramento, California, USA). The test is based on the determination of the minimum inhibitory

concentration (MIC) for the determination of resistance and susceptibility patterns of pathogens; it is based on both EUCAST and CLSI breakpoints.

Beckman Coulter developed together with MSF, three AST panels, MicroScan MSF Gram Negative (ref C32699), Dried Overnight Gram Positive (ref C32698), and Fastidious (ref C32670) panels (Figure 25), using the same technology described for the ID panel. Each was designed specifically to include all antibiotics of interest for clinical and surveillance purposes in LRS settings and as required by the GLASS initiative[101].



Figure 25: MSF Microscan AST panels (see further description in Chapter 3), Mini-Lab (France)

An evaluation study was performed on these panels to verify their performance using organisms from LRSs as well as challenging organisms as determined by members of the Scientific Committee. In the study, the accuracy of the final interpretation (Resistant, Susceptible or Intermediate / Susceptible Increased exposure) was evaluated versus methods as proposed by EUCAST (e.g. disk diffusion method, etc.), and applying EUCAST breakpoints for interpretation. Acceptance criteria, definitions of essential and categorical agreement, plus classification of errors into Minor (MIN), Major (MAJ) and Very Major (VMJ) followed ISO 20776-2:2007. The results of this study showed:

- Gram positive panel: overall categorical agreement (CA) was >90% for all organisms/antibiotic combinations and VMJ were $\leq 3\%$ for all antibiotics except for fosfomycin/Staphylococcus spp. and *Enterococcus* spp./quinupristin-dalfopristin combinations[101].
- Gram-negative panel: overall CA was >90% for all molecules/ organism combinations and VMJ were $\leq 3\%$ for all molecules/ organism combinations[101].
- Fastidious panel: overall CA was > 90% and VMJ was $\leq 3\%$ for all molecules/ organism combinations[101].

5. Quality Management System

As previously described, the Mini-Lab concept is the interleaving of different layers of adapted-, optimised-components, processes and usage scenarios. To ensure that this complex puzzle met quality requirements to ensure that accurate results in the absence of an on-site expert microbiologist were provided and to provide standardisation of processes and procedures to end-users (within MSF operations), a robust and comprehensive Quality Management System (QMS) was necessary. The integrated QMS was designed following relevant recommendations from the WHO Stepwise Laboratory Improvement Process Towards Accreditation criteria³ (SLIPTA) and ISO 15189. It was also designed to address the 12 pillars of the Quality System Essential structure developed as outlined by CLSI for WHO[105]. Usually a QMS for a standard medical laboratory only applies when the laboratory is installed, as the Mini-Lab is a deployable laboratory, the Mini-Lab QMS takes into account the “operational life cycle” of the Mini-Lab as described in **Erreur ! Source du renvoi introuvable.**

The Mini-Lab QMS is accessible from an MSF SharePoint platform (Microsoft Inc, Redmond, Washington, USA) within the QMS Mini-Lab tool box, referred as tool box (Figure 26), accessible upon demand at this address <https://msfintl.sharepoint.com/sites/GRP-PAR-MINILAB2> . The Mini-Lab SharePoint platform gives access to the Mini-Lab e-learning courses on the MSF Tembo platform and to the LIMS sand-box (access to a test server).

Tool Box (EN)



Figure 26: Snapshot of the QMS Mini-Lab Tool Box structure as available, snapshot taken on the 03rd February 2022, Mini-Lab (France)

³ <https://www.who.int/tb/laboratory/afro-slipta-checklist-guidance.pdf>

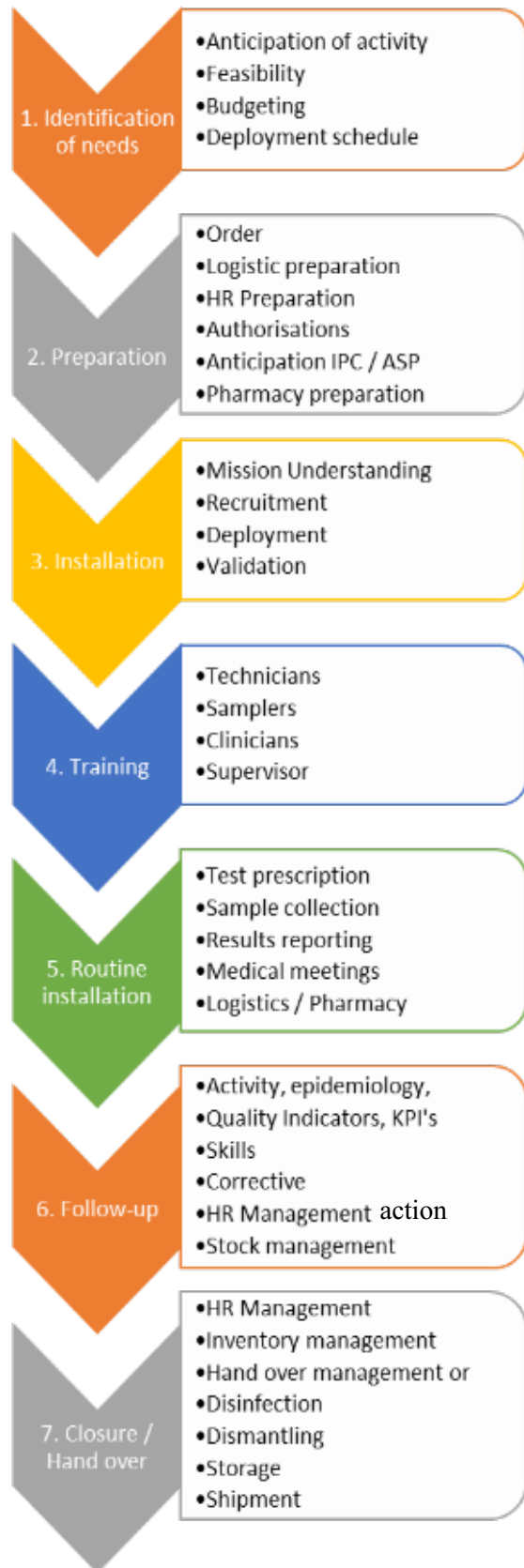


Figure 27: Operational Life cycle of the Mini-Lab installation on MSF fields. This life cycle does not take into account the production cycle, Mini-Lab (France).

The tool box is composed of nineteen folders, all embedding documents, didactic material, training, IT tools, etc. that are necessary for all actors intervening in the operational life cycle of the Mini-Lab. Also classification is done per type of activity, specific features on the SharePoint allow some staff to be directly guided to useful documents to perform the activities required (e.g. water and sanitation specialist, decision makers at the headquarters, pharmacist, etc.).

As an example medical supply managers, also referred to as pharmacists in the field, are generally involved in different aspects, during the preparation phase with the ordering of supplies, custom clearance, preparing the storing space at the central pharmacy, etc. During installation and routine phases they can be involved with procurement of reagents and consumables, implementation of the stock management, and in charge of destroying expired reagents.

Also, for some personnel such as the microbiologist involved in the implementation (Mini-Lab implementer), or the on-site manager, they can access a ready to use check list with proposed sequencing timing for multiple phase-based actions (Figure 27).

5.1.1. Facilities and safety

As the Mini-Lab has been developed to be used by trained technicians but non-experts in microbiology, it was conceived with an objective to minimise and mitigate risk.

A complete risk analysis was done, using WHO guidance[106], identifying five risks (biological, fire, chemicals, ergonomic and electrical) and different probabilities of occurrence. As described previously, safety has been one of the main development focuses for the “box-benches”, potential risks identified were mitigated with embedded safety equipment for electric, fire, biological, ergonomic risk mitigation (extinguishers, eye shower, etc.). Biological risk is mitigated by workflow measures, by the choice of techniques (sealed transfer system from identified hazardous steps), upstream equipment, by wearing of PPE, by the information and training of staff as well as by the maintenance and cleaning of the laboratory and equipment as well as good waste management. Ergonomic risk of accident and spillage have been reduced through the use of specific furniture and collective protective equipment (e.g. rack to transport slides, removal of the sharp edge of equipment, etc.). Specific modules for 1 day on site training and e-learning have been developed concerning hygiene, security and safety, with case studies, simulations for the use of safety equipment (e.g. extinguisher, spillage kit, etc.), emergency management and first aid. Also, laboratory design and spatial organisation has been addressed by the development of standard recommendations for the room space for deployment of the Mini-Lab, and with the provision of several installation scenarios based on the physical aspects of premises and rooms. Also a specific program for waste management and maintenance of the Mini-Lab has been developed from the segregation solid biohazard waste to the waste treatment plant of the hospital (specific recommendations). Procedures have been developed for the laboratory staff and the site hygienist. Also, a treatment plan has been provided for the safe handling of and disposal of expired reagents using available equipment in the field (i.e. high temperatures incinerator, co-processed incineration), all based on experimentation and experience in the field.

5.1.2. Equipment

All equipment was selected based on safety, low maintenance requirements, and robustness in tropical conditions where power outages, dust, humidity, and other challenges are common. For example, the incubators developed with Global Goods and JP Selecta can withstand a 12-hour electricity cut without having the internal temperature fluctuating within 1°C of the set temperature. The autoclave from Tuttnauer has an embedded automatically defined cycle for waste sterilisation with safety measures to secure the door when electricity is cut. Installation, calibration, equipment setup, usage, preventive and corrective maintenance procedures are available. In addition, maintenance plans and troubleshooting FAQs are provided with the Mini-Lab. All documents are available for troubleshooting, service, repair and retirement of equipment,

equipment maintenance and are accessible for the onsite biomedical engineer team which is in charge of curative maintenance. Several check list and log books are also available to track equipment maintenance and to ease the daily workload of the laboratory personnel concerning daily preventive maintenance.

5.1.3. Purchasing and inventory

The Mini-Lab concept simplified the logistics concerning reagents and consumables, by first reducing the number of reagents and labware required. Secondly, the number of reagents requiring cold chain has been reduced and most of the tests can be stored at 25°C or controlled ambient temperatures.

Reagents are supplied in a kit form or are predefined on the order form, this embeds all necessary consumables required to process a predefined number of blood cultures. Inventory management is done using the MSF “Easystock” information system or with dedicated excel based tools. Forms, logs, receipts and storage of supplies, monitoring of inventory are all addressed within specific SOPs or sections of the tool box. Also several check lists are available for the inventory and stock management programs. Implementing a laboratory in a remote area requires strong supply chain management; MSF is known to have effective supply chain management for medical items. However, supply chain management related to a clinical bacteriology laboratory is relatively new to MSF and field workers. A set of documents has been made available for the supply management teams to explain the specification of the different reagents, as well as trouble-shooting documents that embed lessons learned from past deployment.

5.1.4. Process control—sample management

Sample management has been partly addressed in section 4. All aspects, from collection, preservation, sample processing, sample storage, retention, disposal, and sample transport have been addressed within simple and clear SOPs. Bench aids are also available and have been compiled into a laboratory handbook. Multiple colour atlases are available containing a collection of pictures taken for the expected results (Gram stain, colony morphology, etc.). For reading of ID and AST plates, several bench aids with colour guided interpretation are also provided.



Figure 28: Laboratory technician in Carnot MSF performing routine testing under supervision of a trainer microbiologist, October 2021, Mini-Lab (France)

5.1.5. Process control—quality control / Occurrence management / Process improvement

Internal Quality Controls (IQC) allow for the detection of systematic as well as random errors, and aid in the identification of invalid and non-acceptable results. In the Mini-Lab, twelve standardised bacterial ATCC (American Type Culture Collection) strains, have been chosen for either routine or test/specific quality controls and a troubleshooting FAQ document is available to guide users if nonconformity in QC is occurring. Three kits of ATCC strains were developed with Microbiologics, Inc (St. Cloud, Minnesota, USA) based on the KWIK-STIK™ technology. The first two kits are composed of 12 ATCC strains advised by EUCAST and the third kit is composed of a few other ATCC strains for training purposes (contaminants mainly). All QC strains have been validated with 20 repetitions on each of the reagents to calculate the standard deviations for the quantitative methods (MIC) and standard growth behaviour. All information is made available by strain on a QC Strain Identification sheet (see 8.1.1 QC Strain Identification Sheet EUCAST V11-DOC-V2.2 or Annex 5) and in the database of the QC follow-up tools. SOP (8.1 Internal Quality Control-SOP-V1.1) describes all the steps of the QC program and a resolution plan is available to support the technician in the root cause analysis if any discrepancy arises (8.3 Resolution Plan-DOC-V1.1). QC results are electronically followed and automatic alerts warn the user via an excel based tool for QC follow-up. The user enters results, the tool warns the user if the value is out of the defined range, the tool provides QC follow-up indicators. Sources and consequences of laboratory errors, investigation of occurrences, rectifying and managing occurrences. Tools for process improvement, quality indicators, sources and consequences of laboratory errors, investigation of occurrences, rectifying and managing occurrences are all addressed in the tool box and the personnel is trained on it use.

5.1.6. Assessment—audits, external quality assessment, norms, and accreditation

During the development of the Mini-Lab, the Mini-Lab team and external consultants have strongly advised that the Mini-Lab participate in External Quality Assessment (EQA) proficiency either as proposed by MSF or by local authorities. Inspections and Mentoring by an experienced microbiologist every 9 months has also been advised. International standards and standardisation bodies, national standards and technical guidelines, used to develop the QMS are all presented in the Mini-Lab toolbox. The Mini-Lab cannot be ISO 15189 accredited as this only applied to fixed facilities and has not been objective for the Mini-Lab project. However, in a recent but unpublished

evaluation of the second Mini-Lab prototype deployed in Carnot MSF in Central African Republic, the evaluator rated the Mini-Lab 4 stars out of 5 utilising the WHO SLIPTA evaluation process (result presented in Annex 4).

5.1.7. Personnel / Organisational

A set of tools for recruitment, orientation (Job description, questionnaire, recruitment process follow-up, etc.) and duty follow-up (task allocation tables, duty roster, competency assessment program and check list) are available within the Mini-Lab toolbox. The Mini-Lab supervisor always has access and will be trained on the use of competency and competency assessments, and the recording of the competency in a designated log. Specific onsite training has been developed for lab technicians, lab supervisors, practitioners and nurses collecting blood for testing in the Mini-Lab (from test prescriptions to test results), with theoretical, interactive, practical modules and teaching guides to help the microbiologist facilitate onsite training. An e-learning version of the training for laboratory technicians has been developed for continuous educational purposes. Training is provided prior to the start of laboratory activities, this includes competency evaluation at the end of training to verify the effectiveness of training and the competency of the technicians. Table 5 provides an overview of the different training courses offered.

Table 5: Description of the trainings available for different target audiences intervening in the Operational Mini-Lab Life cycle

Training Name	Nb Hours	Target audience	Objective
Mini-Lab Technician (pBMLT)	135 hours / 20 days	Laboratory technicians and supervisors recruited for set-up	To enable learners to provide quality results to clinicians, to use the various equipment that is in the Mini-Lab, to develop a critical sense of their workflows and to manage the laboratory optimally.
Mini-Lab Supervisor (BMLS)	30 hours / 20 days	Mini-Lab Laboratory Supervisor	To enable learners to solve problems or find help to resolve non-conformities in analyses or in the operation of the laboratory, validate and communicate test results to clinicians, collect and analyse activity data, quality monitoring, epidemiological surveillance, manage inventory, support the pharmacist in pharmacy inventory management and international ordering, assess the competence of technicians and provide adequate training to newcomers.
Mini-Lab Sampler (BMLC)	4 h or 2 times 2 h	Nurses or medical staff authorised to take samples	To enable learners to understand the challenges of quality sampling and the impacts on the individual and downstream testing, identify the analyses in line with the indications (justify the purpose of the

			prescription), master and carry out the different procedures for collecting and routing samples.
Mini-Lab Diagnostic tools Stewardship (MLDS)	3 h	Clinicians	To allow learners to understand the challenges of the proper use of diagnostic tools made available, understanding the test utilisation criteria, to acquire an understanding and a critical sense of the results provided by the Mini-Lab.
Mini-Lab Technician (Distance) (eBMLT)	20 -25 h 32 Modules	Laboratory technicians in the field, prerequisites for future implementers	To support Mini-Lab users with different e-learning modules. Before the face-to-face training for the first time and in their daily use of the Mini-Lab and allow them to understand more quickly the use of the different techniques and equipment used at the Mini-Lab, to develop a critical sense of their work and to manage the laboratory optimally.
Mini-Lab Implementer (BMLI)	70 hours, 10-day	future implementers (microbiologist or experienced lab tech)	To be able to deploy, implement the Mini-Lab and its activities, train lab techs, supervisors, and nurses to use properly all the available procedures. To integrate the activities of the Mini-Lab into the processes of the site

From a QMS organisational requirement, management roles, organisational structure, organisational functions are all set and available within different check lists and procedures. At MSF Headquarters, a support team, composed of experienced microbiologist and engineers has been assigned to provide troubleshooting, update users of any change and improvement into the QMS, Mini-LIMS and Mini-Lab related components.

5.1.8. Customer service

Assessing and monitoring customer satisfaction, customer satisfaction surveys have not been addressed as they are not an objective that was set by MSF.

5.1.9. Documents and records

The QMS Mini-Lab toolbox presented in Figure 26 is composed of 484 documents, all addressing the different aspect of the operational life cycle described in **Erreur ! Source du renvoi introuvable.**

A printed detailed Mini-Lab handbook is also provided with the Mini-Lab, its intent is to empower end users to be fully autonomous for preparation, deployment, using and closing of the Mini-Lab. It contains some of the elements present in the QMS Mini-Lab toolbox such as: (i) guide and book of laboratory forms for set-up procedures, organisational management; ii) good laboratory practices on stock and document management systems, health and safety and laboratory forms; (iii) equipment monitoring and quality control; (iv) SOPs for the pre-analytical, analytical and post-analytical

steps; (v) bench-aids, i.e. visual synthesis of SOP to guide the lab technicians while working at their bench. In addition, a set of templates is available for the technicians outlining their daily duties and how to provide results to clinicians in the case of LIMS failure.

5.1.10. Information management

The Mini-LIMS is a tablet based LIMS that allows for simplified data entry, process follow-ups, sample management, and validate results. The tablet-based format reduces data entry errors, helps lab technicians adhere to the workflow, and provides accident and error reports. A microbiology decision support system, further described in this chapter, is based on international guidelines (European Manual of Clinical Microbiology [107], Clinical Laboratory Standard Institute [108], European Committee on AST[109], [110]) and provides guidance on results interpretation, flag errors and biosafety risks. The Mini-LIMS makes it possible to follow the analyses that are carried out by the Mini-Lab, the accompanying interpretation of the test results of the Mini-Lab and to direct the laboratory technicians towards the different actions that are to be carried out according to a sample's interpretations.

The Mini-LIMS keeps all the results, interpretations and reports for clinicians for a period of 5 years and is GDPR (General Data Protection Regulation) compliant⁴.

Mini-LIMS can generate activity reporting and send anonymised data to WHONET[111] for surveillance purposes. The data is then utilised to generate epidemiology surveillance reporting on the trending of AMR organisms, compile data to generate hospital based antibiogram or generate the indicators requested from GLASS guidance[112].

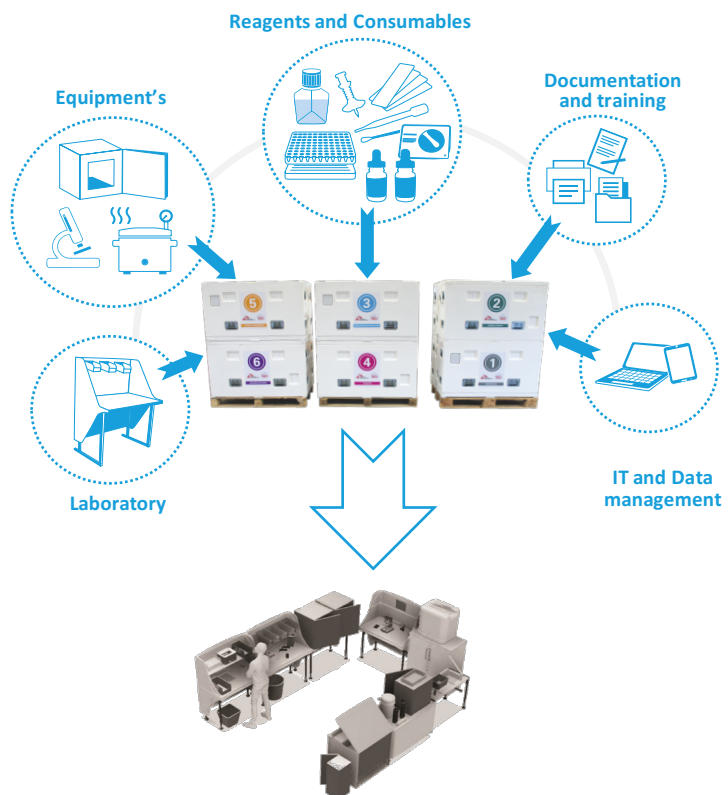


Figure 29: Schematic view of the Mini-Lab concept, a deployable Clinical Bacteriology Laboratory developed to provide access to blood culture capacities in difficult to reach settings, Mini-Lab (France)

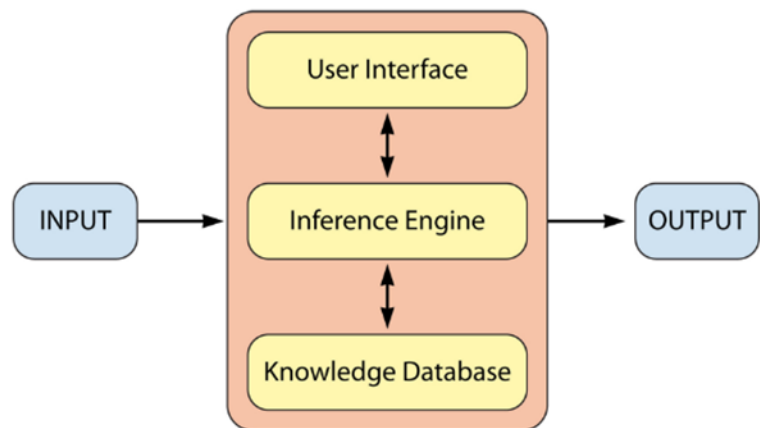
⁴ <https://gdpr.eu/compliance/?cn-reloaded=1> assessed on the 06/02/2023 at 15 :45 CET

6. Development of a Microbiology Decision Support System: focus on the pre-ID Experts systems component

6.1. Introduction

Clinical bacteriology testing from processing of the specimen to the interpretation of results is a complex process that requires extensive expertise and knowledge in microbiology[113]. LIMS have improved the quality of work done by laboratories. As highlighted by Turner et al. in a recent review, several requirements are unique to microbiology labs, and therefore the LIMS require a considerably more complex design than what is typically used for the blood sciences (biochemistry and haematology)[113]. In a microbiology laboratory multiple results are generated per specimen, electronic notes on each specimen must be taken at each step, tests are added dynamically during the process, variability of microorganism phenotypic test results, and the possibility of multiple resistance mechanisms increase the complexity and requirements for an LIMS fit-for-purpose in a clinical microbiology laboratory[113].

A designed for purpose LIMS can facilitate the daily work of laboratory technician, with features such as sample management, and automated report generation.[113]. However the difficulties of culture-based bacteriology diagnostics are based upon dichotomic choices made by experienced microbiologists based upon results and observations during the testing process. Traditionally, simple rules have been used by microbiologists for linking the detected phenotype of an organism to a clinically actionable finding, however a simple flow chart or table can quickly evolve into a complex algorithm[114].



To overcome this complexity, expert system, defined by Rhoads et al. as a software that combines a database of information with a set of rules to

help make a conclusion about an input (Figure 30)[114], have been created. In clinical bacteriology laboratory, expert systems has often been focused on AST interpretation. This is due to: (i) result reporting is complex (e.g. antimicrobial susceptibility (AST) data

Figure 30: Schematic representation of an expert system. According to Rhoads et al. « An expert system is a form of artificial intelligence that allows a user to use software rules (inference engine) together with a knowledge database to make a conclusion (output) about an input » [114]

with multiple reference ranges depending on pathogen–drug combination, (ii) bacterial nomenclature changes over time as new species are identified and existing ones are reclassified on the basis of newly generated sequencing data, (iii) AST interpretation with multiple resistance mechanisms is complex and evolving, (iv) rules for reporting results can be dependent upon the demographics of the individuals being tested, and (vi) the specimen’s source, or antimicrobial resistance[114]. Instead of relying on humans to investigate these criteria, recall associated rules, and accurately implement the rules, an automated system can be built to selectively interpret input data and provide results which are adherent to set standards.

It is not surprising that microbiology laboratories have turned to expert systems (aka knowledge-based systems) to attempt to systematise algorithms into more usable and useful systems. Despite adaptation made to simple blood culture workflows in the Mini-Lab process, there are several steps that required extended expertise for test output interpretation, choosing of next steps and results reporting. It was soon realised during the development process that to provide access to clinical microbiology in the absence of expert microbiology a multilayer approach was needed, which embedded different expert systems, to cover the entire analytical process (Figure 31), and which could be adapted into a LIMS.

6.2. Brief description of the development methodology

The methodology used to develop the expert system was complex. It was initiated prior to the development of the LIMS with the goal of interrogation of LIMS software and not just an extension of existing expert system such as WHONET[111]. Firstly, all the steps which required complex decision-making and all the steps that could impact the quality of the result, therefore requiring an expert system, were mapped. There are many, and the extent of the work done will not be presented in this section. Figure 33 provides an overview of the different types of expert systems embedded as a microbiology decision support system into the Mini-LIMS. In this chapter, only the work done around the pre-ID will be presented. It was then realized that different types of expert systems with different inference engine and knowledge databases would be required, with the objectives to: (i) transform a result of one or more test reactions into a result of identification of a bacteria (genus, species, family, etc.), (ii) transform an MIC value into clinical category results (R/I/S), (iii) verify the concordance and quality of the results, (iv) interpret resistance mechanisms, the absence of mechanism, (v) provide understandable results to the technician or to the clinician and allow them to use the result and learn from them.

9.1.1 Blood culture : Flowchart

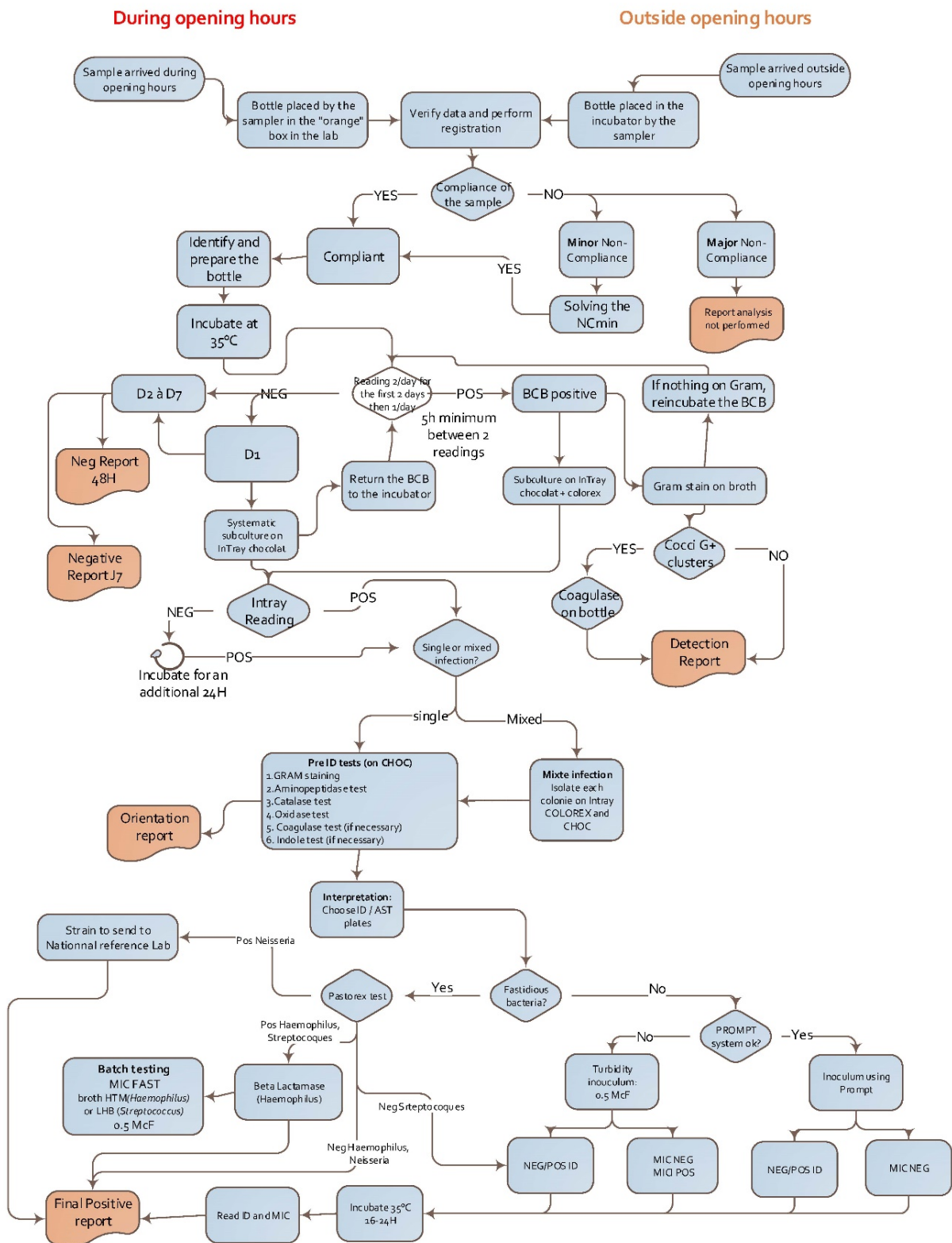


Figure 31: Diagram of the Mini-Lab blood culture workflow, extract of the document DOC-9.1.1-HEMLOGI V1, 16/11/2021, page 1, Mini-Lab (France)

While knowledge databases for the first level of interpretation are available for ID and AST, for Pre-ID tests it was realised that it would have to be built. For example, while it is known that *Staphylococcus aureus* is catalase positive, and that *Enterococci* are not all aminopeptidase negative, there is literature that provides the behavioural probability of a given bacterial species, genus, family to a phenotypic reaction for such enzymatic tests.

Different statistical models and existing knowledge databases from manufacturers who have developed phenotypic identification (Biotypes) such as BioMerieux API (Marcy l'étoile, France) or Beckman Coulter Microscan (West Sacramento, California, USA) were searched. During meetings and discussions with multiple academic specialists on medical algorithms and specialists from industry, it became clear that for pre-ID a model would have to be built to take into account the intrinsic analytical performance of the selected pre-ID tests. As well, the study done to evaluate the performance of the BCBs[97] showed that this database should include the expected behaviour of bacteria on the type of growth in blood culture vials and subculture as well. A dichotomic tree was then designed to visualised one part of the algorithm (SCS, pre-ID) and tested during the first field pilot in Haiti (Figure 32). A probability-based biotypes table by genus or bacterial family using the different studies carried out on the systems involved in the orientation of the results (BCB, SCS, pre-ID) was then created. This work was carried out during the performance studies of the subculture system [99] at the Bicêtre teaching hospital's laboratory (see chapter 2), for BCBs during the performance evaluation[97] and for pre-ID tests (catalase, oxidase, aminopeptidase, Gram) during the performance evaluation made for the ID panels[100] (n=130) and during a proof of concept study (n=59) done by the Institute of Tropical Medicine of Antwerp (Belgium) not published (data provided in Annex 9).

To establish the knowledge databases for level two, during the various laboratory evaluations of analytical components (BCB, SCS, pre-ID, ID, AST), different scenarios of errors or mishandling that could occur in the field were tested. For example, the use of the Gram-positive side of the ID plate on a Gram-negative bacterium. As an example when *Chromobacterium violaceum* is identified by the system, Ombelet et al. found that it is likely that the wrong microplate was inoculated. *Chromobacterium violaceum* could be a misidentification of certain Gram-positive organisms such as *Staphylococcus* spp.[100]. Another example highlighted by the study done by Ombelet et al., if *Chryseobacterium indologenes*, it is likely that the wrong microplate was inoculated. *Chryseobacterium indologenes* is the result of misidentification of certain yeast. In addition, the large number of repetitions or bacteria tested on these different systems (n=367 for ID) has made it possible to highlight repeated patterns of

systematic errors or to, at a minimum calculate a probability of occurrence. Thus, making it possible to highlight patterns of repeated errors specific to the reagents and tests. For the AST, patterns of systematic errors were captured during the evaluation study presented in chapter 3, and following a literature review, different rules of interpretation were incorporated such as resistance mechanisms, intrinsic resistance, unusual phenotypes detailed by EUCAST[[109], [115]. We also included manufacturer instructions and expert consensus.

10.10 Pre-ID: Decision Tree

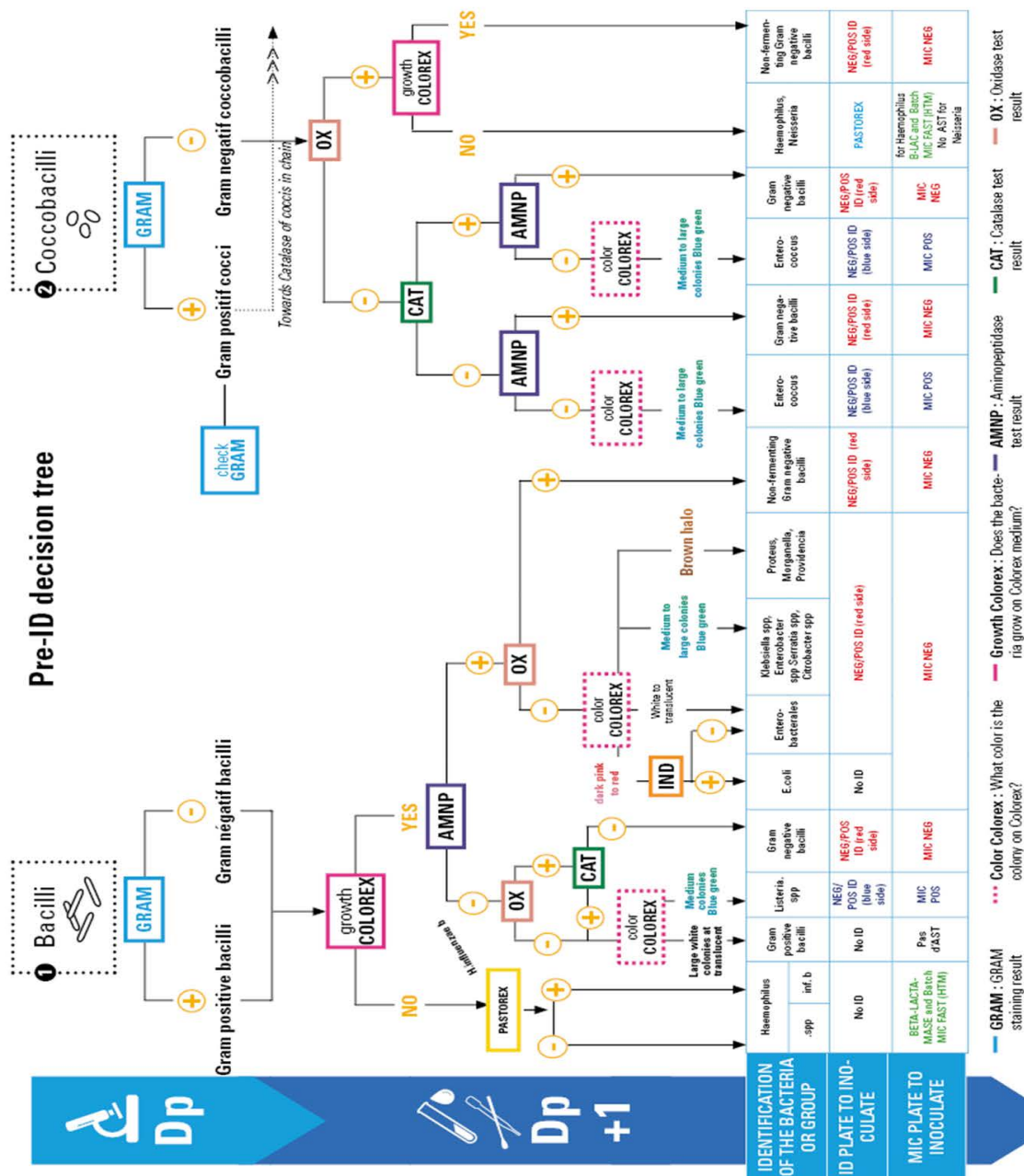


Figure 32: Mini-Lab pre-ID decision tree for bacilli, this algorithm does not take into consideration growth information obtained from the BCB. Extracted from the document DOC-10.10-PIDADEC, V1.1, 10/08/2022, page1, Mini-Lab (France)

To develop the level three knowledge database, a panel of experts in microbiology, infectious disease, IPC, epidemiology, was pulled together. The expert panel then established revisions for rules and formulated sentences based on the expertise of the group and various existing guidance's [107], [116], including MSF's therapeutic guides⁵. Key rules used by the expert panel were; (i) messages for technicians must be actionable, (ii) messages must be concise, (iii) explain to the technicians "the problem" which generated the message (do not give the raw action to be carried out), (iv) if there is a discrepancy on identification, provide information such as, grouping of bacteria, and (v) results to clinician must explain the origin of organism and provide relevant information for treatment.

Following this work, all inference engines were developed by our engineers and for the pre-ID, using statistical models derived from the Microscan Biotypes database. The expert system and the various databases were initially integrated into an Excel -based database using Virtual Basic for Application (VBA). This was done as a mockup to simulate the expected results from the data entry.

This mockup expert system was initially tested using the results of the Haiti study, including all the results of the different tests carried out and the results from the reference methods. This was done to adapt and modify the statistical models, the thresholds for triggering of rules, among other things. In total more than 150 results were tested with the mockup. The mockup was also tested during the first 4 months of the study in Carnot before being integrated into the Mini-LIMS software.

6.3. Description of the pre-ID expert systems

Figure 33 describe the structure of the Mini-LIMS software and the interleaving of the different layers of expert systems. Red rectangles in the diagram indicated with numbers 1 to 8, represent the different expert systems with (1) addressing all expert rules and features related to an individual and their sample data management (e.g. recall of an individual's ID, demographic data quality check, volume sampled verification, etc.), (2) covering expert rules related to sample management (e.g. incubation time, pathway to next step, etc.), (3) focused on the Assisted Reading System rules (e.g. collection of gram pictures, colour atlas for ID plate reading, atlas of type of growth for AST panels, etc.), (4) pre-ID biotypes interpretation mechanism, (5) ID biotype lookup to interpret results of the Neg/Pos ID Microscan panel (knowledge database is provided by a direct interface with the Lab Pro software of Beckman Coulter), (6) AST breakpoint interpretation to transform MIC into clinical category (knowledge database

⁵ <https://medicalguidelines.msf.org/en>, assessed 23rd April 2020

is provided through a partnership with WHONET to yearly update our database, based on current EUCAST or CLSI updates), (7) pre-ID and ID expert rules to check results and provide alert or message to technician, clinicians, and (8) AST expert rules to interpret resistance mechanisms, interpretative antibiogram, display and/or edit alert messages or recommendations for technicians or clinicians.

All of these expert systems together, constitute the microbiology decision support system (MDSS) component of the Mini-LIMS (in the light red rectangle in the figure 33). Finally the MDSS is embed into the Mini-LIMS (blue rectangles in the figure 33) along with the user and administrative interface. Other inference engines allow the Mini-LIMS, to be connected to the different cameras (microscope and microplate viewer box), to provide reports (activity, results, etc.), and to extract the result database into a format that can be used by surveillance software (WHONET, DHIS2).

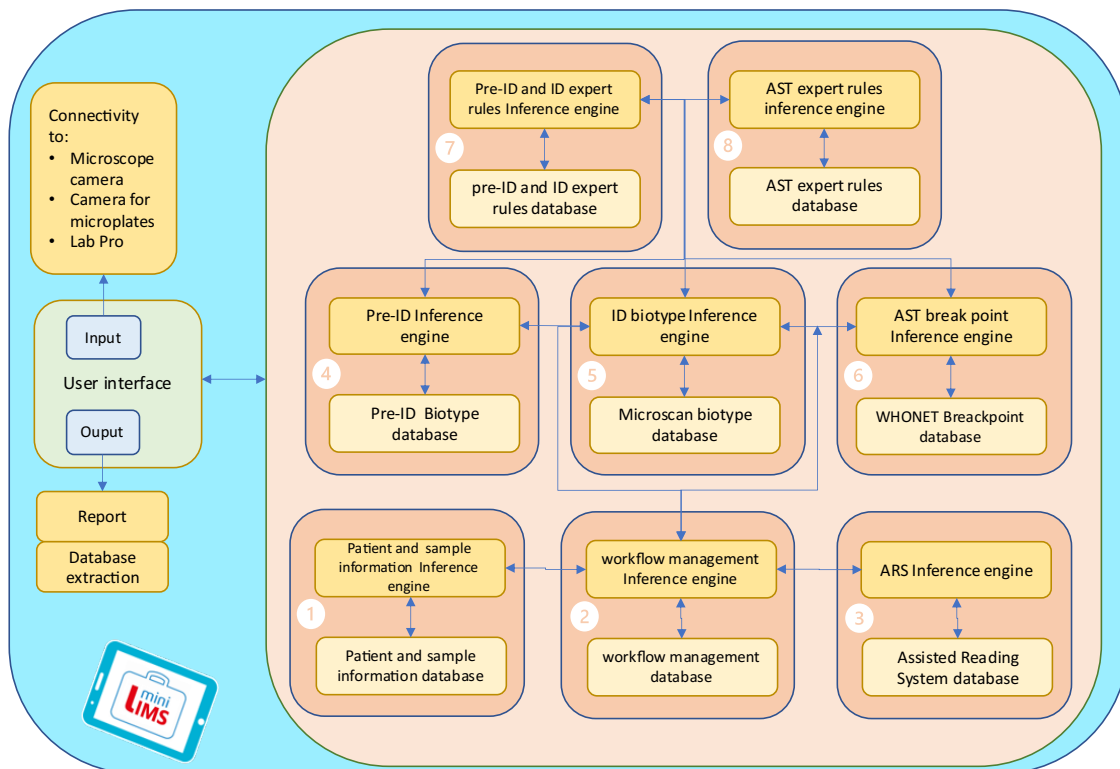


Figure 33: Diagram describing the different layers of the expert systems (red rectangle indicated with numbers 1 to 8) assembled as a unique microbiology decision support system (all diagrams in the light red rectangle) and embed into the Mini-LIMS (all diagrams in the blue rectangle, Mini-Lab (France))

6.3.1. Description of the pre-ID workflow

To understand the results and the complexity of the pre-ID expert systems developed, the process as it is at the final version of the Mini-Lab will be described. In the Mini-Lab process, pre-ID is the phase that provides to the clinician, early

information concerning the organism present in the BCB and an ID orientation of the organism. This pre-ID is carried out in two steps; detection and orientation. Detection is possible after a minimum of a 2 hour incubation, when a BCB is read as positive (or subculture for QC). A Gram stain can then be performed on the sample, followed by microscopic observation. To read the Gram-stained slide, a camera attached to the microscope displays a picture on the Mini-LIMS, the technician scrolls through a library of predefined Gram pictures to select the one that corresponds to the samples, picture and enters further testing results. This step captures; (i) the presence of a bacteria in the sample, (ii) the Gram status of the bacteria, (iii) the morphotype of the bacteria (bacillus, cocci, etc.), and (iv) particular types of growth (turbidity, hemolysis, etc.). At the end of this step, the Mini-LIMS publishes a report for the clinician to confirm the presence of bacteria, its morphotype and its Gram status and to explain the possible origin of the bacterium and therapeutic options. The technician must then prepare two subcultures, InTray Chocolate and InTray Colorex, this allows the second step of the pre-ID to be carried out. For the orientation, this is done between 16 to 24 hours of the incubation of the subcultures (first stage), when the bacterial growth of these subcultures is confirmed, the Mini-LIMS ask the user to take a picture of the colonies on the plate and to enter the results of colony morphology. The technician then carries out tests on the colonies taken from the subcultures (i.e. oxidase, catalase, coagulase, aminopeptidase, and indole). The results of these tests will make it possible to; (i) refine precisely the identification of the bacteria, (ii) determine in certain cases if the bacteria is a suspected pathogen or a contaminat which might have occurred during the sampling, and (iii) initiate the preparation of ID and AST microplates that will finalise the identification and obtain an antibiogram. When the orientation tests are completed, a new report is edited for the clinician to confirm the previous report and provide guidance on the identification of the bacteria, allowing the clinician to adapt treatment plans based upon the detected organism.

6.3.2. Pre-ID Biotype expert system

6.3.2.1. Pre-ID Biotype Knowledge database

The first knowledge table, also refered to as the pre-ID biotype database; (i) list the organism and groups of organism that can be identified during Pre-ID, (ii) informs per test the expected probability response as percentage of each of these organisms to the detection and orientation tests and (iii) weighs the importance of each of these tests for each organism.

It is presented in the form of a table where the organisms are indicated in rows and the tests (with their probability results and weighting) are indicated in columns (Annex 10).

The database is composed of 19 lines of organisms and each line is composed of 40 parameters (probability results and weighting).

Sur groupe	Groupe	Organisme	Det Chainet	Proba Grn	Det Grn	Proba Ox	Det E
Levure	Yeast	Yeast	Mogén	0,825	Majeur	0,01	Majeur
Bacille Gram neg	Enterobacterales	Siella spp, Enterobacter spp, Citrobacter spp, Serratia	NC	0,015	Mogén	0,01	Majeur
Bacille Gram neg	Enterobacterales	Escherichia coli	NC	0,015	Mogén	0,01	Majeur
Cocci Gram pos	Streptococcus spp / Enterococcus spp	Enterococcus spp	Mogén	0,985	Majeur	0,01	Majeur
Bacille Gram neg	Haemophilus spp, Neisseria spp, Moraxella spp	Haemophilus spp	Mogén	0,01	Majeur	0,39	Majeur
Bacille Gram pos	Bacilli gram pos	Bacillus spp	Mogén	0,025	Mogén	0,01	NC
Gram neg Cocci	Haemophilus spp, Neisseria spp, Moraxella spp	Neisseria spp	Mogén	0	Majeur	0,39	Majeur
Bacille Gram neg	GN non-fermenter	Pseudomonas spp	Mogén	0,025	Mogén	0,39	Majeur
Bacille Gram neg	Enterobacterales	Salmonella spp	Mogén	0,015	Mogén	0,01	Majeur
Cocci Gram pos	Micrococcus spp / Staphylococcus spp	Staphylococcus aureus	Mogén	1	Majeur	0,01	Majeur
Cocci Gram pos	Micrococcus spp / Staphylococcus spp	Staphylococcus coag neg	Mogén	1	Majeur	0,01	Majeur
Bacille Gram neg	GN non-fermenter	Stenotrophomonas spp	Mogén	0,025	Mogén	0,6	Majeur
Cocci Gram pos	Streptococcus spp / Enterococcus spp	Streptococcus spp	Mogén	0,985	Majeur	0,01	Majeur
Bacille Gram neg	Enterobacterales	Proteus spp, Morganella spp, Providencia spp	Mogén	0,015	Mogén	0,01	Majeur

Figure 34: Snapshot of the mock-up database for Pre-ID tests are fixed, but this can be modified, added to or items removed by the Mini-LIMS administrator, Mini-Lab (France).

Each preselected organism is classified by; (i) a class (or supergroup): often consisting of the concatenation of its morphotype and its Gram, (ii) a group by genera and species according to their clinical relevance to refine the orientation as proposed by Ombelet et al.[32], and (iii) a more precise orientation at genus level or for some at species level (e.g. *Escherichia coli*).

There are 4 categories of tests, which are treated in different ways, **morphotype tests**, **orientation tests**, **growth type testing**, **growth on InTray Colorex**. For each test, it is necessary to consider 3 parameters, Value (for morphotype class and Colorex growth only), the probability of positive detection of this test, between 0.01 and 0.99 (for all tests except Colorex growth), the determinant, which is used to weigh the importance of a test for a given organism, this determinant can take 4 values: Non Communicate/Minor/Medium/Major.

Morphotype test:

For each line of organism, the morphotype is described with defined probability and determinant (between 0.01 and 0.99) according to 2 parameters; (i) the general morphotype class (i.e. Bacilli/Yeast/Cocci), and (ii) the specific morphotype characteristic (i.e. coccobacillus, cocci in clusters, isolated cocci, cocci chain).

B		C		D	E	F
				Classe de morphotype		
Groupe	Organisme	Morphotype Maj	Proba Morpho	Det Morpho		
Haemophilus spp, Neisseria spp, Moraxella spp	Neisseria spp	Cocci	0,96	Majeur		
GN non-fermenter	Pseudomonas spp	Bacilli	0,98	Majeur		
Enterobacterales	Salmonella spp	Bacilli	0,98	Majeur		
Micrococcus spp /Staphylococcus spp	Staphylococcus aureus	Cocci	0,96	Majeur		

C		F	G	H	I	J
		Caractères				
Organisme	Det Morpho	Proba Coccobacille	Det Coco	Proba Coccis en amas	Det Amas	
Pseudomonas spp	Majeur	0,1	Moyen	0	Moyen	
Salmonella spp	Majeur	0,05	Moyen	0	Moyen	
Staphylococcus aureus	Majeur	0,01	Moyen	0,56	Moyen	
Staphylococcus coag neg	Majeur	0,01	Moyen	0,56	Moyen	
Stenotrophomonas spp	Majeur	0,1	Moyen	0	Moyen	

Figure 35: Snapshot of the pre-ID mock-up database, the top snapshot presents the part of (i) morphotypes class and bottom snapshot presents the (ii) specific morphotypes, Mini-Lab (France)

Orientation tests

These tests are performed on colonies from the blood culture bottle or subculture. They can only take 2 values: positive or negative (or empty if they are not filled in). The tests include: Gram stain, oxidase, catalase, aminopeptidase, coagulase. For each test a probability and determinant are assigned.

Growth Type Tests

These tests describe the observation of colony growth that is observed in BCBs such as; turbidity, haemolysis, growth granulation, white film, isolated settlements, bacterial carpet, gas production. For each test a probability and determinant is assigned. The

difference between these tests is how their results influence the calculation of the organism's probability of match.

Colorex growth

Unlike the other tests, the Colorex growth for each organism cannot be quantified, so there is no probability of positive detection for this test but only a value. The result of this test does not influence the match rate but does influence the expert system suggestion for guidance on the identification of the bacteria. Colorex growth can take 7 values, white to translucent, blue-green, dark red to pink, brown halo, creamy golden, no growth, not concerned (if this test has not been referenced on this organism)

6.3.2.2. Inference engine

6.3.2.2.1. Match rate

The inference engine is composed of a cascade of calculations and rules. The probability of positive detection and the determinants assigned to each test for each organism allowed. Each time pre-ID results are entered a match rate is calculated. This rate describes the response of the organism being analysed to the pre-ID tests, seen across the spectrum of expected results for each organism. The following example will be utilised to explain the cascade calculation. For an organism to be analysed, the laboratory technician must observe the results for the following tests:

- Morphotype class: Bacilli
- Gram: negative
- Aminopeptidase: positive
- Oxidase: positive
- Other tests are not filled in (unobservable, unavailable or other).

When these results are entered into the Mini-LIMS, the system displays the following match rates (Figure 36)

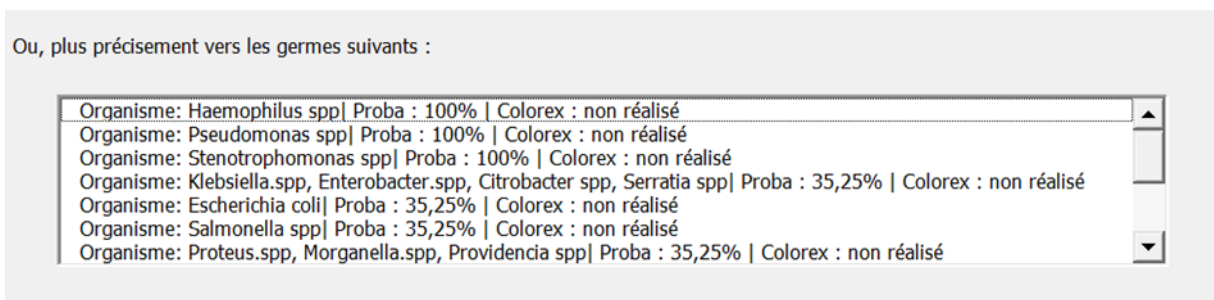


Figure 36: Results provided by the inference engine for the example used for a gram negative, aminopeptidase positive, oxidase positive bacilli, Mini-Lab (France).

These results indicate that the organism being analysed had exactly the same results in the tests reported (morphotype/Gram/aminopeptidase/oxidase) as *Haemophilus* spp., *Pseudomonas* spp. and *Stenotrophomonas* spp., the meaning of a 100% match rate.

Other organisms visible on these results have only a 35% probability of being the organism being analysed based upon the results obtained from testing. To calculate this match rate, it is necessary to know how the probability and determinant of each test is taken into account. When the inference engine starts calculating match rates, it performs the following operations for each organism listed in the knowledge database. Then it will calculate 3 rates; (i) minor match rate (ii) average match rates, and (iii) major match rates. At the initialisation of the calculation, all these rates are worth 1.

If the resulting test entered responded positively, the calculation multiplies the probability of a positive response of this organism with the rate corresponding to its determinant (minor/medium/major).

If the resulting test entered responded negatively, the calculation multiplies 1- the probability of positive response of this organism with the rate corresponding to its determinant (minor/medium/major).

e.g. the following results are observed for the sample analysed:

- Morphotype class: Bacilli
- Gram: negative
- Aminopeptidase: positive
- Oxidase: positive

When these results are compared to those expected for *Salmonella* spp. (knowing inference engine will do so for all bacteria listed). The expected results of *Salmonella* spp. are as follows in Table 6.

Table 6: Expected results found in the pre-ID knowledge database for *Salmonella* spp. organism

Type of test	Results entered	Probability	Determinant
Morphotype class	Bacilli	0.98	major
Gram	negative	0.01	medium
Aminopeptidase	positive	0.01	major
Oxidase	positive	0.96	major

Major (T%Maj) and average (T%Avg) match rates can be calculated for this sample compared to *Salmonella* spp:

Major rate:

$T\%Maj = 1 \times 0.98(\text{morphotype OK}) \times 0.01(\text{Oxidase pos}) \times 0.96 (\text{Aminopeptidase pos})$

$$T\%Maj = 0.009$$

This rate is very low because of the oxidase test whose result should be positive while *Salmonella* expects a negative result (probability of positive detection at 0.01).

Average rate:

$$T\%Avg = 1 \times (1-0.015) (\text{gram negative})$$

$$T\%Avg = 0.985$$

The minor rate is not considered since no test with a minor determinant has been entered.

These 3 rates are then compiled to obtain an overall rate (T%Glob):

$$\mathbf{T\%Glob = (T\%Maj \times 10 + T\%Avg \times 5 + T\%Min \times 2) / 17}$$

In the case of our example, we do not have a minor rate, so the calculation becomes:

$$T\%Glob = (T\%Maj \times 10 + T\%Avg \times 5) / 15$$

$$T\%Glob = (0.009 \times 10 + 0.985 \times 5) / 15 = 0.33$$

This rate alone does not give us information, it must be compared with the maximum result expected for *Salmonella spp.*

$$T\%Maj \text{ Max} = 1 \times 0.98(\text{morphotype OK}) \times (1-0.01)(\text{Oxidase neg}) \times 0.96 (\text{Aminopeptidase pos}) = 0.93$$

That is to say the same as for our sample but with a negative oxidase.

$$T\%Avg \text{ Max} = 1 \times (1-0.015)(\text{gram negative}) = 0.985$$

This gives us:

$$T\%Glob \text{ Max} = (0.93 \times 10 + 0.985 \times 5) / 15 = 0.948$$

We can now calculate the match rate of *Salmonella spp.* with our sample:

$$\mathbf{\underline{T\%match = (T\%Glob / T\%Glob \text{ Max}) \times 100 = 34.8\%}}$$

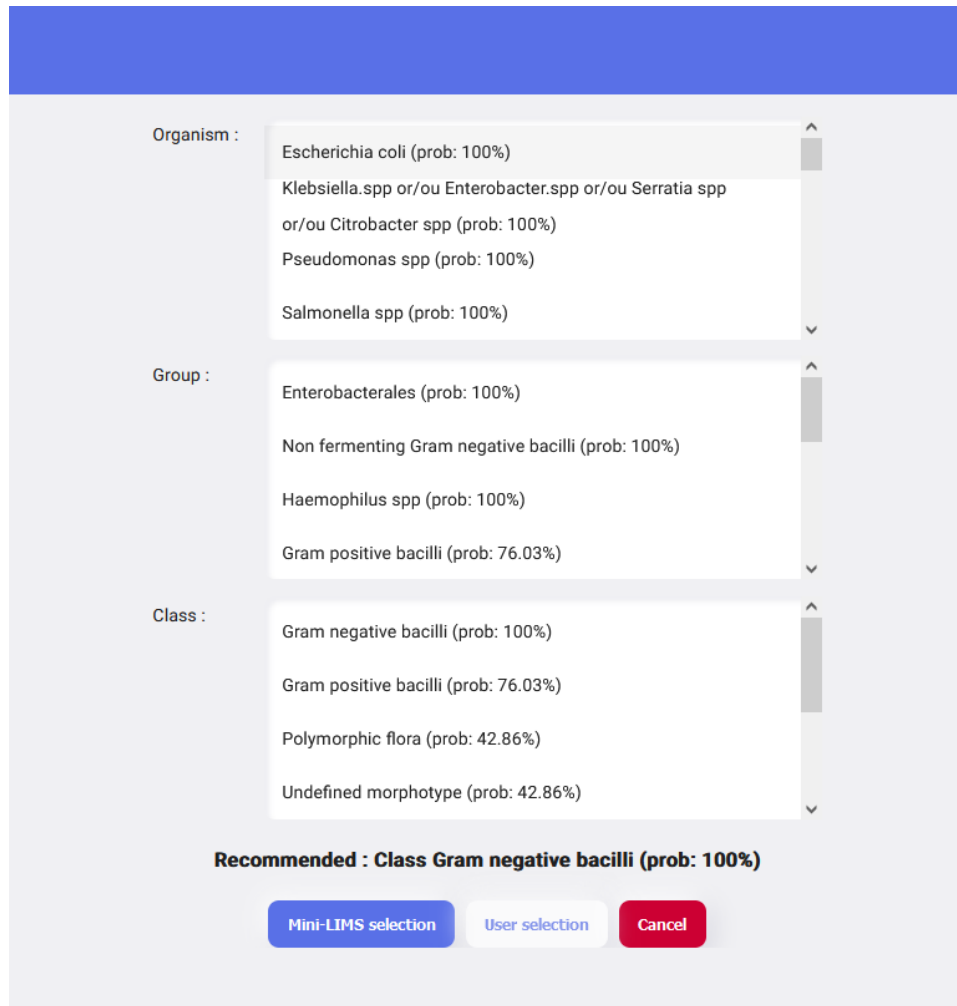
This therefore reflects the comparison between the results obtained by results entered and the results obtained by a "typical" *Salmonella* spp. from the knowledge database.

Special cases of growth types. When a test is negative, the calculation will integrate this test by taking as its coefficients "1- probability positive". However for types of growth (turbidity, haemolysis, etc.), there is no systematic verification, so a negative result is simply not considered.

The analysis results obtained in the laboratory, can at times, not be sufficient for the inference engine to suggest an identification. In the example (Figure 36) we are not able to determine whether the bacteria being analysed is a *Haemophilus* spp., *Pseudomonas* spp. or *Stenotrophomonas* spp., based on the organism's match rate:

This is where the idea of group and class of organism has come into play. Based on the results for the probability of an organism match, the inference engine will group the organisms into the corresponding groups by assigning the highest match rate among the organisms

Then the inference engine does this operation again by grouping of organisms into a class. For our example, this gives the following results (Figure 37).



Organism :

- Escherichia coli (prob: 100%)
- Klebsiella.spp or/ou Enterobacter.spp or/ou Serratia spp or/ou Citrobacter spp (prob: 100%)
- Pseudomonas spp (prob: 100%)
- Salmonella spp (prob: 100%)

Group :

- Enterobacterales (prob: 100%)
- Non fermenting Gram negative bacilli (prob: 100%)
- Haemophilus spp (prob: 100%)
- Gram positive bacilli (prob: 76.03%)

Class :

- Gram negative bacilli (prob: 100%)
- Gram positive bacilli (prob: 76.03%)
- Polymorphic flora (prob: 42.86%)
- Undefined morphotype (prob: 42.86%)

Recommended : Class Gram negative bacilli (prob: 100%)

Mini-LIMS selection User selection Cancel

Figure 37: Cascade calculation results done by the inference engine for gram negative bacilli, aminopeptidase positive, oxidase positive test results. Extract from the actual Mini-LIMS interface, Mini-Lab (France)

Focusing on the organisms that had a 100% match rate, *Haemophilus spp.* was included in the group "*Haemophilus spp., Neisseria spp., Moraxella spp.*", and *Pseudomonas spp.* and *Stenotrophomonas spp.* in the group "Gram Negative (GN) non-fermenter". Since these two groups have the same match rate, they have been grouped into the organism class "Gram-negative bacilli".

6.3.2.2.2. Determination of suggestion:

The inference engine seeks to suggest the most specific identification probability. It does this by comparing the highest matching rate of the probability with the second-highest rate. If this ratio is greater than "1.3x", then the inference engine proposes the organism with the highest rate or probability. Otherwise it defaults back to the group level and makes the same comparison. If a classification is still not possible, it goes up to the level of the organism class. If the match rate of the largest class is still no higher than 1.3x, the rate of the second class with the best match rate, then the inference

engine displays an error message stating that it cannot suggest a classification for the organism ("Tests to be refined/ repeated"). In the example used, inference engine cannot render an organism or group because the match rates are equiprobable. It therefore suggest the organism class "Gram-negative bacilli" as a classification for the Pre-ID.

6.3.2.2.3. Particular cases of the Colorex screen test

Chromogenic media such as the InTray Colorex Screen are quite reliable tests that can differentiate bacteria in some cases based on the colour of the colony but the results probability have not been quantified in the literature. To date, there is no reliable statistical study to quantify the response of different organisms on chromogenic media. The Colorex therefore serves to differentiate bacteria with identical match rates. In the previous example if the Colorex the colony was white to translucent colony in colour, the resulting happens:

- *Haemophilus* spp. does not grow on Colorex
- *Stenotrophomonas* spp. and *Pseudomonas* spp. growth with colonies either white to translucent or greenish on Colorex.

It is therefore one of either *Stenotrophomonas.spp* or *Pseudomonas.spp*. Since the inference engine cannot differentiate between these two bacteria, it will suggest the group of organisms to which they belong too, "non-fermenting gram-negative bacilli".

Morphotype et Gram observé (à sélectionner si le groupe et le germe ne sont pas interprétables)

Morphotype: Bacille Gram neg Proba : 100%
Morphotype: Bacille Gram pos Proba : 6,65%
Morphotype: Gram neg Cocci Proba : 4,17%
Morphotype: Levure Proba : 0%
Morphotype: Cocci Gram pos Proba : 0%

Les résultats de la Pre-ID orientent vers les groupes de germes suivants :

Groupe: Haemophilus spp, Neisseria spp, Moraxella spp Proba : 100%
-->Groupe: GN non-fermenter Proba : 100%
Groupe: Enterobacterales Proba : 35,25%
Groupe: Bacilli gram pos Proba : 6,65%
Groupe: Yeast Proba : 0%
Groupe: Streptococcus spp / Enterococcus spp Proba : 0%

Ou, plus précisément vers les germes suivants :

Organisme: Haemophilus spp Proba : 100% Colorex : incohérent :Blanc à translucide au lieu de Pas de pousse
Organisme: Pseudomonas spp Proba : 100% Colorex : cohérent
Organisme: Stenotrophomonas spp Proba : 100% Colorex : cohérent
Organisme: Klebsiella.spp, Enterobacter.spp, Citrobacter spp, Serratia spp Proba : 35,25% Colorex : incohérent :Blanc
Organisme: Escherichia coli Proba : 35,25% Colorex : incohérent :Blanc à translucide au lieu de Rouge foncé à rose
Organisme: Salmonella spp Proba : 35,25% Colorex : cohérent
Organisme: Proteus.spp, Morganella.spp, Providencia spp Proba : 35,25% Colorex : incohérent :Blanc à translucide a

Veuillez sélectionner un groupe et/ou un germe pour valider les résultats de la Pre-ID

Figure 38: Cascade calculation results done by the inference engine for a gram-negative bacillus, aminopeptidase positive, oxidase positive and colour of colonies white on Colorex, test results entered. Extract from the mock-up interface, Mini-Lab (France)

6.3.2.2.4. Selection threshold

If after calculating match rates, no organism has a match rate greater than 50%, the inference engine will display a message questioning the entry of results: "Test results are very unlikely, please check Pre-ID test results".

6.3.2.2.5. Posting conclusions

Based on these results, the user must then decide on the classification of the organism. The user can either choose the classification suggested by the inference engine or go up to the level of the group or class of organism. This can be done if there is doubt concerning one of the results for. When the choice is confirmed, the Mini-LIMS will then go to next step of expert rule verification.

6.3.3. Pre-ID and ID expert rules

In the process of defining expert rules that can interpret results and provide understandable results to technicians and clinicians, a total of 258 rules that can trigger 401 messages (including pre-ID, ID and AST) were developed. For pre-ID, 35 rules can trigger 96 messages, for ID 55 rules can trigger 169 messages and for AST 168 rules can trigger 233 messages.

6.3.3.1. Pre-ID and ID expert rules database

Pre-ID and ID expert rules trigger conditions that are similar, which is why they are grouped in the same expert system. Pre-ID rules trigger the display of technician messages in the interface of the Gram and Pre-ID results forms when the user has chosen an interpretation.

These Pre-ID rules also trigger the display of messages and recommendations to clinicians for detection and/or classification reports. Sentences are displayed in the report editing interface and the supervisor can decide to incorporate or not the sentences or edit them as required. ID rules will trigger the display of technician messages in the ID plate reader interface when the user has chosen an interpretation.

ID rules also trigger messages and recommendations to be displayed to clinicians for final positive reports. The same as for the pre-ID, sentences are displayed in the report editing interface and the supervisor can decide to incorporate or not the sentences or edit them as required

There are 3 types of messages that can be provided by the expert rules:

- Messages to technicians: displayed in the Mini-LIMS. These messages are often alerts that suggest the technician to take certain precautions or take certain actions given the bacteria identified.
- Messages to clinicians: displayed in positive reports (Detection/Referral/Final). These messages alert the clinician to the risk of an outbreak, or the presence of pathogen of hospital origin and recommendation of actions, warning and/or alerts to be carried out.
- Recommendations to clinicians: displayed in positive reports (Detection/Classification/Final). These messages suggest to clinicians actions for the treatment of the individual in relation to the bacteria or the identified resistance profile.

All messages are available in French, English and Arabic and an example is shown in Table 7 and all messages are classified by:

- Rule number (required): Alphanumeric characters typed by hand by the user. These numbers must be unique (verified by the system). They are uploaded for each analysis in the register of analyses and allow technicians to check which rules have been triggered for each analysis.
- Technician messages (optional): Messages developed by the expert panel. These message are displayed if the rule is triggered in the Gram/Pre-ID Results Entry Forms Monitor or in the ID Plate Reader form. It is displayed when the user chooses an interpretation.
- Clinician messages (optional): Messages developed by the expert panel. These messages are displayed if the rule is triggered in the positive reports editing form. It appears when the supervisor starts editing a positive report.
- Clinician recommendation (optional): Messages developed by the expert panel. These messages will be displayed if the rule is triggered in the positive reports editing form. It appears when the supervisor starts editing a positive report.

Table 7: Examples of messages triggered at the pre-ID step when a Gram-negative bacilli is observed at the microscope from a positive blood culture bottle

Categories	ID	English	French	عربي
------------	----	---------	--------	------

Message to clinicians	PID-I-1	Gram-negative bacilli including non-fermentative bacilli or enterobacterales or <i>Haemophilus</i> spp. – If suspicion of meningitis: it is probably haemophilus spp. – If healthcare-associated infection suspected: it is probably a non-fermentative Gram-negative bacillus. – If suspicion of urinary or intestinal infection: it is probably an enterobacterales.	Bacilles à Gram négatif dont bacilles non fermentaires ou Enterobacterales ou Haemophilus – Si suspicion de méningite : il s’agit probablement d’ <i>Haemophilus</i> spp. – Si suspicion d’infection associée aux soins de santé : il s’agit probablement d’un bacille à Gram négatif non fermentaire. – Si suspicion d’infection urinaire ou intestinale : il s’agit probablement d’un Enterobacterales.	العصيات سلبية الغرام بما في ذلك العصيات غير المخمرة أو Enterobacterales أو Haemophilus - في حالة الاشتباه في التهاب السحايا: من المحتمل أن يكون Haemophilus spp. - في حالة الاشتباه في وجود عدوى مرتبطة بالرعاية الصحية: من المحتمل أن تكون عصية غير مخمرة سلبية الغرام. - في حالة الاشتباه في وجود عدوى بولية أو معوية: من المحتمل أن تكون Enterobacterales.
Message to technicians	PID-I-1	Rule PID-I-1 (Gram-negative bacilli) : Message for the clinician to edit.	PID-I-1 (Gram-negative bacilli) : Message clinicien à éditer	PID-I-1: (عصيات سلبية الغرام): تحرير رسالة للطبيب.
Recommendations	PID-I-1	Assess the patient's condition and identify the source of infection. Check that at least one molecule of the antimicrobial treatment is active against this type of bacteria.	Évaluer l'état du patient et identifier la source de l'infection. Vérifier qu'au moins une molécule du traitement antimicrobien est active contre ce type de bactéries.	قيم حالة المريض وحدد المصدر المحتمل للعدوى. تأكد من أن جزئياً واحداً على الأقل من العلاج بمضادات الميكروبات فعال ضد هذا النوع من البكتيريا.

6.3.3.2. Pre-ID and ID expert rules inference engine

The database for pre-ID and ID expert rules have rules in line, with several columns with different parameters for application of the rules

- Rule number (required): This is the same alphanumeric characters present in the knowledge database as key unique identifier to link both database.
- Category (required): Two fields possible:
 - Pre-ID: Rule actions in the Gram and Pre-ID interface and in detection and orientation report editing forms.
 - ID: Rule actions in the ID interface and in the final positive report editing form.
- Type of identification (mandatory): Used to verify if the identification entered exists in the pre-ID databases and makes it possible to trace the identification. 4 possible fields:
 - Pre-ID class: reserved for the "Pre-ID" category (verified by the system). This is the highest hierarchy of Pre-ID interpretation.
 - Pre-ID groups: reserved for the "Pre-ID" category (verified by the system). This is the middle hierarchy of the Pre-ID interpretation.
 - Pre-ID organisms: reserved for the "Pre-ID" category (verified by the system). This is the lowest hierarchy of Pre-ID interpretation.

- Organism code: reserved for the "ID" category (verified by the system). Allows linking result provided by the Biotype ID expert system with rules to be triggered.
- Identification (required): Depends on the type of identification. This is the first parameter that allows checking the triggering of the rule based on the interpretation in the form.
- Single samples (check box): Basic checkmark. Allows triggering this rule if the sample concerned only one sample in its batch, i.e. if there are no other samples with the same batch number in the analysis register.
- Multi-sample batch (checkbox): Basic checkmark. Allows triggering this rule if the sample concerned is not the only sample in its batch, i.e. if there are other samples with the same batch number in the analysis log.

In positive reports, depending on the category of rules triggered during the analysis (Pre-ID, ID, AST), messages and recommendations will be added to the report editing form for each isolate in the sample. Each message is in this form preceded by a box, checked by default that the supervisor can uncheck to remove the message in question from the report. These messages can also be edited manually by the supervisor if desired. In the edited form, the selected messages are added as follows (for each isolate): (i) clinical messages are added to the "Interpretation" cartridge of the isolate concerned, (ii) the recommendations are added in the "Recommendations" section for the isolate concerned.

Résultat	⚠ DETECTION D'UNE CROISSANCE ⚠
Infection mixte probable <input type="checkbox"/>	
Micro-organisme <input type="text" value="-_/_"/>	
Détection probable : _____ <i>En cours d'identification</i>	
Des tests complémentaires sont en cours pour vous donner une orientation plus précise.	
Interprétation :	

Recommandations :	

Figure 39: Extract of the report form provided to clinicians with the different sections were the sentences are automatically filled by the expert system, Mini-Lab (France)

Chapter 2- Adaptation And Validation Of An Innovative Technique Adapted To LRS Constraints For The Subculture Of Blood Culture Bottles

1. Introduction

Culture media are fundamental in clinical bacteriology, especially for the diagnosis of bloodstream infections which largely still relies on culture of large volumes of blood in culture bottles (BCB) and on further isolation of bacterial species by subculturing on agar plates[68], [117]. For many decades, laboratories in high-income countries procure pre-poured ready-to-use culture media from specialised manufacturers, a practice which is integrated in the laboratory quality management system [118]. Ready-to-use plates are not adapted to logistics constraints in low-resource settings because of their short shelf life, usually under six months, and even less with blood-containing plates[119]. In contrast, LMIC laboratories mostly buy dehydrated culture media and prepare them in-house, a time-consuming solution that requires trained personnel and a strong quality control system, achievable under strict procedures in certain laboratories of LMICs. Additionally, preparing in-house requires the addition of several fresh animal products for the culture, identification and determination of susceptibility to antibiotics of fastidious organisms [120]. Those animal products such as defibrinated sheep or horse blood, contains different concentrations of specific nutriment (β -NAD, Adenin, etc.) that to date have never been dehydrated[121]. Furthermore, in liquid format the blood has a short shelf life[122] and therefore the use of this formulation doesn't meet the Mini-Lab specifications. In developing countries, microbiologists instead of animal blood, often use human blood agar because of the high cost and inhospitable conditions for raising sheep or horses to supply blood. Many pathogens either fail to grow entirely or exhibit morphologies and haemolytic patterns on human blood agar that confound colony recognition and can be hazardous to handle [119], [123]

In this chapter and in the context of the laboratory of the LMIC first-level referral hospital, where clinical bacteriology services should be expanded according to the inaugural WHO Model List of Essential In Vitro Diagnostics, we will firstly, review the culture media selection and processing in LMIC, compile best practices for in-house preparation, discuss ways to improve access to quality-assured products and formulate research questions to improve these practices. Then in the second part of the chapter and in target setting of low resource areas (e.g. little availability of skilled HR- mostly laboratory technicians, scarcity of supply, unfriendly environment, no access to referral

lab, etc.), we explore the selection, from the market of ready to use systems from veterinary, food safety microbiology, of a novel ready to use a type of culture media and perform several proof of principle studies to explore it for use as subculturing system from BCB. We then evaluate the analytical performance of this system on a larger scale to confirm the findings of the proof of principles studies (supplementary results to the published result can be found in Annex 14 and 15).

The review and the work done in the different experiments described in the next section were published in the following peer reviewed journals.

- J. Orekan, B. Barbé, S. Oeng, **J.-B. Ronat**, J. Letchford, J. Jacobs, D. Affolabi, L. Hardy, "Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access," *Clinical Microbiology and Infection*. 2021, doi: 10.1016/j.cmi.2021.05.016.
- A. Natale, S. Oueslati, A. Rochard, D. Lopez-Baez, S. Ombelet, L. Hardy, J. Cunningham, O. Vandenberg, C. Franquesa, **J.-B. Ronat***, T. Naas*, "Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media," **Diagnos-tics**, vol. 13, no. 3, p. 523, Jan. 2023, doi: 10.3390/diagnostics13030523.

* Both authors contributed equally to the manuscript

2. Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access

Clinical Microbiology and Infection 27 (2021) 1400–1408



Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com



Review

Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access

Jeanne Orekan^{1,†}, Barbara Barbé^{2,*}, Sopheap Oeng³, Jean-Baptiste Ronat^{4,5,6}, Joanne Letchford³, Jan Jacobs^{2,7}, Dissou Affolabi¹, Liselotte Hardy²

¹ Clinical Microbiology, Centre National Hospitalier Universitaire Hubert Koutoukou Maga, Cotonou, Benin

² Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

³ Laboratory Department, Diagnostic Microbiology Development Program, Phnom Penh, Cambodia

⁴ Mini-Lab Project, Médecins Sans Frontières, Paris, France

⁵ Team ReSIST, INSERM U1184, School of Medicine University Paris-Saclay, France

⁶ Bacteriology-Hygiene Unit, Assistance Publique – Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

⁷ Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

ARTICLE INFO

Article history:

Received 21 December 2020

Received in revised form

20 April 2021

Accepted 4 May 2021

Available online 18 May 2021

Editor: E.J. Kuipers

Keywords:

Best practices

Clinical bacteriology

Culture media

Dehydrated culture media

Low- and middle-income countries

ABSTRACT

Background: Culture media are fundamental in clinical microbiology. In laboratories in low- and middle-income countries (LMICs), they are mostly prepared in-house, which is challenging.

Objectives: This narrative review describes challenges related to culture media in LMICs, compiles best practices for in-house media preparation, gives recommendations to improve access to quality-assured culture media products in LMICs and formulates outstanding questions for further research.

Sources: Scientific literature was searched using PubMed and predefined MeSH terms. In addition, grey literature was screened, including manufacturer's websites and manuals as well as microbiology textbooks.

Content: Bacteriology laboratories in LMICs often face challenges at multiple levels: lack of clean water and uninterrupted power supply, high environmental temperatures and humidity, dust, inexperienced and poorly trained staff, and a variable supply of consumables (often of poor quality). To deal with this at a base level, one should be very careful in selecting culture media. It is recommended to look for products supported by the national reference laboratory that are being distributed by an in-country supplier. Correct storage is key, as is appropriate preparation and waste management. Centralized media acquisition has been advocated for LMICs, a role that can be taken up by the national reference laboratories, next to guidance and support of the local laboratories. In addition, there is an important role in tropicalization and customization of culture media formulations for private *in vitro* diagnostic manufacturers, who are often still unfamiliar with the LMIC market and the plethora of bacteriology products.

Implication: The present narrative review will assist clinical microbiology laboratories in LMICs to establish best practices for handling culture media by defining quality, regulatory and research paths.

Jeanne Orekan, Clin Microbiol Infect 2021;27:1400

© 2021 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Low- and middle-income countries (LMICs) are seriously hit by the worldwide antimicrobial resistance crisis [1,2]. Clinical bacteriology contributes to patient care and antimicrobial resistance surveillance and links to antibiotic stewardship and infection prevention and control, thereby tackling three out of the five domains

* Corresponding author: Barbara Barbé, Nationalestraat 155, 2000 Antwerp, Belgium.

E-mail address: bbarbe@itg.be (B. Barbé).

† Jeanne Orekan and Barbara Barbé share first authorship.

<https://doi.org/10.1016/j.cmi.2021.05.016>

1198-743X/© 2021 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

of the WHO action plan to contain antimicrobial resistance [3]. Pending the arrival of new-generation diagnostics, culture-based techniques remain the standard for clinical bacteriology in LMICs and should be included in the services of LMIC first-level referral hospitals according to the WHO Model List of Essential *In Vitro* Diagnostics (WHO EDL), launched in 2018 [4,5].

Culture media are fundamental in clinical bacteriology. They are complex products based on a buffered solution of biological nutrients (animal-, plant- or yeast-based). They comprise peptones and extracts (N source) as well as (poly)saccharides (C source), mineral salts, growth factors and vitamins. To obtain solid media, a gelling agent is added, mostly bacteriological agar [6]. Given the biological components, variations in media composition may occur [7].

For many decades, laboratories in high-income countries have been procuring pre-poured ready-to-use culture media from specialized manufacturers, a practice that is integrated in the laboratory quality management system [8]. In contrast, LMIC laboratories mostly buy dehydrated culture media and prepare them in-house [3]. This is not without its challenges: the organizers of the 2011–2016 WHO external quality assessment of African Public Health Laboratories noted in-house preparation of culture media as a major problem [9]. Worldwide, the dehydrated culture media segment represents the biggest market share (44%) of the booming culture media market (next to prepared media (31%) and chromogenic culture media (25%)). A further growth is expected in the coming years (8.1% compound annual growth rate 2020–2027; with highest growth in the Asia Pacific region) [10].

Scope

This narrative review describes challenges related to culture media in LMICs, compiles best practices for in-house media preparation, gives recommendations to improve access to quality-assured culture media products in LMICs and formulates outstanding questions for future research.

Search strategy and source documents used

PubMed was searched using the MeSH terms 'microbiological techniques' AND 'bacteria/growth and development' AND 'culture media'. Grey literature was also screened, including manufacturer's websites and manuals [7,11–13], as well as microbiology textbooks.

Challenges related to culture media in low- and middle-income countries

The complexity of culture media and their preparation presents a challenge for quality [14]. Adding to the complexity are the plethora of culture media used, the requirements for product stability and—in view of long shipment delays—the need for extended shelf life [3,4]. As an example, numerous variants of MacConkey agar exist with different levels of selectivity complicating selection (see Supplementary material, Document S1). Moreover, similar product names may convey substantial differences in composition, which may lead to confusion (see Supplementary material, Fig. S1). Furthermore, information on the product labels is often printed in small font sizes hampering readability. Instructions for preparations may be difficult to understand, particularly by non-native English- or French-speaking staff.

Challenges at the laboratory level: infrastructure, environment, staff

Laboratories in LMIC referral hospitals are basically equipped, staffed by laboratory technicians with little expertise in microbiology and have a poorly implemented quality management system

[3,15]. Clean water and uninterrupted power supply are not assured. High environmental temperature, humidity and dust challenge the storage of dehydrated media and poured plates [3,4]. Using human blood as a substitute for sheep or horse blood is commonplace [16,17]. Laboratory orders are frequently processed by the hospital procurement service, which prioritizes low price over quality and consistency. Media preparation is frequently carried out in the 'laboratory kitchen', close to autoclaves and sinks (high temperature, high humidity), by in-house-trained and poorly supervised auxiliary staff (authors' personal observations).

Challenges at the national level: market factors, regulation

Diagnostic manufacturers concentrate on high-income markets. Despite the predicted increase of the *in vitro* diagnostic (IVD) market and more specifically the culture media market [10,18], manufacturers are reluctant to engage in LMICs, and local production of IVDs is rare [1,4]. Most manufacturers of culture media have a low to moderate presence in Africa and Latin America, but are better represented in the Asia Pacific region [10]. Brazil, India and China, as well as other emerging market economies, could fill this gap. In the absence of effective regulation, product quality is variable. Country demands are inconsistent and price setting is non-transparent [18–20]. Unlike malaria, human immunodeficiency virus (HIV) and tuberculosis, clinical bacteriology is not supported by national control programmes. There are no supranational alliances (e.g. Roll Back Malaria) or international donors with bulk procurement expertise [18]. In terms of international regulations for IVDs, most culture media are considered as 'low risk' (Class A) IVDs with consequent minimal regulation and higher variations in product quality [3,18,21,22].

Best practices for media procurement and preparation in resource-constrained settings

Fig. 1 depicts the different steps of media preparation starting from dehydrated media. Table 1 lists the best practices for culture media preparation. Supplementary Figures S2–S5 depict examples of best practices and common errors. Table 2 lists the quality control requirements. Table 3 gives an overview of the most common errors and their possible causes (trouble-shooting guide). Supplementary Table S1 lists basic equipment and materials needed for culture media preparation in LMIC.

Choice of media, selection of brand and formulation

When selecting culture media, define a small panel of well-described media that is supported by the national reference laboratory and look for products from reputable manufacturers [23]. In case of changing to another brand, carefully check the candidate product's name and ingredients, as compositions may differ. Look for humidity-proof packaging (screw cap, peel-off seal) [11,23]. Verify the Safety Data Sheet, instructions for use and the shelf life.

The clinical microbiologist can give guidance on the selection of appropriate culture media taking into account the prioritization of clinical specimens. At a minimum, next to basic nutrient media, selective media for Gram-positive and Gram-negative bacteria should be available. Stool cultures are generally not recommended for clinical care in first level referral laboratories, but—depending on the setting—Thiosulphate Citrate Bile salts Sucrose agar can be kept in stock in view of epidemic preparedness. Note that different types of primary culture media are available with similar applications (e.g. MacConkey versus Eosin Methylene Blue agar) and their use can even be culturally dependent (e.g. French-speaking versus English-speaking countries).

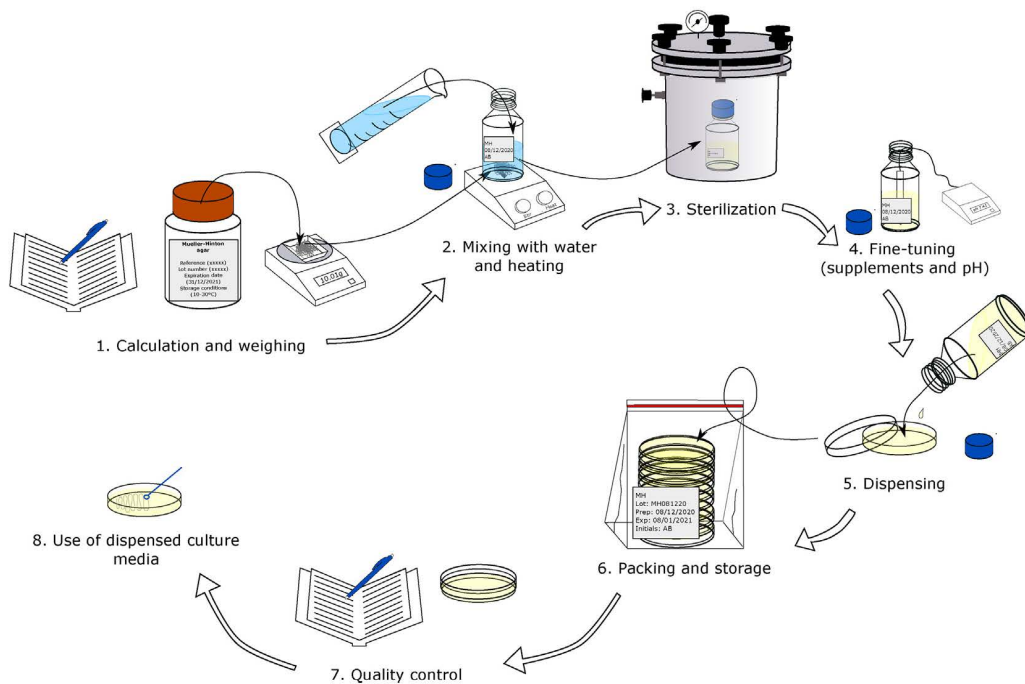


Fig. 1. Preparation of a non-supplemented dehydrated agar medium. Figure inspired by Barry and Fay [14]. Note that the media preparation can only be interrupted at the following steps: (a) for non-selective agar media without supplements, the flask can be stored as solidified agar after autoclaving and re-melted later; (b) the dispensed agar plates can be left closed on the bench to dry overnight (provided there are no insects in the laboratory) and packed and stored the day after.

Procurement, reception, storage and stock management

Select an in-country supplier, providing after-sale support and advice, as well as a back-up supplier; do not rely on non-professional vendors offering occasional sales. Check and record, upon reception, the ordering processing time, correctness of the order delivery, product name and lot number, quantity received, package integrity, expiry date and remaining shelf life [24].

Store dehydrated culture media in a cool, dry place, protected from light and dust [24]. At all times, check the manufacturer's instructions because in rare cases (e.g. Urea Broth powder) storage at 2–8°C is recommended. Monitor the storage area's temperature and humidity. Organize the stock according to a first-in, first-out system, rank media in a logical order and separate opened from closed containers (two-shelf system).

The shelf life of dehydrated culture media is usually long (several years). However, dehydrated media are hygroscopic and quickly deteriorate when exposed to moisture [23]. Therefore, record the date of opening on the container and use the product for maximum 6 months after opening unless otherwise specified by the manufacturer [13]. After this period, visual and performance checks are required [14]. When not in use, keep containers tightly closed and seal the caps with adhesive tape [23]. Discard products when expired, when the powder is clumped, discoloured or not free flowing, or in case of quality issues; record product details and reasons for discarding [24].

Waste management

For disposal of used culture media, follow the in-country guidelines. They are mostly based on WHO guidelines, which recommend inactivation and subsequent incineration before disposal. Inactivation (so-called 'destruction' or Level III inactivation) should be done in the laboratory, in preference by autoclaving [25]. Details about

autoclave and incinerator types, validation and controls are described elsewhere [26]. The African Society of Laboratory Medicine recommends, in cases where an autoclave is not available, an overnight treatment with 2% chlorine solution [27]; however, this practice is less effective compared with autoclaving [26].

Recommendations to improve access to high-quality culture media in LMICs

Centralized local production of media allows for harmonization and bulk procurement

In high-income countries, commercially prepared media have been shown to be beneficial for quality [28]. In line with this, centralized media acquisition has been advocated for LMICs [29]. Centralized production has the advantage of dedicated media processing areas (controlled for dust and humidity), cost-efficiency, standardization (single lot productions) and validation of shelf-life, shipment and storage conditions [28,29]. An example is the Central Media-Making Laboratory in Phnom Penh, Cambodia, hosted by a governmental tertiary-level medical university [30]. The Central Media-Making Laboratory is ISO9001 certified and currently produces 23 types of culture media delivered to 28 clients in Cambodia.

If overseen by the National Reference Laboratory or the Ministry of Health, the centralized media production can also contribute to harmonization. Harmonization will lead to a more predictable demand and allows for bulk purchasing and supply and pricing arrangements [18,23,29]. Besides pricing agreements, selected pooled procurement mechanisms may contribute to a viable and dynamic market, as has been shown for HIV diagnostics: offering multi-year contracts with committed annual product volumes and sourcing to different manufacturers has fostered innovation and competition [31,32].

Table 1
Best practices for culture media preparation in resource-constrained settings

1. Calculation and weighing of dehydrated media

- a. *Location and conditions:*
- ✓ Damp-free environment (low humidity), if available a laboratory fume hood
 - ✓ Table, balance & materials: clean, dust-free and powder-free
- b. *Balance:*
- ✓ Top loading (precision) balance
 - ✓ Positioned on a level, stable, vibration-free table
 - ✓ Calibrated at regular intervals (e.g. annually)
 - ✓ Verify control weights before use or on a daily basis
- c. *Materials and PPE:*
- ✓ To avoid inhalation and skin contact: use a safety mask and gloves
 - ✓ Wash hands before and after working with the dehydrated culture media
 - ✓ If required (hazard warnings on the MSDS sheets or labels, particularly 'skin and eye irritation'): wear safety goggles (e.g. for sodium deoxycholate)
- d. *Calculate:*
- ✓ Follow the manufacturer's instructions
 - ✓ Prepare a table with pre-defined weights and volumes for a fixed number of plates: to generate solid medium with a depth of 4 mm in a Petri plate of 9-cm diameter, a volume of ~20–25 mL is needed
 - ✓ Record product name, lot number, preparation date, weight and volume of water, balance used and operator identification in logbook
- e. *Weighing:*
- ✓ To facilitate weighing: use materials with pre-defined volume
 - ✓ Open the container and take the required amount of powder
 - ✓ Close the container immediately to protect it from humidity
 - ✓ Do not put excess powder back into the bottle!

2. Mixing with water and heating

- a. *Water:*
- ✓ Fresh water prepared by distillation, deionization or reverse osmosis. No tap water (affects selectivity and pH). Store in clean, sealed glass container.
 - ✓ Verify purity before use (no bacterial growth!)
 - ✓ Optionally, verify the quality before use (recommended by some references):
 - pH: 5.5–7.7 (Note: to measure pH, add 0.3 mL of saturated potassium chloride to 100 mL of water)
 - Conductivity: <15 microSiemens
- b. *Materials:*
- ✓ Heater-stirrer, heating mantle (for large volumes) or bain-marie
 - ✓ Heat-resistant gloves
 - ✓ Glassware: borosilicate, resistant to chemicals and heat
 - Rinse with distilled/deionized water after cleaning to avoid detergent residue
 - Store in cupboard. Discard broken, etched or chipped glassware
 - Use Erlenmeyers (wide base for uniform heating, small top to avoid evaporation), bottles/flasks/tubes with screw caps, graduated cylinder to measure water
 - If required (e.g. glass Petri plates), sterilize at 160°C for 2 hours in hot air oven. Allow oven to cool to 50°C before opening (to avoid cracking of glassware)
- c. *Mixing with water (preparation):*
- ✓ Pre-heating the water to 50–60°C may facilitate dissolution
 - ✓ Fill to max ½ flask capacity (to avoid spills), max 1 L per flask (for mixing)
- d. *Procedure for dissolving powder in water:*
- ✓ Pour half of the required water in the flask, and add the pre-weighed powder
 - ✓ Stir or rotate for a few minutes (do not shake!)
 - ✓ Pour the rest of the water down the sides to dissolve any excess powder sticking to the flask walls (dry powder may not be sterilized in the autoclave)
- e. *Heating (required for agar-containing media):*
- ✓ Do not close flasks tightly
 - ✓ Heat up to boiling, with frequent stirring, until solution becomes clear
 - ✓ Avoid boiling, overheating and foaming, scorching and burning, clumping and inconsistent mixing
 - ✓ Only autoclave when completely dissolved. Dissolution during sterilization can cause uneven dissolution, pH drifts and browning

3. Sterilization

- a. *Autoclaving:* (usually at 121 ± 2°C for 15 ± 1 minutes)
- ✓ Note that manufacturer's instructions refer to volumes of up to 1 L
 - ✓ Some media cannot be autoclaved (e.g. SS agar, Cary Blair agar)
 - ✓ For liquid media in tubes or bottles: first dispense in tubes/bottles, then autoclave!
- b. *Avoid:*
- ✓ Over-sterilization causing precipitation, pH change, component destruction
 - ✓ Under-sterilization resulting in contaminated medium
 - ✓ Direct contact with water or autoclave and water accumulation in recipients
- c. *Ensure:*
- ✓ The wrapping allows for steam penetration
 - ✓ The lids/caps are not completely tightened
 - ✓ There is enough space between the items to allow steam circulation
- d. *Identification of sterilized items:*
- ✓ Use indicator tape to label the flask (medium name/code, preparation date, initials)
- e. *Verification of autoclave cycles:*
- ✓ Chemical indicators with each cycle (e.g. time–steam–temperature strips)
 - ✓ Biological indicators on interval basis
- f. *After sterilization:*
- ✓ Use a 'cool down program' or wait until the pressure drops sufficiently before opening (~70–80°C) to avoid a fast pressure drop (liquid can boil over, caps can be blown off)
 - ✓ Do not wait too long before unloading the autoclave, overheating may destroy media ingredients

4. Fine-tuning: Adding supplements, pH verification

- a. *Adding supplements:*
- ✓ Let the media cool down to 45–50°C before adding heat-labile supplements
 - ✓ Filter-sterilize heat-labile supplements (not in the case of blood)
 - ✓ Let supplements come to room temperature before adding
 - ✓ Add sterile supplements aseptically
 - ✓ Ensure adequate mixing
- b. *Verification of pH after sterilization (not before):*
- ✓ Verify pH (usually at 25°C) with pH meter before dispensing
 - ✓ Take 20 mL out of the flask, let cool down and measure pH. Keep flask in water bath
 - ✓ Compare to the pH range specified in the manufacturer's instructions. If pH is ok, continue with dispensing
 - ✓ The pH of ready-made dehydrated media should not require adjustment. In case of deviation, verify water, dehydrated medium, glassware, procedure and start again

5. Dispensing of prepared culture media

- a. *Cool down media in water bath (45–50°C) before dispensing to minimize condensation:*
- ✓ Dispensing at too high temperature → excessive evaporation
 - ✓ If media stay too long in water bath → precipitation (reheat, but do not overheat)
 - ✓ Do not use cold water to cool down agar media → flakes/cloud formation
- b. *Dispense the media aseptically:*
- ✓ Work close to a flame or in a biological safety cabinet
 - ✓ Flame sterilize the neck of the flask before and between pouring
- c. *Drying of plates:*
- ✓ Dry for several hours at room temperature (up to 24 hours) to remove condensation
 - ✓ Selective media: ±30 minutes with lid ajar. If contamination risk: keep lids closed
 - ✓ Dry before packing to prevent condensation on the lids. Avoid over-drying (cracks)
- d. *Dispensing of agar or liquid media in tubes:*
- ✓ Use tubes with lids that allow ventilation (e.g. screw caps), do not tighten completely

(continued on next page)

Table 1 (continued)

<ul style="list-style-type: none"> ✓ Dispense in a draught-free room (with closed windows), avoid fans or climate control 	<ul style="list-style-type: none"> ✓ Put tubes in an autoclavable rack ✓ Mix gently by rotating the flask before dispensing ✓ Dispense the correct volume per tube. Use sterile, graduated pipettes or a media distribution syringe/pump ✓ Close screw caps tightly after autoclaving ✓ Drying depends on the type of media: <ul style="list-style-type: none"> ○ For agar slants, let dry in a sloped position to give a butt of 2.5–3 cm deep and a slope of 2–2.5 cm long. Use a standardized and validated rack ○ For agar, semi-solid tubes, liquid media: let dry in rack (vertical position)
<p>c. <i>Dispensing of agar media in plates:</i></p> <ul style="list-style-type: none"> ✓ Mix gently by rotating the flask before dispensing ✓ For antimicrobial susceptibility testing, agar depth should be 4 ± 0.5 mm: <ul style="list-style-type: none"> ○ Circular Petri plate of 90 mm = ~25 mL ○ Use sterile, graduated pipettes or media distribution syringe/pump ✓ Avoid air bubbles (flame surface or use heated loop to remove them) ✓ Dispense on a level surface 	
<p>6. Packing and storage</p> <p>a. <i>Packing and storage of plates:</i></p> <ul style="list-style-type: none"> ✓ Label individual plates: name prepared medium/code, preparation date, lot number ✓ Wrap plates in sealed, labelled, plastic bags, maximum ten plates/bag (to avoid moisture) ✓ Store upside down, at 2–8°C, in the dark ✓ Close packaging again after opening to take out some plates ✓ Shelf life [13]: <ul style="list-style-type: none"> ○ Blood agar: 7 days; with unstable additives: 2–5 days ○ Most selective media: 5–7 days ○ Nutrient agar without blood: 2–4 weeks <p>b. <i>Storage of prepared but not dispensed agar (solidified):</i></p> <ul style="list-style-type: none"> ✓ Only for autoclaved, non-selective media (without heat-labile components) ✓ In tightly closed flasks, at room temperature or 2–8°C, in the dark ✓ Shelf life up to 6 months ✓ Add supplements after re-melting 	<p>c. <i>Packing and storage of tubes:</i></p> <ul style="list-style-type: none"> ✓ Store the tubes in a labelled rack in the dark, following manufacturer's instructions ✓ Ensure that the screw caps are tightly closed ✓ Shelf life [13]: <ul style="list-style-type: none"> ○ Simple, non-selective broths and agars: 6 months ○ Selective media: 3 weeks (2–8°C) ○ Selenite broth: 2–3 months <p>d. <i>Labelling of outer packaging of plates, racks of tubes and prepared agar:</i></p> <ul style="list-style-type: none"> ✓ Medium name (abbreviated)/code ✓ Lot number—simple with preparation date included (e.g. MC110220—a/b/c in case of multiple lots for the same medium) ✓ Date of preparation ✓ Date of expiration ✓ Initials
<p>7. Quality control (refer to Table 2)</p> <p>8. Use of dispensed culture media</p> <p>a. <i>Bring to room temperature before use</i></p> <ul style="list-style-type: none"> ✓ No visible drops of water on the agar surface or inside the lid ✓ Do not shake off condensation water from the lid! ✓ Dry plates for 20–30 minutes at 35–37°C with agar plates upside down, agar base resting at an angle on the lid 	<ul style="list-style-type: none"> ✓ If necessary, dry plates at 20–25°C overnight ✓ Do not over-dry plates (cracks in surface, surface wrinkled) <p>b. <i>Visual sterility check before use</i></p> <ul style="list-style-type: none"> ✓ Check the plates for contamination/growth of colonies

According to references [11–14,23,24,29,56]. The focus is put on dehydrated agar-containing solid media. These best practices are complementary to the manufacturer's instructions and the laboratory Quality Management System. For some practices, different options were found according to different references (e.g. stacking plates after pouring, leaving them on the bench with the lid open, etc.). In these cases, we selected the option that is most adapted to settings in low- and middle-income countries. Abbreviations: MSDS, material safety data sheet; PPE, personal protective equipment.

Table 2
Quality control requirements for culture media preparation

Quality control	
<p>a. <i>Quality control (QC):</i></p> <ul style="list-style-type: none"> ✓ Should be based on a pragmatic, risk-based approach ✓ Perform QC for each newly prepared batch ✓ Quarantine newly prepared and dispensed media until they pass QC <p>b. <i>pH verification (refer to Table 1)</i></p> <p>c. <i>Visual inspection</i></p> <ul style="list-style-type: none"> ✓ Cracked or damaged plates ✓ Agar detached from the plates ✓ Frozen or melted agar ✓ Unequal filling of the plates ✓ Insufficient amount of agar (<3 mm) (Note: For Mueller–Hinton agar, the agar depth should be 4 ± 0.5 mm) ✓ Haemolysis of blood-containing media ✓ Changes in the expected colour of the media ✓ Excessive bubbles or rough surfaces ✓ Excessive moisture or dehydration ✓ Obvious contamination ✓ Presence of precipitates ✓ Integrity of the packaging ✓ Presence of broken or cracked Petri plates or tubes ✓ Presence of leakage from the Petri plates or tubes ✓ Accuracy of the labelling 	<p>d. <i>Sterility check</i></p> <ul style="list-style-type: none"> ✓ 48 h incubation at 35–37°C (varying from 24 h to 5 days depending on the reference) ✓ Batch with <100 units: 2% sample; batch with >100 units: ten random units ✓ In case of contamination, reject the batch and prepare a new one ✓ Do not re-use the plates used for sterility check ✓ Note that an additional visual check should always be done right before use <p>e. <i>Performance check:</i></p> <ul style="list-style-type: none"> ✓ Use QC organisms: <ul style="list-style-type: none"> ○ Choose appropriate QC organisms based on standards, guidelines or manufacturer's instructions ○ Type culture collection organisms (e.g. ATCC) are recommended but previously characterized clinical organisms or EQA strains shown to be phenotypically stable (documentation required) are also accepted ○ QC organisms should be correctly maintained and stored (refer to CLSI M22-A3 [8]) ✓ Use a standardized suspension to inoculate the culture media ✓ Check and record growth (colony size and morphology), selectivity and differentiation: <ul style="list-style-type: none"> ○ Support of target organism: Use at least one organism, record growth and biochemical reaction (e.g. colour on the MacConkey) ○ Selective media: Use at least one organism that is expected to grow and at least one organism that is not expected to grow ○ Differential medium: Use organisms which will display the intended growth and reactions (e.g. MacConkey: <i>Shigella flexneri</i>, <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>) ○ Biochemical media (e.g. urease-test): Use at least one organism that will produce a negative reaction and one organism that will produce a positive reaction ✓ Perform disc diffusion to check Mueller–Hinton agar plates

According to references [8,13,23,24,29,56–59].

Abbreviations: ATCC, American Type Culture Collection; EQA, external quality assessment; QC, quality control.

Table 3
Troubleshooting: overview of the most common errors and possible causes

Error	Possible causes
Clumping of dehydrated culture media	<ul style="list-style-type: none"> ✓ Humidity too high during storage ✓ Container left open for too long ✓ Container not tightly closed after opening ✓ Dehydrated culture medium beyond shelf life
Wrong pH	<ul style="list-style-type: none"> ✓ pH meter not calibrated ✓ pH verification done on too hot medium (generally to be done at 25°C) ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Use of poor-quality water or container ✓ Use of chemically contaminated containers ✓ Incomplete dissolution/mixture of medium ✓ Dehydrated medium stored incorrectly (e.g. not tightly closed) or beyond shelf life
Incomplete solubility	<ul style="list-style-type: none"> ✓ Use of inadequate water ✓ Inadequate heating/inadequate timing for dissolution ✓ Insufficient soaking or incomplete mixing ✓ Flask too small to allow adequate mixing and/or convection
Darkening, caramelization	<ul style="list-style-type: none"> ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Incomplete dissolution of medium
Incomplete gelling or soft agar	<ul style="list-style-type: none"> ✓ Incorrect proportions of product to water: error in weighing or over-dilution ✓ Agar not properly dissolved: poor mixing, prolonged storage at 50°C ✓ Overheating of culture medium, possibly at low pH ✓ Repeated re-melting causing overheating
Turbidity, precipitation	<ul style="list-style-type: none"> ✓ Poor quality of dehydrated media ✓ Use of poor quality of water or container ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Wrong pH ✓ Incomplete dissolution/mixture of medium ✓ Loss of water of the prepared culture medium due to evaporation
Poor growth or loss of differential properties	<ul style="list-style-type: none"> ✓ Incorrect or improperly maintained QC organisms used ✓ Overheating: excessive sterilization, heterogeneous mixing, medium kept at 50°C for too long, repeated re-melting or at too high temperature ✓ Incomplete dissolution/mixture of medium ✓ Inhibitory substances in water, container or inoculum ✓ Wrong pH

According to references [11–13,60].

Abbreviation: QC, quality control.

Anticoagulated hair sheep blood as a substitute for defibrinated wool sheep or horse blood

Citrated blood of hair sheep (the short-haired sheep resistant to tropical climates) is a practical alternative for defibrinated wool sheep and horse blood [16,33]. It may be obtained in partnership with a local farmer, slaughterhouse or veterinary school.

Role of the national reference laboratories and clinical microbiologists

As part of their cardinal role in the antimicrobial resistance surveillance, National Reference Laboratories provide guidance, technical support, training and supervision to the tiered national laboratory network [15,34]. For culture media, this comprises guidance in selection and supply, procedures for storage and preparation, quality control (including lot-to-lot variation) and post-market surveillance of culture media. In addition, services include distribution of strains for quality control and antibiotic susceptibility testing for those organisms requiring more complex media (e.g. fastidious organisms) [23,29].

The pivotal role of the clinical microbiologist in the day-to-day working of a clinical bacteriology laboratory is apparent. Clinical microbiologists give advice on prioritization of specimens,

selection of culture media and diagnostic tests, choice of antibiotics for susceptibility testing, validation of results and safeguarding quality. Moreover, they play a central role in the strengthening of diagnostic capacity in LMICs [35]. In many LMICs, however, equivalents to clinical microbiologists are rare and ill-defined and most clinical bacteriology laboratories are overseen by managers with little expertise in clinical bacteriology [3]. There is an urgent need for more qualified professionals in LMICs to fill this gap.

The WHO essential diagnostics list

The WHO EDL promotes and prioritizes access to IVDs. It is modelled on and complementary to the WHO List of Essential Medicines [36], which has been crucial for access to affordable treatment in LMICs [35]. The WHO EDL creates political will and calls for manufacturers' commitment [18]. WHO member states are invited to adapt the WHO EDL to their local disease burden. As an example, the Cambodian National Essential Medicine List included a list of essential culture media and diagnostic testing devices even before the WHO EDL launch [37]. Through the WHO EDL and their member state adaptations, manufacturers are informed that listed products (including culture media) are considered as essential and so must be quality assured [18].

Professional societies in clinical microbiology and academia

Professional societies issue evidence-based guidelines and algorithms about IVDs and some of them are very rationalized practices in LMICs. As an example, for quality control of culture media outside antibiotic susceptibility testing, the CLSI M22-A3 guideline authorizes the use of well-characterized clinical organisms [8]. This pragmatic recommendation allows the National Reference Laboratories to biobank and distribute phenotypically stable quality control strains instead of procuring expensive culture collection strains. Even more impact can be expected from independent product evaluations such as those recently carried out by the European Committee on Antimicrobial Susceptibility Testing for Mueller–Hinton media and antibiotic discs [38,39]. Apart from guidance, independent evaluation studies create awareness among users and manufacturers and incite product improvement [40], as was demonstrated for the antibiotic discs [39].

A more stringent and harmonized regulation of in vitro diagnostics

The Mueller–Hinton and antibiotic disc evaluation studies performed by the European Committee on Antimicrobial Susceptibility Testing highlighted the need for more stringent regulatory criteria [39]. In the absence of implemented regulations in some LMICs, laboratories frequently refer to regulatory approval in high-income countries, for example the CE (*Conformité Européenne*) mark of the European Union. The current EU IVD 98/79 Directive will be superseded by the more stringent EU Regulation 2017/746, which will be effective from 2022 onwards, but the exact place of culture media is still unclear [41]. The Pan-African Harmonization Working Party is working on a harmonized IVD regulatory framework, with emphasis on a common registration file, clinical evidence and post-marketing [42,43].

In vitro diagnostic manufacturers

The landscape of diagnostic manufacturers in LMICs is changing, as manufacturers from booming economies are entering [4,10,18]. However, for emerging economies to be competitive, a higher demand from LMICs, North–South technology transfer and an improved diagnostics production and supply chain are required [18]. In addition, diagnostic manufacturers must become familiar with the aspects of clinical bacteriology and the multitude and diversity of consumables [3]. Public–Private Partnerships or similar can be explored to enable laboratories or networks of laboratories to strengthen media production, including pooled procurement and training [44]. An example in the context of HIV and tuberculosis is the Public–Private Partnership between the US President's Emergency Plan for AIDS Relief (PEPFAR) and Becton Dickinson to strengthen laboratory systems in several African countries, which resulted in improved specimen referral systems in Ethiopia [45]. To be successful, clear communication and explicit division of the roles and responsibilities of each partner must be ensured, and the autonomy and flexibility of the laboratory must be safeguarded [3,45].

Further research to be done

Rational use of resources and culture media

Cost-effective use of culture media is key to building sustainable clinical microbiology services. A tiered identification system with basic (genus or group level) identification on site and referral to reference laboratories for species identification can reduce costs and complexity [4]. Further, adapted culture media should be studied in a clinical setting. Examples are glucose-topped

MacConkey agar for urine cultures as well as chromogenic media (provided it is affordable and stable) [46,47]. The high investment needed to obtain sheep blood in remote settings calls for alternatives, such as blood-free media for large-colony haemolytic streptococci [48]. Re-usable culture tubes and Petri plates made of borosilicate glass can reduce costs as well as biplates, which are cost-effective, but may be difficult to find and are more complex to prepare. The clinical microbiologist can give recommendations after a risk- and cost-based evaluation.

Bacteriological agar is obtained from sea algae at only a few harvesting sites and its increasing use may imply higher cost and shortage. In addition, it is expensive (estimated at US\$500/kg in 2012) and its cost represents more than 90% of the cost of common solid media [49]. In food and water microbiology, ready-to-use absorbent pads impregnated with culture media are used but they have not yet been adapted to the need for subculture in clinical bacteriology [50]. Alternatives for bacteriological agar are underway but need further research; they include low-cost food-grade agar [49], cellulose produced by engineered bacteria [51] and alternative gelling agents [6,52].

Tropicalization and customization of culture media formulations

Marketed products typically display stability at temperatures up to 25°C or 30°C but actual storage temperatures in LMICs may be much higher [53]. Therefore, research also needs to address the temperature stability of culture media and the humidity resistance of their packaging. Examples of low-tech low-cost formulations are pre-weighed sealed packages (e.g. ready-to-use for 1 litre) offered by several suppliers and prepared media stored in bottles that can be re-melted and poured. Further, granulated formulations of dehydrated media may have, in addition to their other advantages (less dust, longer shelf life), the potential of better temperature and humidity resistance [12]. More demanding questions are about extending the shelf life of poured products (e.g. by vacuum-sealed gas-flushed packaging) and the implementation of biodegradable plastic materials [54,55].

Transparency declaration

The authors have no conflicts of interest to declare.

Funding

This work was funded by the Belgian Directorate of Development Cooperation (DGD) through the Multi-Year Programme (2017-2021) between the Belgian DGD and the Institute of Tropical Medicine, Belgium, and by the Research Foundation Flanders (FWO) project G0C5520.

Acknowledgements

We thank David Dolinger for providing information on regulations of *in vitro* diagnostics; Vera Kühne from the Institute of Tropical Medicine (Antwerp, Belgium) for designing Fig. 1; and Chrea Makteyka from the Central Media Making Laboratory, University of Health Sciences, Cambodia and Vivian Fensham from Integrated Quality Laboratory Services, Lyon for providing valuable inputs. We thank the Instituto Nacional de Saúde, Mozambique, for the pictures that were used to compose Figures S2-S5.

Author contributions

JO, SO, J-BR and JL contributed to the methodology, formal analysis, and to reviewing and editing; BB contributed to the

methodology, formal analysis, writing the original draft, and to reviewing and editing; JJ contributed to the conceptualization, methodology, formal analysis, writing the original draft, reviewing and editing, and supervision; DA contributed to the conceptualization, reviewing and editing, and supervision; and LH contributed to the conceptualization, methodology, formal analysis, writing the original draft, reviewing and editing, visualization and supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2021.05.016>.

References

- [1] Bebell LM, Muiru AN. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? *Glob Heart* 2014;9:347.
- [2] Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. *PLoS ONE* 2017;12.
- [3] Jacobs J, Hardy L, Semret M, Lunguya O, Phe T, Affolabi D, et al. Diagnostic bacteriology in district hospitals in sub-Saharan Africa: at the forefront of the containment of antimicrobial resistance. *Front Med* 2019;6:205.
- [4] Ombelet S, Ronat J-B, Walsh T, Yansouni CP, Cox J, Vlieghe E, et al. Clinical bacteriology in low-resource settings: today's solutions. *Lancet Infect Dis* 2018;18.
- [5] World Health Organization. WHO model list of essential in vitro diagnostics. Available at: 1st ed. Geneva: WHO; 2018. https://www.who.int/medicines/publications/essentialmedicines/18th_EML.pdf. [Accessed 17 December 2020].
- [6] Bonnet M, Lagier JC, Raoult D, Khelaifa S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microb New Infect* 2020;34. <https://doi.org/10.1016/j.nmni.2019.100622>.
- [7] Zimbro MJ, Power DA, Miller SM, Wilson GE, Johnson JA. Difco™ & BBL™ manual. Manual of microbiological culture media. Available at: 2nd ed. Sparks, MD: Becton Dickinson; 2009. https://www.bd.com/documents/guides/user-guides/DS_CM_Difco-and-BBL-culture-media-manual_UG_EN.pdf. [Accessed 17 December 2020].
- [8] Clinical and Laboratory Standards Institute (CLSI). Quality control for commercially prepared microbiological culture media; approved standard. CLSI Document M22-A3. 3rd ed. Wayne, PA: CLSI; 2004.
- [9] Perovic O, Yahaya AA, Viljoen C, Ndiokubwayo JB, Smith M, Coulibaly SO, et al. External quality assessment of bacterial identification and antimicrobial susceptibility testing in African national public health laboratories, 2011–2016. *Trop Med Infect Dis* 2019;4.
- [10] Up Market Research. Global culture media market analysis & forecast, 2017–2027. Maharashtra. Up Market Research; 2020.
- [11] Valls JS, Nacente RB. Handbook of microbiological culture media, 11th ed. Barcelona. Available at: 2011. https://www.scharlabmagyarorszag.hu/katalogus/scharlau_mikrobiologiai_kezikonyv.pdf. [Accessed 17 December 2020].
- [12] Merck. Microbiology manual, 12th ed. Darmstadt: Merck. Available at: http://www.labquimia.es/pdf_catalogo/MERCK_Manual_de_mikrobiologia_12a_edicion.pdf. [Accessed 17 December 2020].
- [13] Oxoid Limited. Technical Support—FAQs on culture media, supplements and raw materials. Available at: <http://www.oxoid.com/UK/blue/techsupport/its.asp?itsp=faq&faq=tsfaq011&cat=culture+media%2C+supplements+and+raw+materials&lang=EN&c=UK>. [Accessed 17 December 2020].
- [14] Barry AL, Fay GD. A review of some common sources of error in the preparation of agar media. *Am J Med Technol* 1972;38:241–5.
- [15] Barbé B, Yansouni CP, Affolabi D, Jacobs J. Implementation of quality management for clinical bacteriology in low-resource settings. *Clin Microbiol Infect* 2017;23:426–33.
- [16] Russell FM, Biribo SSN, Selvaraj G, Oppedisano F, Warren S, Seduadua A, et al. As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries. *J Clin Microbiol* 2006;44:3346–51.
- [17] Ferguson JK, Joseph J, Kangapu S, Zoleveke H, Townell N, Duke T, et al. Quality microbiological diagnostics and antimicrobial susceptibility testing, an essential component of antimicrobial resistance surveillance and control efforts in Pacific island nations. *West Pac Surveill Response J* 2020;11:41–6.
- [18] Stevenson M. A situation analysis of the state of supply of in vitro diagnostics in Low-Income Countries. *Glob Public Health* 2020;15:1836–46.
- [19] Daily J. Fever diagnostic technology landscape, 1st ed. Vernier. Available at: 2018. https://unitaid.org/assets/Fever_diagnostic_technology_and_market_landscape.pdf. [Accessed 17 December 2020].
- [20] Daily J. Malaria diagnostics technology and market landscape, 3rd ed. Vernier. Available at: 2016. <https://www.ghdonline.org/uploads/Unitaid-Malaria-Dx-Tech-Mkt-Landscape-3rd-Ed-April-2016.pdf>. [Accessed 17 December 2020].
- [21] International medical device regulators forum (IMDRF). Available at: <http://www.imdrf.org/about/about.asp>. [Accessed 17 December 2020].
- [22] World Health Organization (WHO). WHO global model regulatory framework for medical devices including in vitro diagnostic medical devices. Geneva. Available at: 2017. https://www.who.int/medical_devices/publications/global_model_regulatory_framework_meddev/en/. [Accessed 17 December 2020].
- [23] Cheesbrough M. District laboratory practice in tropical countries. Part 2. 2nd ed. Cambridge: Cambridge University Press; 2006.
- [24] Kaur T, Gupta S, Kaur M. Establishment and application of ideal media room in microbiology laboratory. *Int J Med Microbiol Trop Dis* 2016;2:22–3.
- [25] World Health Organization. Laboratory biosafety manual. Available at: 4th ed. Geneva: WHO; 2020. <https://www.who.int/publications/i/item/9789240011311>. [Accessed 26 February 2021].
- [26] World Health Organization. Safe management of wastes from health-care activities. Available at: 2nd ed. Geneva: WHO; 2014. https://www.who.int/water_sanitation_health/publications/safe-management-of-wastes-from-healthcare-activities/en/. [Accessed 17 December 2020].
- [27] MLO Staff. The fundamental challenge of laboratory associated waste management. Available at: African Association for Laboratory Medicine—Lab Culture; 2018. p. 14–8. https://aslm.org/wp-content/uploads/2018/05/ASLM_LabCulture_Issue_18.pdf?x10839. [Accessed 17 December 2020].
- [28] Nagel JG, Kunz IJ. Needless retesting of quality-assured, commercially prepared culture media. *Appl Microbiol* 1973;26:31–7.
- [29] Perilla MJ, Ajello G, Bopp C, Elliott J, Facklam R, Knapp JS, et al. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Atlanta. Available at: 2003. https://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_RMD_2003_6/en/. [Accessed 17 December 2020].
- [30] University of Health Sciences Cambodia—Central Media Making Laboratory. Available at: <https://www.uhs.edu.kh/cmml/>. [Accessed 17 December 2020].
- [31] Unitaid. Market and Technology Landscape. HIV rapid diagnostic tests for self-testing, 3rd ed. Vernier. Available at: 2017. <https://www.who.int/hiv/pub/vct/hiv-self-testing-2017-thirdedition/en/>. [Accessed 17 December 2020].
- [32] The Global Fund. Market shaping strategy. Available at: <https://www.theglobalfund.org/en/sourcing-management/market-shaping-strategy/>. [Accessed 17 December 2020].
- [33] Yeh E, Pinsky BA, Banaei N, Baron EJ. Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. *PLoS ONE* 2009;4.
- [34] World Health Organization. GLASS Guidance for national reference laboratories. Available at: Geneva: WHO; 2020. <https://www.who.int/publications/i/item/9789240010581>. [Accessed 17 December 2020].
- [35] Yustus E, Hamers RL. What the WHO's List of Essential Diagnostics means for clinical microbiology laboratories and antimicrobial stewardship practice worldwide. *Clin Microbiol Infect* 2019;25:6–9.
- [36] Schroeder LF, Guarner J, Amukele TK. Essential diagnostics for the use of world health organization essential medicines. *Clin Chem* 2018;64:1148–57.
- [37] Available at: Essential medicine list, 9th ed. 2018. <https://www.ddfcambodia.com/bureau/essential-drug-bureau.html>. [Accessed 17 December 2020].
- [38] Åhman J, Matuschek E, Kahlmeter G. EUCAST evaluation of 21 brands of Mueller–Hinton dehydrated media for disc diffusion testing. *Clin Microbiol Infect* 2020;26:1412. e1–1412.e5.
- [39] Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers—EUCAST evaluations in 2014 and 2017. *Clin Microbiol Infect* 2019;25:346–52.
- [40] Cunningham J, Jones S, Gatton ML, Barnwell JW, Cheng Q, Chiodini PL, et al. A review of the WHO malaria rapid diagnostic test product testing programme (2008–2018): performance, procurement and policy. *Malar J* 2019;18:387.
- [41] Reinikainen M, Suppo M. Explaining IVD classification issues. London. Available at: https://www.bsigroup.com/globalassets/meddev/localfiles/fr-fr/whitepapers/md-classification_issues_for_ivd.pdf. [Accessed 17 December 2020].
- [42] Pan African harmonisation working party. Available at: <http://www.pahwp.org/>. [Accessed 17 December 2020].
- [43] McNerney R, Peeling RW. Regulatory in vitro diagnostics landscape in Africa: update on regional activities. *Clin Infect Dis* 2015;61:S135–40.
- [44] Ravishankar N, Lehmann J. Opportunities abound: public private partnerships for laboratory services in East Africa. Available at: 2015. <https://openknowledge.worldbank.org/handle/10986/22483>. [Accessed 17 December 2020].
- [45] Nkengasong JN, Mbopi-Keou FX, Peeling RW, Yao K, Zeh CE, Schneidman M, et al. Laboratory medicine in Africa since 2008: then, now, and the future. *Lancet Infect Dis* 2018;18:e362–7.
- [46] Karah N, Rafei R, Elamin W, Ghazy A, Abbara A, Hamze M, et al. Guideline for urine culture and biochemical identification of bacterial urinary pathogens in low-resource settings. *Diagnostics* 2020;10.
- [47] Perry JD. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. *Clin Microbiol Rev* 2017;30:449–79.

- [48] Niederstebruch N, Sixt D. Standard Nutrient Agar 1 as a substitute for blood-supplemented Müller–Hinton agar for antibiograms in developing countries. *Eur J Clin Microbiol Infect Dis* 2013;32:237–41.
- [49] Petrovski S, Tillett D. Back to the kitchen: food-grade agar is a low-cost alternative to bacteriological agar. *Anal Biochem* 2012;429:140–1.
- [50] Liofilchem. Easy dry media. Available at: <https://www.liofilchem.com/products/liofilchem-products/category.html?classe=012>. [Accessed 17 December 2020].
- [51] Yin N, Santos TMA, Auer GK, Crooks JA, Oliver PM, Weibel DB. Bacterial cellulose as a substrate for microbial cell culture. *Appl Environ Microbiol* 2014;80:1926–32.
- [52] Das N, Triparthi N, Basu S, Bose C, Maitra S, Khurana S. Progress in the development of gelling agents for improved culturability of microorganisms. *Front Microbiol* 2015;6. <https://doi.org/10.3389/fmicb.2015.00698>.
- [53] Jacobs J, Kühne V, Lunguya O, Affolabi D, Hardy L, Vandenberg O. Implementing COVID-19 (SARS-CoV-2) rapid diagnostic tests in sub-Saharan Africa: a review. *Front Med* 2020;7. <https://doi.org/10.3389/fmed.2020.557797>.
- [54] Choi YW, Rogers J v. Shelf-life assessment of agar plates vacuum-sealed in nylon/polyethylene packaging flushed with nitrogen. *J Microbiol Exp* 2015;2. <https://doi.org/10.15406/jmen.2015.02.00075>.
- [55] Dittrich S, Tadesse BT, Moussy F, Chua A, Zorzet A, Tängdén T, et al. Target product profile for a diagnostic assay to differentiate between bacterial and non-bacterial infections and reduce antimicrobial overuse in resource-limited settings: an expert consensus. *PLoS ONE* 2016;11. <https://doi.org/10.1371/journal.pone.0161721>.
- [56] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 6.0. Available at: 2020. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2020_manuals/Media_preparation_v_6.0_EUCAST_AST.pdf. [Accessed 17 December 2020].
- [57] Jones RN, Krisher K, Bird DS, College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. Update from the College of American Pathologists Microbiology Surveys Program (2001). *Arch Pathol Lab Med* 2003;127(6):661–5. <https://doi.org/10.5858/2003-127-661-ROTSOT>.
- [58] Cantarelli VV, Inamine E, Zenobin Brodt TC, Secchi C, de Souza Pereira F, Amaro MC, et al. Quality control for microbiological culture media. Is it enough to follow the NCCLS M22-A2 procedures? *Braz J Microbiol* 2003;34:8–10.
- [59] The Australian Society for Microbiology. Guidelines for assuring quality of medical microbiological culture media. Available at: 2nd ed. 2012. <https://biomeddiagnostics.com/laravel-filemanager/files/5/Guidelines%20for%20Assuring%20Quality%20of%20Medical%20Microbiological%20Culture%20Media.pdf>. [Accessed 17 December 2020].
- [60] Basu S, Pal A, Desai PK. Quality control of culture media in a microbiology laboratory. *Ind J Med Microbiol* 2005;23:159–63. <https://doi.org/10.4103/0255-0857.16586>.

3. Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media.



diagnostics



Article

Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media

Alessandra Natale ^{1,*}, Saoussen Oueslati ^{2,3} , Alice Rochard ¹, Sien Ombelet ⁴, Daniel Lopez-Baez ⁵, Liselotte Hardy ⁴ , Jane Cunningham ⁶, Céline Franquesa ¹, Olivier Vandenberg ^{7,8,9,†} on behalf of the Mini-Lab Scientific Committee, Jean-Baptiste Ronat ^{1,2,3} and Thierry Naas ^{2,3}

- ¹ Médecins Sans Frontières, Operational Center Paris, 75019 Paris, France
² Team ReSIST, INSERM U1184, Université Paris Saclay, CEA, Inserm, Immunologie des Maladies Virales, Auto-Immunes, Hématologiques et Bactériennes (IMVA-HB/IDMIT), 92265 Fontenay-aux-Roses & Kremlin Bicêtre, France
³ Service de Bactériologie-Hygiène, Hôpital Bicêtre, AP-HP, 94270 Le Kremlin-Bicêtre, France
⁴ Department of Clinical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium
⁵ Hampshire Hospitals Foundation Trust, Basingstoke RG24 9NA, UK
⁶ Access Campaign, Médecins Sans Frontières, 1211 Geneva, Switzerland
⁷ Center for Environmental Health and Occupational Health, School of Public Health, Université Libre de Bruxelles (ULB), 1070 Brussels, Belgium
⁸ Innovation and Business Development Unit, Laboratoire Hospitalier Universitaire de Bruxelles—Universitair Laboratorium Brussel, Université Libre de Bruxelles (LHUB-ULB), 1000 Brussels, Belgium
⁹ Division of Infection and Immunity, Faculty of Medical Sciences, University College London, London WC1E 6BT, UK
 * Correspondence: alessandra.natale@paris.msf.org
 † Members of the Mini-Lab Scientific Committee are listed in the Acknowledgments.



Citation: Natale, A.; Oueslati, S.; Rochard, A.; Ombelet, S.; Lopez-Baez, D.; Hardy, L.; Cunningham, J.; Franquesa, C.; Vandenberg, O., on behalf of the Mini-Lab Scientific Committee; Ronat, J.-B.; et al. Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media. *Diagnostics* **2023**, *13*, 523. <https://doi.org/10.3390/diagnostics13030523>

Academic Editor: Timothy E. Sweeney

Received: 25 November 2022
 Revised: 3 January 2023
 Accepted: 14 January 2023
 Published: 31 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Culture media is fundamental in clinical bacteriology for the detection and isolation of bacterial pathogens. However, in-house media preparation could be challenging in low-resource settings. InTray[®] cassettes (Biomed Diagnostics) could be a valid alternative as they are compact, ready-to-use media preparations. In this study, we evaluate the use of two InTray media as a subculture alternative for the diagnosis of bloodstream infections: the InTray[®] Müller-Hinton (MH) chocolate and the InTray[®] Colorex[™] Screen. The InTray MH chocolate was evaluated in 2 steps: firstly, using simulated positive blood cultures (reference evaluation study), and secondly, using positive blood cultures from a routine clinical laboratory (clinical evaluation study). The Colorex Screen was tested using simulated poly-microbial blood cultures. The sensitivity and specificity of the InTray MH chocolate were respectively 99.2% and 90% in the reference evaluation study and 97.1% and 88.2% in the clinical evaluation study. The time to detection (TTD) was ≤20 h in most positive blood cultures (99.8% and 97% in the two studies, respectively). The InTray[®] MH Chocolate agar showed good performance when used directly from clinical blood cultures for single bacterial infections. However, mixed flora is more challenging to interpret on this media than on Colorex[™] Screen, even for an experienced microbiologist.

Keywords: InTray cassettes; InTray[®] Müller-Hinton (MH) chocolate; Colorex[™] screen; bloodstream infection; diagnosis of sepsis; clinical bacteriology in low resource settings; polymicrobial infections; blood cultures; subcultures; LMICs

1. Introduction

Culture media are fundamental in any clinical bacteriology laboratory. Conventional culturing methods in clinical laboratories generally consist of using standard agar poured plates or commercially available pre-poured ready-to-use agar plates. However, ready-to-use plates are not adapted to the logistics of low-resource settings because of their short

shelf life, usually under six months, or even less when containing blood components. Therefore, laboratories in low- and middle-income countries (LMICs) rely on dehydrated culture media and in-house preparation (rehydration of powder with sterile water). This is challenging, time-consuming and requires specific equipment, space (a dedicated autoclave for sterilisation and space for plating), well-trained personnel and a strong quality control system [1]. Moreover, the addition of sterile animal blood or specific substrates may be difficult to obtain or challenging to supply (short shelf-life, cold chain requirement, price). Centralised media preparation, acquisition, tropicalization and customised solutions by in-vitro diagnostics manufacturers are among the suggested solutions [1].

Bloodstream infection is the presence of an infectious agent in blood. If not treated properly, this can cause sepsis, a deregulated response of the body to the infection, subsequently causing life-threatening organ failure with negative outcomes. It has been estimated that the incidence of sepsis worldwide is 48.9 million, with 11.0 million sepsis-related deaths in 2017 [2]. LMICs are the most affected, in particular by sepsis-related deaths, with 8.2 million deaths estimated in 2017 (84.8% of the total) [2]. Sepsis can be treated, and deaths avoided with a timely and accurate diagnosis. To combat this important global health threat, the WHO responded with a WHO Secretariat Report and, in May 2017, the Seventieth World Health Assembly (WHA) adopted the WHA70.7 Resolution [3].

In the clinical diagnostic process, the detection of bloodstream infections starts with the sampling of a small volume of blood from the patient. After overnight incubation in the laboratory in a specific bottle with nutrient media (blood culture bottle), pathogens can hopefully be analysed by subculturing the positive blood culture on agar plates [4,5]. The type of culture media is important to facilitate the growth of pathogens. Usually, a combination of selective and non-selective media helps the technicians to predict the type of bacteria that were present in the original blood sample by grouping them by their general characteristics (Gram-positive or negative, bacilli or cocci etc.) [6]. This step is crucial to give clinicians an orientation for initial treatment [7] but also to help lab technicians choose the most appropriate tests required for full pathogen identification and antibiotic resistance patterns. The capacity to recover the pathogen, plus time to results and accuracy are therefore important quality indicators in the first step of the process. Beyond the clinical relevance at the patient level, blood culture data analysis can further shed light on microbiological surveillance and antibiotic stewardship, both essential components of the WHO action plan to contain antibiotic resistance [8].

Bloodstream infections are generally caused by a single bacterial pathogen, but co-infections with multiple pathogens may occur. The causative agents include gram-positive and gram-negative bacteria, anaerobes, fungi, and parasites, depending on settings, patient population, infection entry site etc. [9,10]. Polymicrobial infections might occur, depending on the patient population, in 1–2% of patients, particularly with severe immunodeficiency and with a polymicrobial focus such as abdominal (perforation, abscesses) and gynaecological infections [11]. MSF internal data from conventional microbiology laboratories in low-resource settings show that in these locations, mixed infections can reach 10% of cases, especially in pediatric populations.

Within the context of the Mini-Lab project, an MSF initiative to realise a small-scale, transportable, clinical microbiology laboratory adapted to low-resource settings [12,13], we tested whether an alternative subculturing solution, the InTray™ cassettes (BioMed Diagnostics, Inc., White City, OR, USA), could be used for bloodstream infection diagnosis directly from blood cultures. These in-vitro culture-media cassettes have several advantages: FDA and CE-marked, ready-to-use, small size for easy transport and storage and long shelf-life even with blood-containing media (12 months for most agar formulations). They can also be customised with nearly any media and are recommended for the isolation, cultivation and counting of fastidious and non-fastidious organisms [14,15]. For this evaluation, firstly, we performed a proof-of-concept study with the following objectives: (a) test the use of InTray cassettes directly from blood cultures, and (b) select the most convenient media for our application. Then, we evaluated the two selected InTray

media compositions; the InTray MH (Muller Hinton) chocolate agar for the detection of the most frequent pathogens causing bloodstream infections and the InTray Colorex Screen for the detection of polymicrobial infections. The selected media were evaluated in two different studies in two European laboratories: a reference validation study at the Institute of Tropical Medicine in Antwerp and a clinical evaluation study at the microbiology and hygiene laboratories at Bicêtre University Hospital.

2. Materials and Methods

2.1. Proof-of-Concept Study

To test the use of InTrays cassettes directly from blood cultures and to select the media composition best suited for our application, we previously carried out a small proof-of-concept study. Briefly, 4 different formulations of InTray™ cassette media were compared (Tryptic Soy Agar -TSA-, Tryptic Soy Agar with 5% sheep blood, Mueller Hinton -MH- Chocolate Agar, and Colorex Screen) to 3 respective reference media: Columbia agar with 5% sheep blood and the Chocolate agar with PolyViteX (bioMérieux, Marcy-l'Étoile, France), and UriSelect™4 (Bio-Rad Laboratories, Marnes la Coquette, France). A total of 30 blood cultures were directly inoculated on all types of InTray cassettes and reference media. Half of these (n = 14) were clinically positive blood cultures retrieved from a clinical laboratory, and 16 were simulated positive blood cultures (prepared as further described in the clinical evaluation study).

2.2. Reference Validation Study

A total of 490 BacTAlert bottles (bioMérieux), BacT/ALERT FA Plus Aerobic and BacT/ALERT PF Plus were used to evaluate the performance of InTray MH chocolate agars, compared with reference chocolate agars (Becton-Dickinson, Franklin Lakes, NJ, USA) at the Laboratory of the Institute of Tropical Medicine (Antwerp, Belgium). The protocol for the preparation of the simulated blood cultures and processing of positive cultures has been described elsewhere [16–18]. A selection of 20 species was inoculated in the blood cultures. These strains were isolated during research studies based in different tropical or LMICs like the Democratic Republic of Congo (DRC), Ecuador, Benin, Burkina Faso, Cambodia, Gambia, but also France and Peru (Appendix C, Table A2). For the subculturing of the InTray cassettes, an optimised subculturing protocol (Appendix A) was used. After inoculation, InTray cassettes were placed in a normal ambient incubator, and reference media were incubated in a normal ambient incubator for all non-fastidious organisms and in a CO₂ incubator for fastidious organisms, both at 35 ± 1 °C. The choice of air atmosphere for the incubation of InTray cassettes independently from the type of organisms was justified by a small proof of concept study (Appendix B). Reading was done after 16 h or up to 48 h if no detection of growth was detected earlier. Data collection and analysis were the same as described elsewhere [16].

2.3. Clinical Evaluation Study

During the study period between November 2018 and March 2019, 70 positive BacT/ALERT FA Plus blood cultures, as confirmed by a positive signal by the automated detection system and by the clinical laboratory, with less than 7 days from date to positivity were retrieved from the clinical laboratory of the Bicêtre University Hospital (Le Kremlin-Bicêtre, France). The 70 blood cultures corresponded to 70 patients, of which 65 were adults and 5 were paediatric patients. The spectrum of organisms retrieved in this context was mainly composed of *Staphylococcus aureus* (30%) and coagulase-negative Staphylococci (CNS; 25.7%; Table 1). For the subculturing of the InTray cassettes, the optimised subculturing protocol of the reference validation study was used (Appendix A). After inoculation, according to routine clinical laboratory results, InTray MH chocolate cassettes were placed in a conventional air incubator, and reference media were incubated in a conventional air incubator for all non-fastidious organisms and in a CO₂ incubator for fastidious organisms at 35 ± 1 °C. Reading was done after 16 h or up to 48 h if no growth

was detected earlier. Data and pictures were collected and analysed with Excel (Office365, Microsoft Corporation, Washington, DC, USA).

Table 1. Organism distribution retrieved from blood cultures during the clinical evaluation study.

Organism	N° of Strains
<i>Staphylococcus aureus</i>	21
<i>Staphylococcus epidermidis</i>	10
<i>Klebsiella pneumoniae</i>	9
<i>Pseudomonas aeruginosa</i>	7
<i>Enterobacter cloacae</i>	5
<i>Escherichia coli</i>	3
<i>Staphylococcus hominis</i>	3
<i>Staphylococcus capitis</i>	2
<i>Staphylococcus haemolyticus</i>	2
<i>Klebsiella oxytoca</i>	1
<i>Micrococcus luteus</i>	1
<i>Morganella morganii</i>	1
<i>Staphylococcus pettenkoferi</i>	1
<i>Streptococcus agalactiae</i>	1
<i>Streptococcus constellatus</i>	1
<i>Streptococcus mitis</i>	1
<i>Streptococcus pneumoniae</i>	1
Total	70

2.4. Detection of Poly-Microbial Cultures

For the detection of polymicrobial cultures, simulated blood cultures were inoculated with a mix of 2 bacterial species prepared in a saline solution. The procedure was as follows. Each clinical isolate was first plated onto standard chocolate agar for colony counting. Then dilutions from fresh subcultures of both strains were mixed in a saline solution. A volume of 100 µL of the mix was inoculated together with 2 mL of human blood into a blood culture bottle to obtain a final bacterial concentration of 5–50 CFU/species/bottle. The same volume of saline solution mix was also directly plated onto reference media (UriSelect™4) to check growth prior to incubation. After overnight incubation in a normal air incubator at 35 ± 1 °C, one drop of the blood culture was inoculated onto InTray MH chocolate agar, InTray Colorex Screen and reference UriSelect. The optimised subculturing protocol (Appendix A) was used. In total, 11 types of mixes were prepared, divided into 3 main categories: clinically relevant mixes (as by MSF internal data), colour challenge mixes, including species colonies with an expected similar colour, and contaminant mixes, including a pathogen and a contaminant (Table 2). Each mixture was prepared using 3 different stains of the same species and plated in triplicate on each media (for a total of 99 inoculated mixes).

In all studies, sensitivity was calculated as the number of plates with any growth over total inoculated plates, while the specificity at the species level was the number of species with “expected growth characteristics” on InTray over the total species analysed. In “discrepancy of growth,” we included a slower growth or other growth variations, like colony colour, colony aspect etc., compared to the same species on reference media (“expected growth”). The ease of use of the InTray cassette was evaluated using the possibility of picking single colonies and the identification of polymicrobial cultures on the agar. Feedback on the usability of the cassettes was also collected from the users.

Table 2. Microbial types of mixes used for the study.

Types of Mixes	Combination
Clinically relevant	<i>Acinetobacter baumannii</i> — <i>Klebsiella pneumoniae</i>
	<i>Escherichia coli</i> — <i>Streptococcus pneumoniae</i>
	<i>Enterococcus faecalis</i> — <i>Escherichia coli</i>
	<i>Enterococcus faecalis</i> — <i>Staphylococcus aureus</i>
	<i>Klebsiella pneumoniae</i> — <i>Streptococcus pneumoniae</i>
Colony colour challenge	<i>Pseudomonas aeruginosa</i> — <i>Salmonella</i> spp.
	<i>Staphylococcus aureus</i> — <i>Staphylococcus epidermidis</i>
	<i>Klebsiella pneumoniae</i> — <i>Enterococcus faecalis</i>
Contaminants	<i>Escherichia coli</i> — <i>Candida albicans</i>
	<i>Escherichia coli</i> — <i>Staphylococcus epidermidis</i>
	<i>Enterobacter cloacae</i> — <i>Corynebacterium striatum</i>

3. Results

3.1. Proof-of-Concept Study Results

After 16 h, the incubation recovery rates on InTray cassettes were: 24/30 on TSA, 27/30 on TSA + 5% sheep blood agar, 28/30 on MH chocolate agar, and 23/30 on Colorex Screen, compared to 27/30 on the reference Columbia + 5% sheep blood, 29/30 on chocolate agar with PVX, and 23/30 on UriSelect media. The difference between the InTray MH chocolate agar and the reference chocolate agar with PVX was due to the lack of growth of one strain of *Streptococcus* spp, a fastidious organism. The 2 main conclusions of this proof-of-concept were that; (a) overall, the InTray cassettes were comparable to reference media for subculturing directly from blood cultures; (b) the best InTray media for recovering a wide range of pathogens from mono-microbial cultures and for detection of poly-microbial cultures were the InTray MH chocolate and the Colorex Screen, respectively.

3.2. Reference Validation Study Results

In this study, out of a total of 490 blood culture bottles inoculated with 20 bacterial species, 486 grew on the InTray MH chocolate agar after 24 h incubation, while all 490 grew on the reference media. The only two species that showed growth discrepancy between the InTray MH chocolate and the reference media were *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In this study, the sensitivity was, therefore, 99.2% (CI 95%; 98.3–99.9%). Also, only two out of 20 species showed a growth variation (suboptimal growth) on InTray cassettes compared to the reference method, giving a specificity of 90% (CI 95%; 76.8–100%).

Additional testing of several *S. pneumoniae* and *S. pyogenes* strains showed that a longer incubation time of the InTray cassettes (up to 48 h) and incubation in a conventional incubator instead of in a CO₂ incubator improved the growth of the two *Streptococcus* species. Extended incubation time (>24 h) improved growth for *S. pneumoniae* but not for all *S. pyogenes* tested: out of a total of 12 *S. pyogenes* strains tested, 11 showed good growth after 48 h, indicating that growth variation was strain-specific more than species-specific (Table 3).

The time to detection in this study was, therefore, 24 h for 99.2% of cultures and 48 h for all the strains except for 1 *S. pyogenes* strain. Furthermore, the presence of single colonies occurred in 86% of the InTray cassettes compared to 99% of the reference media, with more than 10 usable colonies for downstream analyses in 31% of cases compared to 68% for the reference method. The median was five colonies (min = 0, max = 48) for the InTray and 15 (min = 0, max = 214) for the reference media.

Table 3. Results of the reference validation study expressed in terms of the percentage of positivity at 24 h and 48 h of incubation.

	Total (n)	Growth on Both Agars	InTray Only	Reference Only	Percentage of Positive InTrays after 24 h Incubation	Percentage of Positive InTray after 48 h Incubation
All other bacteria/yeast *	433	433	0	0	100%	na
<i>Streptococcus pneumoniae</i>	49	47	0	2	98.0%	100%
<i>Streptococcus pyogenes</i>	8	6	0	2	75.0%	90%
Total	490	486	0	4	99.2%	

* *Burkholderia cepacia*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella Typhimurium*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Candida tropicalis*, *K. oxytoca*, *E. cloacae*, *C. freundii*, *Salmonella Typhi*, *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, *S. agalactiae*, *S. suis* and *Enterococcus faecalis*.

3.3. Clinical Evaluation Study Results

In this study, 70 positive blood cultures were inoculated on the InTray MH chocolate agar and on the reference agar plates. No difference in terms of positivity was observed between the InTray cassettes and the reference media, with a concordance of 100%. The sensitivity of the InTray cassette was 97.1% with any growth, as it was for the reference. In both cases, 1 strain of *Streptococcus pneumoniae* and 1 of *Streptococcus constellatus* did not grow on the culture media (Table 4).

Table 4. Results of the clinical evaluation study expressed in terms of percentage of positivity.

	Total (n)	Growth on Both Agars	InTray Only	Reference Only	Percentage of Positive InTray
All other bacteria/yeast *	66	66	0	0	100%
<i>Streptococcus pneumoniae</i>	1	0	0	0	0%
Other <i>Streptococcus</i> spp **	3	2	0	0	66%
Total	70	68	0	0	97.1%

* *E. cloacae*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *M. luteus*, *M. morgani*, *P. aeruginosa*, *S. aureus*, *S. capitis*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. pettenkoferi*. ** *S. constellatus*, *S. mitis*, *S. agalactiae*.

Time to detection was 16 h in 81% of cultures and 20 h in 100% of cultures on both media. The slowest strains to grow were one *Micrococcus luteus*, one *Staphylococcus aureus*, one *Streptococcus mitis*, one *Staphylococcus pettenkoferi*, two *Staphylococcus capitis*, and five *Staphylococcus epidermidis* strains. Strains with variation compared to the reference are reported in Table 5.

Sensitivity and specificity at the species level on the InTray MH chocolate were 97.1% (CI 95%; 93.2–100%) and 88.2% (CI 95%; 72.9–100%), respectively, as two species out of the 17 inoculated showed a discrepancy with growth observed on the reference media. In this study, of all the species successfully cultured on InTray cassettes, a difference in growth characteristics was observed in two species, *Micrococcus luteus* and *Staphylococcus epidermidis*; in the first case, the colonies were of considerably smaller size than on the reference media, while in one strain of *S. epidermidis* the overall growth was very weak on InTray cassette opposite to the reference media, even after prolonged incubation (Table 5). Isolated colonies were available on 62 InTray cassettes (62/68, 91.2% of grown cultures), with more than 10 isolated colonies available on 36 of them (36/68, 53% of grown cultures), compared to 100% on reference media.

Table 5. Table of discrepancies of growth on InTray MH chocolate with reference media, including the number of isolated colonies. n/a = information not available.

	N of Strains	Time to Detection	N of Isolated Colonies	N of Colonies on Reference	Growth Characteristics	Discordance with Reference (Y, N)
<i>E. coli</i>	2	16 h	No isolated colonies	>10	n/a	N
<i>M. luteus</i>	1	20 h	>10	>10	Much smaller colonies on InTray	Y
<i>M. morgani</i>	1	16 h	No isolated colonies	>10	n/a	N
<i>P. aeruginosa</i>	3	16 h	No isolated colonies	>10	n/a	N
<i>S. aureus</i>	2	20 h; 16 h	>10	>10	Presence of white colonies also on reference	N
<i>S. capitis</i>	2	20 h	1-10; >10	>10	n/a	N
<i>S. epidermidis</i>	5	20 h	>10	>10	One strain with very faint growth	Y
<i>S. hominis</i>	1	16 h	>10	>10	two types of colonies also on reference	N

3.4. Detection of Polymicrobial Cultures Results

Of the 33 total mixed cultures (11 mixes, each in triplicate) inoculated on both InTray MH chocolate agar and on InTray Colorex Screen, 100% showed any growth both on Colorex Screen and on MH chocolate agar. However, a clearly detectable mixed culture was detectable in 17 of the 33 mixes inoculated on Colorex Screen (51.5%) and only in 8 of the 33 mixes (24.2%) of MH chocolate agar.

All the strains classified as “contaminants” (*Candida albicans*, *S. epidermidis* and *Corynebacterium striatum*) plus *S. pneumoniae*, in the mix with *E. coli* and with *K. pneumoniae*, had difficulty growing, both on InTray cassettes and on the reference media. Growth controls of the mix inoculated onto reference media before incubation in the blood culture bottle were always positive, and the two strains were clearly detectable. Including in the analysis only those mixes with both inoculated species were recovered from the reference media, a mixed flora was discerned on a total of 51 (94.4%) of 54 grown plates (CI 95%; 88–100%) on Colorex Screen, and in 24 (44.4%) of the 54 (CI 95%; 31.2–57.7%) MH chocolate agar InTray cassettes.

However, these results were not consistent among replicates; for example, the combination of *Enterococcus faecalis* and *E. coli* was detected on Colorex Screen thanks to colour differentiation (*E. coli* pink and *E. faecalis* blue) but missed on 2/3 of replicates on InTray MH chocolate because *E. faecalis* colonies were too small. Again, only in one of the three replicates of mixes made with *Salmonella spp.* and *Pseudomonas aeruginosa*, the colonies of the two species were so similar in morphology and colour that it was not possible to distinguish among them, while in the other two mixes made of different strains of the same species, colour and morphology were sufficiently different to detect the mixed flora (Figure 1).

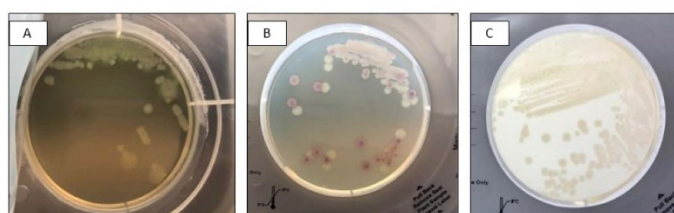


Figure 1. Mixed culture of *Salmonella* spp. (pink) and *Pseudomonas aeruginosa* (white) after subculture on InTray Colorex Screen (A) Visualization of mixed flora based on morphology; (B) Detection based on colony colour; (C) Mixed infection not detected.

Of the total number of strains grown (including replicates, 9×18 strains = 162), the presence of single colonies occurred in 132 or 81.5% (95% CI; 75.5–87.4%) on InTray Colorex Screen compared to 100% on the reference media, while only in 67.9% (95% CI; 60.7–75.1%) on MH chocolate InTray cassettes (Table 6).

Table 6. Detection of mixed cultures and presence of single colonies on InTray MH chocolate and Colorex Screen media cassettes. * the total number of mixed cultures included only the number of replicates of mixes successfully recovered from the reference media (in bold in the table).

	Types of Mixes	Detection of Mixed Culture on InTray (Comment, n of Mixes Replicates)		Presence of Single Colonies on InTray(Comment, n of Single Replicates)	
		Colorex Screen	MH Chocolate	Colorex Screen	MH Chocolate
Clinically relevant	<i>Acinetobacter baumannii</i> <i>Klebsiella pneumoniae</i>	3/3	Possible but difficult to detect based on morphology only, 3/3	2/9 Predominance of <i>K. pneumoniae</i>	Mixed layers, 0/9 Mixed layers, 0/9
	<i>Escherichia coli</i> <i>Streptococcus pneumoniae</i>	No growth of <i>S. pneumoniae</i> also on reference, 0/3	No growth of <i>S. pneumoniae</i> also on reference, 0/3	9/9 No growth (0/9)	9/9 No growth (0/9)
	<i>Enterococcus faecalis</i> <i>Escherichia coli</i>	3/3	Size of colonies was different on some InTrays, 1/3	9/9 9/9	3/9 Overgrowth, 9/9
	<i>Enterococcus faecalis</i> <i>Staphylococcus aureus</i>	3/3	Not possible to detect based on morphology only, 0/3	9/9 7/9	6/9 6/9
	<i>Klebsiella pneumoniae</i> <i>Streptococcus pneumoniae</i>	No growth of <i>S. pneumoniae</i> on reference, 0/3	No growth of <i>S. pneumoniae</i> on reference, 0/3	9/9 No growth, 0/9	9/9 No growth, 0/9
Colony colour challenge	<i>Pseudomonas aeruginosa</i> <i>Salmonella</i> spp.	2/3	Possible but difficult to detect based on morphology only, 1/3	3/9 3/9	3/9 3/9
	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>	3/3	Not possible to detect based on morphology only, 0/3	9/9 9/9	9/9 9/9
	<i>Klebsiella pneumoniae</i> <i>Enterococcus faecalis</i>	3/3	3/3	9/9 9/9	8/9 9/9
Contaminants	<i>Escherichia coli</i> <i>Candida albicans</i>	No growth of <i>C. albicans</i> on reference, 0/3	No growth of <i>C. albicans</i> on reference, 0/3	9/9 No growth, 0/9	9/9 No growth, 0/9
	<i>Escherichia coli</i> <i>Staphylococcus epidermidis</i>	No growth of <i>S. epidermidis</i> on reference, 0/3	No growth of <i>S. epidermidis</i> on reference, 0/3	9/9 No growth, 0/9	9/9 No growth, 0/9
	<i>Enterobacter cloacae</i> <i>Corynebacterium striatum</i>	No growth of <i>C. striatum</i> on reference, 0/3	No growth of <i>C. striatum</i> on reference, 0/3	9/9 No growth, 0/9	9/9 No growth, 0/9
	Tot mixed infections included * = 54 Tot grown strains = 162	51/54 (91.4%)	24/54 (44.4%)	132/162 (81.5%)	110/162 (67.9%)

Of all the species grown on MH chocolate and on the Colorex Screen, the colour and aspect of colonies were comparable to their aspect on the reference media. Only in the case of *Acinetobacter baumannii* were colonies more difficult to recognize on the InTray MH chocolate than on the reference media (Table 7). Overall, the colonies were less defined and with more irregular margins on the InTray cassettes than on the reference media; nevertheless, they were recognizable by an expert microbiologist's eye.

Table 7. Table of discrepancies between InTray Colorex Screen and reference media for polymicrobial cultures. Expected colours are either from the manufacturer IFU (* Biomed Diagnostics), or as shown on reference media.

	Time to Detection (h)	Expected Colour	Colony Colour on Colorex	Colony Aspect on MH Chocolate	Discordance with Reference (Y, N, Description)
<i>Acinetobacter baumannii</i>	16 h	White beige	White	Difficult to distinguish, small and wrinkled colonies	Y mix clearly visible on reference compared to Colorex Screen
<i>Enterobacter cloacae</i>	16 h	Metallic blue *	Blue	Round with irregular margins	N
<i>Enterococcus faecalis</i>	16 h	Teal blue *	Blue	Round and small	N
<i>Escherichia coli</i>	16 h	Dark pink/reddish *	Pink	Round, smooth colonies	N
<i>Klebsiella pneumoniae</i>	16 h	Metallic blue *	Blue	Round, smooth colonies	N
<i>Pseudomonas aeruginosa</i>	16 h	Cream, translucent *	White/cream	Round with irregular margins	N
<i>Salmonella</i> spp.	16 h	White	White/light pink	Round small with regular margins	N
<i>Staphylococcus aureus</i>	16 h	Golden, small, matt *	White/gold	Round small with regular margins	N

4. Discussion

The InTray cassettes have already been successfully used in LMICs, targeting uropathogens from urine specimens [19] and ESBL Enterobacterales from blood cultures, where the combination of Colorex Screen and ESBL chromogenic media was able to reduce turnaround time significantly [20]. In this study, we tested the combination of InTray™ MH chocolate and Colorex Screen media for a wider application, in early orientation and diagnosis of sepsis, as a simple alternative to the classic combination of selective and non-selective media. Given the variability of bloodstream infection pathogens, media characteristics were chosen to allow the detection of the widest possible range of pathogens using the smallest and easiest possible combination of media. Pre-identification tests, such as Gram staining and other simple biochemical tests (oxidase, catalase etc.), can be directly performed on single colonies on chocolate agar, while colony characteristics on Colorex Screen assist preliminary species identification and give additional information about mono and poly-microbial cultures. Early reporting has a high impact on the early selection of effective antimicrobials for patient treatments [7,21].

In both the reference validation and the clinical evaluation study, most of the organisms inoculated were able to grow on the InTray™ MH chocolate agar after overnight incubation in a normal atmosphere incubator. Overall, only *Streptococcus* spp. showed delayed or no growth on InTray™ cassettes compared to the reference media. However, these organisms are often called “fastidious organisms” because they require special conditions for optimal growth, such as a 5% carbon dioxide atmosphere [22]. Surprisingly, we found that the growth of these fastidious organisms on InTray cassettes was optimized by incubation in a normal atmospheric incubator instead of in a CO₂ atmosphere. The InTray™ GC cassette with an integrated CO₂ disk, appositely designed by the manufacturer for fastidious organisms [23], showed no advantage, in our experience, compared to regular cassettes

incubated in a conventional incubator (Appendix B). This is actually advantageous, as the possibility to incubate “fastidious organisms” in a normal air incubator instead of under a CO₂ atmosphere reduces equipment and makes laboratory workflow easier for non-expert microbiologists.

In fact, in the context of the Mini-Lab project in which this study was carried out, the ability of the laboratory to be operated by non-expert microbiologists was a key aspect. Therefore, during development, the diagnostic test selection was also based on ease of use. Because the diameter and depth of the InTray cassettes’ agar are reduced compared to standard agar plates (6 cm versus 9 cm in diameter, respectively), although media content remains similar [24], colony growth characteristics could be affected. Growth in a lawn may occur, obstructing the collection of single colonies for pre-identification tests and downstream analyses. Also, poorly discernible colony colour or morphology might delay accurate identification. In this study, single colonies were easily detectable on InTray™ MH chocolate agar when a single pathogen was responsible for the infection, but not in the case of a polymicrobial infection. In this case, instead, Colorex Screen showed well-defined and easily discernible single colonies, allowing recognition of polymicrobial growth. Colony characteristics and colours on Colorex Screen matched the reference media, facilitating its use for non-experts. On the contrary, on InTray™ MH chocolate, polymicrobial cultures were difficult to detect, and pathogens were not easily recognisable by colony morphology alone, even by an expert microbiologist. On Colorex Screen, only the types of mixed cultures using “contaminants” didn’t give the expected results. However, since missing growth also on reference media occurred after overnight incubation in blood culture bottles, we deduced that in the blood cultures, these species were over-competed and died off. We are aware that this was a limitation of this study. Interestingly, this occurred with all the species usually representing “contaminants” or skin flora; unfortunately, we didn’t perform any tests without incubation in blood cultures, as we wanted to recreate conditions as similar as possible to real samples. Other study limitations include a small sample size and lack of testing in a laboratory based in LMICs.

The use of the InTray™ cassette was favourably evaluated by the user with positive feedback, in particular on the safety provided by the resealable label with its anti-fog window, which also helps avoid spillover. However, two factors were identified as potentially impeding good isolation of colonies: the presence of condensation inside the cassette and the need for better control of drop size from blood cultures when dispensing the drop in order to avoid overgrowth of bacterial cultures. Protocols were optimised following up on this feedback (Appendices A and B).

However, before considering to implement diagnostics in LMICs, a consideration about costs is deemed necessary. In this case, a general consideration of costs could reveal that whilst initial medium costs are higher for InTray™ cassettes, when we account for other fixed and variable costs the final per unit cost of specimen and isolate might not be that different. In the context of the Mini-Lab project, a comparison should be made between costs traditionally incurring in LMICs settings using reference culture and Mini-Labs using InTray™ cassettes. This might show that the initial higher medium costs could be offset by lower initial set-up, maintenance, rent and labour costs when using conventional in-house media preparation. The degree to which this is offset should be properly established but, in general, could take into consideration: (a) set-up costs, manual vs. automated processing blood cultures, manual for the Mini-Lab; (b) labour, the simpler nature of InTray™ cassettes allows for greater savings in labour costs; (c) rent of space/facilities, for the Mini-Lab not yet identified as so far deployed only in MSF operations. However, current estimations of specimen costs in the Mini-Lab by using the InTray cassettes fall not far off estimations considered still high for low resource settings [25].

5. Conclusions

The advantages of using InTray™ cassette devices are numerous: they show good performance, are easy and safe to use, easy to stock and have a long shelf-life. The

combination of the MH chocolate and Colorex Screen InTray™ cassettes is a valid alternative to classic media for the detection of pathogens from bloodstream infections, in particular in low-resource settings where classic media preparation could be challenging.

However, other than developing tropicalised and customised solutions, in-vitro diagnostics manufacturers should also focus in parallel on making them more cost-effective for LMICs; otherwise, they will remain accessible only in a few privileged conditions.

Author Contributions: Conceptualisation, A.N., S.O. (Saoussen Oueslati), A.R., S.O. (Sien Ombelet), L.H., O.V., J.-B.R. and T.N.; methodology, A.N., A.R., S.O. (Saoussen Oueslati), S.O. (Sien Ombelet) and D.L.-B.; visualization, A.N., S.O. (Saoussen Oueslati), A.R., S.O. (Sien Ombelet); formal analysis, A.N., S.O. (Saoussen Oueslati), A.R. and S.O. (Sien Ombelet); validation, J.C., C.F., T.N. and J.-B.R.; investigation, S.O. (Saoussen Oueslati), A.R. and S.O. (Sien Ombelet); resources, C.F., J.-B.R. and T.N.; data curation, A.N., A.R., S.O. (Saoussen Oueslati) and S.O. (Sien Ombelet); writing—original draft preparation, A.N., D.L.-B. and J.-B.R.; writing—review and editing, L.H., J.C., C.F., O.V. and T.N.; supervision, L.H., T.N., O.V. and J.C.; project administration, C.F. and T.N.; resources, L.H., J.-B.R., C.F. and T.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank for their support; the Mini-Lab development team: Albane Mazoyer, Bernard Baillet, Alice Rochard, Julie Hubert, Baptiste Boillot, Baptiste Le Corre, Delphine Berger, Saoussen Oueslati, Florica Ratiu, Léa Courtier and many others; the teams at the Institute of Tropical Medicine and at Bicetre Hospital who worked on the two studies described in this paper; the Mini-Lab Scientific Committee members who are ensuring scientific robustness of this concept: Antoine Andreumont, Stijn Deborggraeve, David Dolinger, Wael Elamin, Jan Jacobs, Rupa Kanapathipillai, Thomas Kesteman, Céline Langendorf, Tjalling Leenstra, Justine Michel, Nada Malu, Arlene Chua, Thierry Naas, Maurice Page, John Stelling, Elsa Tran, Olivier Vandenberg, Timothy Walsh; the MSF internal Steering Committee members and all MSF staff that supported the Mini-Lab project during all these years.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

During a proof-of-concept study, different InTray™ cassettes (Biomed Diagnostics Inc., White City, OR, USA) agar compositions were tested against reference media. Isolation of single colonies was one of the indicators for ease of use, but we found that when the preparation of the InTray cassettes was performed as recommended by the manufacturer, an excess of condensation inside the cassettes could cause overgrowth on the agar surface with few single colonies. We, therefore, optimized, by making small adjustments, to both the preparation and inoculation steps.

Preparation of the InTray cassette was done as follows: (a) after the cassettes were removed from the fridge, they were tilted to concentrate the liquid on one side of the agar chamber, (b) the protective seal of the chamber was carefully removed while holding the cassette tilted, (c) the liquid on the side was carefully withdrawn with a sterile pipette; (d) the cassette was then resealed using the external protective label; (e) the cassette was incubated to dry out in upward position in an incubator at 35 ± 1 °C for at least 1 h. Drying time was defined based on the procedure routinely used in the research laboratory by technicians pouring standard plates.

Inoculation on the InTray cassettes was performed using a Safety Subculture Unit (SCU) (ITL BioMedical, Reston, VA, USA). The SCU offers many advantages; other than avoiding needlestick injuries, it also has a transparent plastic tip to visually control the blood flow during subculturing and a filter cap to safely exhaust the gas produced by growth inside positive blood cultures when pricking the bottle cap. After the drop was

deposited on the InTray cassettes, a 1 μL sterile loop was used to streak the sample with a variation of the classic streaking method as follows; (a) streak the drop on half of the surface with a 1 μL loop; (b) repeat streaking on a new quarter of the InTray with the same loop, without touching the first quarter, to completely unload the loop; (c) repeat striking on the last quarter with the same loop, this time touching the last lines of the previous quarter. (Figure A1)

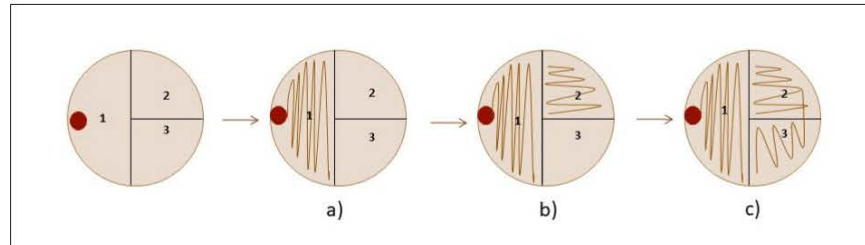


Figure A1. Inoculation technique for InTray cassettes: (a) expand the drop by streaking on half the surface (1, first quarter); (b) streak on a new quarter (2, second quarter) without touching the previous one; (c) repeat on the last quarter (3, third quarter) by touching the previous one.

These small adjustments to the protocol improved the growth of single isolated colonies on InTray cassettes. The Figure below shows growth on InTray before and after protocol optimization. (Figure A2).



Figure A2. Comparison of subcultures of *S. aureus* on InTray MH Chocolate agar before (left) and after (right) optimisation of InTray subculturing protocol.

Appendix B

For optimal growth, some fastidious organisms such as *Streptococcus* spp., *Neisseria* spp and *Haemophilus* spp. etc., require a 5% CO_2 atmosphere. Based on the InTray™ cassette technology, Biomed Diagnostics has developed and validated a self-contained system for the production of carbon dioxide, the InTray GC. The InTray GC device consists of a rectangular tray with an inner well containing any agar formulation. Since in a previous proof of concept study, results showed that the growth of some *Streptococcus* species was improved by incubation in a normal air incubator, we designed a small test comparing the normal InTray cassettes incubated in a normal incubator or in a CO_2 incubator, versus the InTray GC device. In this small study, 3 fastidious species (1 *Haemophilus influenzae*, 1 *Streptococcus pneumoniae*, and 1 *Neisseria subflava*), 2 non-fermenters (1 *Acinetobacter baumannii*, 1 *Pseudomonas aeruginosa*) and 1 *Klebsiella pneumoniae* were cultured from clinical samples; 1 ATCC *H. influenzae* was used as control. The clinical strains and the control strain were cultured overnight in blood culture bottles and then subcultured in triplicate under the 3 growth conditions: on InTray MH chocolate agar with normal incubator, on InTray

GC MH chocolate with normal incubator and on InTray MH chocolate agar incubated in a CO₂ incubator. The incubation temperature was 35 ± 1 °C, and growth was recorded after 16 h, 24 h and 48 h incubation. The results are shown in the table below (Table A1).

Table A1. Cumulative growth of all strains on InTray Chocolate Agar at different times of growth (N = 19).

Time of Growth	Cumulative Growth (N InTray, %)		
	CO ₂ Incubator		Air Incubator
	InTray GC MH Chocolate	InTray MH Chocolate	InTray MH Chocolate
16 h	32 (56%)	42 (74%)	45 (79%)
24 h	54 (95%)	57 (100%)	57 (100%)
48 h	57 (100%)	57 (100%)	57 (100%)

After 48 h of incubation, all strains grew on all InTray cassettes and incubating conditions. The integrated CO₂ device doesn't seem to confer any advantage on growth, instead causing a slower growth rate for certain organisms, in this case, the two non-fermenting species. In the case of incubation in a normal air incubator, in contrast, after 16 h, growth was visible in 45 compared to the 42 plates incubated in the CO₂ incubator, suggesting a comparable or even faster growth under air conditions. Single colonies were visible in 100% of InTray MH chocolate, both incubated in CO₂ and in an air incubator, compared to 93% of the InTray GC device. In conclusion, the InTray MH chocolate agar (without the CO₂ device) incubated in a normal air incubator was the optimal combination for the growth of fastidious and non-fermenting organisms.

Appendix C

Table A2. List of bacterial species used in the reference validation study and their origin.

Bacterial/Yeast Species Tested	Origin
<i>Acinetobacter baumannii</i>	DRC, Ecuador
<i>Burkholderia cepacia</i>	DRC, Ecuador, Benin
<i>Candida tropicalis</i>	DRC, Burkina Faso
<i>Citrobacter freundii</i>	DRC, Cambodia
<i>Enterobacter cloacae</i>	Ecuador, Benin, Cambodia
<i>Enterococcus faecalis</i>	DRC, Benin
<i>Escherichia coli</i>	DRC, Burkina Faso, Cambodia, Ecuador, Benin
<i>Haemophilus influenzae</i>	Burkina Faso, France, Belgium
<i>Klebsiella oxytoca</i>	Benin, Ecuador
<i>Klebsiella pneumoniae</i>	DRC, Benin, Burkina Faso, Cambodia
<i>Neisseria meningitidis</i>	Burkina Faso
<i>Pseudomonas aeruginosa</i>	DRC, Benin, Cambodia
<i>Salmonella Typhi</i>	Peru, DRC, Cambodia
<i>Salmonella Typhimurium</i>	DRC, Cambodia, Burkina Faso, Benin, Gambia
<i>Staphylococcus aureus</i>	DRC, Benin, Burkina Faso, Cambodia
<i>Stenotrophomonas maltophilia</i>	DRC, Cambodia
<i>Streptococcus agalactiae</i>	Cambodia
<i>Streptococcus pneumoniae</i>	DRC, Burkina Faso, Cambodia
<i>Streptococcus pyogenes</i>	DRC, Burkina Faso, Cambodia
<i>Streptococcus suis</i>	Cambodia

References

1. Orekan, J.; Barbé, B.; Oeng, S.; Ronat, J.B.; Letchford, J.; Jacobs, J.; Affolabi, D.; Hardy, L. Culture media for clinical bacteriology in low- and middle-income countries: Challenges, best practices for preparation and recommendations for improved access. *Clin. Microbiol. Infect.* **2021**, *27*, 1400–1408. [CrossRef] [PubMed]
2. Rudd, K.E.; Johnson, S.C.; Agesa, K.M.; Shackelford, K.A.; Tsoi, D.; Rhodes Kievlan, D.; Colombara, D.; Ikuta, K.; Kisson, K.; Finfer, S.; et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. *Lancet* **2020**, *395*, 200–211. [CrossRef] [PubMed]
3. PAHO/WHO. *WHA70.7 Resolution: Improving the Prevention, Diagnosis and Clinical Management of Sepsis*; Agenda Item 12.2; 2017-PAHO/WHO; Pan American Health Organization: Washington, DC, USA, 2017. Available online: <https://www.paho.org/en/documents/wha707-resolution-improving-prevention-diagnosis-and-clinical-management-sepsis-2017> (accessed on 21 November 2022).
4. Franco-Paredes, C. Chapter 2-Bloodstream Infections. In *Core Concepts in Clinical Infectious Diseases (CCCID)*; Academic Press: Cambridge, MA, USA, 2016; pp. 9–15. Available online: <https://www.sciencedirect.com/science/article/pii/B978012804423000020> (accessed on 11 November 2022).
5. Bennett, J.; Dolin, R.; Blaser, M. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-book, 8 ed. 2019. Available online: <https://books.google.co.uk/books?hl=en&lr=&id=8pKqDwAAQBAJ&oi=fnd&pg=PP1&dq=John+E.+Bennett+MD,+in+Mandell,+Douglas,+and+Bennett%27s+Principles+and+Practice+of+Infectious+Diseases,+2020&ots=YjFJRwgiZK&sig=Lan60kQgpJpr71LdW-qDXdhNMdl> (accessed on 11 November 2022).
6. Steinbach, W.J.; Shetty, A.K. Use of the diagnostic bacteriology laboratory: A practical review for the clinician. *Postgrad. Med. J.* **2001**, *77*, 148. [CrossRef] [PubMed]
7. Stone, R.B.; Steele, J.C.H. Impact of Reporting Gram Stain Results From Blood Cultures on the Selection of Antimicrobial Agents. *Am. J. Clin. Pathol.* **2009**, *132*, 5–6. [CrossRef]
8. WHO. *Global Action Plan on Antimicrobial Resistance*; World Health Organization: Geneva, Switzerland, 2017; pp. 1–28.
9. Chun, K.; Syndergaard, C.; Damas, C.; Trubey, R.; Mukindaraj, A.; Qian, S.; Jin, X.; Breslow, S.; Niemi, A. Sepsis Pathogen Identification. *SLAS Technol.* **2015**, *20*, 539–561. [CrossRef]
10. Dellinger, R.P.; Levy, M.M.; Rhodes, A.; Annane, D.; Gerlach, H.; Opal, S.M.; Sevransky, J.E.; Sprung, C.L.; Douglas, I.S.; Jaeschke, R.; et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med.* **2013**, *39*, 165–228. [CrossRef]
11. Hansen, S.L.; Hetmanski, J. Enhanced detection of polymicrobial bacteremia by repeat subculture of previously positive blood cultures. *J. Clin. Microbiol.* **1983**, *18*, 208–210. [CrossRef]
12. Ronat, J.B.; Natale, A.; Kesteman, T.; Andremon, A.; Elamin, W.; Hardy, L.; Kanapathipillai, R.; Michel, J.; Langendorf, C.; Vandenberg, O.; et al. AMR in low-resource settings: Médecins Sans Frontières bridges surveillance gaps by developing a turnkey solution, the Mini-Lab. *Clin. Microbiol. Infect.* **2021**. [CrossRef]
13. Natale, A.; Ronat, J.-B.; Mazoyer, A.; Rochard, A.; Boillot, B.; Hubert, J.; Baillet, B.; Ducasse, M.; Mantelet, F.; Oueslati, S.; et al. The Mini-Lab: Accessible clinical bacteriology for low-resource settings. *Lancet Microbe* **2020**, *1*, e56–e58. [CrossRef]
14. Papp, J.R.; Henning, T.; Khubbar, M.; Kalve, V.; Bhattacharyya, S.; Travanty, E.; Xavier, K.; Jones, K.; Rudrik, J.T.; Gaynor, A.; et al. Recovery of *Neisseria gonorrhoeae* from 4 commercially available transport systems. *Diagn. Microbiol. Infect. Dis.* **2016**, *86*, 144–147. [CrossRef]
15. Bijlani, K.H.; Gomez, M.; Matias, R.; Najafi, A.; Najafi, R.; Arumugam, S. Comparative Evaluation of Biomed InTray® Colorex MRSA with BD ESwab Collection Kit/BBL™ CHROMagar® MRSA II. *J. Med. Microbiol. Diagn.* **2018**, *7*, 1–6. [CrossRef]
16. Ombelet, S.; Natale, A.; Ronat, J.-B.; Kesteman, T.; Vandenberg, O.; Jacobs, J.; Hardy, L. Biphasic versus monophasic manual blood culture bottles for low-resource settings: An in-vitro study. *Lancet Microbe* **2022**, *3*, e124–e132. [CrossRef]
17. Ombelet, S.; Natale, A.; Ronat, J.B.; Kesteman, T.; Vandenberg, O.; Jacobs, J.; Hardy, L. Biphasic Versus Monophasic Manual Blood Culture Bottles for Low-Resource Settings: An In-Depth in vitro Evaluation Using Simulated Blood Cultures. *SSRN Electron J.* **2021**. [CrossRef]
18. Ombelet Id, S.; Natale, A.; Ronat, J.-B.; Vandenberg, O.; Jacobs, J.; Hardyid, L. Considerations in evaluating equipment-free blood culture bottles: A short protocol for use in low-resource settings. *PLoS ONE* **2022**, *17*, e0267491. [CrossRef]
19. Olaru, I.D.; Elamin, W.; Chisenga, M.; Malou, N.; Piton, J.; Yeung, S.; Ferrand, R.A.; Hopkins, H.; Chonzi, P.; Masunda, K.P.; et al. Evaluation of the InTray and Compact Dry culture systems for the diagnosis of urinary tract infections in patients presenting to primary health clinics in Harare, Zimbabwe. *Eur. J. Clin. Microbiol. Infect. Dis.* **2021**, *40*, 2543–2550. [CrossRef]
20. Chimhini, G.; Olaru, I.D.; Fitzgerald, F.; Chisenga, M.; Ferreyra, C.; Malou, N.; Piton, J.; Chimhuya, S.; Yeung, S.; De, S.; et al. Evaluation of a Novel Culture System for Rapid Pathogen Identification and Detection of Cephalosporin Resistance in Neonatal Gram-negative Sepsis at a Tertiary Referral Unit in Harare, Zimbabwe. *Pediatr. Infect. Dis. J.* **2022**, *40*, 785–791, Erratum in *Pediatr. Infect. Dis. J.* **2022**, *41*, 360. [CrossRef]
21. Uehara, Y.; Yagoshi, M.; Tanimichi, Y.; Yamada, H.; Shimoguchi, K.; Yamamoto, S.; Yanai, M.; Kumasaka, K. Impact of Reporting Gram Stain Results From Blood Culture Bottles on the Selection of Antimicrobial Agents. *Am. J. Clin. Pathol.* **2009**, *132*, 18–25. [CrossRef]
22. Gera, K.; McIver, K.S. Laboratory Growth and Maintenance of *Streptococcus pyogenes* (The Group A Streptococcus, GAS). *Curr. Protoc. Microbiol.* **2013**, *30* (Suppl. 30), 9D.2.1. [CrossRef]

23. Beverly, A.; Bailey-Griffin, J.R.; Schwebke, J.R. InTray GC Medium Versus Modified Thayer-Martin Agar Plates for Diagnosis of Gonorrhea from Endocervical Specimens. *J. Clin. Microbiol.* **2000**, *38*, 3825–3826. [[CrossRef](#)]
24. Samra, Z.; Heifetz, M.; Talmor, J.; Bain, E.; Bahar, J. Evaluation of use of a new chromogenic agar in detection of urinary tract pathogens. *J. Clin. Microbiol.* **1998**, *36*, 990–994. [[CrossRef](#)]
25. Roberts, T.; Luangsanatip, N.; Ling, C.L.; Hopkins, J.; Jaksuwan, R.; Lubell, Y.; Vongsouvath, M.; van Doorn, H.R.; Ashley, E.A.; Turner, P. Antimicrobial resistance detection in Southeast Asian hospitals is critically important from both patient and societal perspectives, but what is its cost? *PLoS Glob. Public Health* **2021**, *1*, e0000018. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 3- What Are The Performances Of Three New Types Of Innovative AST Panel Adapted To LRS?

1. Introduction

Determination of the antimicrobial susceptibility patterns of significant bacterial isolates is among the primary responsibilities of the clinical microbiology laboratory. From a practical perspective, clinicians often perceive such test results to be at least as important as determination of the aetiologic agents of patients' infections[124], [125]. In the face of ever escalating antimicrobial resistance and the frequent need for treatment with newer, often more expensive antibiotics, antibacterial susceptibility testing (AST) results take on an increasingly important role[126].

The goal of *in vitro* AST is to provide a reliable predictor of how an organism is likely to respond to antimicrobial therapy in the infected host. This type of information helps the clinician select the appropriate antimicrobial agent, supports the development of antimicrobial clinical guidelines, and provides data for epidemiological surveillance[127]. Such epidemiological surveillance data provide a base to choose the appropriate empirical treatment (first-line therapy), to detect the emergence and/or the dissemination of resistant bacterial strains or resistance determinants in different bacterial species, and can document the impact of new protocol (hygiene, treatment, etc.) through operational studies [128].

There are many different methodologies available for detecting organism resistance to antimicrobials. Susceptibility testing methods include disk diffusion (Kirby-Bauer), broth microdilution (both manual and automated), agar dilution, and antibiotic gradient methods [127], [129] and all have advantages and disadvantages depending if used in high-resource or in low-resource settings.

The Mini-Lab main target settings for deployment are low resource areas (e.g. little availability of skilled HR- mostly laboratory technicians- , scarcity of supply, unfriendly environment, no access to referral lab, etc.), therefore the technical workflow for identification and AST should take those constraints into consideration and should be adapted accordingly[32]. In contexts in which the Mini-Lab is operated, the simplicity of the AST system, ease of use, reproducibility of results and feasibility in the hands of the "operator" are key to its success. This AST system should be easy to read manually or with the use of a simple reading apparatus for accurate and reproducible reading, particularly when coupled with an open access "expert" system which, in addition to editing reports, can assist in quality assurance, guide users in interpretation; provide

interpreted results to clinicians and give an alert when a rare resistance phenotype occurs.

In this chapter, we will start in section 2 by describing the selection, adaptation and analytical performance verification of the Mini-Lab AST system. Then in section 3, we will explore the selection and in laboratory evaluation of a reading apparatus adapted for these panels. The work done in the experimentation described after has been published in peer-reviewed journals or presented during the conferences below:

Section 2: Validation of three MicroScan® Antibiotic Susceptibility Testing (AST) plates designed for Low Resource Settings

- **J. B. Ronat**, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vanden-berg, T. Naas, "Validation of three MicroScan® Antibiotic Susceptibility Testing microplates designed for Low Resource Settings Principal objective of this study was to verify the accuracy of the MicroScan Conclusions Our results indicate that performance of the 3," in **Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse**, 2020, poster presentation, CA-SFM.
- **J. B. Ronat**, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vanden-berg, T. Naas, "Evaluation of the PROMPT inoculation system on the MicroScan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings (LRS)," in **Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse**, 2020, poster presentation, CA-SFM.
- **J. B. Ronat**, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vandenberg, T. Naas, "Validation of Three MicroScan® Antimicrobial Susceptibility Testing Plates Designed for Low - Resource Settings," **Diagnostics**, vol. 12, no. 2106, 2022, doi: <https://doi.org/10.3390/diagnostics12092106>.

Section 3: Evaluation of the BIOMIC Video Reader System for Determining Interpretive Categories of Isolates using Microscan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings

- **J. B. Ronat**, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, Liselotte, O. Vanden-berg, T. Naas, "Evaluation of the BIOMIC Video Reader System for Determining Interpretive Categories of Isolates using Microscan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings)," in **European Congress on Clinical Microbiology and Infectious Diseases**, 2020, poster presentation, ESCMID

2. Validation of three MicroScan® Antibiotic Susceptibility Testing (AST) plates designed for Low Resource Settings



Article

Validation of Three MicroScan® Antimicrobial Susceptibility Testing Plates Designed for Low-Resource Settings

Jean-Baptiste Ronat ^{1,2,3,*}, Saoussen Oueslati ², Alessandra Natale ¹, Thomas Kesteman ⁴, Wael Elamin ^{5,6}, Céline Langendorf ⁷, Liselotte Hardy ⁸, Olivier Vandenberg ^{9,10,11} and Thierry Naas ^{2,3}

¹ Médecins Sans Frontières, 75019 Paris, France

² Team ReSIST, INSERM U1184, School of Medicine University Paris-Saclay, 94270 Le Kremlin-Bicêtre, France

³ Bacteriology-Hygiene Unit, Assistance Publique-Hôpitaux de Paris, Bicêtre Hospital, 94270 Le Kremlin-Bicêtre, France

⁴ Oxford University Clinical Research Unit, Hanoi 10000, Vietnam

⁵ London School of Medicine, Queen Mary University, London CB105, UK

⁶ School of Medicine, Elrazi University, GH6V East Azhari Road, Khartoum North, Sudan

⁷ Epicentre, 75019 Paris, France

⁸ Unit Tropical Bacteriology, Department of Clinical Sciences, Institute of Tropical Medicine, 2000 Antwerp, Belgium

⁹ Center for Environmental Health and Occupational Health, School of Public Health, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium

¹⁰ Innovation and Business Development Unit, Laboratoire Hospitalier Universitaire de Bruxelles (LHUB-ULB), Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium

¹¹ Division of Infection and Immunity, University College London, London WC1E 6BT, UK

* Correspondence: jean-baptiste.ronat@paris.msf.org

Citation: Ronat, J.-B.; Oueslati, S.; Natale, A.; Kesteman, T.; Elamin, W.; Langendorf C.; Hardy, L.; Vandenberg, O.; Naas, T. Validation of three MicroScan® Antimicrobial Susceptibility Testing Plates Designed for Low-Resource Settings. *Diagnostics* **2022**, *12*, 2106. <https://doi.org/10.3390/diagnostics12092106>

Academic Editor: Andreas Kjaer

Received: 21 July 2022

Accepted: 26 August 2022

Published: 30 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Easy and robust antimicrobial susceptibility testing (AST) methods are essential in clinical bacteriology laboratories (CBL) in low-resource settings (LRS). We evaluated the Beckman Coulter MicroScan lyophilized broth microdilution panel designed to support Médecins Sans Frontières (MSF) CBL activity in difficult settings, in particular with the Mini-Lab. We evaluated the custom-designed MSF MicroScan Gram-pos microplate (MICPOS1) for *Staphylococcus* and *Enterococcus* species, MSF MicroScan Gram-neg microplate (MICNEG1) for Gram-negative bacilli, and MSF MicroScan Fastidious microplate (MICFAST1) for Streptococci and *Haemophilus* species using 387 isolates from routine CBLs from LRS against the reference methods. Results showed that, for all selected antibiotics on the three panels, the proportion of the category agreement was above 90% and the proportion of major and very major errors was below 3%, as per ISO standards. The use of the Prompt inoculation system was found to increase the MIC and the major error rate for some antibiotics when testing Staphylococci. The readability of the manufacturer's user manual was considered challenging for low-skilled staff. The inoculations and readings of the panels were estimated as easy to use. In conclusion, the three MSF MicroScan MIC panels performed well against clinical isolates from LRS and provided a convenient, robust, and standardized AST method for use in CBL in LRS.

Keywords: antibiotic susceptibility testing; low-resource settings; clinical bacteriology; MicroScan

1. Introduction

Antimicrobial resistance (AMR) is today universally recognized as a global threat, because of the rapid emergence and dissemination of resistant bacteria and genes among humans, animals, and the environment on a global scale. It represents a heavy burden for healthcare systems all over the world [1–4]; however, the situations in low- and middle-income countries (LMICs) are particularly concerning because of the limited availability of diagnostic/surveillance and clinical/control resources [5,6].

1.1. Antibiotic Susceptibility Testing in Low-Resource Settings

Developing evidence-based treatment guidelines and measuring the impacts of AMR control efforts require representative and comparable data for drug-resistant bacterial infections [7]. Yet, such data have proven extremely difficult to obtain in LMICs, despite the increasing evidence that AMR is rapidly escalating in these contexts [4,8–12]. Available data on AMR in LMICs lack standardized laboratory and data collection practices and are often not representative of populations outside of the main cities [13]. The limited access to adequate laboratory support, in some settings with low resources, contributes to the increase in antibiotic resistance and complicates the management of infections [14]. AMR poses a unique threat in LMICs, with the potential to reverse recent progress toward infectious disease control, to damage healthcare provision generally, and threaten the safety of essential health services, such as surgery to the most vulnerable and underserved populations [8,9,12].

In this article, we define low-resource setting(s) (LRS) as an area within a country with limited access to medication, equipment, supplies, and devices, with less-developed infrastructure (electrical power, transportation, controlled environment/buildings), fewer or less-trained laboratory personnel, basic diagnostic laboratory, no expert microbiologist, and with no (or hardly introduced) clinical bacteriology [15–17].

The deployment of conventional microbiology laboratories in LRS is challenging. It requires complex infrastructure, logistics, equipment, and specialized human resources, often lacking in LRS [17–21]. Affordable and effective point-of-care (POC) diagnostics, especially those that distinguish between viral and bacterial infections, identify pathogens, and provide antibiotic susceptibility testing (AST) are yet to materialize [12,22]. As a result, Médecins Sans Frontières (MSF) developed its own solution: the Mini-Lab. The Mini-Lab is a transportable, self-contained, quality-assured, stand-alone CBL adapted to low-resource settings. It can be operated by laboratory technicians without prior microbiology expertise except for a short, one-month training [23–26].

1.2. Development of Adapted AST Solution for LRS within the Context of Mini-Lab

AST techniques have been embarked in the Mini-Lab in order for their users to (1) improve case management of bloodstream infections, (2) support antimicrobial stewardship, and (3) capture data from decentralized rural areas for AMR surveillance [27]. First, technical specifications (target product profile) were defined, a market analysis was performed, and a call for proposal was launched. The MicroScan® (Beckman Coulter, Inc., West Sacramento CA, USA) platform and the PROMPT™ inoculation system (Beckman Coulter, Inc., West Sacramento CA, USA) were selected for their ready-to-use and sealed/packaged formats. Moreover, lyophilized MIC AST micro-broth dilution systems were considered less error-prone than disc diffusion methods. They provide first-rate information [28,29], such as MIC, which can be read manually or with an automatic reader, and produces high-reproducibility and standardized results thanks to its pre-prepared panels. MSF partnered with Beckman Coulter to tailor the MIC panel. The selection of antibiotics was based on (i) the list of antibiotics available as CE-IVD from Beckman Coulter, (ii) the list of antibiotics used in MSF facilities, and (iii) the WHO's essential drug lists [30]. Those panels were tailored to the needs of the patients, the local epidemiology, and expected antibiotic resistance (ABR) patterns (Supplementary Table S1).

Special attention was given to commonly-used antibiotics, antibiotics of last resort, and proxy indicators of resistance mechanisms as per GLASS requirements [27] and AWaRe classifications [31]. Drug dilutions were chosen to match both CLSI and EUCAST breakpoints in 2019 [32,33] and were embedded by Beckman Coulter on the MicroScan panels (Figure 1 and Supplementary Table S2); antibiotics abbreviations were defined as per EUCAST recommendations [34].

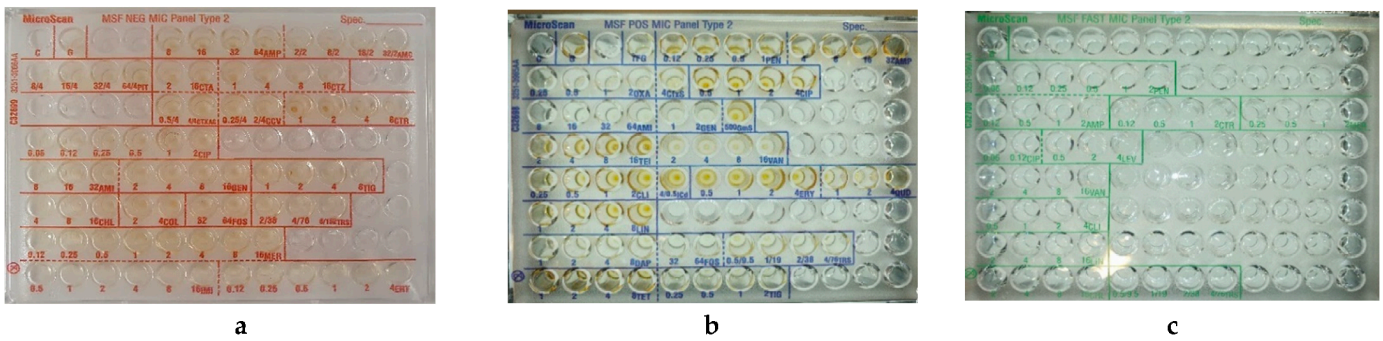


Figure 1. Example of the three MSF MicroScan MIC panels. (a) MicroScan MSF dried overnight Gram-positive panel, (b) MicroScan MSF dried overnight Gram-negative panel, (c) MSF dried overnight fastidious panel.

Three CE-IVD AST microplates were developed: one for rapidly-growing aerobic and facultatively anaerobic gram-positive cocci (MicroScan MSF dried overnight Gram-positive panel, C32698), one for aerobic and facultatively anaerobic gram-negative bacilli (MicroScan MSF dried overnight Gram-negative panel, C32699), and one for aerobic non-enterococcal streptococci (including *Streptococcus pneumoniae* and *Haemophilus* spp.) (MicroScan MSF dried overnight fastidious panel, C32700).

During the CE-IVD certification process, most of the performance testing by manufacturers relies on isolates from high-income countries, where etiologies of sepsis are usually different from those in LMICs [27–30]. Therefore, our study aimed (i) to verify the accuracy of the three panels used with the Prompt inoculation methods and isolates either from LRS or challenging strains following ISO20776-2:2007 recommendations [35], and (ii) to verify the inter-observer variability in manually reading the panels

2. Materials and Methods

2.1. MicroScan MSF MIC Panels

We evaluated the MicroScan MSF dried overnight Gram-positive type 1 (MICPOS1), MicroScan MSF dried overnight Gram-negative type 1 (MICNEG1), and MSF dried overnight fastidious type 1 (MICFAST1) panels. In the current manuscript, we will further use the terms MICPOS1, MICNEG1, and MICFAST1 panels, respectively, to refer to each of these panels. When mentioning all types of panels, we will refer to “MSF MIC panels”. The breakpoints table of EUCAST version 9.1 (2019) [33] was used to interpret the MIC results. The lot numbers of the panels and reagents used in this experiment are listed in Supplementary Table S3.

2.2. Clinical Isolates and Reference Strains

A total of 387 anonymized clinical isolates, either fresh, recently frozen, or from stock, were tested. These included 332 isolates corresponding to the most common bloodstream pathogens or contaminants in LRS. Of the clinical isolates, 47.4% originated from sub-Saharan Africa, 28.5% from Asia, 12.2% from South America, and 11.4% from Europe (See Table 1 for details per species). On the MICPOS1, 123 Gram-positive strains were tested, of which, 60% (74) were *Staphylococcus* spp. isolates and 40% (49) were *Enterococcus* spp. isolates. On the MICNEG1, 157 Gram-negative rod isolates were tested, of which 72% (112) were Enterobacterales and 28% (45) were non-fermenting Gram-negative rods. On the MICFAST1, 107 fastidious isolates were tested, of which 82% (87) were *Streptococcus* spp. isolates and 18% (20) were *Haemophilus influenzae* isolates. Bacterial isolates were obtained from microbiological surveillance studies in LRS, from the strain collections of the Institute of Tropical Medicine (ITM), Antwerp, Belgium, of the Bicêtre University Hospital, French National Reference Laboratory for Antimicrobial Resistance (French AMR NRI.) Paris France and of the Hôpital Universitaire Saint-Pierre Université Libre

Bruxelles (LHUB-ULB), Brussels, Belgium. As per ISO recommendations for evaluating the performance of AST [36], at least 25% of the isolates in the entire study were from fresh clinical samples.

Table 1. Geographical origins of the isolates. Anonymized isolates were obtained from surveillance studies of several partners (ITM, French AMR NRL, LHUB-ULB).

Species	Total Number of Isolates Tested	Africa	Asia	South America	Europe
Species Tested on the MSF Pos MIC Panel					
<i>Staphylococcus aureus</i>	47	23	13	6	5
<i>Staphylococcus epidermidis</i>	11	5	3	1	2
<i>Staphylococcus hominis</i>	15	7	4	2	2
<i>Staphylococcus haemolyticus</i>	10	5	3	1	1
<i>Staphylococcus warneri</i>	1	1	-	-	-
<i>Enterococcus faecium</i>	35	14	10	4	4
<i>Enterococcus faecalis</i>	14	6	4	2	2
Total isolates tested on the panel	133	61	37	16	16
Species Tested on the MSF Neg MIC Panel					
<i>Escherichia coli</i>	25	12	7	3	3
<i>Klebsiella pneumoniae</i>	29	14	8	4	3
<i>Klebsiella oxytoca</i>	9	4	3	1	1
<i>Klebsiella ozaena</i>	1	1	-	-	-
<i>Morganella morganii</i>	1	1	-	-	-
<i>Salmonella</i> Paratyphi A	8	4	2	1	1
<i>Salmonella</i> Typhimurium ^a	8	4	2	1	1
<i>Salmonella</i> Choleraesuis	7	3	2	1	1
<i>Enterobacter cloacae</i>	16	7	5	2	2
<i>Enterobacter hermannii</i>	1	1	-	-	-
<i>Enterobacter kobei</i>	1	1	-	-	-
<i>Enterobacter asburiae</i>	1	1	-	-	-
<i>Citrobacter freundii</i> complex	5	2	1	1	1
<i>Pseudomonas aeruginosa</i>	15	7	4	2	2
<i>Acinetobacter baumannii</i> complex	14	6	4	2	2
<i>Burkholderia cepacia</i>	10	5	3	1	1
<i>Stenotrophomonas maltophilia</i>	6	3	2	1	-
Total isolates tested on the panel	157	76	43	20	18
Species Tested on the MSF FAST MIC Panel					
<i>Streptococcus pneumoniae</i>	54	26	15	7	6
<i>Streptococcus agalactiae</i>	37	17	12	4	4
<i>Streptococcus pyogenes</i>	30	14	9	4	3
<i>Streptococcus mitis</i>	22	10	6	3	2
<i>Streptococcus oralis</i>	6	3	2	1	1
<i>Streptococcus anginosus</i>	10	5	3	1	1
<i>Streptococcus constellatus</i>	2	1	1	-	-
<i>Haemophilus influenzae</i>	20	10	6	2	2
Total isolates tested on the panel	181	86	54	22	19

(a) *S. enterica* subsp. *enterica* serovar Typhimurium (hereafter, *S. Typhimurium*).

2.3. Reference Antimicrobial Susceptibility Testing Methods.

The antimicrobial susceptibility profiles of the isolates were determined in most cases by disc diffusion (Kirby Bauer) following the EUCAST standard method [37]. Exceptions were: agar gradient diffusion (Liofilchem, Roseto degli Abruzzi, Italy) for teicoplanin and vancomycin with all staphylococci (84), broth microdilution (dried panels from Sensititre, Thermo Fisher Scientific, East Grinstead, UK) for colistin with all Gram-negative bacilli (157) and daptomycin with all staphylococci (84), and agar dilution (Liofilchem, Roseto degli Abruzzi, Italy) for fosfomycin with all staphylococci (84), as per EUCAST guidelines (the complete list of reagents can be found in Supplementary Table S3). Reference testing was performed at the same time as MSF panels testing, according to the manufacturer's instructions, and will be referred to in this article as AST reference panels or reference method.

2.4. Inoculum Preparation

Prior to testing, frozen isolates were subcultured twice and fresh isolates were subcultured once on tryptic soy agar plates containing 5% sheep blood (blood agar plate (BAP)) (or chocolate agar for *H. influenzae*) and incubated at 35 °C for 18 to 24 h under aerobic or CO₂ atmosphere as per isolate requirement.

For the turbidity methods (MSF MIC Panels), in accordance with the manufacturer instruction for users (IFU), four to five large, or five to ten small well-isolated colonies were collected from an 18–24 h BAP or chocolate agar using a cotton swab and resuspended in 3 mL of Inoculum Water (B1015-2, Beckman Coulter, Inc., West Sacramento CA, USA) for 2–3 s using a vortex. Turbidity was measured using a turbidimeter (Den 1B, Biosan, Riga, Latvia,) and adjusted as needed to reach the final turbidity of 0.5 +/- 0.02 McFarland. For the MICPOS1 and MICNEG1, 100 µL (0.1 ml) of the suspension was transferred into a 25 mL tube of Inoculum Water with Pluronic (B1015-7, Beckman Coulter, Inc., West Sacramento CA, USA) and mixed 8–10 times. For the MICFAST1, 100 µL (0.1 ml) of the suspension was transferred into a 25 mL tube of *Haemophilus* Test Medium (HTM) (B1015-26, Beckman Coulter, Inc., West Sacramento CA, USA) for *Haemophilus* spp. isolates or into a 25 mL tube of cation-adjusted Mueller–Hinton Broth with 3% Lysed Horse Blood (LHB) (B1015-25, Beckman Coulter, Inc., West Sacramento CA, USA) for *Streptococcus* spp. isolates and mixed 8–10 times.

2.5. Comparison between Standard Inoculum Method and Prompt Inoculation Method

All isolates inoculum-tested with MICPOS1 and MICNEG1 panels were prepared using the turbidity method and the Prompt inoculum method [38]. The Prompt Inoculation System-D (reference B1026-10D, Beckman Coulter, Inc., West Sacramento CA, USA) consists of a rod with a groove at its tip, it is designed to hold a specific number of bacteria (“wand”) and a bottle of diluent for the resuspension of bacteria. A breakaway collar is a small cylinder placed along the wand that serves as a wiping mechanism. Here, the wand was used to touch three different colonies as large as (or larger than) the tip, holding the wand perpendicular to the agar surface, and then the collar was slid down to wipe the wand before placing it into the bottle, pressing down to ensure a tight seal. The bottle was then vigorously shaken 8 to 10 times to resuspend the bacteria from the wand tip. The Prompt microbial suspension was used within 4 h, as stated by IFU.

2.6. Panel Inoculation and Incubation

Purity check plates were performed on all isolates tested using Mueller–Hinton agar or chocolate agar. MSF MIC panels were inoculated using the Renok Rehydrator/Inoculator, a manual pipettor that simultaneously rehydrates and inoculates all 96 wells of MicroScan panels. Contents of the inoculated Pluronic tube used with the turbidity method or of the Prompt bottle were poured into the Seed Tray Renok disposable D-inoculation set (B1013-4, Beckman Coulter, Inc., West Sacramento CA, USA), an inoculator set

consisting of a transfer lid (to hold and dispense the inoculum), and a seed trough (to contain the inoculum). The solution was transferred to the MSF MIC panels using MicroScan Renok (B1018-18, Beckman Coulter), which delivered $115 \pm 10 \mu\text{L}$ of broth suspension to each well. Reference panels were inoculated and incubated according to EUCAST and IFU from the manufacturer. All MSF MIC panels were incubated at $35 \pm 2^\circ\text{C}$ in an off-line, ambient air non- CO_2 incubator.

2.7. Manual Panel Reading and Inter Observer Variability

The MSF MIC panels were read 16–20 h after incubation. The panels were read manually against a black, indirectly lighted background using a viewer box prototype (Figure 2) adapted for this purpose (Ref. 9999400, JP Selecta, Barcelona, Spain) with interchangeable white or black backgrounds according to the type of panels. Growth in a well was defined as turbidity in the form of haze throughout the well, a button in the center of the well, or fine granular growth throughout the well as per EUCAST [39]. MSF MIC panels were read manually by two technicians. For inter-observer variability calculation, laboratory technicians were blinded to each other's results. If a discrepancy in reading was found, a consensus was made among readers for the final reading results. All results were recorded onto specific bench sheets and imported into WHONET version 5.6, freely available software for the interpretation of AST using MIC or inhibition zone diameter data [40,41].

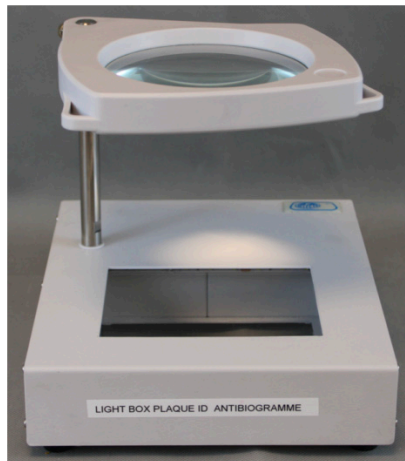


Figure 2. Prototype of the microplate viewer box by JP Selecta, used for visual reading. The background at the bottom can be changed from black to white [26].

2.8. Ease of Use

Assessment of the ease of use was done by surveying the operators with a questionnaire for feedback on each of the components of the system. The readability level of the IFU was assessed using Flesch–Kincaid Grade levels (https://www.online-utility.org/english/readability_test_and_improve.jsp; accessed on 20 March 2021) [13].

2.9. Data Analysis.

The a priori sample size calculation was not performed before the start of the study. Data were collected on worksheets and entered into Microsoft Excel 2019 (version 2110). The sample size was determined following ISO 20776:2 2007 standard [35] recommendations. Statistical analyses were performed in R (version 4.0.2) using RStudio or Microsoft Excel 2019 (version 2110). The essential agreement (EA) was not calculated as most of the reference testing consisted of disc diffusion methods giving only interpretative category results. Categorical agreement (CA), very major errors (VMEs), major errors (MEs), and minor errors (mEs) were calculated as described in the ISO 20776:2 2007 standard [35]. The CA was defined as susceptible (S), susceptible to increased exposure (I), or resistant

(R), as per the EUCAST definition V9, which was the same with both methods. A VME was defined as a false susceptible result with the MSF MIC panels, whereas an ME was a false R or non-susceptible result with the MSF MIC panels; a mE was identified when one method reported an I result while the other method reported S or R results. The acceptance criteria for the study are based on ISO 20776-2:2007 and are as follows: CA \geq 90%; ME \leq 3%, VME \leq 3%.

For inter-observer agreement, two indicators were calculated. First, a measure of the reliability of reading the MIC by a reader against the final reading (an agreement made by both readers if there was a discrepancy) with the calculation of Cohen's kappa (CK) coefficient [42]. A CK $>$ 0.8 was considered as a very good agreement; $0.6 <$ CK \leq 0.8 as a good agreement; $0.4 <$ CK \leq 0.6 as a moderate agreement; $0.2 <$ CK \leq 0.4 as a fair agreement; and CK \leq 0.2 as a poor agreement. Second, CA against the AST reference method was calculated by each reader to determine the impact on the result interpretations.

2.10. Quality Control

Daily QC was done on the MSF MIC panel tested according to the Beckman recommendations (*E. coli* ATCC1 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC1 29213, *E. faecalis* ATCC 29212, *H. influenzae* ATCC 49766, and *S. agalactiae* ATCC 13813). For out-of-control QC results, after a careful panel examination, QC testing was repeated and if the "out-of-control" occurred again, testing was stopped to identify the problem.

2.11. Resolution of Discrepancies

Isolates with a VME or ME were retested using both methods, as were selected isolates with specific drug/organism combinations resulting in \geq 10% mEs. Calculations of CA, VMEs, MEs, and mEs were obtained following resolutions of discrepant results after repeated testing. If an error persisted after repeated testing, it was included in the calculations. If the error was resolved after repeated testing, it was not counted as an error, and the initial result was disregarded.

3. Results

3.1. Evaluation of the MSF MicroScan MIC Panels

3.1.1. MSF Gram-Pos Panel Results

Individual antimicrobial data are presented in Table 2 for *Staphylococcus* spp. and Table 3 for *Enterococcus* spp., CA and error rates were within acceptable limits. Of the 74 *Staphylococcus* spp. tested, after repeated testing, VME occurred in 2 isolates (3%) for teicoplanin and 1 isolate each (1%) for erythromycin and clindamycin. ME occurred in two isolates (3%) for ciprofloxacin, gentamicin, and tetracycline, and one isolate each (1%) for the cefoxitin screening test, amikacin, erythromycin, fosfomycin, trimethoprim/sulfamethoxazole, and tigecycline. Of the 49 *Enterococcus* spp. tested, after repeated testing, 1 (2%) VME was noted for ampicillin, gentamicin high level, and tigecycline. One (2%) ME occurred for ampicillin, ciprofloxacin, gentamicin high level, and tigecycline.

Table 2. Results for Staphylococci tested with the MICPOS1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a				No. of Isolates MICPOS1 ^b				CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])
	Total	R	I	S	R	I	S					
Penicillin	63	59	0	4	59	0	4	63 (100)	0 (0)	0 (0)	0 (0)	
Ciprofloxacin	74	37	0	37	39	0	35	72 (97)	0 (0)	2 (3)	0 (0)	
Amikacin	74	17	4	53	16	8	50	69 (93)	4 (5)	1 (1)	0 (0)	
Gentamicin	74	28	0	46	30	0	44	72 (97)	0 (0)	2 (3)	0 (0)	

Teicoplanin	74	3	0	71	1	0	73	72 (97)	0 (0)	0 (0)	2 (3)
Vancomycin	74	2	0	72	2	0	72	74 (100)	0 (0)	0 (0)	0 (0)
Quinupristin-dalfopristin	74	0	7	67	0	7	67	74 (100)	0 (0)	0 (0)	0 (0)
Erythromycin	74	30	0	44	30	1	43	71 (96)	1 (1)	1 (1)	1 (1)
Clindamycin	74	14	0	60	12	1	61	72 (97)	1 (1)	0 (0)	1 (1)
Daptomycin	74	1	0	73	1	0	73	74 (100)	0 (0)	0 (0)	0 (0)
Fosfomycin	74	29	0	45	30	0	44	73 (99)	0 (0)	1 (1)	0 (0)
Trimethoprim/Sulfamethoxazole	74	18	0	56	19	3	52	70 (95)	3 (4)	1 (1)	0 (0)
Linezolid	74	5	0	69	5	0	69	74 (100)	0 (0)	0 (0)	0 (0)
Tetracycline	74	37	0	37	39	0	35	72 (97)	0 (0)	2 (3)	0 (0)
Tigecycline	74	0	0	74	1	0	73	73 (99)	0 (0)	1 (1)	0 (0)

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.

Table 3. Results for Enterococci tested with the MICPOS1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a				No. of Isolates MICPOS1 ^b				CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])
	Total	R	I	S	R	I	S					
Ampicillin	49	30	0	19	29	1	18	47 (96)	0 (0)	1 (2)	1 (2)	
Ciprofloxacin	49	29	0	20	30	0	19	48 (98)	0 (0)	1 (2)	0 (0)	
Teicoplanin	49	23	0	26	23	0	26	49 (100)	0 (0)	0 (0)	0 (0)	
Vancomycin	49	23	0	26	23	0	26	49 (100)	0 (0)	0 (0)	0 (0)	
Quinupristin-dalfopristin	49	19	16	14	19	16	14	45 (91)	4 (9)	0 (0)	0 (0)	
Linezolid	49	2	0	47	2	0	47	49 (100)	0 (0)	0 (0)	0 (0)	
Tigecycline	49	4	0	45	4	0	45	47 (96)	0 (0)	1 (2)	1 (2)	

^a Number of isolates tested with the reference method and classified as R resistant; I, intermediate; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, intermediate; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.

3.1.2. MSF Gram-Neg Panel Results

Individual antimicrobial data are presented in Table 4 for Enterobacterales and Table 5 for non-fermenting Gram-negative rods. For all isolates tested, CA and error rates were within acceptable limits. Of the 112 Enterobacterales tested, VME occurred in two isolates (3%) for gentamicin and trimethoprim/sulfamethoxazole, and one isolate each (1%) for ampicillin, amoxicillin–clavulanate, amikacin, colistin, and ertapenem. Two isolates (3%) were found to have ME for amoxicillin–clavulanate, ciprofloxacin, and gentamicin, and one (1%) isolate each for ceftazidime, ceftriaxone, trimethoprim/sulfamethoxazole, colistin, and meropenem.

Table 4. Results for Enterobacterales tested with the MICNEG1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a				No. of Isolates MICNEG1 ^b				CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])
	Total	R	I	S	R	I	S					
Ampicillin	112	100	0	12	99	0	13	111 (99)	0 (0)	0 (0)	1 (1)	
Amoxicillin/Clavulanic Acid	112	72	0	40	73	0	39	109 (97)	0 (0)	2 (2)	1 (1)	

Ceftazidime	112	47	2	63	49	0	64	108 (96)	3 (3)	1 (1)	0 (0)
Ceftriaxone	112	50	0	62	51	1	60	110 (98)	1 (1)	1 (1)	0 (0)
Piperacillin/Tazobactam	112	39	4	69	39	4	69	106 (93)	6 (7)	0 (0)	0 (0)
Ciprofloxacin	112	51	4	57	53	4	55	106 (93)	4 (4)	2 (2)	0 (0)
Amikacin	112	16	4	92	14	5	93	106 (93)	5 (6)	0 (0)	1 (1)
Gentamicin	112	40	5	67	40	4	68	104 (92)	4 (4)	2 (2)	2 (2)
Trimethoprim/Sulfamethoxazole	112	69	1	42	69	0	43	108 (96)	1 (1)	1 (1)	2 (2)
Chloramphenicol	112	47	0	65	47	0	65	112 (100)	0 (0)	0 (0)	0 (0)
Colistin	112	12	0	77	12	0	77	110 (98)	0 (0)	1 (1)	1 (1)
Fosfomycin	112	1	0	111	1	0	111	112 (100)	0 (0)	0 (0)	0 (0)
Tigecycline	112	0	0	112	0	0	112	112 (100)	0 (0)	0 (0)	0 (0)
Meropenem	112	16	6	90	17	6	79	106 (93)	5 (6)	1 (1)	0 (0)
Imipenem	112	19	7	86	19	7	86	112 (100)	0 (0)	0 (0)	0 (0)
Ertapenem	112	33	0	79	32	0	80	111 (99)	0 (0)	0 (0)	1 (1)

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure.; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.

Table 5. Results for non-fermenting Gram-neg bacilli tested with the MICNEG1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a				No. of Isolates MICPNEG1 ^b				CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])
	Total	R	I	S	R	I	S					
Ceftazidime	15	8	3	4	8	2	5	14 (96)	1 (4)	0 (0)	0 (0)	
Piperacillin/Tazobactam	15	4	0	11	4	0	11	15 (100)	0 (0)	0 (0)	0 (0)	
Ciprofloxacin	29	12	6	11	12	6	11	29 (100)	0 (0)	0 (0)	0 (0)	
Amikacin	29	7	2	20	7	2	20	29 (100)	0 (0)	0 (0)	0 (0)	
Gentamicin	29	15	0	14	15	0	14	29 (100)	0 (0)	0 (0)	0 (0)	
Trimethoprim/Sulfamethoxazole	29	10	0	20	9	1	20	28 (99)	1 (1)	0 (0)	0 (0)	
Chloramphenicol	9	4	3	2	4	3	2	9 (100)	0 (0)	0 (0)	0 (0)	
Colistin	15	3	0	12	3	0	12	15 (100)	0 (0)	0 (0)	0 (0)	
Meropenem	45	14	6	25	14	6	25	43 (96)	2 (4)	0 (0)	0 (0)	
Imipenem	29	12	0	17	10	1	18	27 (94)	1 (3)	0 (0)	1 (3)	

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.

3.1.3. MSF FAST Panel Results

Individual antimicrobial data are presented in Table 6 for Streptococci and Table 7 for *H. influenzae*. For all isolates tested, CA and error rates were within acceptable limits. Of the 87 Streptococci tested, VME was found in one isolate (1%) for vancomycin, clindamycin, trimethoprim/sulfamethoxazole, and chloramphenicol. ME was observed in one (2%) isolate of *Streptococcus pneumoniae* for meropenem and one isolate of *Streptococcus mitis* for penicillin, clindamycin, and trimethoprim/sulfamethoxazole. Of the 20 *Haemophilus influenzae* tested, only 1 was found to be discrepant between I and R (i.e., mE) for trimethoprim/sulfamethoxazole.

Specific resistance mechanism detection data, tested with the MICPOS1 or MICNEG1 panels, are presented in Table 8.

Table 6. Results for *Streptococcus* spp. test tested with the MICFAST1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a			No. of Isolates MICPFAST1 ^b			CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])	
	Total	R	I	S	R	I					S
Penicillin	87	10	9	68	12	8	67	84 (97)	2 (2)	1 (1)	0 (0)
Meropenem ^g	53	0	0	53	1	0	52	52 (98)	0 (0)	1 (2)	0 (0)
Ceftriaxone ^f	-	-	-	-	-	-	-	-	-	-	-
Ampicillin ^g	53	12	6	35	14	2	37	49 (92)	4 (8)	0 (0)	0 (0)
Levofloxacin ^h	63	1	0	62	1	0	62	63 (100)	0 (0)	0 (0)	0 (0)
Vancomycin	87	2	0	85	1	0	86	86 (99)	0 (0)	0 (0)	1 (1)
Clindamycin	87	16	0	71	16	0	71	85 (98)	0 (0)	1 (1)	1 (1)
Trimethoprim/Sulfamethoxazole	87	4	0	83	4	0	83	85 (98)	0 (0)	1 (1)	1 (1)
Chloramphenicol	87	3	0	84	2	0	85	86 (99)	0 (0)	0 (0)	1 (1)
Linezolid ⁱ	63	0	0	63	0	0	63	63 (100)	0 (0)	0 (0)	0 (0)

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.; ^g The susceptibility of streptococcus group A, B, C, G to cephalosporins is inferred from the benzylpenicillin susceptibility, no breakpoint available on disc diffusion.; ^h Only interpretation for *S. pneumoniae* and *S. Viridans* group.; ⁱ Only interpretation for *S. pneumoniae*, *S. Viridans* group, and *S. Anginosus* group.

Table 7. Results for the H. influenzae test with the MICFAST1 standard turbidity inoculum method and visual reading versus AST reference methods.

Antimicrobial	No. of Isolates AST Reference ^a			No. of Isolates MICPFAST1 ^b			CA ^c (no. [%])	mE ^d (no. [%])	ME ^e (no. [%])	VME ^f (no. [%])	
	Total	R	I	S	R	I					S
Meropenem	20	0	0	20	0	0	20	20 (100)	0 (0)	0 (0)	0 (0)
Ceftriaxone	20	3	0	17	3	0	17	20 (100)	0 (0)	0 (0)	0 (0)
Ampicillin	20	2	0	18	2	0	18	20 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	20	3	0	17	3	0	17	20 (100)	0 (0)	0 (0)	0 (0)
Levofloxacin	20	2	0	18	2	0	18	20 (100)	0 (0)	0 (0)	0 (0)
Trimethoprim/Sulfamethoxazole	20	6	1	13	7	0	13	19 (95)	1 (5)	0 (0)	0 (0)
Chloramphenicol	20	0	0	20	0	0	20	20 (100)	0 (0)	0 (0)	0 (0)

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d mE, minor error.; ^e ME, major error.; ^f VME, very major error.

Table 8. Results for specific resistance tests using MICNEG1 or MICPOS1 standard turbidity inoculum methods and visual reading versus AST reference methods.

Multidrug Resistant Organism	No. of Isolates AST Reference ^a			MSF Panel ^b		CA ^c (no. [%])	ME ^d (no. [%])	VME ^e (no. [%])
	Total	R	S	R	S			
Methicillin-resistant <i>Staphylococcus aureus</i> ^f	47	33	14	33	14	47 (100)	0 (0)	0 (0)
Inducible clindamycin-resistant <i>Staphylococci</i> ^f	74	26	48	26	48	74 (100)	0(0)	0 (0)
Vancomycin-resistant <i>Staphylococcus aureus</i> ^f	47	2	45	2	45	47 (100)	0 (0)	0 (0)
High-level gentamicin resistance <i>Staphylococci</i> ^f	49	25	24	25	24	47 (96)	1 (2)	1 (2)
Vancomycin-resistant <i>Enterococci</i> ^f	49	23	26	23	26	49 (100)	0(0)	0 (0)

Extended-spectrum beta-lactamase-producing Enterobacterales _g	112	19	93	19	93	112 (100)	0 (0)	0 (0)
Carbapenem-resistant Enterobacterales _g	112	33	79	32	80	111 (99)	0 (0)	1 (1)
Colistin-resistant Enterobacterales _g	112	12	77	12	77	110 (98)	1 (1)	1 (1)
Carbapenem-resistant <i>Pseudomonas aeruginosa</i> _g	15	4	11	4	11	15 (100)	0 (0)	0 (0)
Colistin-resistant <i>Pseudomonas aeruginosa</i> _g	15	4	11	4	11	15 (100)	0 (0)	0 (0)
Carbapenem-resistant <i>Acinetobacter baumannii</i> _g	14	8	6	8	6	15 (100)	0 (0)	0 (0)

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b number of isolates tested with the evaluated method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^c CA, categorical agreement.; ^d ME, major error.; ^e VME, very major error.; ^f Resistance test evaluated on the MICPOS1 panel.; ^g Resistance test evaluated on the MICNEG1 panel.

3.2. Results of the Evaluation of the Prompt Performance

Results of the evaluation of MSF MIC panels using the Prompt inoculation method can be found in Supplementary Table S4–S6, S7 for Staphylococci, Enterococci, Enterobacterales, and non-fermenting gram-negative rods, respectively. Specific resistance mechanism detection data are presented in Supplementary Table S8. When Staphylococci were tested with Prompt, the amikacin molecule had the largest number of discrepancies with a CA of 78%, of which 11 and 5 isolates were found to have mE and ME, respectively. Compared with the turbidity inoculum method, more ME in Staphylococci were observed for ciprofloxacin (9%), gentamicin (9%), vancomycin (4%), erythromycin (9%), trimethoprim/sulfamethoxazole (14%), and linezolid (5%), all above the 3% threshold of ISO 20776-2: 2007. For Enterococci, four (9%) isolates had mEs for quinupristin–dalfopristin and two (4%) isolates had VMEs for tigecycline.

3.3. Inter-Observer Variability

The results of the inter-observer agreement between the two independent readers who read the panels visually are presented in Table 9. Overall, the reading agreements for MICNEG1 and MICFAST1 were both classified as very good (CK 0.94 and 0.95, respectively). The reading agreement of MICPOS1 was classified as good (CK 0.82). This was mainly due to the lower agreement in reading for ampicillin (0.76), amikacin (0.78), teicoplanin (0.79), and tigecycline (0.82), and especially for daptomycin (0.37), fosfomycin (0.63), and linezolid (0.37). However, the discrepancies in MIC reading did not affect the CA between each of the two readers and the reference method, with CAs of 96% and 96% for daptomycin, 76% and 77% for fosfomycin, and 97% and 96% for linezolid.

Table 9. Results of the inter-observer variability test using MSF MIC panels with the Prompt inoculation method versus the standard AST method.

Antimicrobial	<i>Staphylococci and Enterococci</i>				<i>Gram-negative bacilli</i>				<i>Fastidious organisms</i>			
	n ^a	Kappa Cohen	R1 CA ^b [%]	R2 CA ^c [%]	n ^a	Kappa Cohen	R1 CA ^b [%]	R2 CA ^c [%]	n ^a	Kappa Cohen	R1 CA ^b [%]	R2 CA ^c [%]
Penicillin	63	0.95	98	98					87	0.99	97	97
Ampicillin	123	0.76	96	92	112	0.91	99	98	107	1.00	98	98
Amoxicillin/Clavulanic Ac					112	0.97	99	98				
Cefoxitin screening/Oxacillin	74	0.95	92	93								
Ceftazidime					127	0.95	97	96				
Ceftriaxone					112	0.89	97	98	20	1.00	100	100
ESBL test					112	0.97	99	97				
Piperacillin/Tazobactam					127	0.97	99	93				
Levofloxacin									83	0.98	100	100
Ciprofloxacin	49	0.89	99	99	141	0.95	94	96				

Amikacin	123	0.78	78	76	141	0.99	99	93				
Gentamicin	123	0.92	100	96	141	1.00	92	92				
Gentamicin (high level)	49	0.90	90	90								
Teicoplanin	123	0.79	99	96								
Vancomycin	123	1.00	97	97					87	1.00	98	98
Quinupristin-dalfopristin	123	0.93	89	91								
Erythromycin	74	0.97	96	96								
Clindamycin	74	0.99	97	97					87	0.64	100	100
Inducible clindamycin resistance	74	0.96	97	95								
Daptomycin	74	0.37	96	96								
Fosfomycin	74	0.63	76	77	112	0.76	100	100				
Trimethoprim/Sulfamethoxazole	74	0.62	95	95	142	0.87	96	96	107	0.92	98	93
Linezolid	123	0.37	97	96					63	1.00	100	100
Tetracycline	74	0.94	97	97								
Tigecycline	123	0.82	95	94	112	0.97	100	100				
Chloramphenicol					121	0.90	99	99	107	1.00	98	98
Colistin					127	0.97	98	98				
Meropenem					157	0.96	93	93	73	0.97	98	98
Imipenem					141	0.96	100	100				
Ertapenem					112	0.97	99	99				
Average all molecules		0.82	94	93		0.94	98	97		0.95	99	98

^a Total number of isolates tested by the molecule. ^b R1CA categorical agreement between Reader 1 and reference methods. ^c R2CA categorical agreement between Reader 1 and reference methods.

3.4. Ease of Use

The instructions for interpretation of growth results were considered by the laboratory technicians as understandable but, as no pictures were included to ease the comprehension, the color atlas document made by EUCAST was much appreciated [39]. The Fleisch–Kincaid grade levels (FKGL) of the three MSF MIC panels IFU were nine each. FKGL refers to US grade levels (i.e., years of schooling) necessary to understand the text. The FKGL of the first part of the IFU of the Prompt method, with all instructions and limitations (Figure 3a), was 11. The FKGL dropped to six in the last part, where instructions for the Prompt are explained as bench aids with black and white figures (Figure 3b). The FKGL of the IFU of the Renok, which included bench aids as well, was rated 6.

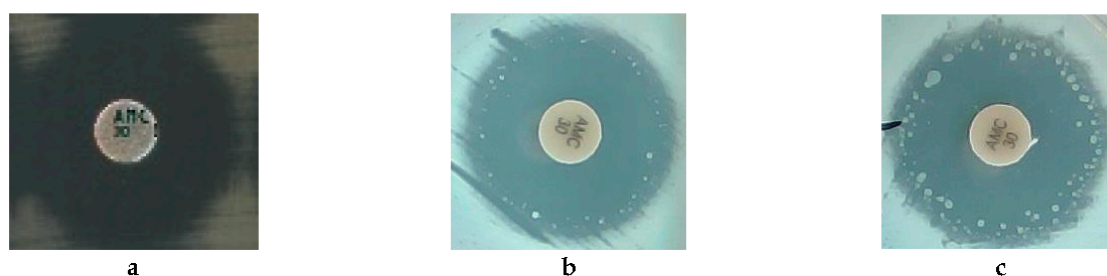


Figure 3. Photo of the inhibition zone produced by *Salmonella* Typhimurium with: (a) initial reference method with Mueller–Hinton agar from Becton, Dickinson and a disc from Bio-Rad with no sign of heteroresistance; (b,c) sign of heteroresistance when using bioMérieux Mueller–Hinton Enterobacterales agar (bioMérieux Inc., Marcy l'Étoile, France) and an AMC disc from i2a (i2a, Montpellier, France).

Both the Prompt™ and the RENOK systems were considered user-friendly and time-efficient by both users, particularly compared to other inoculation methods using 0.5 McFarland standards and single-pipette dispensers. Interpreting the panels was not

considered difficult, except for trimethoprim/sulfamethoxazole because of the “trailing effect”, a typical reading of this antibiotic where the MIC should be read as: (1) approximately 80% reduction of growth, (2) a white button less than 2 mm in diameter, or (3) a white button that is semi-translucent.

The packaging of individual panels was of very good quality, providing air-sealed individual aluminum–plastic pouches with humidity indicators; they were easy to open. Other components were provided within sturdy cardboard boxes fit for difficult transport conditions in LRS. Concerning the shelf life of the MSF MIC panels, Prompt and Pluronic water were within the limits predefined by the Mini-Lab target product profile (minimally 12 months), and the storage conditions (2–25 °C) were within the acceptable Mini-Lab specifications (2–40 °C). However, the shelf life of the HTM and LHB broth needed to rehydrate MICFAST1 was 6 months at 2–8 °C.

4. Discussion

4.1. Performance Evaluation of the MSF MIC Panels

For all drug/organism combinations, our study showed that MICPOS1, MICNEG1, and MICFAST1 panels performed satisfactorily, in agreement with the previous evaluation using the MicroScan technology [43–47], and when testing isolates from LMICs. In addition, they performed as expected with challenging strains for confirmation of resistance mechanisms, such as extended-spectrum beta-lactamase-producing Enterobacterales, methicillin-resistant *Staphylococcus aureus*, induced resistance to clindamycin among Staphylococci, high-level aminoglycoside resistance among Enterococci, and screening of carbapenemase-producing Enterobacterales, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. As mentioned by the manufacturer, a limitation of the MICFAST1 panel is its inability to detect resistance to levofloxacin in *Streptococcus* spp. and resistance to ciprofloxacin and meropenem in *Haemophilus influenzae*, due to the lack of resistant strains at the time of comparative testing.

However, before repeated testing for the resolution of discrepant results, amoxicillin–clavulanate (AMC) of the MICNEG1 was found to have a CA of 89% with 11% ME. All MEs (12/112) on the AMC were found in *Salmonella* species, 5 *Salmonella enterica* serotype Choleraesuis (from Cambodia, Ecuador), and 7 *S. Typhimurium* (from DRC, Burkina Faso). When these strains were removed from the analysis, the CA for AMC was 100%. We suspected that the presence of monoclonal heteroresistance was not captured by the disc diffusion methods we used as reference. This has previously been described for other organisms/drug combinations [48–50] and colistin, polymyxin, and carbapenems among *S. Typhimurium* [51]. Therefore, we re-evaluated the 12 discrepant isolates using various combinations of discs and media from different manufacturers in triplicate with the addition of another broth microdilution (BMD) panel (Sensititre, Thermo Fisher Scientific, East Grinstead, UK) and gradient diffusion strip (E-test, bioMérieux, Marcy-l'Étoile, France). The results are presented in Supplementary Table S7 and photos of the inhibition zone produced by *Salmonella Typhimurium* are presented in Figure 3. Sensititre BMD and MICNEG1 gave similar MIC results for AMC. Only the combination of BioMérieux specific Mueller–Hinton agar for Enterobacterales (bioMérieux, Marcy-l'Étoile, France) with a disc from i2a (Montpellier, France) gave similar results to the MICNEG1. To our knowledge, heteroresistance for amoxicillin–clavulanate within the *Salmonella* species has not been described in the literature and should be further studied using a population analysis profile and other methods, as proposed by Andersson et al. in their recent review on the impact of heteroresistance [52].

4.2. Performance Evaluation of the Prompt Inoculation Method

The manufacturer IFU stated that the Prompt System demonstrated elevated MICs with fluoroquinolones (e.g., gatifloxacin), lincosamides (e.g., clindamycin), and macrolides (e.g., erythromycin), and a potential ME with tigecycline and Staphylococci, when

compared with the reference method, for reasons that were not able to be identified from the literature. Our experiment showed a similar increase in MIC when using the Prompt inoculation method with ciprofloxacin and erythromycin, which impacted the ME rates for Staphylococci coming from LRS but we did not find an increase in the ME rate with tigecycline and clindamycin. Moreover, our results suggest that the Prompt inoculation methods increased MIC and MEs on aminoglycosides, such as gentamicin, amikacin, vancomycin, trimethoprim–sulfamethoxazole, and linezolid for Staphylococci

Other studies suggest and confirm that the Prompt inoculation method has no effect on the MIC for the aerobic and facultatively anaerobic Gram-negative bacilli and Enterococci when compared to the standard inoculum method [38,53].

4.3. Inter-Observer Variability

Overall, the agreements between readers were very good, with four different readers during the entire duration of the experiment. No studies reporting agreements between visual readers using Dried overnight MicroScan panels have been published. Despite the first impression of reading difficulties and some disagreements in the reading of some antibiotic MICs, reader discrepancies had no impact on the final clinical category result interpretations.

4.4. Adaptation to LRS: Stability, Ease-of-Use

The temperature stability of the MicroScan panels, currently assured up to 25 °C, does not entirely fulfill the requirements for tropical settings because cool storage (<30 °C) is not always feasible [54]; however, when compared to disc diffusion that requires cold chain storage of the disc, it is more achievable for the MSF supply system in a district hospital to have access to storage facilities with air conditioning than shipping and storing in temperatures between 2 and 8 °C. Regarding the ease-of-use, the MicroScan inoculation system and panel reading (Renok and Prompt for Gram-negative bacilli) is positive; it does not require multiple steps to inoculate panels and the interpretation of panels is quite easy, with the exception of the trimethoprim–sulfamethoxazole (TRS) wells that require some practice. The dedicated prototype microplate viewer box greatly facilitated the reading process. The Flesch–Kincaid grade level scores of the IFU suggested that for MICPOS1, MSFNEG1, and MSFFAST1, a fair level of schooling is required to understand the IFU. Although the language used in professional documents may be at a slightly higher level, Flesch–Kincaid levels below six are desirable for IFU [55,56] as well as for the bench aid parts of the Prompt and Renok systems.

4.5. Recommendations for Use and Further Development of the MicroScan System

The current limitations of the Prompt and IFU panels explained above were tackled within the Mini-Lab project, by developing training material, such as videos, adapted laboratory procedures, bench aids, a color atlas of the different types of growth, and a microplate viewer for visual reading (see Supplementary Figure S2). This evaluation allowed us to adapt our recommendations to field workers, as, for example, to avoid the Prompt when suspecting Staphylococci. In addition, we encourage the manufacturer to mitigate (to the best possible extent) the issues described above. Bench aids could be included with the product as well as video training (available on their website) showing the different growth types from the wells. Lastly, extended shelf-life testing and stability testing in tropical environments are necessary to assure product quality in LRS.

4.6. Strengths and Limitations of the Study

To our knowledge, this is the first study that evaluated the MicroScan Dried MICs on clinical isolates with typical LRS pathogens [57,58]. Furthermore, we assessed robustness and ease of use. We have several limitations to the verification of some drug/organism combinations as we were lacking a number of resistant isolates; amikacin, teicoplanin,

vancomycin, quinupristin–dalfopristin, clindamycin, daptomycin, linezolid, tigecycline for Staphylococci; fosfomycin, tigecycline for Enterobacterales; amikacin, piperacillin-tazobactam, chloramphenicol, and colistin for non-fermenting Gram-negative bacilli. Because we did not dispose of the Biosafety Level-3 (BSL-3) facilities, we could not test pathogens such as *Burkholderia pseudomallei*. No inter-user, intra-lot, or intra-method repetition was done. Furthermore, comparing disc diffusion to MIC values is by itself a limitation of this study, but would have not been financially possible.

5. Conclusions

Confronted with clinical isolates from LRS, MicroScan dried overnight MIC tailored for MSF had excellent performance for Gram-negative, Gram-positive, and fastidious organisms. The Prompt inoculation system together with the Renok transfer system is very convenient but cannot be used for *Staphylococci*. The study additionally identified potential improvements in stability, robustness, and ease of use to ensure adaptation of the MicroScan system to the constraints of LRS for use outside of the MSF Mini-Lab setting and highlight underseen heteroresistance with the disc diffusion method used to test amoxicillin–clavulanate with *Salmonella* species, which should be further studied.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/diagnostics12092106/s1>, Table S1: List of antibiotics or resistance mechanisms tests included onto the different MSF MIC panels as per WHO AWaRe category and based on the list of essential medicine, Table S2. (a) MicroScan MSF Dried Overnight Gram-Negative panel are designed for use in determining antimicrobial agent susceptibility of aerobic and facultatively anaerobic gram-negative bacilli (Enterobacteriaceae and non-fermenting Gram negative bacilli), Table S2. (b) MicroScan MSF Dried Overnight Gram Positive panel are designed for the determination of antimicrobial agent susceptibility of rapidly growing aerobic and facultative gram-positive cocci (*Staphylococcus.spp* and *Enterococcus.spp*), Table S2. (c) MicroScan MSF Dried Overnight Fastidious panel are designed for use in determining antimicrobial agent susceptibility of aerobic non-enterococcal streptococci (including *Streptococcus pneumoniae*) and *Haemophilus spp.* Not to be used for testing of *Neisseria*, Table S3; Technical information on the different panel, disc, media used for the experiment (Lot number, reference, expiry date, etc.), Table S4; Results for Staphylococci tested with MICPOS1 using Prompt inoculum method and visual reading versus AST reference panels, Table S5; Results for Enterococci tested with MICPOS1 using Prompt inoculum method and visual reading versus AST reference panels, Table S6; Results for Enterobacterales tested with MICNEG1 using Prompt inoculum method and visual reading versus AST reference panels, Table S7; Results for Non-fermenting Gram negative bacilli tested with MICNEG1 using Prompt inoculum method and visual reading versus AST reference panels, Table S8; Results for specific resistance test using MICNEG1 or MICPOS1 using Prompt inoculum method and visual reading versus AST reference methods, Figure S1: Extraction of the Prompt® Inoculation System-D Instruction For Use with Microdilution Susceptibility Tests with, (a) general instructions and (b) bench aid like part with more comprehensive instructions, Table S9; Results of the repetition done for AMC discrepant results using different AST methods and brand for disc, media, Figure S2: Extraction of Mini-Lab Bench aid user manual Version1 2022 with, (a) description of type of growth and (b) visual description of the identification of MIC and mechanism of resistance.

Author Contributions: Conceptualization, J.-B.R., S.O., C.L., T.K., A.N., O.V., and T.N.; data curation, J.-B.R.; formal analysis, J.-B.R., A.N.; funding acquisition, J.-B.R.; methodology, J.-B.R., S.O., C.L., T.K., A.N., W.E., L.H., O.V., and T.N.; project administration, J.-B.R.; supervision, A.N., O.V., and T.N.; visualization, J.-B.R.; writing—original draft preparation, J.-B.R.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Médecins Sans Frontières (Mini-Lab project) under the transformational investment committee, funding number: STATIC001.

Institutional Review Board Statement: Because the study did not involve research on human or animal subjects, data, or material, ethical clearance was waived. Bacterial isolates were obtained from microbiological surveillance studies in LRS (surveillance of antimicrobial resistance among consecutive blood culture isolates in tropical settings, Institutional Review Board number 613/08,

Ethical Committee Antwerp University Hospital 8/20/96) or from anonymous strain collections of Institute of Tropical Medicine (ITM) travel clinic, Antwerp, Belgium or the Bicêtre University Hospital, French National Referral Laboratory for Antibiotic Resistance (French AMR NRL), and of the Saint Pierre Laboratoire Hospitalo-Universitaire Brussel-Université Libre Bruxelles (LHUB-ULB).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be available upon reasonable request from the corresponding author.

Acknowledgments: We thank Cécile Emeraud, Delphine Girlich, Sandrine Bernabeu, Remy Bonnin, Laura Biez, and Camille Gonzalez for their advice concerning the laboratory technical section, and our surveillance study partners for providing the clinical isolates. Furthermore, we are indebted to Barbara Zimmer for her valuable feedback on the use of the panels, comments provided on the article, and intellectual support. Finally, we would like to thank all people involved in MSF, especially the Mini-Lab team, the Mini-Lab scientific committee, and the Mini-Lab Steering committee for their continued and valued scientific and practical input.

Conflicts of Interest: The authors declare no conflict of interest. The manufacturer of the panels had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

- Nadimpalli, M.; Delarocque-Astagneau, E.; Love, D.C.; Price, L.B.; Huynh, B.-T.; Collard, J.-M.; Lay, K.S.; Borand, L.; Ndir, A.; Walsh, T.; et al. Combating Global Antibiotic Resistance: Emerging One Health Concerns in Lower-and Middle-Income Countries. *Clin. Infect. Dis.* **2018**, *66*, 963–969. <https://doi.org/10.1093/cid/cix879>.
- Iskandar, K.; Molinier, L.; Hallit, S.; Sartelli, M.; Catena, F.; Coccolini, F.; Hardcastle, T.C.; Roques, C.; Salameh, P. Drivers of antibiotic resistance transmission in low-and middle-income countries from a ‘one health’ perspective—A review. *Antibiotics* **2020**, *9*, 372. <https://doi.org/10.3390/antibiotics9070372>.
- O’Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*; The Review on Antimicrobial Resistance: London, UK, May 2016.
- Murray, C.J.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* **2022**, *399*, 629–655. [https://doi.org/10.1016/s0140-6736\(21\)02724-0](https://doi.org/10.1016/s0140-6736(21)02724-0).
- Founou, R.C.; Founou, L.L.; Essack, S.Y. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. *PLoS ONE* **2017**, *12*, e0189621. <https://doi.org/10.1371/journal.pone.0189621>.
- Bebell, L.M.; Muir, A.N. Antibiotic use and emerging resistance: How can resource-limited countries turn the tide? *Glob. Heart* **2014**, *9*, 347–58. <https://doi.org/10.1016/j.gheart.2014.08.009>.
- Clift, C. Review of Progress on Antimicrobial Resistance. In *The Royal Institute of International Affairs*; Forest Stewardship Council: Bonn, Germany, 2019.
- Gelband, H.; Molly Miller, P.; Pant, S.; Gandra, S.; Levinson, J.; Barter, D.; White, A.; Laxminarayan, R. State of the World’s Antibiotics 2015. *Wound Health S. Afr.* **2015**, *8*, 30–34.
- World Health Organization. *Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early Implementation 2016–2017*; WHO: Geneva, Switzerland, 2017.
- World Health Organization. *Presentation WHO Report on Antibacterial Resistance*; WHO: Geneva, Switzerland, 2014.
- Ashley, E.A.; Recht, J.; Chua, A.; Dance, D.; Dhorda, M.; Thomas, N.; Ranganathan, N.; Turner, P.; Guerin, P. J.; White, N. J.; Antimicrobial Resistance in Low and Middle Income Countries. In *An Analysis of Surveillance Networks*; IDDO: Oxford, UK, 2016.
- Wellcome Trust. *The Global Response to AMR: Momentum, Success, and Critical Gaps*; Wellcome Trust: London, UK, 2020.
- Gandra, S.; Alvarez-Uria, G.; Turner, P.; Joshi, J.; Limmathurotsakul, D.; van Doorn, H.R. Antimicrobial resistance surveillance in low-and middle-income countries: Progress and challenges in eight South Asian and Southeast Asian countries. *Clin. Microbiol. Rev.* **2020**, *33*, e00048-19. <https://doi.org/10.1128/CMR.00048-19>.
- Lewis, J.M.; Feasey, N.A.; Rylance, J. Aetiology and outcomes of sepsis in adults in sub-Saharan Africa: A systematic review and meta-analysis. *Crit. Care* **2019**, *23*, 212. <https://doi.org/10.1186/s13054-019-2501-y>.
- Girosi, F.; Olmsted, S.S.; Keeler, E.; Burgess, D.C.H.; Lim, Y.-W.; Aledort, J.E.; Rafael, M.E.; Ricci, K.A.; Boer, R.; Hilborne, L.; et al. Developing and interpreting models to improve diagnostics in developing countries. *Nature* **2006**, *444*, 3–8. <https://doi.org/10.1038/nature05441>.
- Jacobs, J.; Hardy, L.; Semret, M.; Lunguya, O.; Phe, T.; Affolabi, D.; Yansouni, C.; Vandenberg, O. Diagnostic Bacteriology in District Hospitals in Sub-Saharan Africa: At the Forefront of the Containment of Antimicrobial Resistance. *Front. Med.* **2019**, *6*, 205. <https://doi.org/10.3389/fmed.2019.00205>.
- Ombelet, S.; Barbé, B.; Affolabi, D.; Ronat, J.-B.; Lompo, P.; Lunguya, O.; Jacobs, J.; Hardy, L. Best Practices of Blood Cultures in Low- and Middle-Income Countries. *Front. Med.* **2019**, *6*, 131. <https://doi.org/10.3389/fmed.2019.00131>.

18. Saeed, D.K.; Hasan, R.; Naim, M.; Zafar, A.; Khan, E.; Jabeen, K.; Irfan, S.; Ahmed, I.; Zeeshan, M.; Wajidali, Z.; et al. Readiness for antimicrobial resistance (AMR) surveillance in Pakistan; a model for laboratory strengthening. *Antimicrob. Resist. Infect. Control* **2017**, *6*, 101. <https://doi.org/10.1186/s13756-017-0260-6>.
19. Hazim, C.; Ibrahim, R.A.; Westercamp, M.; Belete, G.A.; Kibret, B.A.; Kanter, T.; Yimer, G.; Adem, T.S.; Stevenson, K.B.; Urrego, M.; et al. Establishment of a Sentinel Laboratory-Based Antimicrobial Resistance Surveillance Network in Ethiopia. *Health Secur.* **2018**, *16* (Suppl. S1), S30–S36. <https://doi.org/10.1089/hs.2018.0052>.
20. Ombelet, S.; Ronat, J.-B.; Walsh, T.; Yansouni, C.P.; Cox, J.; Vlieghe, E.; Martiny, D.; Semret, M.; Vandenberg, O.; Jacobs, J.; et al. Clinical bacteriology in low-resource settings: today's solutions. *Lancet Infect. Dis.* **2018**, *18*, e248–e258. [https://doi.org/10.1016/S1473-3099\(18\)30093-8](https://doi.org/10.1016/S1473-3099(18)30093-8).
21. Baron, E.J. Clinical Microbiology in Underresourced Settings. *Clin. Lab. Med.* **2019**, *39*, 359–369. <https://doi.org/10.1016/j.cll.2019.05.001>.
22. Okeke, I.N.; Feasey, N.; Parkhill, J.; Turner, P.; Limmathurotsakul, D.; Georgiou, P.; Holmes, A.; Peacock, S.J. Leapfrogging laboratories: The promise tech solutions for and pitfalls of high-tech solutions for antimicrobial resistance surveillance in low-income settings. *BMJ Glob. Health* **2020**, *5*, e003622. <https://doi.org/10.1136/bmjgh-2020-003622>.
23. Ronat, J.-B.; Natale, A.; Kesteman, T.; Andremont, A.; Elamin, W.; Hardy, L.; Kanapathipillai, R.; Michel, J.; Langendorf, C.; Vandenberg, O.; et al. AMR in low-resource settings: Médecins Sans Frontières bridges surveillance gaps by developing a turn-key solution, the Mini-Lab. *Clin. Microbiol. Infect.* **2021**, *27*, 1414–1421. <https://doi.org/10.1016/j.cmi.2021.04.015>.
24. Natale, A.; Ronat, J.-B.; Mazoyer, A.; Rochard, A.; Boillot, B.; Hubert, J.; Baillet, B.; Ducasse, M.; Mantelet, F.; Oueslati, S.; et al. The Mini-Lab: Accessible clinical bacteriology for low-resource settings. *Lancet Microbe* **2020**, *1*, e56–e58. [https://doi.org/10.1016/s2666-5247\(20\)30012-4](https://doi.org/10.1016/s2666-5247(20)30012-4).
25. Ombelet, S.; Natale, A.; Ronat, J.-B.; Kesteman, T.; Vandenberg, O.; Jacobs, J.; Hardy, L. Biphasic versus monophasic manual blood culture bottles for low-resource settings: an in-vitro study. *The Lancet Microbe* **2022**, *3*, e124–e132. [https://doi.org/10.1016/S2666-5247\(21\)00241-X](https://doi.org/10.1016/S2666-5247(21)00241-X).
26. Ombelet, S.; Natale, A.; Ronat, J.-B.; Vandenberg, O.; Hardy, L.; Jacobs, J. Evaluation of MicroScan Bacterial Identification Panels for Low-Resource Settings. *Diagnostics* **2021**, *11*, 349. <https://doi.org/10.3390/diagnostics11020349>.
27. World Health Organization. *GLASS Manual for Early Implementation*; WHO: Geneva, Switzerland, 2015.
28. Jorgensen, J.H.; Ferraro, M.J. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clin. Infect. Dis.* **2009**, *49*, 1749–1755. <https://doi.org/10.1086/647952>.
29. The European committee and Antimicrobial susceptibility testing. *Testing Breakpoint Tables for Interpretation of MICs and Zone Diameters*; EUCAST: Växjö, Sweden, 2020; Volume 10.
30. World Health Organization. World health organization model list of essential medicines. In *Mental and Holistic Health: Some International Perspectives*; WHO: Geneva, Switzerland, 2019; Volume 21, pp. 119–134.
31. Sharland, M.; Pulcini, C.; Harbarth, S.; Zeng, M.; Gandra, S.; Mathur, S.; Magrini, N. Classifying antibiotics in the WHO Essential Medicines List for optimal use—Be AWaRe. *Lancet Infect. Dis.* **2018**, *18*, 18–20. [https://doi.org/10.1016/S1473-3099\(17\)30724-7](https://doi.org/10.1016/S1473-3099(17)30724-7).
32. Clinical and Laboratory Standards Institute (CLSI). *M100 Performance Standards for Antimicrobial Susceptibility Testing M100-Ed29*; CLSI: Wayne, PA, USA, 2019.
33. EUCAST. *Breakpoint Tables for Interpretation of MICs and Zone Diameters*; EUCAST: Växjö, Sweden, 2019; Volume 9.
34. EUCAST. *EUCAST System for Antimicrobial Abbreviations*; EUCAST: Växjö, Sweden, 2018.
35. ISO. *Clinical Laboratory Testing and In Vitro Diagnostic Test Systems—Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices—Part 2: Evaluation of Performance of Antimicrobial Susceptibility Test Devices Against Reference Broth Micro-Dilution*; ISO: Geneva, Switzerland, 2007.
36. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition 32*; CLSI: Wayne, PA, USA, 2012.
37. Matuschek, E.; Brown, D.F.J.; Kahlmeter, G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin. Microbiol. Infect.* **2014**, *20*, O255–O266. <https://doi.org/10.1111/1469-0691.12373>.
38. Flejzor, B.; Bokkenheuser, V.D. Performance of the prompt system in identification and antimicrobial susceptibility testing of clinical isolates. *J. Clin. Microbiol.* **1985**, *21*, 267–268. <https://doi.org/10.1128/jcm.21.2.267-268.1985>.
39. EUCAST. EUCAST reading guide for broth microdilution (version 2.0). In *European Committee on Antimicrobial Susceptibility Testing*; EUCAST: Växjö, Sweden, 2020; pp. 1–20.
40. Stelling, J.M. News and Notes WHONET: An Information System for Monitoring Recommendations for Preventing the Spread of Antimicrobial resistance. *Emerg. Infect. Dis.* **1995**, *1*, 66–67.
41. Hindler, J.; Stelling, J. Analysis and presentation of cumulative antibiograms: A new consensus guideline from the Clinical and Laboratory Standards Institute. *Clin. Infect. Dis.* **2007**, *44*, 867–873. <https://doi.org/10.1086/511864>.
42. Cohen, J. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* **1960**, *20*, 37–46. <https://doi.org/10.1177/001316446002000104>.
43. Bankert, D.A.; Crist, A.E.; George, W.; Eastman, J.T.; Han, A.; Homing, A. *Multicenter Evaluation of Four Automated ID/AST Systems for Antimicrobial Susceptibility Testing of Commonly Isolated Enterococcus spp. and Staphylococcus spp.*; American Society of Microbiology: Washington, DC, USA, 2007; pp. 1–4.

44. Anderson, K.F.; Lonsway, D.R.; Rasheed, J.K.; Biddle, J.; Jensen, B.; McDougal, L.K.; Carey, R.B.; Thompson, A.; Stocker, S.; Limbago, B.; et al. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. *J. Clin. Microbiol.* **2007**, *45*, 2723–2725. <https://doi.org/10.1128/JCM.00015-07>.
45. Bankert, D.A.; et al. C-122 Multicenter Evaluation of Four Automated ID/AST Systems for Antimicrobial Susceptibility Testing of Enterobacteriaceae and Commonly Isolated Glucose Non-Fermenting Gram-Negative Bacilli. In *ASM2*; American Society of Microbiology: Washington, DC, USA, 2006; p. 122.
46. Roe, D.; et al. *A Multicenter Evaluation of a MicroScan Dried Overnight Panel for Screening E. coli and Klebsiella spp. for the Presence of ESBLs*; American Society of Microbiology, Washington DC; 1999; pp. 1–5.
47. Rennie, R. et al. *Multicenter Evaluation of Daptomycin and Ertapenem in a MicroScan Dried Overnight Panel with Gram-Positive Bacteria*; American Society of Microbiology, Washington DC; 2005; pp. 1–6.
48. Creely, D.; Zambardi, G.; Van Belkum, A.; Dunne, W.M.; Peyret, M.; Gayral, J.P.; Shortridge, D.; Shubert, C. International dissemination of *Escherichia coli* strains with discrepant behaviour in phenotypic antimicrobial susceptibility tests. *Eur. J. Clin. Microbiol. Infect. Dis.* **2013**, *32*, 997–1002. <https://doi.org/10.1007/s10096-013-1837-5>.
49. Shubert, C.; Slaughter, J.; Creely, D.; van Belkum, A.; Gayral, J.P.; Dunne, W.M.; Zambardi, G.; Shortridge, D. Population analysis of *Escherichia coli* isolates with discordant resistance levels by piperacillin-tazobactam broth microdilution and agar dilution testing. *Antimicrob. Agents Chemother.* **2014**, *58*, 1779–1781. <https://doi.org/10.1128/AAC.02181-13>.
50. Lo-Ten-Foe, J.R.; de Smet, A.M.G.A.; Diederens, B.M.W.; Kluytmans, J.A.J.W.; van Keulen, P.H.J. Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. *Antimicrob. Agents Chemother.* **2007**, *51*, 3726–3730. <https://doi.org/10.1128/AAC.01406-06>.
51. Nicoloff, H.; Hjort, K.; Levin, B.R.; Andersson, D.I. The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification. *Nat. Microbiol.* **2019**, *4*, 504–514. <https://doi.org/10.1038/s41564-018-0342-0>.
52. Andersson, D.I.; Nicoloff, H.; Hjort, K. Mechanisms and clinical relevance of bacterial heteroresistance. *Nat. Rev. Microbiol.* **2019**, *17*, 479–496. <https://doi.org/10.1038/s41579-019-0218-1>.
53. Lund, M.E.; Hawkinson, R.W. Evaluation of the prompt inoculation system for preparation of standardized bacterial inocula. *J. Clin. Microbiol.* **1983**, *18*, 84–91. <https://doi.org/10.1128/jcm.18.1.84-91.1983>.
54. World Health Organization. *COVID-19 Target Product Profiles for Priority Diagnostics to Support Response to the COVID-19 Pandemic*; WHO: Geneva, Switzerland, 2020; Volume 0.1.
55. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification—Diagnostic Assessment Guidance on Test Guidance on Test Method Validation for In Vitro Diagnostic Medical Devices TGS-4; Tech. Guid. Ser. WHO Prequalification—Diagnostic Assess 2017. Available online: <http://apps.who.int/bookorders.%0A>, <http://apps.who.int/iris/bitstream/10665/258971/1/WHO-EMP-RHT-PQT-TGS4-2017.04-eng.pdf?ua=1> (accessed on 5 May 2022).
56. Gillet, P.; Maltha, J.; Hermans, V.; Ravinetto, R.; Bruggeman, C.; Jacobs, J. Malaria rapid diagnostic kits: Quality of packaging, design and labelling of boxes and components and readability and accuracy of information inserts. *Malar. J.* **2011**, *10*, 39. <https://doi.org/10.1186/1475-2875-10-39>.
57. A Reddy, E.; Shaw, A.V.; A Crump, J. Community-acquired bloodstream infections in Africa: A systematic review and meta-analysis. *Lancet Infect. Dis.* **2010**, *10*, 417–432. [https://doi.org/10.1016/S1473-3099\(10\)70072-4](https://doi.org/10.1016/S1473-3099(10)70072-4).
58. Kern, W.V.; Rieg, S. Burden of bacterial bloodstream infection—A brief update on epidemiology and significance of multidrug-resistant pathogens. *Clin. Microbiol. Infect.* **2019**, *26*, 151–157. <https://doi.org/10.1016/j.cmi.2019.10.031>.

Table S1. List of antibiotics or resistance mechanisms tests included onto the different MSF MIC panels as per WHO AWaRe category and based on the list of essential medicine

Antibiotic	Class	AWaRe Category	MIC POS Panel ¹		MIC NEG Panel ²		MIC FAST Panel ³	
			<i>Staphylo-</i> <i>cocci</i>	<i>Entero-</i> <i>cocci</i>	<i>Enterobac-</i> <i>terales</i>	<i>Non-Fer-</i> <i>menting</i> <i>bacilli</i>	<i>Strepto-</i> <i>cocci</i>	<i>Haemoph-</i> <i>ilus</i>
Ampicillin	Penicillins	Access	X	X	X		X	
Benzylpenicillin	Penicillins	Access	X				X	
Oxacillin	Penicillins	Access	X					
Amoxicillin/clavulanic Acid	Beta lactam - beta lactamase inhibitor	Access			X			
Chloramphenicol	Amphenicols	Access			X		X	X
Gentamicin	Aminoglycosides	Access	X		X	X		
Gentamicin (high level)	Aminoglycosides	Access		X				
Amikacin	Aminoglycosides	Access	X		X	X		
Clindamycin	Lincosamides	Access	X					
Inducible clindamycin resistance	Lincosamides	Access	X					
Tetracycline	Tetracyclines	Access	X					
Trimethoprim/sulfamethoxazole	Trimethoprim	Access	X		X	X	X	X
Cefoxitin screen	Second-generation cephalosporins	Watch	X					
Ceftazidime	Third generation cephalosporins	Watch			X	X		
Ceftriaxone	Third generation cephalosporins	Watch			X		X	X
ESBL test	Third generation cephalosporins	Watch			X	X		
Piperacillin/tazobactam	Beta lactam - beta lactamase inhibitor	Watch			X	X		
Ertapenem	Carbapenems	Watch			X	X		
Imipenem/cilastatin	Carbapenems	Watch			X	X		
Meropenem	Carbapenems	Watch			X	X	X	X
Ciprofloxacin	Fluoroquinolones	Watch	X	X	X	X		X
Levofloxacin	Fluoroquinolones	Watch					X	X
Teicoplanin	Glycopeptides	Watch	X	X				
Vancomycin (IV)	Glycopeptides	Watch	X	X			X	
Erythromycin	Macrolides	Watch	X					
Tigecycline	Glycylcyclines	Reserve	X	X				
Daptomycin	Lipopeptides	Reserve	X					
Linezolid	Oxazolidinones	Reserve	X				X	
Fosfomycin (IV)	Phosphonics	Reserve	X		X			
Colistin	Polymyxins	Reserve			X	X		
Dalfopristin-quinupristin	Streptogramins	Reserve	X	X				

¹ MicroScan Inducible Clindamycin test is intended to detect inducible resistance for staphylococci with the antimicrobial agent clindamycin. The MicroScan Cefoxitin Screen is intended to determine the susceptibility of Staphylococcus species to the penicillinase-stable beta-lactams. The Cefoxitin Screen uses the 16-20 hour result from a well containing cefoxitin at 4 mcg/mL and growth media, labeled CfxS, and the oxacillin MIC at 16-20 hours..

² Trimethoprim, and Trimethoprim/Sulfamethoxazole broth contain thymidine phosphorylase to reduce thymidine levels in the medium. Ceftazidime, cefotaxime, ceftazidime/ clavulanic acid and cefotaxime/clavulanic acid are used to confirm the presence of ESBLs. The confirmation test is a ≥ 3 twofold dilution decrease in MICs of suspected organisms to ceftazidime or cefotaxime in the presence of a fixed concentration of clavulanic acid, versus its MIC when tested alone.

³ Panels are rehydrated with Mueller-Hinton broth supplemented with 5% lysed horse blood (LHB) for Streptococci as recommended by EUCAST and with Hemophilus Test Medium (HTM) for Hemophilus testing as recommended by CLSI, after inoculation of the broth with a standardized suspension of the organism.

****)

C	G			8 AMP	16 AMP	32 AMP	64 AMP	2/2 AMC	8/2 AMC	16/2 AMC	32/2 AMC
8/4 PIT	16/4 PIT	32/4 PIT	64/4 PIT	2 CTA	16 CTA	1 CTZ	4 CTZ	8 CTZ	16 CTZ		
				0.5/4 CTXAC	4/4 CTXAC	0.25/4 CCV	2/4 CCV	1 CTR	2 CTR	4 CTR	8 CTR
0.06 CIP	0.12 CIP	0.25 CIP	0.5 CIP	1 CIP	2 CIP						
8 AMI	16 AMI	32 AMI	2 GEN	4 GEN	8 GEN	16 GEN	1 TIG	2 TIG	4 TIG	8 TIG	
4 CHL	8 CHL	16 CHL	2 COL	4 COL	32 FOS	64 FOS	2/38 TRS	4/76 TRS	8/152 TRS		
0.12 MER	0.25 MER	0.5 MER	1 MER	2 MER	4 MER	8 MER	16 MER				
0.5 IMI	1 IMI	2 IMI	4 IMI	8 IMI	16 IMI	0.12 ERT	0.25 ERT	0.5 ERT	1 ERT	2 ERT	4 ERT

Abbr.	Antimicrobial Agents	Dilutions
C	Negative Control	
G	Growth Control	
AMI	Amikacin	8-32
AMC	Amoxicillin/Clavulanic Acid	2/2, 8/2 - 32/2
AMP	Ampicillin	8 - 64
CTA	Cefotaxime	2, 16
CTXAC	Cefotaxime/Clavulanic Acid	0.5/7, 4/4
CTZ	Ceftazidime	1, 4 -16
CCV	Ceftazidime/Clavulanic	0.25/4, 2/4
CTR	Ceftriaxone	1 - 8
CHL	Chloramphenicol	4 - 16
CIP	Ciprofloxacin	0.06 - 2
COL	Colistin	2 - 4
ERT	Ertapenem	0.12 - 4
FOS	Fosfomycin	32 - 64
GEN	Gentamicin	2 - 16
IMI	Imipenem	0.5 - 16
MER	Meropenem	0.12 - 16
PIT	Piperacillin/Tazobactam	8/4 - 64/4
TIG	Tigecycline	1 - 8
TRS	Trimethoprim/Sulfamethoxazole	2 / 38 - 8/152

ceptibility of rapidly growing aerobic and facultative gram-positive cocci (*Staphylococcus.spp* and *Enterococcus.spp*)

C	G		TFG	0.12 PEN	0.25 PEN	0.5 PEN	1 PEN	4 AMP	8 AMP	16 AMP	32 AMP
0.25 OXA	0.5 OXA	1 OXA	2 OXA	4 CfxS	0.5 CIP	1 CIP	2 CIP	4 CIP			
8 AMI	16 AMI	32 AMI	64 AMI	1 GEN	2 GEN	500 GmS					
2 TEI	4 TEI	8 TEI	16 TEI	2 VAN	4 VAN	8 VAN	16 VAN				
0.25 CLI	0.5 CLI	1 CLI	2 CLI	4/0.5 ICd	0.5 ERY	1 ERY	2 ERY	4 ERY	1 QUD	2 QUD	4 QUD
1 LIN	2 LIN	4 LIN	8 LIN								
1 DAP	2 DAP	4 DAP	8 DAP	32 FOS	64 FOS	0.5/9.5 TRS	1/19 TRS	2/38 TRS	4/76 TRS		
1 TET	2 TET	4 TET	8 TET	0.25 TIG	0.5 TIG	1 TIG	2 TIG				

Abbr.	Antimicrobial Agents	Dilutions
C	Negative Control	
G	Growth Control	
TFG	Thymidine Free Growth Well	(Comparator for SXT)
AMI	Amikacin	8 - 64
AMP	Ampicillin	4 - 32
CfxS	Cefoxitin Screen	4
CIP	Ciprofloxacin	0.5 - 4
CLI	Clindamycin	0.25 - 2
DAP	Daptomycin	1 - 8
ERY	Erythromycin	0.5 - 4
FOS	Fosfomycin	32 - 64
GEN	Gentamicin	1 - 2
GmS	Gentamicin Synergy Screen	500
ICd	Inducible Clindamycin Test	4 / 0.5
LIN	Linezolid	1 - 8
OXA	Oxacillin	0.25 - 2
PEN	Penicillin	0.12 - 1
QUD	Quinupristin/Dalfopristin	1 - 4
TEI	Teicoplanin	2 - 15
TET	Tetracycline	1 - 8
TIG	Tigecycline	0.25 - 2
TRS	Trimethoprim/Sulfamethoxazole	0.5 / 9.5 - 4 / 76
VAN	Vancomycin	2 - 16

Table S2.c) MicroScan MSF Dried Overnight Fastidious panel are designed for use in determining antimicrobial agent susceptibility of aerobic non-enterococcal streptococci (including *Streptococcus pneumoniae*) and *Haemophilus* spp. Not to be used for testing of *Neisseria*

G													
0.06 PEN	0.12 PEN	0.25 PEN	0.5 PEN	1 PEN	2 PEN								
0.12 AMP	0.5 AMP	1 AMP	2 AMP	0.12 CTR	0.5 CTR	1 CTR	2 CTR	0.25 MER	0.5 MER	1 MER	2 MER		
0.06 CIP	0.12 CIP	0.5 LEV	2 LEV	4 LEV									
2 VAN	4 VAN	8 VAN	16 VAN										
0.5 CLI	1 CLI	2 CLI	4 CLI										
2 LIN	4 LIN	8 LIN	16 LIN										
2 CHL	4 CHL	8 CHL	16 CHL	0.5/9.5 TRS	1/19 TRS	2/38 TRS	4/76 TRS						

Abbr.	Antimicrobial Agents	Dilutions
C	Negative Control	
NOTE = No Negative Control Well (C) because broth is added to the entire panel when inoculated		
AMP	Ampicillin – AMP	0.12 , 0.5 - 2
CTR	Ceftriaxone – CTR	0.12 , 0.5 - 2
CHL	Chloramphenicol – CHL	2 - 16
CIP	Ciprofloxacin – CIP	0.06 - 0.12
CLI	Clindamycin – CLI	0.5 - 4
LEV	Levofloxacin – LEV	0.5, 2 - 4
LIN	Linezolid – LIN	2 - 16
MER	Meropenem – MER	0.25 - 2
PEN	Penicillin – PEN	0.06 - 2
TR5	Trimethoprim/Sulfamethoxazole – TRS	0.5/9.5 - 4/76
VAN	Vancomycin – VAN	2 - 16

Table S3. Technical information on the different panel, disc, media used for the experiment (Lot number, reference, expiry date, etc.)

Item	Method	Cond	Brand	Ref	Batch	Date exp
Pos MIC Panel Type 1	BMD	20/box	BC	B1020-103B	19190329	2020-01-10
Neg MIC MSF Panel Type 1	BMD	20/box	BC	B1020-104B	19190330	2020-01-10
FAST MSF RUO MIC Panel Type 1	BMD	20/box	BC	B1020-105W	19190331	2020-02-10
Prompt	Inoculum	60/box	BC	B1026-10D	19170714	2020-01-10
Cation-adjusted Mueller-Hinton Broth with 3% Lysed Horse Blood, 25mL (BC)	Broth	10/box	BC	B1015-25	19190328	2020-01-10
Haemophilus Test Medium (HTM), 25mL	Broth	10/box	BC	B1015-26	19190326	2020-03-10
Inoculator-D Set, 60/pk (BC)	Consumable	260/box	BC	B1013-5	19190324	2023-04-10
Inoculum Water, 3mL (BC)	Inoculum	60/box	BC	B1015-2	19190907	2020-04-10
Inoculum Water with Pluronic, 25 mL	Inoculum	60/box	BC	B1015-7	19190928	2020-04-10
Amikacin	30µg	4 x 50 Disc	Biorad	66148	64240285	2019-12-10
Amoxicillin/Clavulanic Acid	20-10µg	4 x 50 Disc	Biorad	66178	200417A	2019-10
Ampicillin	2µg	4 x 50 Disc	Biorad	67288	64239659	2019-11-27
Ampicilline	10µg	4 x 50 Disc	Biorad	66128	64239660	2019-11-12
Cefepime	30µg	4 x 50 Disc	Biorad	66098	64261585	2020-03-17
Cefepime/Clavulanic Acid	30µg/10µg	4 x 50 Disc	Biorad	66466	190117G	2020-01
Cefotaxime	5µg	4 x 50 Disc	Biorad	67718	64253166	2020-02-11
Cefotaxime/Clavulanic Acid	5µg/10µg	4 x 50 Disc	Biorad	68456	190117N	2020-01
Cefoxitin Screen	30 µg	4 x 50 Disc	Biorad	66228	200318A	2020-04
Ceftazidime	10µg	4 x 50 Disc	Biorad	67298	64251000	2020-01-27
Ceftazidime/Clavulanic Acid	30µg/10µg	4 x 50 Disc	Biorad	68446	64251001	2020-01-27
Ceftriaxone	30µg	4 x 50 Disc	Biorad	66188	200414C	2020-04
Chloramphenicol	30 µg	4 x 50 Disc	Biorad	66278	64221108	2020-09-11
Ciprofloxacin	5µg	4 x 50 Disc	Biorad	68648	64240327	2020-02-17
Clindamycin	2 µg	4 x 50 Disc	Biorad	66328	64246826	2020-01-03
Colistin	BMD	8*10	Fisher	FRCOL	B1441A	2020-01
Ertapenem	10µg	4 x 50 Disc	Biorad	67518	64248425	2019-02-14
Erythromycin	15 µg	4 x 50 Disc	Biorad	66448	64231553	2019-11-05
AD Fosfomycin 0.25-256	Agar dilution	6 test	Liofilchem	77061	22122083	2020-12-18
Gentamicin	10µg	4 x 50 Disc	Biorad	66608	64246826	2020-03-18
Gentamicin Synergy Screen	30µg	4 x 50 Disc	Biorad	667318	64182638	2020-03-25
Imipenem	10µg	4 x 50 Disc	Biorad	66568	4331644	2020-10-31
Levofloxacin	5µg	4 x 50 Disc	Biorad	66858	4331644	2020-10-31
Linezolid	10 µg	4 x 50 Disc	Biorad	67878	200417D	2020-07
Meropenem	10µg	4 x 50 Disc	Biorad	67048	64261586	2020-03-17
Penicillin	1 unit	4 x 50 Disc	Biorad	67788	64226720	2019-10-08
Piperacillin/Tazobactam	30-6µg	4 x 50 Disc	Biorad	67338	64248373	2020-01-13
Quinupristin/Dalfopristin	15 µg	4 x 50 Disc	Biorad	67528	64221134	2020-10-03
Teico/ Vanco / Dapto MIC	BMD	10	Fisher	FRUNIGP1	B1341B	2020-08
Teicoplanin	30µg	4 x 50 Disc	Biorad	68948	64240292	2019-12-12
Tetracycline	30µg	4 x 50 Disc	Biorad	67448	64240328	2020-01-01
Tigecycline	15µg	4 x 50 Disc	Biorad	67398	64248382	2020-02-10
Trimethoprim/Sulfa	1.25-23.75µg	4 x 50 Disc	Biorad	68898	64240288	2019-12-19
Vancomycin	5µg	4 x 50 Disc	Biorad	68888	64219266	2020-08-28
Mueller-Hinton-F square	Agar	10 plaques	Biorad	63525	64468399	2020-02
Mueller-Hinton square	Agar	10 plaques	Biorad	63901	64460701	2020-05
Chocolate agar w/o Vitox	Agar	20 plates 90 mm	Liofilchem	10601	22122082	2019-10

Table S4: Results for Staphylococci tested with MICPOS1 using Prompt inoculum method and visual reading versus AST reference panels

Antimicrobial	No. of isolates			No. of isolates			PtCA _d	PtmE _e	PtME _f	PtVME _g	
	AST Reference ^b			MICPOS1 ^c							
	Total ^a	R	I	S	R	I					S
							(no. [%])	(no. [%])	(no. [%])	(no. [%])	
Penicillin	63	59	0	4	59	0	4	63 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	74	37	0	37	43	0	31	68 (92)	0 (0)	6 (8)	0 (0)
Amikacin	74	17	4	53	18	4	52	73 (99)	0 (0)	1 (1)	0 (0)
Gentamicin	74	28	0	46	33	11	30	58 (78)	11 (15)	5 (7)	0 (0)
Teicoplanin	74	3	0	71	10	0	64	67 (91)	0 (0)	7 (9)	0 (0)
Vancomycin	74	2	0	72	4	0	70	72 (97)	0 (0)	2 (3)	0 (0)
Quinupristin-dalfopristin	74	0	7	67	2	7	65	70 (95)	0 (0)	3 (4)	1 (1)
Erythromycin	74	30	0	44	30	0	44	74 (100)	0 (0)	0 (0)	0 (0)
Clindamycin	74	14	0	60	20	1	53	65 (88)	1 (1)	7 (9)	1 (1)
Daptomycin	74	1	0	73	1	0	73	72 (97)	0 (0)	1 (1)	1 (1)
Fosfomycin	74	29	0	45	29	0	45	74 (100)	0 (0)	0 (0)	0 (0)
Trimethoprim/Sulfamethoxazole	74	18	0	56	29	0	55	71 (96)	0 (0)	2 (3)	1 (1)
Linezolid	74	5	0	69	6	0	68	73 (99)	0 (0)	1 (1)	0 (0)
Tetracycline	74	37	0	37	47	4	23	60 (81)	4 (5)	10 (14)	0 (0)
Tigecycline	74	0	0	74	4	0	70	70 (95)	0 (0)	4 (5)	0 (0)

a Total number of isolates tested by antibiotic

b Number of isolate tested classified with reference method by R resistant; I, intermediate; S, susceptible.

c Number of isolate tested classified with evaluated method by R resistant; I, intermediate; S, susceptible

d PtCA, categorical agreement with PROMPT inoculum.

e PtmE, minor error with PROMPT inoculum.

f PtME, major error with PROMPT inoculum.

g PtVME, very major error with PROMPT inoculum.

Table S5: Results for Enterococci tested with MICPOS1 using Prompt inoculum method and visual reading versus AST reference panels

Antimicrobial	No. of isolates			No. of isolates			PtCA _d	PtmE _e	PtME _f	PtVME _g	
	AST Reference ^b			MICPOS1 ^c							
	Total ^a	R	I	S	R	I					S
							(no. [%])	(no. [%])	(no. [%])	(no. [%])	
Ampicillin	49	30	0	19	29	1	18	47 (96)	0 (0)	1 (2)	1 (2)
Ciprofloxacin	49	29	0	20	30	0	19	48 (98)	0 (0)	1 (2)	0 (0)
Teicoplanin	49	23	0	26	23	0	26	47 (96)	0 (0)	1 (2)	1 (2)
Vancomycin	49	23	0	26	23	0	26	49 (100)	0 (0)	0 (0)	0 (0)
Quinupristin-dalfopristin	49	19	16	14	19	16	14	49 (100)	0 (0)	0 (0)	0 (0)
Linezolid	49	2	0	47	2	7	40	42 (91)	7 (9)	0 (0)	0 (0)
Tigecycline	49	4	0	45	4	0	45	49 (100)	0 (0)	0 (0)	0 (0)

a Total number of isolates tested by antibiotic

b Number of isolate tested classified with reference method by R resistant; I, intermediate; S, susceptible.

c Number of isolate tested classified with evaluated method by R resistant; I, intermediate; S, susceptible

d PtCA, categorical agreement with PROMPT inoculum.

e PtmE, minor error with PROMPT inoculum.

f PtME, major error with PROMPT inoculum.

g PtVME, very major error with PROMPT inoculum.

Table S6: Results for Enterobacterales tested with MICNEG1 using Prompt inoculum method and visual reading versus AST reference panels

Antimicrobial	No. of isolates			No. of isolates			PtCA _d (no. [%])	PtmE _e (no. [%])	PtME _f (no. [%])	PtVME _g (no. [%])	
	AST Reference ^b			MICPNEG1 ^c							
	Total ^a	R	I	S	R	I					S
Ampicillin	112	100	0	12	99	0	13	111 (99)	0 (0)	0 (0)	1 (1)
Amoxicillin/Clavulanic Acid	112	72	0	40	73	0	39	109 (97)	0 (0)	2 (2)	1 (1)
Ceftazidime	112	47	2	63	49	0	64	108 (96)	3 (3)	1 (1)	0 (0)
Ceftriaxone	112	50	0	62	51	1	60	110 (98)	1 (1)	1 (1)	0 (0)
Piperacillin/Tazobactam	112	39	4	69	39	4	69	112 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	112	51	4	57	54	8	49	107 (95)	3 (3)	3 (3)	0 (0)
Amikacin	112	16	4	92	18	11	83	104 (92)	7 (6)	2 (2)	0 (0)
Gentamicin	112	40	5	67	40	8	64	108 (96)	3 (3)	1 (1)	1 (1)
Trimethoprim/Sulfamethoxazole	112	69	1	42	70	2	40	106 (94)	1 (1)	3 (3)	2 (2)
Chloramphenicol	112	47	0	65	46	1	65	108 (96)	1 (1)	1 (1)	2 (2)
Colistin	112	12	0	77	15	0	74	109 (97)	0 (0)	3 (3)	0 (0)
Fosfomycin	112	1	0	111	2	0	110	109 (97)	0 (0)	2 (2)	1 (1)
Tigecycline	112	0	0	112	0	0	112	112 (100)	0 (0)	0 (0)	0 (0)
Meropenem	112	16	6	90	16	6	90	112 (100)	0 (0)	0 (0)	0 (0)
Imipenem	112	19	7	86	20	12	90	107 (95)	5 (4)	1 (1)	0 (0)
Ertapenem	112	33	0	79	34	6	84	106 (94)	6 (5)	1 (1)	0 (0)

a Total number of isolates tested by antibiotic

b Number of isolate tested classified with reference method by R resistant; I, intermediate; S, susceptible.

c Number of isolate tested classified with evaluated method by R resistant; I, intermediate; S, susceptible

d PtCA, categorical agreement with PROMPT inoculum.

e PtmE, minor error with PROMPT inoculum.

f PtME, major error with PROMPT inoculum.

g PtVME, very major error with PROMPT inoculum.

Table S7: Results for Non-fermenting Gram negative bacilli tested with MICNEG1 using Prompt inoculum method and visual reading versus AST reference panels

Antimicrobial	No. of isolates			No. of isolates			PtCA _d (no. [%])	PtmE _e (no. [%])	PtME _f (no. [%])	PtVME _g (no. [%])	
	AST Reference ^b			MICPNEG1 ^c							
	Total ^a	R	I	S	R	I					S
Ceftazidime	15	8	3	4	8	3	4	15 (100)	0 (0)	0 (0)	0 (0)
Piperacillin/Tazobactam	15	4	0	11	4	0	11	15 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	29	12	6	11	12	6	11	29 (100)	0 (0)	0 (0)	0 (0)
Amikacin	29	7	2	20	7	3	19	28 (97)	1 (3)	0 (0)	0 (0)
Gentamicin	29	15	0	14	15	0	14	29 (100)	0 (0)	0 (0)	0 (0)
Trimethoprim/Sulfamethoxazole	29	10	0	20	10	0	20	30 (100)	0 (0)	0 (0)	0 (0)
Chloramphenicol	9	4	3	2	4	3	2	9 (100)	0 (0)	0 (0)	0 (0)
Colistin	15	3	0	12	3	0	12	15 (100)	0 (0)	0 (0)	0 (0)
Meropenem	45	14	6	25	14	8	23	45 (96)	2 (4)	0 (0)	0 (0)

Imipenem	29	12	0	17	12	0	17	29 (97)	0 (0)	0 (0)	1 (3)
----------	----	----	---	----	----	---	----	---------	-------	-------	-------

a Total number of isolates tested by antibiotic

b Number of isolate tested classified with reference method by R resistant; I, intermediate; S, susceptible.

c Number of isolate tested classified with evaluated method by R resistant; I, intermediate; S, susceptible

d PtCA, categorical agreement with PROMPT inoculum.

e PtME, minor error with PROMPT inoculum.

f PtME, major error with PROMPT inoculum.

g PtVME, very major error with PROMPT inoculum.

Table S8: Results for specific resistance test using MICNEG1 or MICPOS1 using Prompt inoculum method and visual reading versus AST reference methods

Multidrug resistant organism	No. of isolates AST Reference ^b			No. of isolates MSF panel ^c		PtCA ^d (no. [%])	PtME ^e (no. [%])	PtVME ^f (no. [%])
	Total ^a	R	S	R	S			
Methicillin resistant <i>Staphylococcus aureus</i> ^g	47	33	14	34	13	46 (99)	1 (1)	0 (0)
Inducible clindamycin resistant <i>Staphylococci</i> ^g	74	26	48	26	48	74 (100)	0 (0)	0(0)
Vancomycin resistant <i>Staphylococcus aureus</i> ^g	47	2	45	4	43	43 (96)	3 (4)	1 (1)
High-level gentamicin resistance <i>Enterococci</i> ^g	49	25	24	25	24	47 (100)	1 (2)	1 (2)
Vancomycin resistant <i>Enterococci</i> ^g	49	23	26	23	26	49 (100)	0 (0)	0(0)
Extended spectrum beta lactamase <i>Enterobacterales</i> ^h	112	19	93	19	93	112 (100)	0 (0)	0(0)
Carbapenem resistant <i>Enterobacterales</i> ^h	112	33	79	32	80	0 (0)	0 (0)	1 (1)
Colistin resistant <i>Enterobacterales</i> ^h	112	12	77	13	76	112 (97)	2 (2)	1 (1)
Carbapenem resistant <i>Pseudomonas aeruginosa</i> ^h	15	4	11	4	11	15 (100)	0 (0)	0 (0)
Colistin resistant <i>Pseudomonas aeruginosa</i> ^h	15	4	11	4	11	15 (100)	0 (0)	0 (0)
Carbapenem resistant <i>Acinetobacter baumannii</i> ^h	14	8	6	7	7	15 (100)	0 (0)	1 (3)

a Total number of isolates tested by antibiotic

b Number of isolate tested classified with reference method by R resistant; I, intermediate; S, susceptible.

c Number of isolate tested classified with evaluated method by R resistant; I, intermediate; S, susceptible

d PtCA, categorical agreement with PROMPT inoculum.

e PtME, major error with PROMPT inoculum.

f PtVME, very major error with PROMPT inoculum.

g Resistance test evaluated on the MICPOS1 panel.

h Resistance test evaluated on the MICNEG1 panel.

Prompt®* Inoculation System-D For Use with Microdilution Susceptibility Tests

Intended Use
The Prompt Inoculation System-D is used to standardize inocula for microdilution antimicrobial susceptibility tests.

Summary
The microdilution procedure for antimicrobial susceptibility testing has provided the clinical microbiologist with a reliable method for obtaining meaningful quantitative susceptibility test results. This procedure is used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents and has rapidly gained broad acceptance in the clinical laboratory.¹ Accuracy and reproducibility in the MIC procedure depend on use of defined materials and methods.

One of the important requirements in the MIC procedure is control of the bacterial population of the inoculum within defined limits. This step may be accomplished in two ways:

- 1) manual adjustment of the inoculum to match a 0.5 McFarland turbidity standard² followed by appropriate dilution or
- 2) incubation to stationary phase in broth culture³ followed by appropriate dilution.

The Prompt Inoculation System-D provides the microbiologist with a method for obtaining standardized inocula while eliminating the need for incubation and turbidity adjustment.^{4,5,6,7} Microdilution susceptibility results obtained using the Prompt Inoculation System-D show a correlation of 97% or greater with the microdilution susceptibility results obtained with inocula prepared according to the Clinical and Laboratory Standards Institute (CLSI) procedure.²

Principles

The Prompt Inoculation System-D consists of an inoculation wand and a bottle of diluent. The wand is a polypropylene rod with a breakaway collar that serves as a wiping mechanism. The rod is attached to a stopper. At the tip of the wand is a groove designed to hold a specific number of bacteria. Thirty (30) mL of diluent are provided in the plastic bottle. The wand is touched to several bacterial colonies on a primary isolation plate, wiped, then placed in the plastic bottle. The bacteria are suspended by shaking the bottle. The bacterial suspension is stable for four hours. When the Prompt Inoculation System-D is used within four hours after preparation, 97% of MICs are within ± one (1) dilution of the MICs determined by the conventional method.

The Prompt Inoculation System-D facilitates the MIC inoculum preparation by eliminating: 1) the incubation period, and 2) the need to adjust the inoculum concentration.

Suspending Solution

Prompt Inoculation System-D catalog number B1026-100 contains 30 mL (± 1.0 mL) of stabilized, aqueous PLURONIC** surfactants.

Warnings and Precautions

1. For in vitro diagnostic use only.
2. Colonies must be selected from a FRESH culture plate (< 24 hours).
3. The bacterial suspension should be used within four hours of preparation.
4. DO NOT FLAME the plastic inoculation wand under ANY circumstances.
5. To avoid contamination, keep fingers above the ridge of the stopper and on the outer surface of the collar.
6. Keep wands covered when not in use.
7. Do not use if colonies are smaller than the wand tip.

* Registered trademark of 3M Company, St. Paul, MN USA

** Registered trademark of BASF Corp., Parsippany, NJ USA

NOTE:
After inoculation, the Prompt inoculation vial, wand and collar should be considered potential pathogen carriers. Handle and discard the product accordingly.

Storage
Prompt units should be stored at 2-27°C. Avoid direct light. Do not use units beyond the expiration date shown on the package. Inoculated units may be stored for up to four hours at room temperature.

Product Deterioration
Prompt bottles should not be used if the bottle or cap is cracked.

Specimen Collection and Preparation
Specimens received in the laboratory should be treated in the usual manner for the preparation of a primary culture plate. Three isolated colonies larger than the wand tip from an eighteen to twenty-four (18-24) hour culture are needed for use with the Prompt Inoculation System-D.

Procedure

Materials Provided

62 Prompt inoculation wands
60 Prompt inoculation bottles
Prompt Inoculation System-D can be used for preparation of inocula for microdilution MIC testing procedures that use the inoculum suspended in a non-nutritive solution to rehydrate the tray. To use, follow the instructions below:

A. Preparation of bacterial suspension

1. Remove the required number of Prompt inoculation bottles from the box.
2. Remove an inoculation wand from the box.
3. Holding the wand tip perpendicular to the agar surface, touch 3 isolated colonies as large as or larger than the tip. Do not penetrate the agar. Do not scrape or drag the tip across the colonies (Figure 1-1).



Figure 1-1

NOTE:
For very small, pin-point colonies, continue incubation of the primary plate until they reach the diameter of the wand tip. If the colony diameter is not likely to reach this size (for example, some streptococci), an alternate method for inoculum preparation should be used.

4. Holding the wand by the handle with one hand, grasp the collar with the other hand and pull firmly to break the connection between the collar and wand shaft. Do not twist or bend the collar. Keep the tip pointed toward the floor, away from yourself and others (Figure 1-2).



Figure 1-2

5. Slide the collar slowly down and off the wand shaft and discard the collar (Figure 1-3).
6. While holding the inoculation wand with one hand, pick up a Prompt inoculation bottle.

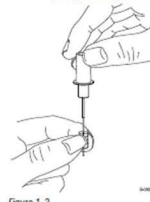


Figure 1-3



Figure 1-4

7. Bend the cap of the bottle sideways until it snaps off (Figure 1-4).
8. Place the inoculation wand into the bottle and press down with a twisting motion to assure a tight seal (Figure 1-5).



Figure 1-5

9. Shake the bottle vigorously 8 to 10 times to release the bacteria from the wand tip. If the organism is not released from the wand, let the solution sit for 5 minutes and shake again (Figure 1-6).



Figure 1-6

The bacterial suspension should be used within four hours of preparation. If not used immediately after preparation, shake vigorously to resuspend the bacteria just prior to use.

B. Addition of Suspension to Seed Tray

1. Remove the inoculation wand from the bottle and discard.
2. Pour the suspension into the seed tray by gently squeezing the bottle with a pumping action (Figure 1-7).



Figure 1-7

3. Proceed with the appropriate microdilution MIC procedure.
NOTE:
The inoculated Prompt bottle, wand and collar should be considered potential pathogen carriers. Handle and discard appropriately.

(a)

(b)

Figure S1. Extraction of the Prompt® Inoculation System-D Instruction For Use with Microdilution Susceptibility Tests with, (a) general instructions and (b) bench aid like part with more comprehensive instructions

Table S9: Results of the repetition done for AMC discrepant results using different AST methods and brand for disc, media. Were used in this experiment disc from Liofilchem(Liofilchem, Roseto degli Abruzzi, Italy), Biorad, I2A; MH agar from Biorad, Liofilchem, Biomérieux MH Enterobacterales, E-test Gradient strip (E-test, bioMérieux, Marcy l'Étoile, France) and for Broth Microdilution Sensititre (Sensititre, Thermo Fisher Scientific, UK), MICNEG1 (Microscan, Beckman Coulter

N° isolat	Type of method Brand Disc / Brand agar	BMD		BMD		GD		KB		KB		KB		KB		KB		KB		KB					
		MIC-	NEG1	Sensi-	Sensi-	E test/	Bio	Lio/Bio	I2A/Bio	Bio/Bio	Lio/Lio	I2A/Lio	Bio/Lio	Lio/BM	I2A/BM	Bio/BM	KB	KB	KB	KB	KB				
128	Salmonella typhimurium	16	R	>8	R	4	S	21	S	21	S	21	S	21	S	19	S	21	S	18	R	17	R	19	S
129	Salmonella typhimurium	16	R	>8	R	6	S	20	S	17	S	20	S	20	S	18	R	21	S	19	S	16	R	19	S
130	Salmonella choleraesuis	16	R	>8	R	8	S	21	S	20	S	21	S	21	S	18	R	21	S	18	R	17	R	20	S
131	Salmonella choleraesuis	16	R	>8	R	3	S	20	S	18	R	21	S	22	S	18	R	22	S	20	S	17	R	23	S
133	Salmonella choleraesuis	16	R	>8	R	3	S	23	S	19	S	24	S	23	S	20	S	24	S	21	S	17	R	22	S
135	Salmonella choleraesuis	8	R	>8	R	3	S	20	S	19	S	22	S	22	S	21	R	22	S	15	R	22	S	23	S
138	Salmonella typhimurium	32	R	>8	R	4	S	18	R	17	R	19	R	18	R	17	S	19	S	19	S	15	R	19	S
139	Salmonella typhimurium	32	R	>8	R	6	S	17	R	15	R	17	R	17	R	15	S	18	R	16	R	14	R	17	R
140	Salmonella typhimurium	32	R	>8	R	4	S	19	S	17	R	20	S	21	S	17	S	21	S	19	S	17	R	19	S
141	Salmonella typhimurium	8	S	>8	R	4	S	20	S	18	R	21	S	21	S	18	S	20	S	19	S	19	S	20	S
142	Salmonella typhimurium	8	S	>8	R	4	S	22	S	20	S	22	S	21	S	19	R	23	S	21	S	18	R	21	S

*Abbreviations: BMD= Broth Micro Dilution; GD= Gradient Diffusion; KB = Kirby Bauer; Bio = Biorad; Lio = Liofilchem; I2A=I2A

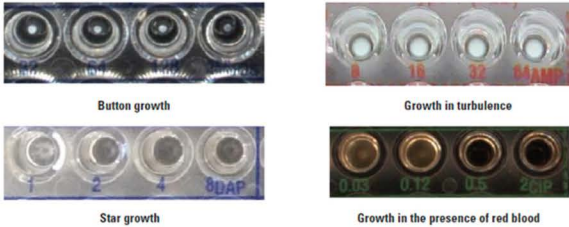
BENCH AID
23
Microbes Sans Frontières
Mini Lab

ID/AST

Plates reading MIC

This document is a summary of the various SOPs + 10.15 Plates reading MIC
* Please read the POS before using this FM.

1 Types of growth

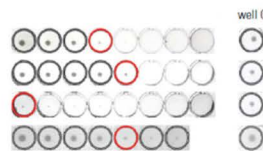


Growth not taken into account



Attention for TRS

positive well only if the haze is ≥ 80% of the well's G



Effective date : 10/11/2021 | Version : V1 | Code : FM-23-LECMIC | 81

(a)

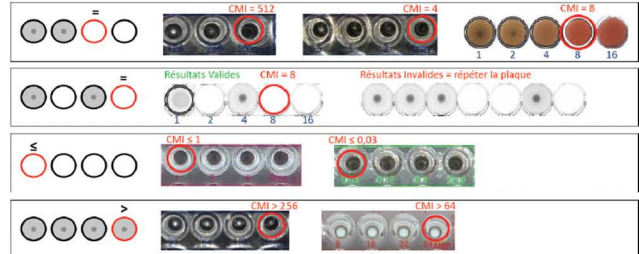
2 Reading assistance – Plates MIC

NEG MIC and POS MIC Plates

Read the plate only if :
C = No growth = clear well AND G = growth = cloudy well

FAST MIC Plates

Read the plate only if :
G = growth = cloudy well



3 Interpretation of resistance mechanisms

	Species	Acronym	Definition	Interpretation (MIC or R/S)
1	Enterobacterales and Pseudomonas aeruginosa	ESBL	Extended Spectrum Béta-lactamase	CTZ > 4 à > 16 et DCV <= 0.25/4 and/or CTA 16 à > 16 et CTXAC <= 0.5/4 and/or CTA > 16 et CTXAC = 4/4
2	Enterobacterales	CRE	Carbapenem-resistant Enterobacterales	MER > 0.125 and/orERT > 0.125
3	Salmonella spp	FRS	Salmonella Resistant to Fluoroquinolones	Salmonella spp. CIP R
4	Pseudomonas aeruginosa	PARC	Pseudomonas aeruginosa Résistante à la Ceftazidime	Pseudomonas Aeruginosa Ceftazidim = R
5	Pseudomonas aeruginosa	IRPA	Ceftazidime resistant Pseudomonas aeruginosa	Pseudomonas Aeruginosa = MER = R and/or IMI = R
6	Acinetobacter baumannii	CRAB	Acinetobacter baumannii Résistant aux Carbapénèmes	Acinetobacter baumannii = MER = R and/or IMI = R
7	Staphylococcus aureus	MRSA	Carbapenem Resistant Acinetobacter baumannii	Staphylococcus aureus test resistant to cefaxin or oxacillin
8	Staphylococcus aureus	VRSA	Vancomycin Resistant Staphylococcus aureus	Staphylococcus aureus – Vancomycin R or Teicoplanin = R
9	Staphylococcus spp	IcL	Clindamycin Inducible	IcL > 4
10	Enterococcus spp	VRE	Vancomycin Resistant Enterococci	Enterococcus.sp = Vancomycin Resistant or Intermediate
11	Enterococcus spp	GmS	High resistance to aminoglycosides	GmS > 500
12	Streptococcus pneumoniae	PRSp	Penicillin Resistant Streptococcus pneumoniae	Streptococcus pneumoniae = R
13	Haemophilus influenzae	ARHi	Haemophilus influenzae influenzae resistant to Ampicillin	Haemophilus influenzae = Béta-lactamase test positive or AMP = R

Effective date : 10/11/2021 | Version : V1 | Code : FM-23-LECMIC

(b)

Figure S2. Extraction of Mini-Lab Bench aid user manual Version1 2022 with, (a) description of type of growth and (b) visual description of the identification of MIC and mechanism of resistance

3. Evaluation of the Biomic Video Reader System for Determining Interpretive Categories of Isolates using Microscan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings

3.1. Introduction.

Timely and accurate AST results are essential but difficult to obtain by inexperienced microbiologists or laboratory technicians. Previous experience described in section 2 of this chapter, supports that the MSF Microscan MIC panels can be read accurately by human eyes, however, the experiment was done by expert readers, far from the reality of the field where the Mini-Lab is deployed and expert microbiologists are rare. Manual reading can be a source of errors especially for non-specialist technicians.

Thus, to achieve the goal of providing accurate result in the Mini-Lab by trained but non-experts in microbiology technicians, an automatic reading system coupled with an expert system was investigated. Automated susceptibility testing with the microdilution method has been used and described most notably with the Vitek (bioMérieux, Marcy l'étoile, France.) and MicroScan WalkAway (Beckman Coulter, West Sacramento, USA.) systems. Potential advantages of automation include standardisation resulting in increased accuracy, more rapid results which may positively affect patient care, technologist time savings in reading and interpreting results, improved data management, and the use of an expert system for automated reviews and verification of the data generated[130]. However, those automatic readers which incubate and read automatically AST MIC panels are quite expensive, heavy and cumbersome, making them difficult to transport on unpaved roads and to accommodate on small laboratory benches. Therefore a smaller, more practical solution was needed for the Mini-Lab set-up.

Bacteria growth in the well of microbroth dilution panel appears as turbidity, either as white cloudiness throughout the well, white spot in the centre of the well, or fine grain growth throughout the well. Many AST automatic readers use spectrophotometer for the detection of the absorbance as main detection mechanisms. However, since a few decades ago, improvements in digital imaging processing has opened up the possibility to use camera-based reading systems, technology better known to be less expensive and more robust[131].

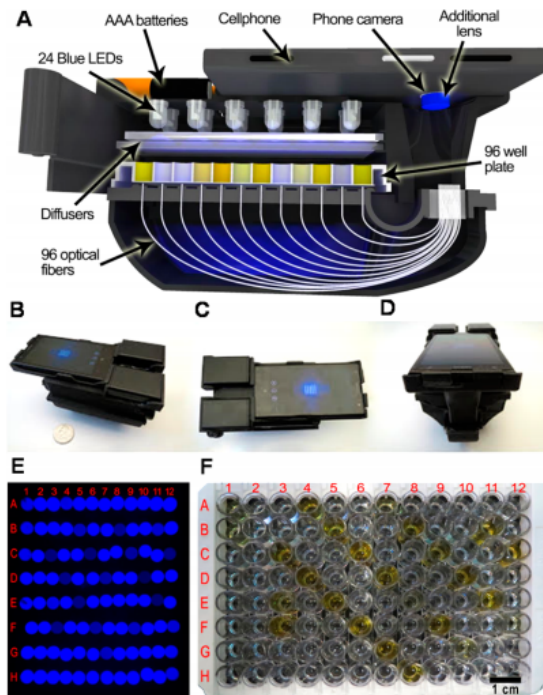


Figure 40: Schematic overview (A) and different perspectives (B-D) of the cell phone based ELISA colorimetric reader. Sample images (E), and sample plate (F). Rows and columns are labelled in (E) to correspond with the plate in (F), UCLA (USA).

ameter or MIC and reports antimicrobial agent disk diffusion, broth microdilution, agar dilution, and antibiotic gradient method susceptibility results [134], [135]. The Biomic V3 requires manual loading and unloading of one microplate at a time.

According to Fader et al., [136] this system provides a more standardised turbidity reading of test well end points, while eliminating inherent variation between different microbiologists. It also saves time related to manual reading and the enlarged screen and image enable to see the turbidity endpoints more clearly. Surprisingly, BIOMIC recommends that each plate or MIC panel be reviewed before and during video reading by an experienced microbiologist skilled in reading susceptibility tests. Since in the fields where MSF intervenes on-site skilled microbiologists are very difficult to find, we scanned for studies describing the difference in accuracy between raw automatic reading and interpretation, and the difference between automatic adjusted reading and interpretation by expert microbiologists, to

We therefore reviewed the market to find an existing and customized camera-based reader with the criteria of being robust, affordable, with low energy requirements and accurate. The particularity of the Microscan MIC panel it that the size of the plate and arrangement of the wells are not standard but a proprietary feature, therefore eliminating the possibility to use the mobile phone-based micro-plate reader (Figure 40) developed by UCLA[132], [133]. We therefore selected the BIOMIC V3 system (Giles Scientific, New York, N.Y.), a semiautomatic video-assisted plate reader, composed of an image capture card, a cabinet with a video camera, and a software. The Biomic video system automatically (Figure 41) reads and interprets zone diameter or MIC and reports antimicrobial agent disk diffusion, broth microdilution, agar dilution, and antibiotic gradient method susceptibility results [134], [135]. The Biomic V3 requires manual loading and unloading of one microplate at a time.



Figure 41: BIOMIC Video Reader System, Giles Scientific (USA)

see if the difference met MSF requirements. Out of five articles found evaluating the accuracy of the Biomic V3 [134]–[138], none of them investigated this difference. Therefore we decided to evaluate the accuracy of the BIOMIC V3 in reading and interpreting the results, automatically and adjusted, from the MSF Microscan MIC panels read visually initially by microbiologist experts.

3.2. Methods

This evaluation has been done in parallel to the experiment previously described in section 2 of this chapter[101] in June 2019, therefore only the specific part of the methodology applied to this experiment will be described in this methodology section. The same 387 anonymized clinical isolates were used, either fresh, recently frozen, or from stock. 123 Gram-positive strains were tested on the MICPOS1, of which, 60% (74) were *Staphylococcus* spp. isolates and 40% (49) were *Enterococcus* spp. isolates. 157 Gram-negative rod isolates were tested on the MICNEG1, of which 72% (112) were Enterobacterales and 28% (45) were non-fermenting Gram-negative rods. On the MICFAST1, 107 fastidious isolates were tested, of which 82% (87) were *Streptococcus* spp. isolates and 18% (20) were *Haemophilus influenzae* isolates. As per ISO recommendations for evaluating the performance of AST [36], at least 25% of the isolates in the entire study were from fresh clinical samples.

3.2.1. Biomic automated systems and visual reading.

A standard commercially available Biomic V3 microbiology system with the Biomic 2018 software was purchased from Giles Scientific Inc. This system consisted of the standard Biomic V3 reader cabinet containing light-emitting diode (LED), visible light with a high-resolution colour digital camera, a personal computer with the Windows operating system, and Biomic 2018 clinical microbiology software. Additionally, each system included the Biomic automated well reader software module. Customisation of the software consisting in developing the three panels layout with the composition of the molecules and dilutions tested, was requested to read the MSF Microscan MIC panels (MICPOS1, MICNEG1 and MICFAST1). The MSF MIC panels inoculated from the evaluation of the MSF Microscan panels were read 16–20 h after incubation. Test panels were firstly read visually by two experts then by a third expert using the automated Biomic reader. Because of a problem during the experiment (mismanagement of the inoculum to be used), for MICPOS1 panels, reading with Biomic was only done on the panels inoculated using the PROMPT system, for other panels (MICNEG1 and MICFAST1) the panel inoculated using standard Mc Farland method were read. For inter-reading variability calculation, laboratory technicians were blinded to each other's

results. If a discrepancy in visual reading was found, a consensus was made among readers for the final reading results. Reader using Biomic was able to change status of MIC proposed by the system from the picture display on the screen only if it disagreed with the proposed reading. All visual results were recorded onto specific bench sheets and imported into WHONET version 5.6, [40,41]. Both Biomic software and WHONET use the breakpoint table of EUCAST version 9.1 (2019) [33] to interpret the MIC results.

3.2.2. Ease of Use

Assessment of the ease of use was done by surveying the operators with a questionnaire for feedback on each of the components of the system. The readability level of the IFU was assessed using Flesch–Kincaid Grade levels (https://www.online-utility.org/english/readability_test_and_improve.jsp; accessed on 20 March 2021) [13].

3.2.3. Data Analysis

Data from the BIOMIC software were extracted to capture, raw and adjusted MIC reading, raw and adjusted interpretation, hereafter mentioned as raw automatic MIC reading (raMIC), operator adjusted MIC reading (adMIC), raw automatic interpretation (raRIS), operator adjusted interpretation (adRIS), together with visual MIC reading (viMIC) and visual interpretation (viRIS); All data were transferred under Microsoft Excel 2019 (version 2110). Statistical analyses were performed in R (version 4.0.2) using RStudio or Microsoft Excel 2019 (version 2110). Categorical agreement (CA), meaning the addition of very major errors (VMEs), major errors (MEs), and minor errors (mEs) were calculated as described in the ISO 20776:2 2007 standard [35]. CA was determined for raRIS, adRIS, viRIS, against referral AST method (previously described in section 2) to capture discrepancy toward AST reference method of each reading. They will be named hereafter as raCA; adCA and viCA. The acceptance criteria for this study were the same than previously described [35]: $CA \geq 90\%$; $ME \leq 3\%$, $VME \leq 3\%$. The essential agreement (EA), MIC reading method is equal to or within +/- 1 dilution of the MIC reference result, was calculated between raMIC and viMIC or between adMIC and viMIC, the last one being the MIC reference result. For inter-observer agreement, measure of the reliability of reading the MIC by a reading method (raMIC or adMIC) against the viMIC, was done using the calculation of Cohen's kappa (CK) coefficient [42]. A $CK > 0.8$ was considered as a very good agreement; $0.6 < CK \leq 0.8$ as a good agreement; $0.4 < CK \leq 0.6$ as a moderate agreement; $0.2 < CK \leq 0.4$ as a fair agreement; and $CK \leq 0.2$ as poor agreement..

3.3. Results

3.3.1. MSF Gram-Pos panel reading methods results

Individual antimicrobial results are presented in Table 8 for *Staphylococcus* spp. and *Enterococcus* spp. For Category Agreement between the AST reference interpretation results and reading methods of the MICPOS1 panel, CA strictly below 90% was observed out of 20 molecules tested for four molecules (20%) with viRIS, five molecules (25%) with adRIS and by four molecules (20%) with raRIS. EA strictly below 90% was observed out of 20 molecules tested for 12 molecules (60%) with adMIC and by 13 molecules (65%) with raMIC. Overall, raCA is higher than viCA and adCA with respectively 94,5%, 93.1% and 91.4%. In general, for the MICPOS1 panel, adjusted and raw automatic MIC reading agreement against visual MIC reading is classified as good agreement with a CK of 0.75 and 0.82 respectively.

Table 8: Results of reading methods for *Staphylococcus* spp. and *Enterococcus* spp. using MICPOS1 panel

Antimicrobial	n _a	Category agreement			Essential agreement		MIC Agreement	
		viCA _b [%]	adCA _c [%]	raCA _d [%]	adEA _e [%]	raEA _f [%]	adCK _g	raCK _h
Penicillin	63	100	98	100	91	90	0.7	0.95
Ampicillin	123	96	96	96	87	86	0.72	0.76
Cefoxitin screen	74	99	99	99	92	92	0.98	0.95
Ciprofloxacin	49	94	87	94	80	81	0.76	0.89
Amikacin	123	78	65	84	78	76	0.73	0.78
Gentamicin	123	91	93	100	89	87	0.87	0.92
Gentamicin (high level)	49	92	96	96	92	92	0.6	0.9
Teicoplanin	123	98	93	98	90	89	0.86	0.79
Vancomycin	123	97	100	100	92	86	0.82	1
Quinupristin-Dalfopristine	123	91	92	93	92	91	0.9	0.93
Erythromycin	74	88	88	87	84	85	0.78	0.97
Clindamycin	74	97	97	97	91	90	0.96	0.99
Inducible clindamycin resistance	74	99	100	100	92	92	0.98	0.96
Daptomycin	74	96	96	100	89	88	0.89	0.37
Fosfomycin	74	81	77	76	85	81	0.45	0.63
Trimethoprim/Sulfamethoxazole	74	81	77	81	83	77	0.68	0.62
Linezolid	123	97	96	100	79	74	0.42	0.37
Tetracycline	74	97	93	97	88	89	0.89	0.94
Tigecycline	123	96	93	98	84	85	0.18	0.82
Average panel tested		93,1	91,4	94,5	87,3	85,8	0,75	0,82

_a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; _b viCA, Category Agreement between visual reading of the MSF Microscan panel and the AST reference method.; _c adCA, Category Agreement between operator adjusted automatic reading of the MSF Microscan panel and the AST reference method.; _d raCA, Category Agreement between raw automatic reading of the MSF Microscan panel and the AST reference method.; _e adEA, Essential Agreement between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; _f raEA, Essential Agreement between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; _g adCK, Cohen Kappa between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; _h raCK, Cohen Kappa between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.;

3.3.1. MSF Gram-Neg panel reading methods results

Individual antimicrobial results are presented in Table 9 for Enterobacterales and Non-fermenting gram-negative bacilli. For Category Agreement between the AST reference interpretation results and reading methods of the MICNEG1 panel, CA strictly below 90% was observed out of 16 molecules tested only for three molecules (5%) with raRIS. We observed a low CA for tigecycline(16%), imipenem (60%), piperacillin-tazobactam with raRIS. EA strictly below 90% was observed out of 16 molecules tested for only two (8%) molecules (ciprofloxacin and ertapenem) with adMIC and by 7 molecules (65%) with raMIC. Overall, raCA is higher than viCA and adCA with respectively 94,5%, 93.1% and 91.4%. In general, for the MICNEG1 panel, adjusted and raw automatic MIC reading agreement against visual MIC reading is classified as good agreement with a CK of 0.89 and 0.75 respectively.

Table 9: Results of reading methods for Enterobacterales and Non-fermenting gram-negative bacilli. using MICNEG1 panel

Antimicrobial	Category agreement				Essential agreement		MIC Agreement	
	n _a	viCA _b [%]	adCA _c [%]	raCA _d [%]	adEA _e [%]	raEA _f [%]	adCK _g	raCK _h
Ampicillin	112	100	100	99	100	99	0.93	0.88
Amoxicillin/Clavulanic Acid	112	98	99	99	99	99	0.98	0.94
Ceftazidime	127	96	95	93	100	98	0.98	0.88
Ceftriaxone	112	99	98	97	96	92	0.95	0.88
Piperacillin/Tazobactam	127	96	95	71	97	68	0.96	0.59
Ciprofloxacin	141	93	93	92	78	82	0.89	0.92
Amikacin	141	95	95	96	99	66	0.98	0.96
Gentamicin	141	94	95	94	92	99	0.91	0.97
Trimethoprim/Sulfamethoxazole	142	96	97	95	99	93	0.98	0.89
Chloramphenicol	121	97	98	92	96	91	0.94	0.85
Colistin	127	97	98	96	94	93	0.89	0.87
Fosfomycin	112	99	98	96	95	93	0.85	0.79
Tigecycline	112	100	100	16	99	47	0.97	0.37
Meropenem	157	93	94	93	90	54	0.57	0.45
Imipenem	141	92	92	60	96	71	0.92	0.36
Ertapenem	112	99	99	97	85	81	0.51	0.4
Average panel tested		96,5	96,6	86,6	94,7	82,9	0,89	0,75

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b viCA, Category Agreement between visual reading of the MSF Microscan panel and the AST reference method.; ^c adCA, Category Agreement between operator adjusted automatic reading of the MSF Microscan panel and the AST reference method.; ^d raCA, Category Agreement between raw automatic reading of the MSF Microscan panel and the AST reference method.; ^e adEA, Essential Agreement between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^f raEA, Essential Agreement between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^g adCK, Cohen Kappa between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^h raCK, Cohen Kappa between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.;

3.3.1. MSF Gram-FAST panel reading methods results

Individual antimicrobial results are presented in Table 10 for *Haemophilus* spp. and *Streptococcus* spp. For Category Agreement between the AST reference interpretation results and reading methods of the MICFAST1 panel, no CA strictly below 90% was observed. Out of 11 molecules tested, we found that EA below 90% was observed for all molecules MIC read by both methods against the visual MIC reading. Overall, viCA is slightly higher than adCA and raCA with respectively 98.5%, 96.9% and 96.9%. In general, for the MICFAST1 panel, adjusted and raw automatic MIC reading agreement against visual MIC reading is classified as moderate agreement with a CK of 0.69 and 0.52 respectively.

Table 10: Results of reading methods for fastidious organisms (*Haemophilus* spp., *Streptococcus* spp.) using MICFAST1 panel

Antimicrobial	n _a	Category agreement			Essential agreement		MIC Agreement	
		viCA _b [%]	adCA _c [%]	raCA _d [%]	adEA _e [%]	raEA _f [%]	adCK _g	raCK _h
Penicillin	53	97	98	94	68	67	0.81	0.33
Meropenem	73	100	100	99	72	72	0.92	0.85
Ceftriaxone	20	100	100	99	70	70	0.48	0.46
Ampicillin	107	100	100	94	72	63	0.77	0.4
Ciprofloxacin	20	100	100	100	72	70	0.8	0.8
Levofloxacin	83	99	99	99	73	73	0.3	0.27
Vancomycin	87	99	99	99	76	74	0.27	0.33
Clindamycin	87	92	91	90	75	69	0.92	0.52
Trimethoprim/Sulfamethoxazole	107	96	96	95	72	54	0.84	0.43
Chloramphenicol	107	100	99	99	75	75	0.71	0.52
Linezolid	63	100	100	98	75	75	0.81	0.79
Average panel tested		98,5	98,4	96,9	72,7	69,3	0,69	0,52

^a Number of isolates tested with the reference method and classified as R resistant; I, susceptible, increased exposure; S, susceptible.; ^b viCA, Category Agreement between visual reading of the MSF Microscan panel and the AST reference method.; ^c adCA, Category Agreement between operator adjusted automatic reading of the MSF Microscan panel and the AST reference method.; ^d raCA, Category Agreement between raw automatic reading of the MSF Microscan panel and the AST reference method.; ^e adEA, Essential Agreement between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^f raEA, Essential Agreement between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^g adCK, Cohen Kappa between operator adjusted automatic MIC reading and the visual MIC reading of the MSF Microscan panel.; ^h raCK, Cohen Kappa between raw automatic MIC reading and the visual MIC reading of the MSF Microscan panel.

3.3.2. Ease of Use

The use of Biomic to load and unload MIC Microscan panel was highly appreciated by the users. However the use of the software was described as not so easy to use by the three operators, all of them mentioned the difficulties in navigating among the different features of the software to find the most adequate process to read the plate. It was noticed that all operators appreciated that user instructions were available on video formats as well as reading format. The Flesch–Kincaid grade levels (FKGL) of the Biomic written IFU were nine each. FKGL refers to US grade levels (i.e., years of

and 60% respectively, with comparable low EA of 68%, 47% and 71% respectively, meropenem was found to have an EA of 54% but with CA of 93%, comparable to the CA of visual reading for this molecule. It was noticed by the operator that for those mentioned molecules often they required to change the MIC interpretation manually as the automatic system was often not targeting the last well with growth. They mentioned as well that for the MICFAST1, the Biomic systematically had difficulties to find the MIC for penicillin, ceftriaxone, ampicillin, levofloxacin and vancomycin proposing lower MIC than expected, therefore increasing the possibility to underestimate resistance results. However, this does not impact the results as suggested by the results.

Despite some difficulties in using the Biomic software, it was noted by the operator that an interesting capability of the Biomic instrument is the ability of the microbiologist to see all tests well results on a video screen, to make interpretive adjustments as needed, and to save test panel images for supervisory review at a later time or for tele-microbiology purpose[113], [114].

3.4.1. Ease of use

Regarding the ease-of-use, operator feedback on their experience was positive regarding the hardware (screen, drawers to insert the MIC panel, etc.); setup does not require multiple steps to be installed. All equipment and the Biomic itself look robust to be transported on a bumpy road, especially with the protection provided within the packaging. However, user feedback was mixed when it comes to the software and interface use. Biomic software is built on old technology (mysql) and user experienced is different from what we can today expect from such device. Although the language used in documents may be at a slightly higher level, Flesch–Kincaid levels below six are desirable for IFU [55,56] but video instruction makes process understanding easiest.

3.4.2. Strengths and Limitations of the Study

To our knowledge this evaluation is the first evaluating the difference of reading performance between fully automatic and operator adjusted reading and interpretation capacity of the Biomic V3[134]–[138]. There are several limitations to the verification of the reading agreement; firstly, we have not been able to evaluate Biomic types of readings using the same inoculation method for all MSF MIC panels, due to an incident in the study process we had to read the MICPOS1 panels inoculated by PROMPT method. Also, because we did not have access to Biosafety Level-3 (BSL-3) facilities, we could not test pathogens such as *Burkholderia pseudomallei*. Furthermore, comparing disc diffusion to MIC values is by itself a limitation of this study, but otherwise would have not been financially possible.

3.5. Conclusion

While it is clear that the Biomic hardware is simple, easy to use, robust, the software use would take time and some training to get used to. In parallel, our results suggest that it is difficult to conclude that automatic reading is at the same level as the visual reading with expert users, results varied from one panel to another, from a molecule to another and we did not find clear pattern of errors that could explain these fluctuations (e.g. different growth according to the bacteria or the molecules). While it is clear that Biomic automatic or adjusted reading is not superior to visual reading, in the hands of an expert microbiologist, however on the fields of MSF intervention, where skills resources are scarce; automatic reading might provide advantages or disadvantages for final results as it is clear that the operator might be willing to change interpretation based on the picture provided. This experience should be repeated on field conditions with the final intended users to understand the extent of the Biomic value in comparison to its price.

Chapter 4- Does a Simplified Clinical Bacteriology Laboratory Provide Quality and Value?

1. Introduction

Key milestones within the development pathway for an innovative diagnostic approach for infectious diseases, is being able to demonstrate at the site of intended use, with the intended population for testing that the diagnostic is robust, easy-to-use and meets all performance requirements.

Prior to evaluating the Mini-Lab as a whole, and under real-use conditions, given the complexity of the concept, it was decided to first “verify” the “basic” operative version of the laboratory, the first Mini-Lab prototype, in a controlled environment and in a modern European laboratory. Then to determine in a field setting whether the procedures were suitable, simple, and easily reproducible by laboratory technicians with ad hoc training who did not have specific prior microbiology skills. Finally, to verify in real use conditions and on clinical specimens to determine the accuracy of the Mini-Lab results, its ease of use and the impact of results to treatment decisions. Each of these steps helped to identify potential improvements to usability, robustness, and performance.

In this chapter the outcome of the initial verification study is discussed. The validation of the procedures for the Mini-Lab prototype version 1 is presented, via a field evaluation in an MSF Haiti hospital (Port au Prince, Haiti), without integration into patient care. Finally, the evaluation of the Mini-Lab prototype version 2 is described where results were integrated into care management decisions within the MSF Carnot hospital (Carnot, Central African Republic). Prototype iteration will be described, focusing on the main improvements.

The work done in the evaluations have been published in peer-reviewed journals or presented at conferences as outlined below:

- A. Natale, **J-B. Ronat**, A. Mazoyer, A. Rochard, B. Boillot, J. Hubert, B. Baillet, M. Ducasse, F. Mantelet, S. Oueslati, S. Ombelet, C. Langendorf, T. Naas, O. Vandenberg, J. Jacobs, “The Mini-Lab: accessible clinical bacteriology for low-resource settings”, **The Lancet Microbe**, 2020 1,2,e56-e58,
- **J-B. Ronat**, A. Natale, T. Kesteman, A. Andreumont, W. Elamin, L. Hardy, R. Kanapathipillai, J. Michel, C. Langendorf, O. Vandenberg, T. Naas*, F. Kouassi*; “AMR in low-resource settings: Médecins Sans Frontières bridges surveillance

gaps by developing a turnkey solution, the Mini-Lab,” *Clin. Microbiol. Infect.*, vol. 27, no. 10, pp. 1414–1421, 2021, doi: 10.1016/j.cmi.2021.04.015.

- **J-B. Ronat**, “Development of an all-in-one transportable clinical bacteriology laboratory: feedback from testing the Mini-Lab prototype in Haiti”, **MSF Scientific day**, 16 Aug 2021, Online, oral presentation
- **J-B. Ronat**, « Development of an all-in-one transportable clinical bacteriology laboratory: feedback from testing the Mini-Lab prototype in Haiti”, **Congress of the African Society of Laboratory Medicine**, 24 Nov 2021, Online, oral presentation
- **J-B. Ronat**, “Early results of the Mini-Lab field evaluation, an all-in-one transportable clinical bacteriology laboratory for low-resource settings”, **European Congress on Clinical Microbiology and Infectious Diseases**, 29 April 2022, Portugal, oral presentations

2. Field Evaluation of Performances of the Mini-Lab – Port-au-Prince Burn Hospital, Haiti

2.1. Introduction and background

During one week from the 4th of February to the 8th of February 2019, in Saint-Pierre Laboratory University Hospital (LHUB-ULB) in Brussels (Belgium), the complete Mini-Lab workflow was verified in a controlled environment. LHUB-ULB represented an ideal site for its space availability, and proximity to a fully equipped microbiology laboratory, availability of experts on site; and all the comforts of a high resource setting. This allowed the Mini-Lab and its components to be tested without difficulties (Figure 43). The first step of the study was the verification of the installation procedure of the box benches modules (version made of plastic sheet boiling assembly). This was undertaken by 2 lab technicians, with the assistance of the Mini-Lab team. It was to verify that all of the components were present for a correct assembly (“Tetris system” tested before with logistics for the arrangement of each equipment packed into the box benches ready to be transported) and to ensure that the instruction manual was adequate. The contribution given by the “inexperienced eyes” of the lab technicians was very important for improvements to the instruction manual. It was also found that the process was quite cumbersome as the “boxes” were quite heavy, but in total it took less than a day to set up the full Mini-Lab, showing the potential of the Mini-Lab to be assembled and be fully functional in a short period of time.

A second aspect tested was the general comfort of the laboratory, including lighting and space. The choice of the light on the manipulation bench has been a point of high interest during the design for its critical role in ensuring that the operator works in an adequately illuminated and safe space[139]. In this study the choice of the lights, module function and distribution in space was



Figure 43: Photo taken during the MVP testing in February 2019. From top to down, all modules in closed position, opening of a module, Tetris like assembly in a module, module assembled, laboratory technician working on a module, Mini-Lab (France)

considered good for the work benches. The main point of concern in terms of comfort was the available space, in particular on two of the work benches, module 3 (sample process) and module 5 (test readings). With 2 lab technicians working full time in such a space, workflow organisation and daily task management becomes essential. It appeared clear that this was an area for improvement for the next development iteration of the Mini-Lab.

During the verification of the analytical workflows, some critical steps were highlighted. Firstly, the Pre-ID system at that time was methylene blue staining, aminopeptidase, catalase, and oxidase tests with results interpreted based upon a printed decision trees to provide classification. While the process provided appropriate results in terms of bacterial group recognition three main problems were highlighted. The problems are described as follows together with the approach to mitigate them:

- The methylene blue staining is not easy to interpret, plus some experience in using a microscope was required. A decision was made to focus on improving the training for use of the microscope and reading of the slides plus the use of an atlas of bacterial shapes as part of training and as a bench aid.
- The aminopeptidase test cannot be performed on the same day as the methylene blue staining, because of the difficulty (and inaccuracy of results) of recovering colonies from the blood culture agar slant. As this test can be performed only from colonies from the subcultures, it causes a delay in reporting Gram results (intermediate results). Some alternative strategies were then taken into consideration, such as the use of a bacterial culture pellet or performance of the test after subcultures with shorter incubation time (4-6hrs). In addition, a guide for reporting intermediate results to clinicians has been planned.
- There was no test to discriminate at an earlier stage between possible contaminants (coagulase-negative Staphylococci, *CNS*) and pathogens, important for the appropriate reporting of results, lowering costs and timeliness in the request of an additional blood culture. A market review was performed and a decision to incorporate the coagulase tests was made.

Secondly, the automated reader (Biomic V3, Gilles Scientifique, Santa Barbara, USA) for the ID panels gave discordant results, for some very common species. The manufacturer was contacted and work on the database was performed by the manufacturer. The update of the database was supposed to reduce these kinds of errors. Manual reading of the panels was considered easy by the lab technicians, although for the ID panels it was highlighted that there was a difficulty in distinguishing the colours of the reactions. This was in agreement with previous experiences and with early results of

the validation studies performed at ITM and led to the development of colour-coded bench aids (Annex 11 and 12)

The following were confirmed for ease-of-use and safety during this study by both the lab technicians and external experts:

- sub-culturing system,
- inoculation system,
- dehydrated ID and AST panels,
- light box for blood culture reading,
- incubators
- autoclave,
- first mock-up of the LIMS system onto a non-code open-source platform (Joget, Columbia, USA),

In this version of the Mini-Lab, the mock-up LIMS was only integrating for sample workflow and data management. It was important to consider that one of the biases of this exercise was the experience of the lab technician, which was probably much higher than of lab technicians often recruited in field conditions in LRSs. Also, their viewpoints could have been influenced towards positivity by their enthusiasm in participating in this study and their wish to not disappoint.

After a few months to incorporate changes and improvement, the evaluation of the Mini-Lab version 1 was initiated. This evaluation was of the Mini-Lab as a whole, under real-use conditions with clinical and simulated samples. The purpose of the evaluation was to; (i) assess the **usability** of the Mini-Lab in field conditions, to (ii) describe the **ease-of use** of each analytical component and functional pre-analytical steps of the Mini-Lab (iii) describe the ease of **compliance by the lab technicians with the SOPs of the analytical phase** (BCB reading, pre-ID, ID, AST), (iv) to evaluate the **inter-operator agreement** on each analytical component between 2 different laboratory technicians reading the results from the same sample, (v) to evaluate visual reading of ID and AST compared to automated reading, and (vi) to evaluate the **agreement and accuracy of each analytical component** (pre-ID, ID and AST) of the Mini-Lab compared to reference methods generated in a European Union (EU) reference laboratory. The clinical specimens analysed in the Mini-Lab during the field evaluation were not used for clinical management decisions, but only to assess the performance and accuracy of the Mini-Lab results.

2.2. Methods

2.2.1. Study design and study site

The study was prospective and was a descriptive field evaluation of the first Mini-Lab prototype. This evaluation took place at MSF's Drouillard Hospital (Burn Unit) in Port-au-Prince, Haiti, opened since 2015 (Figure 44). There are 40 inpatient beds, including 10 in the Intensive Care Unit (ICU). With between 1,000 and 1,500 emergency admissions per year, Drouillard is the primary referral centre for severe burn victims in Haiti. Drouillard Hospital was also one of the pilot projects for the antibiotic stewardship program implemented in 2017. Microbiology tests in Drouillard hospital are outsourced to the clinical service of Gheskio Centre.

2.2.2. Study participants and samples

The evaluation of the Mini-Lab was conducted among:

- 1 *Laboratory technicians* having a certificate and prior work experience, having attended the training for the Mini-Lab implementation or trained later for integration into the Mini-Lab team, hired by MSF to work full-time and work throughout the whole period of the evaluation, and fulfilling all eligibility criteria.
- 2 *Blood samples (one extra BCB compared to the routine)* collected from hospitalised individuals (≥ 1 year of age), presenting with a suspicion of bloodstream infection and for whom the clinician had prescribed a blood culture according to an algorithm currently in place and whose parent(s) or guardian(s) had consented in writing to participation in the study.
- 3 *Blind spiked blood cultures* with clinical strains provided by Bicêtre and prepared blindly by the microbiologist onsite (containing or not-containing clinical strains of bacteria).



Figure 44: Photo of the emergency entrance of the MSF Burn centre, Drouillard, Haiti; © Lunos Saint Brave / MSF

2.2.3. Specimen collection and transfer to the Mini-Lab

For each individual enrolled, a blood culture sample was collected in the burns unit by a nurse trained in BCB collection (20 ml for adults and 5-10 ml for children). The bottles for the study (Autobio© biphasic) were sent to the Mini-Lab within a maximum

of 30 minutes post collection. Once the BCB arrived in the Mini-Lab, it was handled by the Mini-Lab technicians according to the SOP.

2.2.4. Storage of Bacterial Isolates and Strain Transfer

Each bacterial isolate (from the first subculture containing colonies) was stored in triplicate in a cryovial with microbeads and cryopreservation solution (Microbank™, Pro-Lab Diagnostics) at -20°C until it was prepared for shipment to the reference laboratory in Europe. All bacterial strains isolated in the Mini-Lab were shipped to the Bicêtre, Bicêtre teaching university hospital, Paris, France. Bacterial strains for the preparation of simulated samples in the Mini-Lab were selected and prepared in the Bicêtre and shipped in dry ice (-80°C) to the Mini-Lab. These strains were pre-labelled with a unique number. The list of organisms was not known to any person working at the Mini-Lab Haiti site. Therefore, the analyses were done in a blinded manner.

2.2.5. Data Collection

Daily, the study assistant, registered all individuals with blood cultures received in the Mini-Lab during the study period, and evaluated their eligibility.

Data generated by the Mini-Lab regarding blood culture samples and bacteriological results were entered into a pseudonymized, secured, password-protected database by the Mini-Lab-LIMS (designed on the joget.org platform). Data regarding Mini-Lab usability was collected via dedicated questionnaires and then entered into REDCap by a study co-investigator. All gram staining, subcultures and ID/AST plates were photographed and classified in Sharepoint. The **bacteriological results** were not made available to clinicians but were recorded on a LIMS mock-up form.

To assess the **inter-operator agreement**, each reading of ID and AST was performed blindly by two laboratory technicians and by the on-site microbiologist.

To assess if the analytical procedures were executed in accordance with the corresponding SOPs, test of compliance to SOPs were carried out. A checklist evaluating each detailed step of an analytical procedure was completed by the supervisor by observing the laboratory technicians without making any comments to the technicians and allowed to calculate a score. This assessment was completed after each training in September and December of 2019.

To evaluate the **ease-of use** of each component of the Mini-Lab, a self-administered user experience questionnaire, using an Osgood scale/Likert-type scale was used. Ease of use included: the ease of sample reception procedure, ease of test procedures, ease of interpretation, ease of reporting test results and ease of quality control. The

questionnaire was administered after the initial training (in September 2019), after the retraining in December 2019 and then in April and June of 2020.

Competency testing on key analytical, pre- and post-analytical steps were also regularly conducted to assess potential improvements in testing and procedures. Direct observation of the routine work processes and procedures by the supervisor using a checklist was utilised to calculate a proficiency score multiple times during the study, after the initial training (in September 2019), after the retraining in December 2019, and then in April and June 2020.

2.2.6. Laboratory Analysis in the Reference Laboratory

At the EU reference laboratory, all bacterial isolates were identified by a reference method, utilising MALDI-ToF (Matrix Assisted Laser Desorption Ionisation - Time of Flight). AST was performed using the disk diffusion method and E-test or microdilution to determine an organism's MIC according to EUCAST v9 and also by the same method as used in the Mini-Lab (Beckman-Coulter) with reading with the Biomic with visual adjustment (Table 11).

Table 11: Reference techniques in the reference laboratory

	Criteria of comparison	Mini-Lab	Reference methods
BC	Growth/Non-growth	Autobio	Not applicable
Pre-ID	Gram+/-	Oxidase + Catalase + Aminopeptidase + Blue Methylene Blue staining	MALDI-TOF (Bruker)
ID test	Bacteria Genus, species	Commercial ID panel (Beckman-Coulter)	MALDI-TOF (Bruker)
AST	MIC	Commercial AST panel (Beckman-Coulter)	Reference 1: Commercial AST panels (Beckman-Coulter) with adjusted Biomic reading Reference 2: Disc diffusion method or MIC according to EUCAST v9 recommendations

2.2.7. Data analysis

Extracts from the pseudonymized mock-up LIMS Joget and Biomic databases were analysed with Stata 15 software (College Station, Texas, USA). Descriptive data were presented in frequency and percentages. All outcomes collected and calculated to answer objectives of this study are described in the Table 11.

Table 12: Primary and secondary outcome of the study

Type of outcome	Primary outcome s
Ease of use	Ease of sample reception procedures as measured by a questionnaire score (1 questionnaire/ lab personnel after training, after 3 months and at the end of the study)
	Ease of test procedures and interpretation of analytical components as measured by a questionnaire score (1 questionnaire/ lab personnel after training, after 3 months and at the end of the study)
	Ease of results sharing procedures as measured by a questionnaire score (1 questionnaire/ lab personnel after training, after 3 months and at the end of the study)
	Compliance with analytical and post-analytical SOPs (BCB reading, pre-ID, ID, AST, result report) as measured by a competency testing score (1 questionnaire/ lab personnel after training).
	Incident and near-accident reporting
Type of outcome	Secondary outcomes
Agreement and accuracy compared to reference methods	Agreement and misclassification between the visual readings of the 2 laboratory technicians: Visual 1 vs. Visual 2
	Agreement and misclassification between the best visual reading by a technician and automated reading by Biomic
	Agreement and misclassification between the best visual reading by a technician and automated reading corrected (adjusted) by the microbiologist
	Agreement and misclassification between the different readings (visual, automated, adjusted) and reference results of the MALDI TOF method in Bicêtre Laboratory
	Agreement and misclassification between the different readings (visual, automated, adjusted) and reference results of conventional methods in the reference laboratory, Categorical Agreement, Major Error Rate, Very Major Error Rate*

* The error classification was done according to ISO20776-2: 2007 reference and as previously described in Chapter 3, Categorical Agreement: the MIC interpretation (resistant, susceptible, or intermediate or susceptible high dosage) is consistent with the reference; Major Error: Result given as resistant instead of susceptible according to reference; Very important error: Result given as susceptible instead of resistant according to reference

2.2.8. Ethical considerations.

The study was conducted in accordance with the Helsinki Declaration on Ethical Principles for Medical Research Involving Human Subjects. After clearance from the Steering, and Scientific Committees of the Mini-Lab project, the protocol was approved by the National Committee of Bioethics (CNB) of Haiti (Ref 1819-60 on 14 Aug 2019) and the MSF Ethics Review Board (Ref 1913 on 4 June 2019). Inclusion in the study was voluntary and required prior signed informed consent. Participants and participants' parent(s)/caregiver(s) for minors were informed in French or in Creole of the aim of the study and eligibility criteria by a trained health assistant. Eligible individuals interested in study participation received more detailed information about the study objectives, study procedures, and a clear explanation of the risks and benefits of participating in the research.

2.3. Results

The Mini-Lab prototype V1 was installed into a 20²m room close to the main hospital building in June 2019 by members of the Headquarters' Mini-Lab project team with the support of onsite logisticians (Figure 45). An experienced microbiologist from the Mini-Lab team was present onsite for the duration of the study as the study supervisor. The team collected feedback on the deployment phases to improve the process, this will not be described herein.



Figure 45: Deployment of the Mini-Lab V1 by the logistician team, Mini-Lab (France)

2.3.1. Samples Processed in the Mini-Lab



Figure 46 : Training of the laboratory technicians at the Mini-Lab facility, Mini-Lab (France)

The study officially started on September 23, 2019, but was suspended on September 27, 2019, for staff safety reasons. The study resumed on December 5, 2019, after a one-week refresher for the 2 laboratory technicians initially trained in September (Figure 46). The study was terminated early on June 15, 2020 (instead of late July 2020) due to the SARS-CoV-2 pandemic as Drouillard Hospital was converted into a COVID centre. The period from December 2019 to June 15, 2020, was utilized for analysis.

Sample Analysis: During the study period a total of 103 samples were processed. Among them, 30 individuals had blood cultures between December 13 and March 31 (22 had one blood culture, and 8 had 2 blood cultures several days apart). A total of 38 blood cultures from individuals were received at the Mini-Lab, of which only 37 were analysed due to a major protocol deviation for one sample. Among them 16 were positive with the organisms describe (Table 13: List of organisms identified in the blood cultures of patients included in the study. Due to the low number of organisms collected no further description on population characteristics will be provided.

Table 13: List of organisms identified in the blood cultures of patients included in the study

Identified Organisms	n
<i>Klebsiella pneumoniae</i>	4
<i>Staphylococcus epidermidis</i>	4
<i>Staphylococcus haemolyticus</i>	2
<i>Staphylococcus aureus</i>	2
<i>Acinetobacter lwoffii</i> group	1
<i>Enterococcus durans/hirae</i>	1
<i>E.coli</i>	1
<i>Staphylococcus auricularis</i>	1
Total	16

Simulated blood cultures: A total of 105 different strains provided by Bicêtre were tested with the Mini-Lab between February and June 2020: 46 Gram-negative, 44 Gram-positive and 15 fastidious organisms. Each strain was used multiple times to provide a total of 450 blood cultures between February and June 2020 (Table 14 and Table 15). In parallel with these simulated blood cultures, the microbiologist prepared 2 to 3 negative blood cultures per day between February and June 2020. They were inoculated with blood without a bacterial strain. A total of 246 negative control blood cultures were tested. Thirty (30) of them were positive due to contamination.

Table 14: Number of strains used for simulated blood cultures

	Number of different strains tested	Number of repetitions
Gram-negative	46	209
Gram-positive	44	203
Fastidious organisms	15	38

Table 15: List of organisms sent from Bicêtre Laboratory (France) to the Mini-Lab in Haiti in February 2020

Organisms	Number	Resistance Profile
<i>Aerococcus viridans</i>	1	
<i>Citrobacter freundii</i>	2	1 ESBL
<i>Citrobacter youngae</i>	1	
<i>Enterobacter cloacae</i>	9	3 ESBL - 7 Fluoroquinolones resistant - 5 aminoglycoside resistant
<i>Escherichia coli</i>	13	2 ESBL - 3 Carbapenem resistant - 9 Fluoroquinolones resistant
<i>Haemophilus influenzae</i>	6	All Beta-Lactam sensitive
<i>Klebsiella oxytoca</i>	1	
<i>Klebsiella pneumoniae</i>	13	2 ESBL - 8 Fluoroquinolones resistant - 6 Aminoglycoside resistant
<i>Morganella morganii</i>	1	
<i>Pseudomonas aeruginosa</i>	1	Ceftazidime resistant
<i>Salmonella typhimurium</i>	3	All Ampicillin and Amoxicillin-Clavulanate resistant
<i>Salmonella Paratyphi A</i>	1	
<i>Salmonella choleraesuis</i>	1	
<i>Staphylococcus aureus</i>	25	15 Methicillin resistant
<i>Staphylococcus epidermidis</i>	11	4 Methicillin resistant
<i>Staphylococcus haemolyticus</i>	3	3 Methicillin resistant
<i>Staphylococcus hominis</i>	4	2 Methicillin resistant
<i>Staphylococcus warneri</i>	1	
<i>Streptococcus agalactiae</i>	1	
<i>Streptococcus anginosus</i>	1	
<i>Streptococcus constellatus</i>	1	
<i>Streptococcus mitis/oralis</i>	1	
<i>Streptococcus pneumoniae</i>	3	2 Ampicillin resistant
<i>Streptococcus pyogenes</i>	1	

ESBL: Extended Spectrum Beta-lactamases

2.3.2. Ease of Use and Usability of the Mini-Lab

At the end of the initial training, the two lab technicians considered all pre-analytical, analytical and post-analytical aspects of the Mini-Lab to be easy to use (score > 90%). Technician 1 found the reading of blood culture bottles to be complicated after the first training, and technician 2 found it complicated after the second training. However,

blood culture bottle reading was considered to be simple by both technicians 3 months later (score > 98%) and until the end of the study (Table 16).

Table 16: Ease of use score (%) per section of the self-administered questionnaire for 2 laboratory technicians (lab tech 1 and lab tech 2) 4 times during the study period

	Sept-2019 (After initial training)		Dec-2019 (after second training)		Apr-2020		Jun-2020	
	Lab tech	Lab tech	Lab tech	Lab tech	Lab tech	Lab tech	Lab tech	Lab tech
	1	2	1	2	1	2	1	2
Mini-Lab Comfort	97	93	100	92	95	97	97	97
Product Information	100	97	100	100	100	100	100	100
Sample Receipt	98	92	100	90	93	98	100	98
Inoculation System	100	93	98	92	93	100	100	97
Oxidase Test	100	95	97	95	98	100	100	98
Methylene Blue Test	100	97	98	98	93	100	100	100
Aminopeptidase Test	98	93	95	93	98	98	100	98
BCB	75	98	100	68	100	98	99	96
Subculture-InTray System	98	92	97	92	95	100	98	97
ID Panel Preparation and Seeding	100	91	100	94	100	96	100	97
TSA Panel Preparation and Seeding	99	90	100	89	99	96	100	94
Automated Panel Reading	100	100	100	100	100	92	100	100
Waste Autoclaving	100	100	100	98	95	97	100	100
Final Score	97	95	99	92	97	98	99	98

The analytical SOP compliance assessment (BCB, pre-ID, ID, AST reading) of the technicians by the microbiologist after the initial training, gave very high scores (> 90%) for most procedures (Table 17). Both technicians had difficulty preparing inoculum for ID/AST panels (score 73%) and reading AST panels (score 89%) after the first training, but showed good improvement after the second training in December 2019 with very high SOP compliance scores for both activities (97% and 100% respectively).

Table 17: Analytical SOP compliance score (%) assessed by the microbiologist for 2 laboratory technicians (lab tech 1 and lab tech 2), twice during the study period

	September 2019(after initial training)				December 2019 (after second training)			
	Lab tech 1		Lab tech 2		Lab tech 1		Lab tech 2	
	Score (note)	Score (%)	Score (note)	Score (%)	Score (note)	Score (%)	Score (note)	Score (%)
BCB Incubation and Reading	16/16	100	16/16	100	14/14	100	14/14	100
Subculture Inoculation	20/20	100	20/20	100	19/20	95	19/20	95
Subculture Reading	16/16	100	16/16	100	15/16	94	15/16	94
Methylene Blue Staining and Reading	18/18	100	18/18	100	17/18	94	17/18	94
AMP Creation	12/13	92	12/13	92	12/13	92	12/13	92
Oxidase	6/6	100	6/6	100	6/6	100	6/6	100
Catalase	7/7	100	7/7	100	7/7	100	7/7	100
Inoculum Preparation for ID/AST Panels	19/26	73	19/26	73	28/29	97	28/29	97
ID/AST Panel Inoculation	19/20	95	19/20	95	20/20	100	20/20	100
ID/AST Panel Incubation	15/15	100	15/15	100	15/15	100	15/15	100
ID Panel Reading	34/36	94	34/36	94	36/36	100	36/36	100
AST Panel Reading	8/9	89	8/9	89	10/10	100	10/10	100
Final Score		94.4		94.4		98		98

Concerning competency testing, technician 1s' scores were equal to technician 2 for each of the 4 assessments. The fields where the lowest scores were observed for the 4 assessments concerned: problems of identification, documentation of corrective actions, and the ability to solve or resolve issues. Competency testing showed a very good progression: from 68% to 97% (Figure 47: Competency testing final score assessed by the microbiologist for 2 laboratory technicians 4 times during the study period).

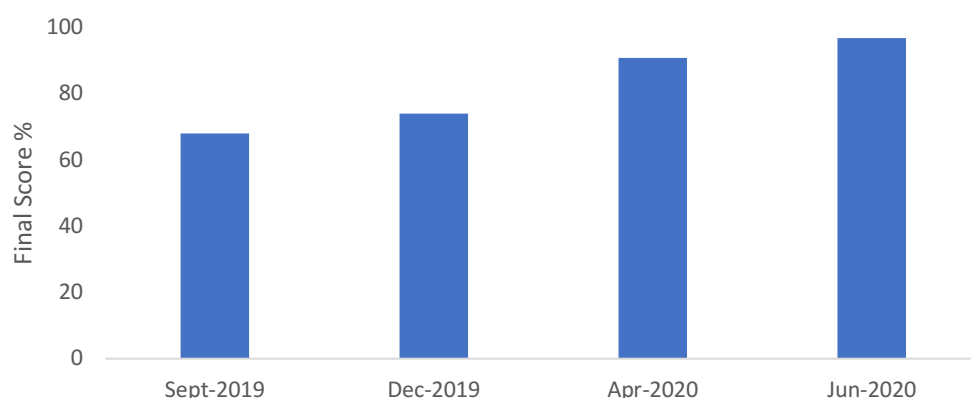


Figure 47: Competency testing final score assessed by the microbiologist for 2 laboratory technicians 4 times during the study period

Nonconformities as determined by the microbiologist described very rare handling errors by the technicians. There was for instance the use of a quality control *Enterococcus* strain instead of *Pneumococcus*, which was resolved, and one error in ID and AST panel numbering with a transcriptional error with another file (simulated strain).

2.3.3. Inter-Operator Agreement on BCB Visual Reading

Agreement on BCB Visual Reading by the laboratory technicians was not performed for technical reasons as the first reading could potentially bias the second reading due to increasing turbidity in the bottle after the first reading. However, the agreement between the BCB visual reading results and the subculture of clinical samples and simulated samples was studied. The agreement between positive BCB visual reading and subculture results was very good. No discrepancies were observed. However, there were doubts concerning the visual reading of 5 BCBs (2 clinical BCBs and 3 simulated BCBs). In two clinical samples at 24 and 48 hours, the methylene blue revealed no organism and subculturing was negative. In three simulated BCBs, the methylene blue revealed no organism and subculturing was negative. It was determined that use of expired blood (i.e. haemolyzed blood to prepare the simulated sample) added confusion to the blood culture visual reading.

The agreement between negative BCB visual readings (broth and agar) for 7 days and the systematic subculture result in D7 showed very good correlation. Among clinical samples, 27 subcultures were made on D6 and 1 was positive at the same time as the broth (reading on D7). It was *Klebsiella pneumoniae*. This clinical sample had received cloxacillin prior to sample collection. For negative simulated BCBs, 209 subcultures were made on D6 and one showed a positive reading on the same day as the broth (D7), the identified organism was *Bacillus* and was considered to be a contaminant.

2.3.4. Pre-ID Agreement and Accuracy

The reference results are gram reaction, oxidase and catalase results based on the identification as determined by MALDI ToF in the reference laboratory.

Methylene Blue

Methylene blue staining was performed from positive blood cultures broth and not from agar. In a comparison analysis with the reference, only strains whose Mini-Lab result gave the same bacterial genus as the reference result given by the laboratory in France were utilized. Minor and major errors were classified as shown in Table 18.

Table 18: Definition of minor and major errors for the interpretation of the results obtained by methylene blue staining as part of pre-ID analyses

Major Errors		Minor Errors	
Results Obtained	Reference Results	Results Obtained	Reference Results
Cocci	Bacilli	Bacilli	Coccobacilli
Cocci for all organisms except <i>Enterococcus faecalis</i>	Coccobacilli	Coccobacilli for all organisms except non-fermentative	Bacilli
Cocci	Yeast	Coccobacilli for <i>Enterococcus faecalis</i>	Cocci
Yeast	Bacilli	Cocci in chain	Cocci in clusters
Yeast	Coccobacilli		

The levels of agreement between both technicians and/or with the supervisor were very high, as was the agreement with the reference lab result (Table 19). The technicians' minor and major errors compared to the supervisor's are presented in Table 18.

Aminopeptidase

The result obtained in the Mini-Lab was a composite between the reading of both laboratory technicians and the supervisor. The agreement with the reference result were almost perfect (Table 19), only one error was observed (AMP+ for *Staphylococcus*) but this could be due to an erroneous data entry.

Oxidase

The result obtained in the Mini-Lab was a composite between the reading of both laboratory technicians and the supervisor. The agreement with the reference result were almost perfect (Table 19), 2 errors were observed:

- Oxidase+ for *Staphylococcus* but it could be due to an erroneous data entry
- Oxidase positive for Enterobacteriaceae in the absence of the supervisor

Catalase

The result obtained in the Mini-Lab was a composite between the reading of both laboratory technicians and the supervisor. The agreement with the reference result were almost perfect (Table 19), only one error was observed (catalase positive for *Streptococcus*) but it was consistent with the final pre-ID result obtained, which was *Staphylococcus*.

Table 19: Inter-operator agreement and agreement with the reference test result for pre-ID testing of the isolated strains of clinical samples or simulated blood cultures

	Methylene Blue			Amino-peptidase	Oxidase	Catalase
	Agreement Correct Result	Minor Error	Major Error			
Inter-Operator Agreement (%)				Not performed	Not performed	Not performed
Tech 1 vs. Tech 2 (n=311)	98.7	0.3	1.0			
Tech lab 1 vs. supervisor (n=466)	96.0	1.9	2.1			
Tech lab 2 vs. supervisor (n=311)	95.2	2.9	1.9			
Agreement with Reference						
Supervisor* vs. reference (n=466)	98.9	0.5	0.6	99.8	99.6	99.8

*In the case of catalase, oxidase and AMP, comparison with reference is made from the consensual results of the technicians and the supervisor

Pre-ID Final Interpretation

Out of the 466 strains analysed in the Mini-Lab, 457 (98%) gave concordant results with the organism identified later by the reference laboratory. However, 9 strains gave discrepant pre-ID results versus the final and/or reference identification (Table 20).

Table 20: List of discrepancies between pre-ID and final identification

Sample	Methylene Blue	AMP	Oxidase	Catalase	Pre-ID Final Interpretation	Organism Identified in the Mini-Lab	Reference Organism
Simulated	Cocci	Pos	Neg	Pos	<i>Acinetobacter</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Simulated	Cocci	Pos	Neg	Pos	<i>Acinetobacter</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Simulated	Cocci	Pos	Neg	Pos	<i>Acinetobacter</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Simulated	Cocci	Pos	Neg	Pos	<i>Acinetobacter</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Simulated	Bacilli	Pos	Pos	Pos	<i>Pseudomonas sp.</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter cloacae</i>
Simulated	Bacilli	Pos	Pos	Pos	<i>Pseudomonas sp.</i>	<i>Haemophilus influenzae</i>	<i>Haemophilus influenzae</i>
Simulated	Cocci in clusters	Neg	Neg	Pos	<i>Staphylococcus sp.</i> , <i>Micrococcus sp.</i>	<i>Staphylococcus hominis</i>	<i>Streptococcus constellatus</i>
Simulated	Cocci in chain	Neg	Neg	Neg	<i>Streptococcus pneumoniae</i>	<i>Streptococcus mitis/oralis</i>	<i>Streptococcus mitis/oralis</i>
Patient	Isolated Cocci	Pos	Neg	Pos	Enterobacteriaceae	<i>Acinetobacter lwoffii</i>	<i>Acinetobacter junii</i>

Non-Conclusive Results or Difficult Result Interpretation

Out of 466 strains tested (clinical and simulated), 4 (0.9%) gave temporary non-conclusive results following all pre-ID testing. All gave conclusive and consistent results after repeat testing. Pre-ID results discrepant with the reference results and giving non-conclusive results for test interpretation were: (i) 2 x *Haemophilus sp.* Cocci in chains and AMP+, (ii) 1 *Enterobacteriales*. Bacilli, AMP-, Oxidase+, (iii) 1 *Streptococcus sp.* Cocci in chain and AMP+

2.3.5. Agreement and Accuracy of Organism Identification (ID)

Several comparisons were made to define ID panel agreement and performance:

For ID testing, result agreement was defined either as absolute agreement (genus + species), or agreement with the genus (genus is concordant, but species is different), or as group agreement according to clinical relevance (the identified species belongs to the same bacteria group according to the Mini-Lab Scientific Committee classification of clinical and therapeutic relevance (Annex 16).

The number of clinical isolated strains is not sufficient to describe ID panel agreements and performances appropriately. Discussion concerning the results will therefore focus on the identification of simulated sample strains. However, all results are shown in Table 21.

The Gram-negative panel showed excellent inter-operator agreement between visual and adjusted readings. Automated reading performance was lower than visual reading performance (87% vs. 97%). Notably, automated reading showed *Shigella* or *Citrobacter* several times instead of *E. coli*, which explains a 87% agreement with the clinical relevance group.

The identification of Gram-positive bacteria (except for *Streptococcus*) showed excellent inter-operator agreement between visual and adjusted readings for the genus and the clinical relevance group (100%). Agreement with the reference was very good for visual and adjusted readings for the genus and the clinical relevance group (> 95%). However, there were many discrepancies concerning *Staphylococcus* species, which explains lower agreement for the genus + species. These discrepancies have no clinical and therapeutic impact if they involve coagulase-negative *Staphylococcus*, but they have an important impact if *S. aureus* is mistaken for coagulase-negative *Staphylococcus* and vice versa. The introduction of coagulase testing to distinguish *S. aureus* from other *Staphylococcus* in the new version of the Mini-Lab should reduce this issue in the future.

The identification of fastidious organisms (*Neisseria*, *Haemophilus* and *Streptococcus*), both visually and adjusted readings have shown good agreement at the genus level. In most cases, visual or adjusted reading did not specify species (in particular for *Streptococcus* in the Gram-positive panel). Automated reading performances were very low (26%) and more than 60% of strains were not identified at all (result obtained "Other"). For adjusted reading, discrepancies were observed for the clinical relevance groups mainly *Streptococcus*, (e.g. *S. anginosus* vs *S. pyogenes*; *S. mutans* vs *S. anginosus*; *S. agalactiae* vs *S. pneumoniae*.)

Table 21: Inter-operator agreement and agreement with reference methods for the identification of isolated organisms from simulated or patient samples, depending on the type of organism (Neg=Gram-negative; Pos=Gram-positive, except for Streptococcus)

	Simulated Sample Strains									Patient Strains						
	Absolute Agreement (Genus + Species)			Agreement Genus only			Agreement with Clinical Group			Absolute Agreement		Agreement Genus only		Agreement with Clinical Group		
	Neg	Pos	Fast	Neg	Pos	Fast	Neg	Pos	Fast	Neg	Pos	Neg	Pos	Neg	Pos	
Visual Reading 1 vs. Visual Reading 2																
n tested	92	108	25	92	108	25	92	108	25	5	3	5	3	5	3	
Agreement (n)	90	96	23	90	108	25	90	108	25	5	3	5	3	5	3	
% Agreement	97.8	88.9	92.0	97.8	100.0	100.0	97.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Visual Reading 1 vs. Automated Reading																
n tested	118	119	17	118	119	17	118	119	17	3	5	3	5	3	5	
Agreement (n)	110	105	4	110	118	5	113	115	5	1	3	2	4	2	3	
% Agreement	93.2	88.2	23.5	93.2	99.2	29.4	95.8	96.6	29.4	33.3	60.0	66.7	80.0	66.7	60.0	
Visual Reading 1 vs. Adjusted Reading																
n tested	127	125	19	127	125	19	127	125	19	3	5	3	5	3	5	
Agreement (n)	127	118	16	127	125	19	127	125	19	3	5	3	5	3	5	
% Agreement	100.0	94.4	84.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Automated Reading vs. Adjusted Reading																
n tested	157	151	23	157	151	23	157	151	23	3	9	3	9	3	9	
Agreement (n)	139	141	5	139	150	6	142	150	5	1	7	2	8	2	7	
% Agreement	88.5	93.4	21.7	88.5	99.3	26.1	90.4	99.3	21.7	33.3	77.8	66.7	88.9	66.7	77.8	
Visual Reading 1 vs. Reference																
n tested	155	163	27	155	163	27	155	163	27	6	6	6	6	6	6	
Agreement (n)	151	139	21	152	162	25	153	155	23	5	4	6	6	6	6	
% Agreement	97.4	85.3	77.8	98.1	99.4	92.6	98.7	95.1	85.2	83.3	66.7	100.0	100.0	100.0	100.0	
Automated Reading vs. Reference																
n tested	157	163	23	157	163	23	157	163	23	3	9	3	9	3	9	
Agreement (n)	137	133	6	137	151	6	137	151	6	1	7	2	8	2	7	
% Agreement	87.3	81.6	26.1	87.3	92.6	26.1	87.3	92.6	26.1	33.3	77.8	66.7	88.9	66.7	77.8	
Adjusted Reading vs. Reference																
n tested	170	162	28	170	162	28	170	162	28	3	9	3	9	3	9	
Agreement (n)	167	143	20	170	162	28	170	162	25	2	7	3	9	3	9	
% Agreement	98.2	88.3	71.4	100.0	100.0	100.0	100.0	100.0	89.3	66.7	77.8	100.0	100.0	100.0	100.0	

Non-Conclusive Results for ID

Automated reading with the Biomic was non-conclusive (result = "Other") for several simulated samples and in particularly for the fastidious organism panels.

- Gram-negative ID Panel: 11/157 (7%), *E. cloacae*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Salmonella* spp.
- Gram-positive ID Panel: 2/153 (1.3%), Coagulase-negative *Staphylococcus*.
- FAST ID Panel: 16/26 (62%), *H. influenzae*, *S. pneumoniae*, *S. pyogenes*

2.3.6. Agreement and Accuracy of Antibiotic Susceptibility Testing (AST)

The definition of extended spectrum beta-lactamase (ESBL) and Methicillin-resistant *Staphylococcus aureus* (MRSA) used for the analysis is described in Table 22.

Table 22: Criteria used for the definition of extended spectrum beta-lactamase (ESBL) and Methicillin-resistant *Staphylococcus aureus* (MRSA) from the minimal inhibitory concentration (MIC) results according to EUCAST and AST Panel manufacturers (Beckman Coulter)

Resistance Mechanism	Organisms	MIC Criteria
Methicillin-resistant	All <i>Staphylococcus</i> spp except <i>S. saprophyticus</i>	CfxS > 4 µg/mL or OXA > 2 µg/mL
Suspected MRSA	Coagulase negative <i>Staphylococcus</i> other than <i>S. saprophyticus</i> or <i>S. lugdunensis</i>	CfxS ≤ 4 µg / mL and OXA = 1 or 2 mg/L
ESBL	Enterobacterales	CTA 16 to > 16 and CTXAC ≤ 0.5/4 or CTZ 4 to > 16 and CCV ≤ 0.25/4 or CTA > 16 and CTXAC = 4/4

CfxS: Cefoxitine; OXA: Oxacillin; CTA: Cefotaxime; CTXAC: Cefotaxime-Clavulanate; CTZ: Ceftazidime; CCV: Ceftazidime-Clavulanate

2.3.6.1. Antibiotics for Clinical Use

2.3.6.1.1. Gram-negative AST Panel

The agreement between the different Gram-negative AST panel readings shows several important results (Table 23): (i) very good agreement between technician 1 and technician 2 visual readings (between 98 and 100% agreement), (ii) very good agreement between the best technician's visual reading and the supervisor's adjusted reading, except for a few major errors for Colistin (4%), (iii) automated reading by Biomic gave low categorical agreements compared with Reference 1 with high rates of major errors for piperacillin-tazobactam (10%), colistin (32%) and fosfomycin (20%), (iv) technician visual readings and the supervisor adjusted reading gave very good categorical

agreements compared with Reference 1 and Reference 2, except for amoxicillin-clavulanate, colistin and fosfomycin.

Table 23: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-negative AST panel for antibiotics used in treatments

Antibiotic	AMP	AMC	CRO	CTZ	CTA	PIT	IMI	MEM	CIP	TIG	AMI	TRS	COL	FOS*	ESBL
Visual Reading 1 vs. Visual Reading 2															
n tested	93	93	92	95	93	94	96	96	96	32	96	92	94	90	96
Agreement (n)	91	93	92	94	92	92	94	96	94	32	95	92	91	89	94
Major Discrepancy (n)	2	0	0	1	0	2	0	0	1	0	1	0	3	1	0
% Agreement	97.8	100.0	100.0	98.9	98.9	97.9	97.9	100.0	97.9	100.0	99.0	100.0	96.8	98.9	97.9
Visual Reading 1 vs. Automated Reading															
n tested	116	112	115	119	117	118	120	117	101	36	120	117	121	116	120
% Categorical Agreement	100.0	98.2	99.1	98.3	100.0	94.9	98.3	100.0	94.1	100.0	96.7	100.0	83.5	80.2	99.2
% Major Error	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	1.7	0.9	0.8
% Very Major Error	0.0	0.0	0.9	0.0	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.0	14.9	19.0	0.0
Visual Reading 1 vs. Adjusted Reading															
n tested	126	126	125	129	126	129	130	130	129	38	130	127	130	125	129
% Categorical Agreement	99.2	100.0	100.0	98.4	99.2	99.2	96.9	100.0	98.4	100.0	100.0	99.2	95.4	99.2	100.0
% Major Error	0.8	0.0	0.0	0.8	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.8	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.8	0.0	0.0
Visual Reading 1 vs. Reference 1															
n tested	151	151	151	155	151	155	155	155	155	-	155	149	153	149	157
% Categorical Agreement	98.0	93.4	94.7	88.4	86.1	87.7	87.7	94.2	90.3		89.7	97.3	83.7	81.9	92.4
% Major Error	0.0	0.7	4.0	3.9	0.0	6.5	0.0	0.0	1.9		1.9	0.0	16.3	8.1	6.4
% Very Major Error	2.0	6.0	0.0	0.6	0.0	3.2	2.6	0.0	0.0		0.6	0.0	0.0	10.1	1.3
Automated Reading vs. Reference 1															
n tested	145	141	145	148	154	146	148	145	126	-	148	145	157	154	159
% Categorical Agreement	97.9	95.0	93.8	91.2	80.5	84.2	87.8	95.9	87.3		90.5	97.2	68.2	74.0	93.7
% Major Error	0.0	0.7	3.4	4.1	0.0	10.3	0.7	0.0	4.0		0.7	0.0	31.8	20.1	5.7
% Very Major Error	2.1	4.3	0.0	0.7	0.0	3.4	0.7	0.0	0.0		0.0	0.0	0.0	5.8	0.6
Adjusted Reading vs. Reference 1															
n tested	167	167	167	170	167	170	170	170	169	-	170	167	170	167	172
% Categorical Agreement	97.6	94.0	94.0	89.4	84.4	90.0	86.5	95.9	90.5		91.8	97.0	88.8	81.4	93.0
% Major Error	0.0	0.6	3.0	4.1	0.0	4.7	0.6	0.0	3.0		0.6	0.0	11.2	7.2	7.0
% Very Major Error	2.4	5.4	0.0	0.0	0.0	3.5	2.4	0.0	0.0		0.6	0.0	0.0	11.4	0.0
Adjusted Reading vs. Reference 2															
n tested	169	169	168	172	169	172	173	173	171	-	173	170	153	46	173
% Categorical Agreement	97.6	93.5	96.4	94.8	56.8	93.0	92.5	97.7	90.6		93.1	90.6	85.6	97.8	93.1
% Major Error	0.0	0.6	3.0	4.1	4.7	6.4	0.6	0.0	4.7		0.6	6.5	12.4	0.0	5.8
% Very Major Error	2.4	5.9	0.0	0.0	0.0	0.6	0.0	0.0	0.0		0.6	0.0	2.0	2.2	1.2
Reference 1 vs. Reference 2															
n tested	45	45	45	46	45	46	46	46	46	-	46	45	41	12	46
% Categorical Agreement	100.0	100.0	97.8	97.8	62.2	93.5	91.3	97.8	95.7		95.7	93.3	97.6	100.0	97.8
% Major Error	0.0	0.0	0.0	0.0	4.4	4.3	2.2	0.0	2.2		0.0	6.7	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	2.4	0.0	2.2

*Used only for urinary tract infections

AMP: ampicillin; AMC: amoxicillin-clavulanate; CRO: ceftriaxone; CTZ: ceftazidime; CTA: cefotaxime; PIT: piperacillin-tazobactam; IMI: imipenem; MEM: meropenem; CIP: ciprofloxacin; TIG: tigecycline; AMI: amikacin; TRS: sulfadoxine-pyrimethamine; COL: colistin; FOS: fosfomycin; ESBL: Extended Spectrum Beta-Lactamase.

2.3.6.1.2. Gram-positive AST Panel

Agreement between the different Gram-positive AST panel readings shows several important results (Table 24): (i) very good agreement between technician 1 and technician 2 visual readings (between 97 and 100% agreement), (ii) very good agreement between the best technician visual reading and the supervisor adjusted reading (between 98 and 100%), (iii) automated reading by Biomic gave low categorical agreements compared with Reference 1 with high rates of major errors for cotrimoxazole (8%), vancomycin (11%) and fosfomicin (8% of major errors and 10% of very major errors); (iv) technicians' visual readings and the supervisor's adjusted reading gave very good categorical agreements compared with Reference 1 and Reference 2, except for fosfomicin which has a 12% very major error rate; (v) discrepancies in the results of oxacillin have no impact on MRSA detection thanks to the use of cefoxitin as alternative detection criteria.

Table 24: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-positive AST panel for antibiotics used in treatments

Antibiotic	PEN	OXA	TRS	CLI	VAN	TEI	TIG	TET	FOS	MRSA
Visual Reading 1 vs. Visual Reading 2										
n tested	105	104	104	105	106	105	104	104	106	105
Agreement	105	104	101	102	105	105	104	102	104	105
Major Discrepancy	0	0	1	1	1	0		0	2	
% Agreement	100.0	100.0	97.1	97.1	99.1	100.0	100.0	98.1	98.1	100.0
Visual Reading 1 vs. Automated Reading										
n tested	124	123	122	121	124	124	123	121	121	122
% Categorical Agreement	100.0	91.9	89.3	99.2	91.1	97.6	100.0	97.5	97.5	100.0
% Major Error	0.0	4.9	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0
% Very Major Error	0.0	3.3	2.5	0.0	8.9	0.8	0.0	0.0	2.5	0.0
Visual Reading 1 vs. Adjusted Reading										
n tested	130	129	129	127	130	130	129	127	127	129
% Categorical Agreement	100.0	98.5	99.2	99.2	99.2	99.2	100.0	99.2	98.4	100.0
% Major Error	0.0	0.0	0.0	0.0	0.8	0.8	0.0	0.0	1.6	0.0
% Very Major Error	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Visual Reading 1 vs. Reference 1										
n tested	123	162	162	163	163	162	162	162	162	162
% Categorical Agreement	99.2	86.4	86.4	95.1	96.3	96.3	97.5	96.3	85.2	100.0
% Major Error	0.8	7.4	3.7	2.5	1.2	3.7	2.5	0.0	3.1	0.0
% Very Major Error	0.0	6.2	0.6	0.0	2.5	0.0	0.0	1.2	11.7	0.0
Automated Reading vs. Reference 1										
n tested	119	149	147	149	149	149	149	149	153	151
% Categorical Agreement	100.0	81.2	85.0	96.0	86.6	97.3	98.0	98.0	81.0	99.3
% Major Error	0.0	7.4	8.2	2.0	10.7	2.7	2.0	0.7	8.5	0.0
% Very Major Error	0.0	11.4	0.0	0.0	2.7	0.0	0.0	0.7	10.5	0.7
Adjusted Reading vs. Reference 1										
n tested	130	162	162	162	162	162	162	162	162	162
% Categorical Agreement	100.0	84.0	85.8	95.1	97.5	96.9	97.5	96.3	83.3	100.0
% Major Error	0.0	8.6	3.1	2.5	0.0	3.1	2.5	0.0	4.9	0.0
% Very Major Error	0.0	7.4	0.6	0.0	2.5	0.0	0.0	1.9	11.7	0.0
Adjusted Reading vs. Reference 2										
n tested	139	-	170	168	171	171	170	168	170	170
% Categorical Agreement	98.6		91.2	94.6	99.4	97.1	97.6	98.2	72.9	97.6
% Major Error	0.0		1.2	2.4	0.0	2.9	2.4	0.0	1.8	2.4
% Very Major Error	1.4		4.1	0.0	0.6	0.0	0.0	0.0	25.3	0.0
Reference 1 vs. Reference 2										
n tested	40	-	44	44	44	44	44	44	-	44
% Categorical Agreement	100.0		93.2	100.0	97.7	100.0	100.0	97.7		97.7
% Major Error	0.0		2.3	0.0	2.3	0.0	0.0	2.3		2.3
% Very Major Error	0.0		0.0	0.0	0.0	0.0	0.0	0.0		0.0

PEN: penicillin; OXA: oxacillin; TRS: sulfadoxine-pyrimethamine; CLI: clindamycin; VAN: vancomycin; TEI: teicoplanin; TIG: tigecycline; TET: tetracycline; FOS: fosfomycin; MRSA: methicillin-resistant *Staphylococcus aureus*

2.3.6.1.3. Fastidious Organism AST Panel

The agreement between the different fastidious organism AST panel readings showed several important results (Table 25): (i) very good agreement between technician 1 and technician 2 visual readings (between 92 and 100% agreement) except for Levofloxacin (86%), but the small number of strains tested does not allow for conclusion (n=7); (ii) very good agreement between the best technician's visual reading and the supervisor's adjusted reading (100%) except for ceftriaxone, ciprofloxacin and levofloxacin, but the small number of strains tested does not allow for conclusion (n < 20); (iii) automated reading by Biomic gave good categorical agreement (100%) compared with Reference 1, high levels of major errors are noted for ceftriaxone (32%) and ciprofloxacin (86%); (iv) technicians' visual readings and the supervisor's adjusted reading gave very good categorical agreement compared with Reference 1, except for ceftriaxone (88%) and levofloxacin (about 70%). The comparison between the supervisor's reading and Reference 2 was better (96% categorical agreement).

Table 25: Inter-operator agreement and agreement with reference methods of the results obtained on fastidious organism AST panel for antibiotics used in treatments

	PEN	AMP	CRO	CIP	CLI	VAN	LEV* except Hi
Visual Reading 1 vs. Visual Reading 2							
n tested	9	22	22	12	13	13	7
Agreement	9.0	22.0	22.0	11.0	13.0	13.0	6.0
Major Discrepancy	0.0	0.0	0.0	1.0	0.0	0.0	1.0
% Agreement	100.0	100.0	100.0	91.7	100.0	100.0	85.7
Visual Reading 1 vs. Automated Reading							
n tested	1	17	17	10	8	8	2
% Categorical Agreement	100.0	100.0	70.6	30.0	100.0	100.0	50.0
% Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	29.4	70.0	0.0	0.0	0.0
Visual Reading 1 vs. Adjusted Reading							
n tested	1	17	17	10	9	9	4
% Categorical Agreement	100.0	100.0	94.1	90.0	100.0	100.0	50.0
% Major Error	0.0	0.0	5.9	10.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Visual Reading 1 vs. Reference 1							
n tested	1	22	23	12	13	13	8
% Categorical Agreement	100.0	100.0	87.0	91.7	100.0	100.0	75.0
% Major Error	0.0	0.0	8.7	8.3	0.0	0.0	0.0
% Very Major Error	0.0	0.0	4.3	0.0	0.0	0.0	0.0
Automated Reading vs. Reference 1							
n tested	1	19	19	14	9	9	2
% Categorical Agreement	100.0	100.0	68.4	14.3	100.0	100.0	100.0
% Major Error	0.0	0.0	31.6	85.7	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adjusted Reading vs. Reference 1							
n tested	1	24	25	14	13	13	7
% Categorical Agreement	100.0	100.0	88.0	100.0	100.0	100.0	71.4
% Major Error	0.0	0.0	4.0	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	8.0	0.0	0.0	0.0	0.0
Adjusted Reading vs. Reference 2							
n tested	1	22	23	14	13	13	7
% Categorical Agreement	100.0	100.0	95.7	100.0	100.0	100.0	71.4
% Major Error	0.0	0.0	4.3	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference 1 vs. Reference 2							
n tested	14	14	14	14	14	14	9
% Categorical Agreement	100.0	100.0	100.0	100.0	100.0	100.0	100.0
% Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0

PEN: penicillin; AMP: ampicillin; CRO: ceftriaxone; CIP: ciprofloxacin; CLI: clindamycin; VAN: vancomycin; LEV: levofloxacin (not tested for *Haemophilus* spp.)

2.3.6.2. Antibiotics Used for Surveillance

Concerning the antibiotics used for ABR surveillance, the results of the different readings for the different panels show multiple important results (Table 26): (i) very good agreement between technician 1 and technician 2 visual readings (between 96 and 100% categorical agreement); (ii) very good agreement between the best technician's visual reading and the supervisor's adjusted reading (between 96 and 100%); (iii) automated reading by Biomic gave good categorical agreements (between 86 and 100%), except for fosfomycin (81%) on the Gram-positive panel and chloramphenicol on the fastidious organism panel (29%); (iv) technicians' visual readings and the supervisor's adjusted reading gave very good categorical agreements compared with Reference 1 (between 90 and 100%), except for fosfomycin on the Gram-positive panel (12% of very major errors). The comparison between the supervisor's reading and Reference 2 was worse (25% of very major errors)

Table 26: Inter-operator agreement and agreement with reference methods of the results obtained on Gram-negative, Gram-positive and fastidious organism AST panels for antibiotics used for monitoring

	Gram-negative Panel				Gram-positive Panel								Fastidious organism Panel			
	ERT	CHL	GEN	CFXS	ERT	LIN	CIP	GEN	AMI	FOS	QUD	DAP	TRS	CHL	MEM	LIN
Visual Reading 1 vs. Visual Reading 2																
n tested	93	91	96	61	104	105	105	106	104	106	104	104	15	15	22	7
Major Discrepancy	0	1	0	0	0	1	0	3		2	0	0	0	0	0	0
% Agreement	100.0	98.9	100.0	100.0	100.0	99.0	100.0	100.0	96.2	98.1	100.0	100.0	100	100	100	100
Visual Reading 1 vs. Automated Reading																
n tested	106	116	117	75	123	124	124	121	123	121	122	121	9	9	17	2
% Categorical Agreement	100.0	100.0	100.0	98.7	99.2	99.2	100.0	100.0	86.2	97.5	99.2	100.0	100	22.2	100	100
% Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.8	2.5	0.0	0.0	0.0	77.8	0.0	0.0
Visual Reading 1 vs. Adjusted Reading																
n tested	126	126	128	81	129	130	130	127	129	127	129	127	12	12	17	4
% Categorical Agreement	100.0	99.2	100.0	98.8	99.2	99.2	50.8	100.0	96.1	98.4	98.4	100.0	100	100	100	100
% Major Error	0.0	0.8	0.0	1.2	0.0	0.8	0.0	0.0	0.8	1.6	0.0	0.0	0.0	0.0	0.0	0.0
% Very Major Error	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Visual Reading 1 vs. Reference 1																
n tested	151	149	153	100	-	162	163	162	162	162	162	162		18	22	
% Categorical Agreement	100.0	96.0	89.5	100.0		99.4	96.3	93.2	91.4	85.2	91.4	100.0		94.4	100	
% Major Error	0.0	0.7	0.7	0.0		0.6	3.1	0.6	2.5	3.1	0.0	0.0		5.6	0.0	
% Very Major Error	0.0	3.4	2.0	0.0		0.0	0.6	3.7	0.6	11.7	0.0	0.0		0.0	0.0	
Automated Reading vs. Reference 1																
n tested	134	145	145	95	-	149	150	153	149	153	148	149		14	19	
% Categorical Agreement	100.0	97.2	91.0	100.0		98.7	97.3	92.2	85.9	81.0	91.2	100.0		28.6	100	
% Major Error	0.0	0.7	0.0	0.0		1.3	2.0	3.3	1.3	8.5	0.0	0.0		71.4	0.0	
% Very Major Error	0.0	2.1	1.4	0.0		0.0	0.7	3.3	0.0	10.5	0.0	0.0		0.0	0.0	
Adjusted Reading vs. Reference 1																
n tested	167	167	168	104	-	162	163	162	162	162	162	162		21	24	
% Categorical Agreement	100.0	96.4	91.7	100.0		100.0	96.9	93.8	93.8	83.3	90.7	100.0		90.5	100	
% Major Error	0.0	0.6	0.0	0.0		0.0	2.5	1.2	1.9	4.9	0.0	0.0		9.5	0.0	
% Very Major Error	0.0	3.0	1.8	0.0		0.0	0.6	3.1	0.0	11.7	0.0	0.0		0.0	0.0	
Adjusted Reading vs. Reference 2																
n tested	169	169	171	107	-	171	171	168	170	170	170	168		21	22	
% Categorical Agreement	97.6	98.8	91.2	85.0		100.0	98.8	95.8	95.3	72.9	100.0	100.0		90.5	100	
% Major Error	0.0	0.6	2.3	0.9		0.0	0.6	1.2	1.8	1.8	0.0	0.0		9.5	0.0	
% Very Major Error	2.4	0.6	1.8	0.0		0.0	0.6	3.0	0.0	25.3	0.0	0.0		0.0	0.0	
Reference 1 vs. Reference 2																
n tested	45	45	46	44	-	44	44	44	44		44	44		14	14	
% Categorical Agreement	97.8	97.8	95.7	97.7		100.0	97.7	97.7	93.2		100.0	100.0		100	100	
% Major Error	0.0	2.2	2.2	2.3		0.0	0.0	0.0	2.3		0.0	0.0		0.0	0.0	
% Very Major Error	2.2	0.0	0.0	0.0		0.0	2.3	0.0	0.0		0.0	0.0		0.0	0.0	

ERT: ertapenem; CHL: chloramphenicol; GEN: gentamycin; CFXS: cefotaxime; LIN: linezolid; CIP: ciprofloxacin; AMI: amikacin; FOS: fosfomycin; QUD: quinupristin; DAP: daptomycin; TRS: cotrimoxazole; MEM: meropenem

2.4. Discussion

This study was the first evaluation of the assembled Mini-Lab prototype (version1) with mainly mock samples but in a use setting in a hospital supported by MSF.

Ease of Use

The evaluation showed that the Mini-Lab was considered easy to use by the main users, (i.e. laboratory technicians with no previous experience in microbiology), and who had attended an initial training 4 weeks before the initiation of the study. At the end of the initial training, most pre-analytical, analytical and post-analytical procedures were considered easy to perform and competency assessments were very good. Quick progress was observed on inoculum preparation, AST reading and blood culture visual reading, and competency scores of greater than 98% were obtained for all fields after 3 months into the study.

QMS

The visual reading of blood cultures is known to be critical and difficult, with possible confusions due to turbidity[68]. Only experience and practice help achieve good performance in this area. The field training and supervised experience helped to adapt and finalise technical procedures and training modules for laboratory technicians. Moreover, many minor nonconformities were recorded during clinical sample reception and helped to amend and revise procedures to minimize their potential.

BCB

Very good agreement was observed between negative BCB visual reading for D7 and systematic subculture result in D6. Thus, systematic subculture on D6 showed no added value and was removed as a procedure in newer versions of the Mini-Lab.

Automated Reading

The assessment of the inter-operator agreement showed very good agreement for bacteria identification and susceptibility testing between panel visual readings by laboratory technicians and the supervisor's adjusted reading. Conversely, the results given by the automated raw reading with the Biomic readers showed an unacceptable proportion of discrepant results with the supervisor's adjusted reading. These results confirm that the reading and interpretation of ID and AST results can be performed by non-expert laboratory technicians without specific equipment. However, for time saving and reliability purposes, an internal system to help with the reading and

interpretation process of ID and AST panels will be developed by the Mini-Lab team (assisted reading system) and will replace the Biomic reader in the subsequent versions of the Mini-Lab.

The comparison of the Mini-Lab results with reference methods for pre-ID, ID and AST generally show very good correlation, as expected from the analytical validations of the different analytical components in reference laboratories (Annex 8). However, a few points of attention and improvement have been identified.

Pre-ID

The use of methylene blue in pre-ID procedures showed 5% of error rate when compared with the supervisor's results. The error rate and the expressed difficulty of interpretation argues in favour of the replacement of methylene blue staining by Gram staining. Moreover, results showed that the pre-ID paper-based decision tree should be automated to improve final interpretation.

ID

The identification panel for Gram-positive bacteria seems to be insufficient in its ability to discriminate *Staphylococcus aureus* from coagulase-negative Staphylococci. To compensate for this, a coagulase test was introduced in the next version of the Mini-Lab to confirm *Staphylococcus aureus*. The identification of *Streptococcus* species also needed to be improved on the Gram-positive panel because the clinical implication is important. The low performance of phenotypic methods for *Streptococcus* has already been documented in other studies on other identification systems compared with molecular methods [140], [141]. The ID panel manufacturer (Beckman-Coulter) has been informed of the low performance found for this genus. Gram-positive ID Panel performance checks for *Streptococcus* will be continued in new field evaluations.

The panel for the identification of fastidious organisms such as *Haemophilus* and *Neisseria* was assessed on a small number of *Haemophilus* strains only. It is therefore difficult to draw conclusions on the performances of this panel.

AST

The agreement of Gram-positive and Gram-negative AST panel results compared with reference methods did not show systematic errors associated with any organism/antibiotic combination. Some discrepancies could be explained by the inherent variability of methods for antibiotic susceptibility testing. Some variability, in particular, is known for amoxicillin-clavulanate [142] and colistin, even with automated reference methods. For example, for colistin, Vitek 2 (bioMérieux®) showed categorical

agreement of 36% to 90% to 36% when compared with microdilution methods [143]–[145]. The *ISO20776-2: 2007* standards on the assessment of AST devices define method acceptability thresholds after advancing and standardised validation (categorical agreement $\geq 90\%$; major error rate $\leq 3\%$; very major error rate $\leq 3\%$) with repetitions in triplicate for discrepant results. These criteria are not appropriate to evaluate the performance of the Mini-Lab AST method because the procedures for formal validation was not followed as it was not the goal of the field evaluation, and no repetition in triplicate was done for discrepant results. The proportion of major errors (result obtained: resistant instead of susceptible) or very major (result obtained: susceptible instead of resistant) between the results given by the supervisor and the reference methods was less than or equal to 5% for all antibiotics used for treatment, except for Fosfomycin for both AST panels, and colistin and amoxicillin-clavulanate for the Gram-negative panel. The AST panels will be monitored, like all other antibiograms, in a reference laboratory using international reference methods during subsequent studies. The AST panel for fastidious organisms was only evaluated on a small number of isolates and therefore it was difficult to draw conclusions.

2.5. Limits

This study has several limitations:

- A small number of clinical samples were used, because of difficulties including participants before blood collection and collecting blood cultures for the Mini-Lab at night or at the weekends. Many efforts were made by the study team to improve procedures, training and communications with the medical team. The study was also interrupted several times due to safety issues and to the SARS-CoV-2 health crisis.
- Some strains sent by Bicêtre for simulated blood cultures were contaminated or nonviable upon arrival in Haiti, in particular some of the fastidious organisms. It was not possible to return strains to France for root cause analysis and verification of the nonviability.
- The strains used for simulated blood cultures lacked diversity. A second shipment of strains was planned but due to logistical reasons linked to the interruption of air traffic during the COVID-19 pandemics the shipment could not occur. This resulted in no *Enterococcus*, only one non-fermentative Gram-negative bacilli and few fastidious organisms being tested.
- Analysis of databases not specifically built for the study to avoid multiple entries (Joget, Biomic or Excel files for the strains of Bicêtre Laboratory) caused major data

management problems. So, a lesson was learnt for the second field evaluation where specific software (REDCap) was used to enter study data in order to simplify database merging and cleaning. Moreover, the regular backup of ID and AST panel pictures was set up to make it possible to use them to explain potential discrepancies with reference methods.

2.6. Conclusion

Although the evaluation was made mostly on simulated samples but under field conditions, the Mini-Lab showed good usability and ease-of-use by unexperienced laboratory technicians (one-month training), which was confirmed by good agreement between the technicians' results and those obtained by the supervisor. Compared with the reference methods, pre-ID, ID and AST methods are acceptable in spite of necessary procedure adjustments to identify *S. aureus* and fastidious organisms. No particular organism-antibiotic combination caused systematic errors on antibiograms, making the results reliable, particularly for antibiotics currently used for treatment. The variability observed was inherent to the method and is within an acceptable range compared with other antibiogram methods. Following the first field evaluation and considering the study results, important procedure adjustments to further improve the usability and reliability of the Mini-Lab results were considered by the Mini-Lab development team for upgrading of the Mini-Lab. During the deployment of the updated version, it will be necessary to continue the evaluation of Gram-positive ID and AST panel performances compared with reference methods to monitor the identification of *Streptococcus* and the variability of some antibiotics in order to evaluate the impact on patient management.

To conclude, the study did not highlight any major malfunctions or errors of the Mini-Lab which would prevent its deployment in a resource-limited countries., especially in light of improvements and adjustments made to the Mini-Lab. The deployment of the new version will be associated with an evaluation, especially concerning the ID and AST panel performances compared with reference methods for the identification of Streptococci and monitoring of variability for some antibiotics in order to evaluate the impact on care management.

3. Evaluation of Performance and Integration in Clinical Practice of the Mini-Lab prototype version 2 in a Carnot District Hospital, Central African Republic

3.1. Introduction and background

The following improvements were made between versions 1 and 2 of the Mini-Lab.

- Box bench now made out of fibreglass with resin to reduce the weight but keeping the same internal arrangement (section 3 of Chapter 1).
- Comprehensive manual describing installations, management, pre-analytical, analytical and post-analytical procedures, as well as all follow-up, reporting and equipment management tools including visual drawing of all the different steps (see Annex 3, 11, 12).
- Special attention in the SOP was given on the workflow management between modules, task assignments schedule, to improve the fluidity of work within a 20 m² space.
- VBA-LIMS (mock-up version) included sample management and expert systems, (section 6 of Chapter 1).
- BCB, it was composed of the Autobio bottles read twice a day for 7 days with no terminal subculture and blind subculturing on to InTray Chocolate for negatively read BCB after 16-24 hours incubation using the BCB light box. Positive BCB were subcultured on InTray Chocolate and InTray Colorex .
- Pre-ID system was composed of a near to the final system (section 4 and section 6 of Chapter 1). Included a gram staining, coagulase tests, catalase, oxidase, indole and aminopeptidase test with the results being entered into an expert system composed of the pre-ID algorithm (section 6 of Chapter 1).
- ID system, the Neg/Pos Microscan panel was used as the main identification test with the PROMPT inoculation system.
- Due to the short shelf life (6 months), HNID Microscan panel was replaced with the Pastorex Meningitis agglutination test for the identification of *Haemophilus influenzae* and *Neisseria meningitidis* and to reinforce identification performance of *Streptococcus agalactiae* and *Streptococcus pneumoniae*.
- AST system, type 2 Neg and Pos MIC panels were used routinely. PROMPT inoculation system was used only for Gram negative bacteria as the evaluation in laboratory had shown discrepant results with the use of PROMPT for *Staphylococcus* spp.

Standard turbidity inoculum preparation was done for *Staphylococcus* spp. and *Enterococcus* spp. Due to the short shelf life (6 months) of the different broth used for the FAST MIC panel, from this version of the Mini-Lab it was advised not be used as routine but rather on a monthly basis as batch testing of all fastidious organism to get resistance profile for surveillance purposes. However, for treatment purposes and for *Haemophilus* spp., Beta-lactamase detection test, a chromogenic cephalosporin test on a disc (Cefinase, Beckton Dickinson, USA), was added

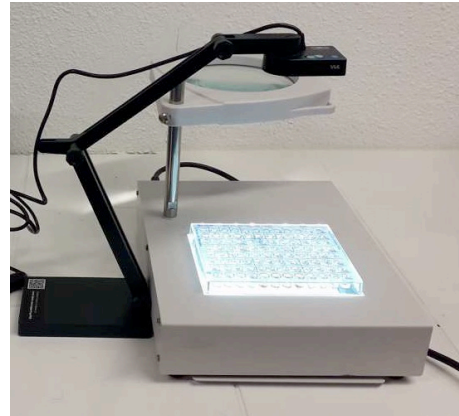


Figure 48: Photo of the microplate viewer box and the attached camera to take the adequate picture and transfer it into the Mini-LIMS, Mini-Lab (France)

- To replace the Biomic V3 reader for ID and AST Microscan plates reading, an assisted reading system (ARS) was developed and incorporated. The Reading and ID or an AST microplate can be done manually on a microplate viewer box with black or with background and where a camera is mounted (Figure 48) take a picture and transfer it into the Mini-LIMS.
- This reading is supported by the LIMS, by means of an interface (Figure 49). The interface asks the user to select a photograph of the plate to be read. The LIMS displays on the same screen a pattern corresponding to the type of plate read, and each well is assigned to an ID reaction or an antibiotic concentration defined by type of plates.
- Mini-LIMS display a colour atlas of growth type atlas to allow the user to choose the right test results. Then the test result information is processed by the different expert system described in section 6 of the Chapter 4.

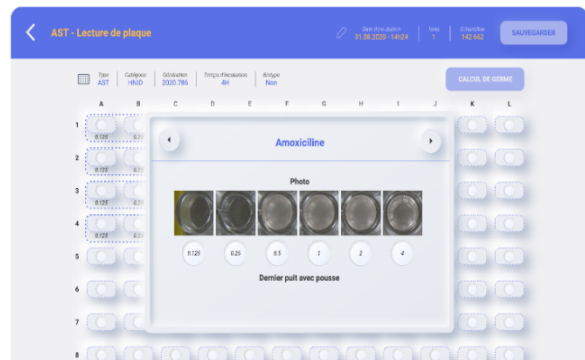


Figure 49: Snapshot of the Mini-LIMS interface to calibrate the reading of ID plate on top and for the AST plates down, Mini-Lab (France)

With the final assembly and improvement of the Mini-Lab (near to the locked product), it was necessary to assess its performance characteristics and; (i) describe the ease-of-use of each analytical component and the pre- and post-analytical steps, (ii) evaluate its usability and robustness, (iii) describe changes in antibiotic prescriptions following

the communication of bacteriological results from the Mini-Lab to the medical team, and (iv) describe pathogens and antibiotic resistance patterns identified in the study population.

3.2. Methods

3.2.1. Study Design and study site

A prospective study was conducted on the field implementation of the Mini-Lab prototype version 2. The Mini-Lab was deployed in Carnot MSF supported district hospital (Central African Republic) in July 2021, the hospital has been supported since 2009 and is located in the South-West of the Central African Republic, in the Mambéré-Kadéï prefecture. This district referral hospital covers a population of approximately 179 480 inhabitants. Most of the industry is mining and the prefecture suffered from multiple episodes of violence in 2013 and 2014. MSF main involvement in Carnot District Hospital (CDH) support is, in paediatrics units including paediatric emergency department, inpatient care, neonatology and nutrition (111 beds) and HIV/TB including internal medicine support and the isolation area for individuals with tuberculosis. The CDH had previously had no bacteriological culture laboratory and no retrospective data was available. The implementation of a formal program of antibiotic stewardship started in April 2021, with the arrival of an infectious disease specialist as antibiotic stewardship internal consultant (AS) for staff training and AS is a focal person for local physicians already working in the hospital.

The study was conducted from the beginning of September 2021 until March 2022. The Mini-Lab was installed in a 20 m² room close to the CDH general laboratory, and run by an experienced



Figure 50: Top to down, from transportation, installation of the Mini-Lab, training of laboratory technicians and treatment of the patient at Carnot Hospital, Central African Republic, Mini-Lab (France)

microbiologist trained on all Mini-Lab procedures, An additional three laboratory technicians were hired and their training conducted for a period of one month. A month was utilized to train nurses, clinicians on relevant modules (sampling, test prescription).

3.2.2. Study Population

The first study population were individuals admitted in the MSF-supported units at CDH who had, on admission or during hospitalization, a blood culture prescribed as per the MSF blood cultures prescription guidelines (Annex 15). In addition, individuals provided written informed consent (selves, guardian or representative for patients under 18 years old or if their clinical condition prevents them from being able to provide consent), provided successful blood culture collection. Second study population was the laboratory technicians working in the Mini-Lab, who had attended the training for the Mini-Lab implementation. During the recruitment process, laboratory technicians with prior work experience in a clinical microbiology laboratory were not selected.

3.2.3. Data Collection

On a daily basis, a study assistant, registered all individuals with blood culture received in the Mini-Lab during the study period, and evaluated their eligibility. The blood cultures from individuals included in the study were managed as per Mini-Lab routine procedures. All gram staining, subcultures and ID/AST plates were photographed and classified and stored in a designated Sharepoint folder. The **bacteriological results** were made available to clinicians as per standard procedures. The results were recorded on the VBA-LIMS mock up (September to December) or the Mini-LIMS (January to March). All bacterial strains isolated by the Mini-Lab from individuals (pathogens or contaminants) included in the study were stored in microbead vials in a -80°C freezer before transfer to the reference laboratory. **Clinical and therapeutic data** from individuals with pathogen positive blood culture was collected by the study coordinator and the antibiotic stewardship internal consultant on a standardised questionnaire using the individuals medical records. To do this, all records from individuals with positive blood culture (regardless of the organism) were evaluated to see if the isolated organism was considered as a pathogen organism or contaminant by the clinician based on clinical and therapeutic considerations.

In order to evaluate the **ease-of use** of each component of the Mini-Lab, a self-administered user experience questionnaire, using Osgood scale/Likert-type scale was used. The questionnaires were the same as used in the evaluation in Haiti. Ease-of-use includes the sample reception procedure, test procedures, interpretation, reporting of test results and internal quality control. The questionnaires were administered after the

initial training, after three months of use and at the end of the study. To evaluate if the analytical procedures were executed in accordance with the corresponding SOPs by the laboratory technicians, the microbiologist carried out **competency testing** at the end of the initial training with a checklist of the main steps of the procedures while observing each lab technician, as defined in the Mini-Lab manual. Following initial competency assessment, the microbiologist repeated the competency assessment of the lab technicians at 3 months and 8 months after the end of the initial study training.

3.2.4. Laboratory Analyses in the Reference Laboratory

Bacterial strains isolated in the Mini-Lab were shipped to Bicêtre University Hospital, Paris, France, in cryovials with microbeads at -20°C. Strains were re-labelled with anonymised numbers and date of the sample. The packing list did not include the names of bacteria isolated at CDH, to ensure that the reference laboratory was blinded from the results obtained by CDH. In the reference laboratory, strains were firstly subcultured and all bacterial isolates were identified by MALDI-ToF. AST was performed, depending on the antibiotics, using disk diffusion method, agar gradient diffusion method (E-test) or micro-broth dilution to determine the minimum inhibitory concentration (MIC) or the diameter of inhibition as per EUCAST V11 standard (Table 27). The results for ID and AST validated by Bicêtre were the reference results used to compare the results at CDH. In the reference laboratory, the strains identified as pathogens by the CDH were also tested for ID by the MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter), and for AST by the MIC Neg Type 2, or MIC Pos Type 2 or MIC FAST Type 2 plates (Beckman-Coulter) used as a comparator against the CDH results. In addition, the first 35 strains shipped were tested with the pre-ID tests as defined in the Mini-Lab procedures. All culture plates and ID/AST plates were photographed for further comparison if discrepancy.

Table 27: Methods in the Mini-Lab and in the reference laboratory

Criteria of Comparison		Mini-Lab	Reference Methods
BC bottles	Growth/ Non-growth	Autobio	Not applicable

Pre-ID	Individual result of each test and pre-ID algorithm result	Oxidase + Catalase + Aminopeptidase + Gram staining + Colorex	Only the first 30 strains: Oxidase + Catalase + Aminopeptidase + Gram staining + Colorex
ID test	Bacteria genus and species	MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter)	All strains: MALDI-TOF (Bruker) Pathogens only: MSF Neg/Pos ID Panel Type 2
AST	R/I/S test results interpretation as per EUCAST V11	MSF Neg/Pos MIC Type 2 (Beckman-Coulter)	All strains: Disc diffusion method, E-test or micro-broth dilution Pathogens only: MSF MIC Type 2 AST plates (Beckman-Coulter),

3.2.5. Data entry and Data analysis

Anonymized sociodemographic and clinical data was entered into the REDCap software program (<http://project-redcap.org/>) on site by an entry operator and the study coordinator. A password-protected laptop was specifically dedicated to this study. The laboratory data (include the bacteriological results) was entered by the Mini-Lab technicians via a secure, password-protected and dedicated database with workflow management: LIMS VBA then Mini-LIMS in January 2021. The Principal Investigator, based in Paris, routinely checked the databases via secure online access. Data was analysed at Epicentre with Stata®16 software (Stata Corporation, College Station, Texas, USA). This analysis was performed for all study participants. The demographic and clinical characteristics of the individuals at the time of inclusion were described. For continuous variables, the average, median, standard deviation, minimum and maximum were given. Categorical variables were described using percentages. Missing data were considered to be missing at random, and no imputation was applied. All outcomes collected and calculated to answer objectives of this study are described in Table 28.

Table 28: Primary and secondary outcome of the study

Type of outcome	Primary outcome
Key Performance Indicators	Proportion of blood cultures positivity: Number of blood cultures showing growth with a pathogen /total number of blood cultures
	Range of pathogens detected: proportion of each major pathogen isolated in blood cultures

	Proportion of contamination: Number of blood cultures showing growth with an organism identified as contaminants by the clinician/ total number of blood cultures.
	Average filling volume of blood cultures bottles: Volume per blood culture bottle (referring to a specific formula based on weight)
	Needle-to-incubator average time: average time interval from blood culture sampling to incubation
	Average time to positivity: Average time interval between blood cultures inoculation and blood culture positivity
	Average turnaround time: Average time interval from registration of the sample in the laboratory to reporting of the final result to the clinician
	ID and AST failure: proportion of panels with no growth or inconclusive results starting from a viable subculture
Agreement and accuracy compared to reference methods	Agreement and misclassification in Mini-Lab vs expected Gram classification based on MALDI TOF result in the reference laboratory
	Agreement at genus and at species level when comparing Mini-Lab against MALDI-TOF in the reference laboratory
	Agreement of AST automated visual reading against conventional methods in the reference laboratory, Categorical Agreement, Major Error Rate, Very Major Error Rate
	Proportion of inconclusive results or difficult result interpretation from the Mini-Lab for pre-ID, ID and AST.
Type of outcome	Secondary outcomes
Ease of use	Ease of sample reception procedures as measured by a questionnaire score (1 questionnaire/ lab personnel after training after 3 months and at the end of the study)
	Ease of test procedures and interpretation of analytical components as measured by a questionnaire score (1 questionnaire/ lab personnel after training after 3 months and at the end of the study)
	Ease of results sharing procedures as measured by a questionnaire score (1 questionnaire/ lab personnel after training after 3 months and at the end of the study)
	Compliance with analytical and post-analytical SOPs (BCB reading, pre-ID, ID, AST, result report) as measured by a competency testing score (1 questionnaire/ lab personnel after training).
	Incident and near-accident reporting
Robustness and Usability	Relative temperature monitoring
	Internal monitoring system for reporting of equipment failures and their consequences on laboratory functioning
Antibiotic prescription practice of clinicians related to patients with positive blood culture(s) with a clinically significant pathogen(s)	Proportion and type of antibiotic changes after communication of Mini-Lab results (preliminary and final results): no change, stop, switch IV-PO, de-escalation, escalation.
	Proportion of antibiotic changes adapted to patient clinical status, targeted clinical guidelines and laboratory results.

3.2.6. Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. After validation by the Steering and Scientific Committees of the Mini-Lab project, the protocol was approved by the “Comité éthique et scientifique de République Centrafricaine” (March 18, 2021, session) and the MSF Ethics Review Board (Ref. 1913a dated June 9, 2020). Inclusion in

the study was voluntary and required prior signed informed consent. Participants and participants' parent(s)/caregiver(s) for minors were informed in French or in Songo with the aim of the study and eligibility criteria by the trained health assistant. Eligible individuals interested in study participation received more detailed information about the study objectives, study procedures, and a clear explanation of the risks and benefits of participating in the research.

3.3. Results

3.3.1. Description of Study Population

Study recruitment took place between September 2021 and February 2022. The study flow chart is presented in Figure 51. Eligible individuals in the study were admitted to CDH between September 13, 2021, and February 13, 2022. Out of 1,960 individuals admitted in CDH during that period, mostly in paediatrics, 1,470 individuals met the criteria for blood culture prescriptions as per MSF guidelines (Annex 15). Among them, 1,042 had at least one blood culture collection during hospitalisation, among whom 948 (91%) were investigated for eligibility by the study health assistant. Approximately a hundred individuals (n=111) were not eligible for study participation with the majority (n=87) being discharged from the hospital before providing their consent. In total, among 837 individuals eligible for the study, 835 were included (2 refused to participate), this involved 960 blood culture bottles processed by the Mini-Lab, including 10 collected in pairs (950 batches). Among the 835 individuals included, 53.3% were males (Table 29). Most (51%) were between 6 months and 5 years of age and approximately 20% were > 15 years (adults treated in internal medicine). A minority of individuals (about 11%) had 2 or more blood cultures (or blood cultures in pairs) during hospitalisation. Blood cultures were more often collected on the day after admission (42%) than on the day of admission (28%), with a mean time of 1.8 days between admission and collection. Only 10% of blood cultures included in the study were collected between mid-September and late October.

There was a major increase in the number of blood cultures prescriptions after November, with more than 200 blood cultures per month, compared with 90 blood cultures in October. There was a peak in December, with 288 recorded blood cultures, i.e. on average 9.6 blood cultures received per day in the Mini-Lab.

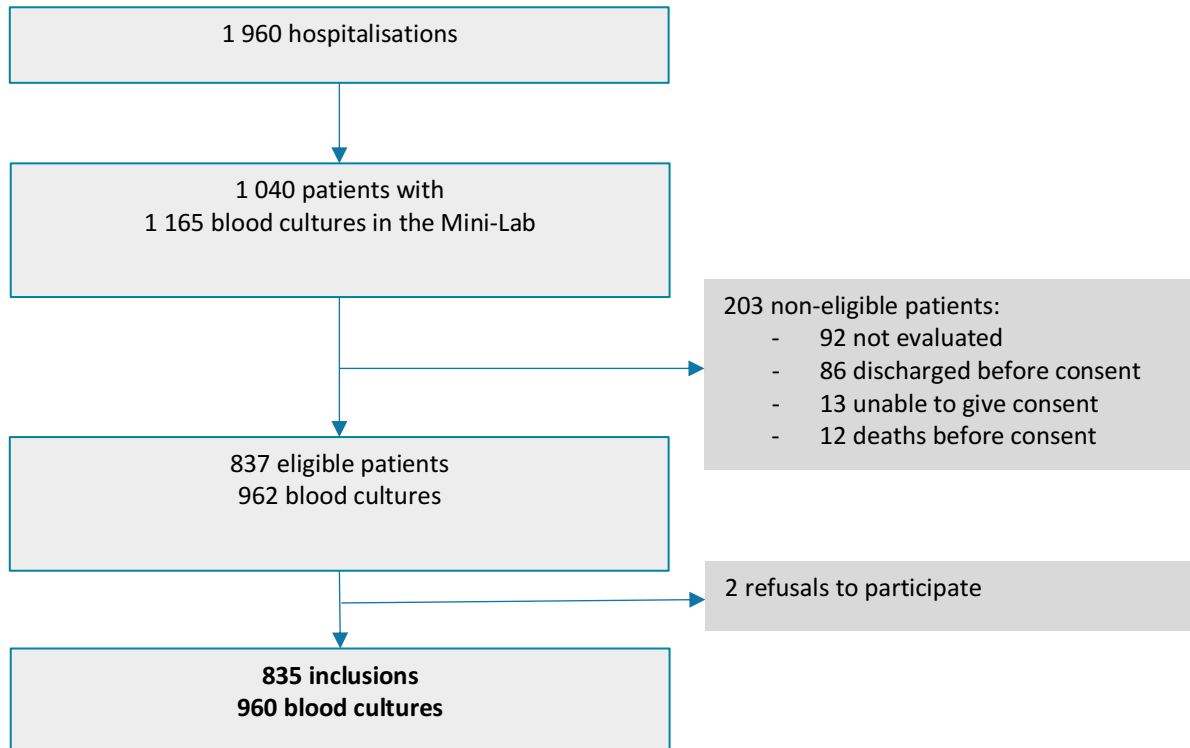


Figure 51: Study flow chart of the study population

Table 29: Participants characteristics, N=835

Characteristics	n	%
Females	388	46.5
Males	445	53.3
	Missing	2
Age at the time of inclusion		
	[0 – 30 days]	69
	[1 month– 6 months]	79
	[6 months– 5 years]	426
	[5 years– 15 years]	109
	> 15 years	148
	Missing	4
1st Blood Culture Prescriber		
	Intensive care	113
	Paediatrics (including paediatric emergency)	424
	CNTH	79
	Neonatology	69
	Internal medicine	147
	Missing	3
Number of blood cultures included		
	Number of blood cultures (single or by pairs)	950
	Number of blood cultures bottles	960
Number of blood cultures per patient		
	1 bottle or 1 pair of bottles	733*
	2 bottle or 2 pairs of bottles	89*
	3 bottles	13
Time of blood culture collection (number of batches)		
	September 2021 (half-month)	38
	October 2021	89
	November 2021	238
	December 2021	288
	January 2022	198
	February 2022 (half-month)	99
Time Interval from Admission to Collection		
	Blood culture collected on day of admission	270
	Blood culture collected D+1	401
	Blood culture collected ≥ D+2	279
Average Time Interval from Admission to Collection, days (DS)		
		1.8
		3.5

3.3.2. Mini-Lab Key Performance Indicators and Comparison with literature data

Among the 960 blood culture bottles received in the Mini-Lab, 123 showed growth with at least one bacterium considered a pathogen by treating clinicians. The proportion of pathogen blood culture positivity was 12.8%. Among the 835 individuals included, 117 had at least one positive blood culture with pathogens, (14.0%). In total, 124 pathogens were identified in 123 blood cultures. In one blood culture bottle, the Mini-Lab identified 2 different pathogens: *Salmonella sp.* + *Klebsiella pneumoniae*. The most frequently isolated pathogens were *Salmonella non Typhi* (44%), *E. coli* (10%) and *Pneumococcus spp.* (9%) (Table 30 and Figure 52). This list of pathogens among the

study population (mostly paediatric) was similar to the one previously published for the same population, particularly the top 5 *Salmonella* non Typhi, *E. coli*, *Pneumococcus* spp., *Staphylococcus aureus* and *Klebsiella* sp.[53].

Table 30: Range of pathogens isolated in the Mini-Lab during the study period, n=124

Organism	n	%
<i>Salmonella</i> sp	55	44.4
<i>Escherichia coli</i>	12	9.7
<i>Streptococcus pneumoniae</i>	11	8.9
<i>Staphylococcus aureus</i>	7	5.6
<i>Klebsiella pneumoniae</i>	6	4.8
<i>Enterococcus faecium</i>	5	4.0
Gram-negative Cocci	4	3.2
<i>Haemophilus influenzae</i>	4	3.2
<i>Haemophilus</i> sp.	2	1.6
<i>Pseudomonas aeruginosa</i>	3	2.4
<i>Acinetobacter lwoffii</i>	2	1.6
<i>Enterobacter cloacae</i>	2	1.6
<i>Neisseria meningitidis</i> Y/W135	2	1.6
<i>Acinetobacter baumannii</i>	1	0.8
<i>Acinetobacter</i> sp.	1	0.8
Gram-positive Bacilli	1	0.8
<i>Comomonas testosteroni</i>	1	0.8
<i>Enterococcus faecalis</i>	1	0.8
<i>Gemella</i> sp.	1	0.8
<i>Klebsiella</i> sp.	1	0.8
<i>Staphylococcus lugdunensis</i>	1	0.8
<i>Staphylococcus paucimobilis</i>	1	0.8
Total	124	100

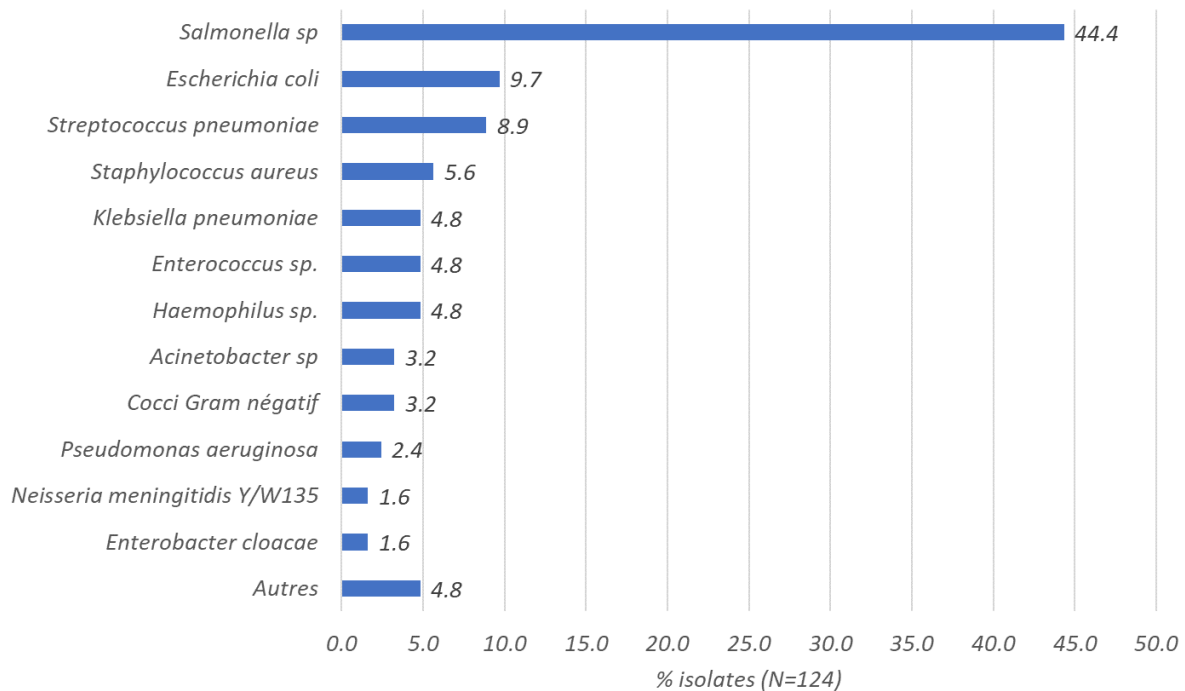


Figure 52: List of pathogens isolated in the Mini-Lab during the study period, N=124

Among the 960 blood cultures received in the Mini-Lab, 150 had at least one organism considered as to be a contaminant by treating clinicians and As internal consultant. This was a contamination rate of 15.6%. In total, 176 contaminants were isolated. Contamination at the site of the collection was mainly due to coagulase-negative *Staphylococci* (38%) and Gram-positive Bacilli (34%) (Table 31 and Figure 53). During the course of the study, the contamination rate increased slightly with no statistical significance, from 13% in September 2021 to 19% in January 2022. This can be explained by the large number of blood cultures collected per day starting from November 2021, giving the overworked staff little time to follow strictly asepsis procedures (Table 32). The highest rates of contamination were observed in neonatology (Table 33). In late January, the arrival of a nurse in charge of healthcare quality helped to resume training sessions for samplers and to improve the blood culture collection quality level. This included among other things, a hand hygiene campaign, contact precautions for multi-resistant bacteria cases, and the quality of urinary catheter positioning.

Table 31: List of isolated organisms in the Mini-Lab considered as contaminants by physicians during the course of the study, n=176

Organism	n	%
Gram-positive Bacilli	57	32.4
<i>Staphylococcus epidermidis</i>	33	18.8
<i>Micrococcus sp.</i>	17	9.7
Polymorphic Flora	8	4.5
Coagulase-Negative Staphylococcus	7	4.0
<i>Staphylococcus haemolyticus</i>	7	4.0
<i>Staphylococcus hominis</i>	7	4.0
<i>Aerococcus viridans</i>	3	1.7
Gram-positive Bacteria	3	1.7
<i>Staphylococcus aureus*</i>	3	1.7
Gram-negative Bacilli	3	1.7
<i>Bacillus sp.</i>	2	1.1
Gram-positive Cocci	2	1.1
Yeast	2	1.1
<i>Pantoea agglomerans</i>	2	1.1
<i>Staphylococcus auricularis</i>	2	1.1
<i>Staphylococcus simulans</i>	2	1.1
<i>Staphylococcus xylosus</i>	2	1.1
<i>Cedecea davisae</i>	1	0.6
<i>Enterococcus faecium*</i>	1	0.6
Gram-positive coccobacilli	1	0.6
<i>Hafnia alvei*</i>	1	0.6
<i>Kocuria kristinae</i>	1	0.6
<i>Providencia rustigianii</i>	1	0.6
<i>Salmonella sp*</i>	1	0.6
<i>Staphylococcus capitis</i>	1	0.6
<i>Staphylococcus cohnii</i>	1	0.6
<i>Staphylococcus intermedius</i>	1	0.6
<i>Staphylococcus lugdunensis</i>	1	0.6
<i>Staphylococcus saprophyticus</i>	1	0.6
<i>Staphylococcus sciuri</i>	1	0.6
<i>Streptococcus salivarius</i>	1	0.6
Total	176	100

*These bacteria are usually classified as potentially pathogenic organisms but, after revision by the clinicians and the AS internal consultant, individual's symptoms were not considered related to these bacteria and thus the isolates were considered as contaminants from a clinical standpoint.

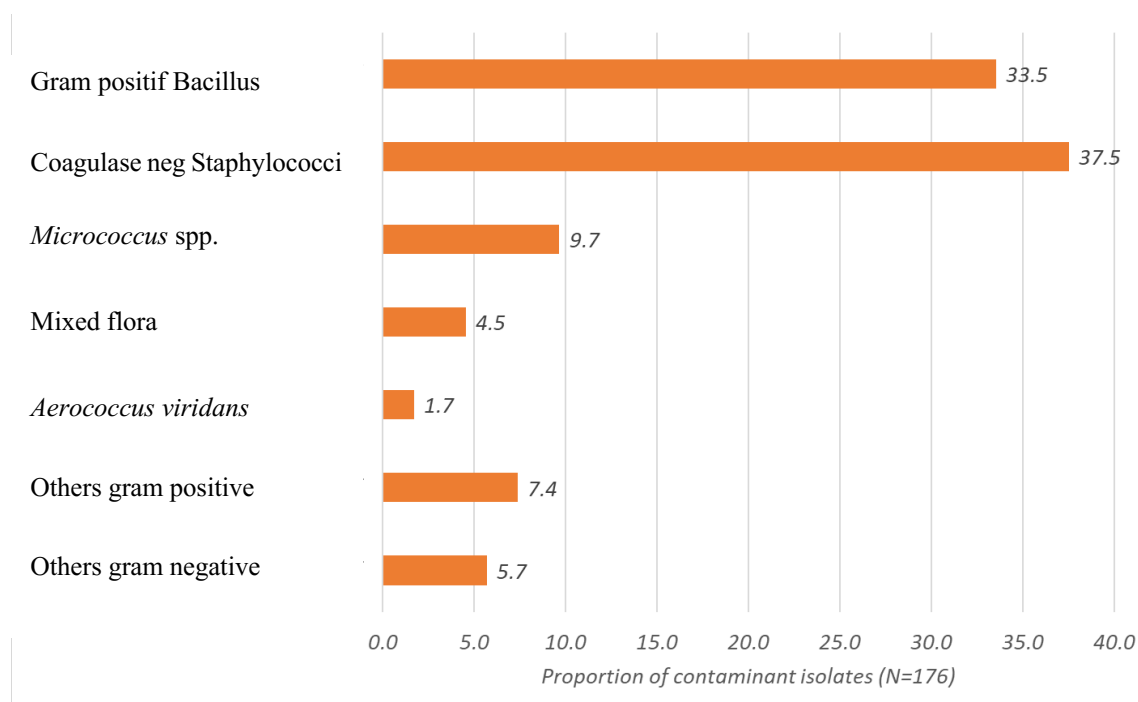


Figure 53: List of isolated contaminants by the Mini-Lab during the study period, N=176

Table 32: Proportion of contamination per month, number of bottles, n=150

Month	N bottles contaminated	N blood cultures analysed	% Contamination
September 2021 (half-month)	5	39	12.8
October 2021	10	93	10.8
November 2021	36	242	14.9
December 2021	45	289	15.6
January 2022	38	198	19.2
February 2022 (half-month)	16	99	16.2

Table 33: Proportion of contamination per prescriber

Wards	N bottles contaminated	N blood cultures analysed	% Contamination
Intensive care	16	143	11.2
Paediatrics (including emergencies)	76	466	16.3
Therapeutic Feeding Center	19	96	19.8
Neonatology	23	75	30.7
Internal medicine	16	176	9.1

Filling volume of blood culture bottles

The average filling volumes for blood culture bottles were in the MSF procedure recommended range based on age, except for the > 15-year age range where the average filling volume was 6.6mL instead of 8mL minimum (n=932) (Table 34).

Table 34: Filling volume of blood culture bottles per age range

Age range	n	Average in mL (SD)	Recommendations as per MSF procedures
Neonates (< 1 month)	74	1.3 (1)	0.5 – 1mL
Infants (< 2 years)	324	2.5 (1.1)	1 – 2 mL
Children (2-15 years)	358	3.2 (1.4)	2.5 – 5mL
Adults (> 15 years)	176	6.6 (2.7)	8 – 10mL

Time Interval from Sampling to Start of Incubation

The time of collection and start of incubation was recorded for 234 samples.

Median time from blood culture sample collection to the start of incubation in the Mini-Lab was 24 minutes for those samples (IQR 14; 45, min = 5, max = 390 (6.5 hours)).

Time to Positivity

Among the 270 positive blood culture bottles, with documented dates, the median time to positivity from blood cultures inoculation to observation of blood cultures positivity (contaminant or pathogen) was 1 day (IQR= 0; 1, min= 0, max = 6). Among the 122 positive blood cultures bottles with pathogens, the median time to positivity was 1 day (IQR= 0; 1; min= 0, max= 5).

Total Turnaround Time

Among the 101 positive blood cultures bottles with pathogens with documented dates, the median turnaround time from sample recordings to final report transmission to the clinician was 2 days (IQR 2; 4, min = 1, max = 9).

ID and AST Test Failures

Among the 271 positive blood cultures bottles, none was left without a final result due to missing subculture or identification. Among 123 isolated pathogens, five could not be identified to genus level: four Gram-negative Cocci, one Gram-positive Bacilli, which was expected since the Mini-Lab procedures do not allow for genus identification of Gram-positive Bacilli (predominantly contaminants) and some Gram-negative Cocci.

Summary of the Mini-Lab Key Performance Indicators

Table 35 outlines all the key indicators used to evaluate the Mini-Lab performance in its routine activity. The study showed that all complied with references as defined in the literature[68] except for the contamination rate which was higher than published acceptable levels.

Table 35: Summary of the Mini-Lab Key Performance Indicators

	MINI-LAB Results	Reference in literature	Reference
Pathogen positivity rate	12.5% (120/960)	5-15%	Baron E, et al. (2013)[146]
Range of pathogens detected	Top 5 = <i>Salmonella</i> non Typhi, <i>E.coli</i> , Pneumococcus, <i>Staphylococcus aureus</i> and <i>Klebsiella sp.</i>		Reddy E, et al. (2010)[53]
Contamination rate during sample collection	15.7% (151/960)	< 3%	Baron E, et al. (2013) [146] Leber AL (2016)[147]
Filling volume of blood cultures bottles (average, mL), n=932		> 80% of recommended volume:	Baron E, et al. (2013) [146] Leber AL (2016)[147]
Neonates (< 1m)	1.3 (SD 1)	0.5 – 1mL	
Children (< 2y)	2.5 (SD 1.1)	1 – 2 mL	
Paediatrics (2y-15y)	3.2 (SD 1.4)	2.5 – 5mL	
Adults (> 15y)	6.6 (SD 2.7)	8 – 10mL	
Median time interval from sampling to start of incubation, n=234	24 min (IQR 14; 45)	< 2 hours	Wilson M, et al. (2007)[148]
Median time to positivity for blood cultures with pathogens, n=122	1 day (IQR= 0; 1; min = 0, max = 5).	1-2 days	
Median turnaround time for blood cultures with pathogens from sample recordings in the laboratory to final report transmission to the clinician, n=101	2 days (IQR 2; 4, min = 1, max = 9).	3 days	Hawkins RC. (2007) ⁶

3.3.3. Agreement of Mini-Lab results vs reference methods or comparator

3.3.3.1. Pre-Identification or Referral Diagnosis (Pre-ID)

The first 35 pathogen strains were utilized for comparison of pre-ID tests performed at Bicêtre. There was a good agreement between the tests performed at the Mini-Lab and at Bicêtre, for Gram staining, AMNP, Oxidase, Catalase and Coagulase. A few discrepancies were identified, particularly for Catalase on Gram-negative, but there was no impact on final identification, and additionally, for oxidase, which led to 3 erroneous referral diagnoses for *Salmonella* spp. (Table 36). For the InTray Colorex media, the

⁶ Hawkins RC. Laboratory Turnaround Time. Clin Biochem Rev. (2007) 28:179–94.

agreement between the Mini-Lab and Bicêtre results was poor (54%) but did not lead to any erroneous referral diagnosis (Table 36 and Table 37).

Table 36: Percentage of agreement of respective pre-ID tests performed at the Mini-Lab versus reference laboratories utilizing identical techniques and reagents.

	Gram Staining		Aminopeptidase (AMNP)		Oxidase		Catalase		Coagulase		Colorex ⁶
	Gram +	Gram -	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Growth and Colour
n tested	16	19	15	17	4	29	12	19	2	6	24
n Agreement	15 ¹	18 ²	15	17	4	24 ³	11 ⁴	15 ⁵	2	6	13
% Agreement	93.8	94.7	100	100	100	82.8	91.7	79.0	100	100	54.2

¹ Discrepancy = Gram-negative Cocci identified at the Mini-Lab and *Micrococcus luteus* identified in Bicêtre

² Discrepancy = Gram-negative Cocci at the Mini-Lab although positive AMNP and pre-ID algorithms shows “Gram-positive Cocci”, photo of the gram showing a slide of over-decolorised gram (decolorised Gram-positive Cocci). *Salmonella* sp. identified in Bicêtre; considered as an erroneous straining for ID agreement interpretation.

³ Discrepancy = Four *Salmonella* sp. issued by the Mini-Lab as final ID and confirmed by the Bicêtre. For three out of these four strains, the pre-ID algorithm had issued a “non-fermentative Gram-negative” result, leading to an erroneous referral diagnosis, but with no impact on final identification.

⁴ Discrepancies = One *Staphylococcus hominis* identified at the Mini-Lab with ID plates (in spite of catalase negativity) and confirmed in the Bicêtre.

⁵ Discrepancies = Two *Salmonella* sp. and two *Pseudomonas* sp with no impact on the algorithm result and final identification confirmed by Bicêtre.

⁶ See more details in the tables below

Table 37: Comparison of InTray Colorex medium results between the Mini-Lab and Bicêtre

Mini-Lab Colorex Results	Reference Laboratory Colorex Results						Total
	White to transparent	Metallic dark blue	Turquoise blue	Creamy gold	Non-Growth	Greenish	
White to transparent	11			2	1	2	16
Metallic dark blue		2	1				3
Turquoise blue	1						1
Creamy gold	1						1
Non-Growth							0
Pink to red				1	1		2
Greenish	1						1
Total	14	2	1	3	2	2	24

Table 38: Details concerning the 11 InTray Colorex result discrepancies between the Mini-Lab and Bicêtre

Colorex Results in the Mini-Lab			Colorex Results in Bicêtre Laboratory		
Pre-ID algorithm	Colorex	Organism ¹	Colorex	Organism ²	
Non fermentative Gram-negative	White transparent	to <i>Pseudomonas aeruginosa</i>	Greenish	<i>Pseudomonas aeruginosa</i>	
Non fermentative Gram-negative	White transparent	to <i>Pseudomonas aeruginosa</i>	Greenish	<i>Pseudomonas aeruginosa</i>	
	Metallic dark blue	<i>Enterococcus faecium</i>	Turquoise blue	<i>Lactococcus garvieae</i>	
<i>Streptococcus</i> spp Proba: 95.23%, Colorex: consistent	Pink to red	<i>Streptococcus pneumoniae</i>	Non-Growth	<i>Streptococcus oralis</i>	
<i>Streptococcus</i> spp Proba: 95.23% , Colorex: consistent	White transparent	to <i>Streptococcus pneumoniae</i>	Non-Growth	<i>Streptococcus pneumoniae</i>	
<i>Micrococcus</i> spp, <i>Staphylococcus</i> spp, Proba: 89.14%	Greenish	<i>Staphylococcus auricularis</i>	White to transparent	<i>Kocuria koreensis</i>	
Coag neg <i>Staphylococcus</i> , Proba: 100%, Colorex: consistent	Pink to red	<i>Kocuria kristinae</i>	Creamy gold	<i>Kocuria palustris</i>	
Gram pos Cocci, Proba: 100%	Creamy gold	<i>Staphylococcus hominis</i>	White to transparent	<i>Staphylococcus hominis</i>	
Group: <i>Streptococcus</i> spp, <i>Enterococcus</i> spp, Proba: 15.14%	Turquoise blue	<i>Staphylococcus sciuri</i>	White to transparent	<i>Staphylococcus sciuri</i>	
Referral impossible (equal probability)	White transparent	to <i>Staphylococcus aureus</i>	Creamy gold	<i>Staphylococcus aureus</i>	
<i>Streptococcus</i> spp Proba: 95.23% Colorex: consistent	White transparent	to <i>Streptococcus pneumoniae</i>	Creamy gold	<i>Micrococcus luteus</i>	

¹ Final identification results provided by the Mini-Lab in Carnot

² Final identification results provided by Bicêtre

3.3.3.2. Identification (ID): Comparison to Reference Method and Comparator

In total, 219 strains isolated at the Mini-Lab was sent to the Bicêtre among the 271 isolates, 88 were considered pathogens and 133 contaminants. All the strains that grew at Bicêtre were tested by MALDI-ToF (reference method). The strains that were found to be pathogens by the clinicians at CDH were also tested in Bicêtre on MSF Neg/Pos ID Panel Type 2 plates (Beckman-Coulter) (comparator) with the same procedure as used at the Mini-Lab.

- Pathogens vs Reference Methods

Among the 88 pathogens tested in Bicêtre by MALDI-ToF, 6 did not regrow, and 6 gave results that were inconsistent with the CDH Mini-Lab findings, suggesting a possible mixed of samples during strain preservation at the Mini-Lab. When the 12 strains are excluded from the analysis, concordance to the genus level was 97.4% (Figure 54). The 3 discrepancies linked with clinical significance are described in Table 39.

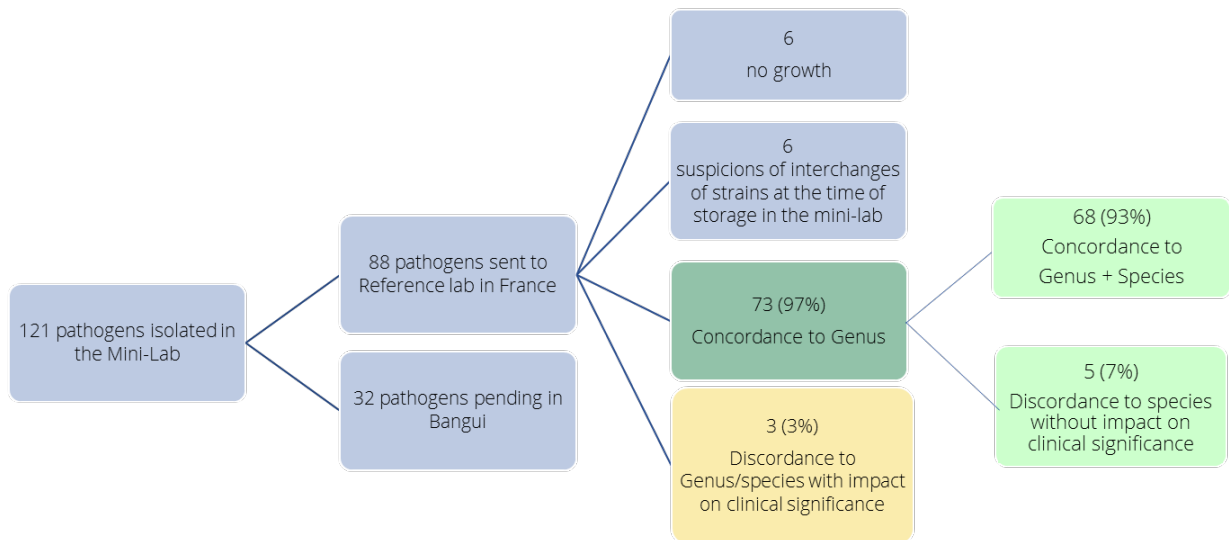


Figure 54: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory

Table 39: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory having a possible impact on clinical significance

Mini-Lab ¹	Reference Method ²	Comparator ³	Comments
<i>S. pneumoniae</i>	<i>S. oralis</i>	<i>Streptococcus sp.</i>	Very similar strains. Problems of agglutination on Pastorex or loss of Pneumococcus characteristics following storage and subculturing. Discrepancies having no impact on patient treatment
<i>E. faecium</i>	<i>Lactococcus garvieae</i>	Inconclusive	Very similar strains. Lack of accuracy of ID plate?
Gram-negative Cocci	<i>Micrococcus luteus</i>	<i>Micrococcus sp.</i>	Problems during pre-ID: Gram over-decolorised? Impact on the rest of the ID procedure: No ID plate used as defined in the procedure for Gram-negative Cocci.

¹ MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter) or pre-ID in the Mini-Lab

² MALDI-ToF in Bicêtre

³ MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter) in Bicêtre

- Contaminant vs Reference Method

Among the 133 contaminants tested at Bicêtre (127 Gram-positive and 6 Gram-negative), 7 did not regrow, and 20 gave results that were inconsistent with the Mini-Lab

results, suggesting a possible error during storage of strains at the Mini-Lab (Figure 55). When these 27 strains are excluded from the analysis agreement to the genus level was 83% (Figure 55) and the 7 discrepancies linked with clinical significance are described in Table 40.

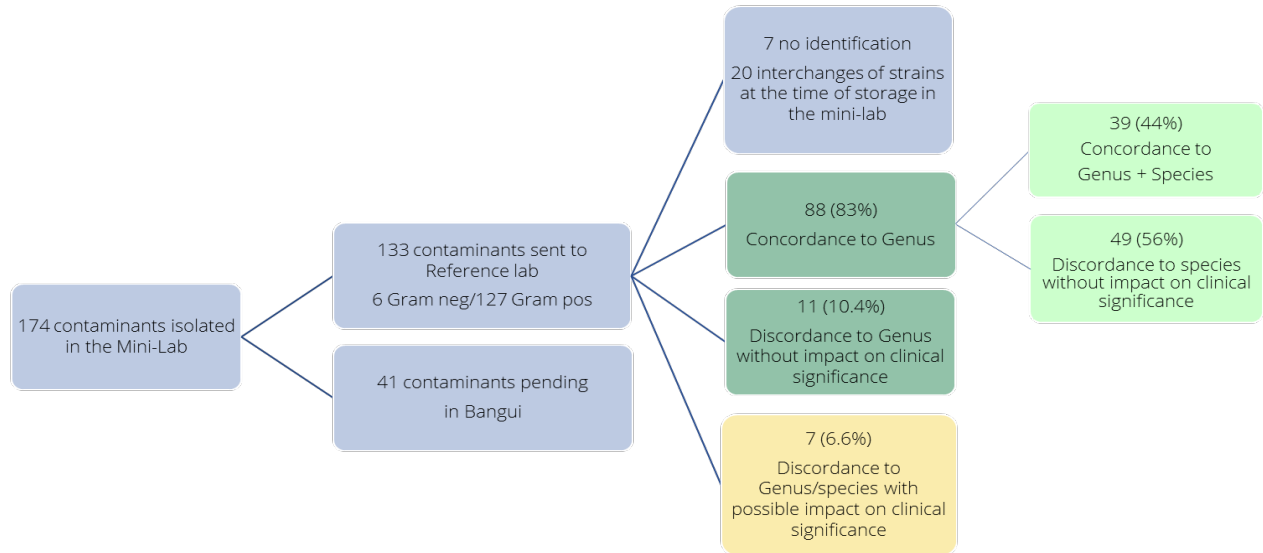


Figure 55: Description of cases of pathogen identification discrepancies between the Mini-Lab and the reference laboratory

Table 40: Description of cases of contaminant identification discrepancies between the Mini-Lab and the reference laboratory having a possible impact on clinical significance

Mini-Lab ¹	Reference Method ²	Comments
Gram-positive Bacteria	<i>Salmonella</i> sp	Pre-ID issue: erroneous Gram interpretation (picture=GNB) and AMNP read as negative; Positive ID Plate Reading; Microbiologist Absent
Gram-positive Bacilli	<i>Salmonella</i> sp	Pre-ID issue: erroneous Gram interpretation (picture=GNB) and AMNP read as positive; Positive ID Plate Reading; Microbiologist Absent
<i>Micrococcus</i> sp.	<i>Enterococcus faecium</i> + <i>Corynebacterium amycolatum</i>	Gram and polymicrobial subculture as per pictures; Rereading of ID plate confirms Micrococcus but probably contaminated.
Yeast	<i>Bacillus cereus</i>	Gram staining difficult to interpret. Unlikely yeast. No picture of subculture available. Do not seem sure of the result since they did an ID plate.
<i>Cedecea davisae</i>	<i>Bacillus subtilis</i>	Pre-ID issue: Over-decolorised gram (picture=GNB); AMNP pos/questionable; Negative ID Plate Reading
<i>Hafnia alvei</i>	<i>Salmonella</i> sp	Pre-ID issue: oxidase read as positive; Microbiologist Absent + glitch with Mini-LIMS
<i>Providencia rustigianii</i>	<i>Bacillus megaterium</i>	Pre-ID issue: Over-decolorised gram (picture=GNB); AMNP?; Gram and polymicrobial culture; Negative ID Plate Reading

¹ MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter) or pre-ID in the Mini-Lab

² MALDI-ToF in Bicêtre

- **Pathogens vs Comparator**

The genus and species agreement between the Microscan results at the Mini-Lab and at Bicêtre were very good for both Gram-negative and Gram-positive bacteria

(Table 41). The 4 genus and species discrepancies are presented in Table 42. In 3 out of 4 cases, the Microscan result at Bicêtre was consistent with that of the Mini-Lab.

Table 41: Genus and species agreement comparing Mini-Lab results to MALDI-TOF and to MSF Neg/Pos ID Panel Type 2 plates (Beckman-Coulter) in the reference laboratory for the identification of organisms depending on the type of organism (Gram-negative; Gram-positive)

	Absolute Agreement Genus + Species		Agreement Genus only		Agreement Clinical Relevance Group		
	Gram+	Gram-	Gram+	Gram-	Gram+	Gram-	
Mini-Lab vs Reference (Maldi-Tof Bicêtre)							
Pathogens							
n tested	15	61	15	61	15	61	
n Agreement	11	57	13	61	12	61	
% Agreement	73.3	93.4	86.7	100.0	80.0	100.0	
Contaminants							
n tested	100	6	100	6	100	6	
n Agreement	37	2	86	2	96	3	
% Agreement	37.0	33.3	86.0	33.3	96.0	50.0	
Mini-Lab vs Comparator (Beckman-Coulter Bicêtre)							
Pathogens							
n tested	14	62	14	62	14	62	
n Agreement	11	61	13	61	14	61	
% Agreement	78.6	98.4	92.9	98.4	100.0	98.4	

Table 42: Description of cases of pathogen identification discrepancies between ID plates in the Mini-Lab and ID plates in the reference laboratory (comparator) vs reference method

Mini-Lab ¹	Comparator ²	Reference Method ³
Gram-neg Cocci	<i>Micrococcus sp.</i>	<i>Micrococcus luteus</i>
<i>Staphylococcus lugdunensis</i>	<i>Staphylococcus haemolyticus</i>	<i>Staphylococcus haemolyticus</i>
<i>Comomonas testosteroni</i>	<i>Roseomonas sp.</i>	<i>Comomonas kerstersii</i>
<i>Streptococcus pneumoniae</i>	<i>Streptococcus sp.</i>	<i>Streptococcus oralis</i>

¹ MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter) or pre-ID at the Mini-Lab

² MSF Neg/Pos ID Panel Type 2 (Beckman-Coulter) in Bicêtre

³ MALDI-ToF in Bicêtre

Summary of Identification Performance Results

- Very good identification agreement for pathogens (genus + species) versus reference methods
- Very good genus agreement for contaminants
- However, some issues requiring attention were identified, especially on pre-ID tests:
 - Gram staining: a few erroneous results due to wrong interpretation or over-decolorised staining, a few discrepancies with AMNP adding confusion
 - In polymicrobial cultures: A few subcultures and polymicrobial Gram status were undetected due to overloaded subcultures at inoculation, therefore non-pure ID plates were inoculated, subculture with purity check should be performed (same routine practice in conventional laboratory)
 - Pneumococci identification: possible erroneous Pastorex interpretation
 - Contaminants: ID plates done even if not recommended by procedures in case of Gram-positive Bacilli or yeast, which raises the issue of technicians trusting their pre-ID results
 - Yeast: lack of experience in gram status and subculture recognition

3.3.3.3. Antibiotic Susceptibility Testing

Microscan Beckman Coulter results were compared to the reference laboratory results obtained by E-test/MIC in a liquid medium and by a diffusion method as per EUCAST 2021 recommendations, in order to define Microscan AST plate agreement and performance. For amoxi-clavulanate and piperacillin-tazobactam, tests were repeated with the MIC method in a liquid medium (Sensititre, Thermo Fisher) for Gram-negative organisms after observing the heterogeneous resistance of several strains as previously observed during the evaluation in the laboratory as described in Chapter 3. The error classification was done as per ISO20776-2: 2007 as described in Chapter 3.

Antibiotics for Clinical Use

- **Gram-negative AST Panel**

The different Gram-negative AST plate readings showed very good categorical agreements (Table 43). Colistin was interpreted on a small number of strains because, due to inconsistencies in MICs when compared with reference methods, Beckman-Coulter, the manufacturer, recommends not to give colistin results for *Salmonella* spp., *Acinetobacter* spp. and *Enterobacter cloacae*. Major errors on colistin concern 2 *Pseudomonas aeruginosa* and 1 *Klebsiella pneumoniae*.

Table 43: Agreement with reference methods of the results obtained on Gram-negative AST panel for

antibiotics used for treatment

Antibiotic	AMP	AMC	CRO	CTZ	PIT	IMI	MER	CIP	TIG	AMI	TRS	COL
n tested	51	51	51	54	54	56	56	56	36	56	53	20
n Minor Error (S <> I, I <> R)				2	3		2					
n Major Error (R instead of S)			1		1			3		2		3
n Very Major Error (S instead of R)					2						1	
% Categorical Agreement	100	100	98	96	89	100	96	95	100	96	98	85
% Major Error	0	0	2	0	2	0	0	5	0	4	0	15
% Very Major Error	0	0	0	0	4	0	0	0	0	0	2	0

AMP: ampicillin; AMC: amoxicillin-clavulanate; CRO: ceftriaxone; CTZ: ceftazidime; CTA: cefotaxime; PIT: piperacillin-tazobactam; IMI: imipenem; MEM: meropenem; CIP: ciprofloxacin; TIG: tigecycline; AMI: amikacin; TRS: cotrimoxazole; COL: colistin;

- **Gram-positive AST Panel**
 - **Genus *Staphylococcus***

The different Gram-positive AST plate readings for *Staphylococci* showed very good categorical agreement, except for cotrimoxazole, clindamycin, erythromycin and fosfomycin (Table 44). For these 4 antibiotics, the results were compared between Gram-positive AST plates (Beckman-Coulter) done at Bicêtre and the reference method. There was a better agreement for clindamycin, erythromycin and fosfomycin than with Gram-positive AST plates done in the Mini-Lab (Table 45). The reading of all the pictures generated of Gram-positive AST plates at the Mini-Lab was repeated which showed discrepancies for erythromycin or clindamycin versus the reference method to check. All readings were consistent with the result obtained at the Mini-Lab. However, a case of “skipped wells” for erythromycin was observed and false interpretation of resistance (MIC 0.5 well without growth, MIC 1 well with growth, MIC 2 well without growth). It was recommended to retest of the plate if similar cases were observed and to reinforce the lab technicians’ training using this occurrence as a case study. No Methicillin-resistant *Staphylococcus aureus* (MRSA) were identified at the Mini-Lab or in the reference laboratory.

Table 44: Agreement between the results obtained on the Gram-positive AST panel in the Mini-Lab for *Staphylococcus sp.* and AST reference methods for antibiotics used for treatments.

Antibiotic	PEN	FOX	TRS	CLI	VAN	TEI	TIG	TET	ERY	FOS
n tested	8	11	21	21	21	21	21	20	21	17
n Minor Error (S <> I, I <> R)				1						
n Major Error (R instead of S)	1		3	2			1	2	5	3
n Very Major Error (S instead of R)			1							1
% Categorical Agreement	88	100	81	86	100	100	95	90	77	76
% Major Error	13	0	14	10	0	0	5	10	23	18
% Very Major Error	0	0	5	0	0	0	0	0	0	6

PEN: penicillin; FOX: ceftaxitin; TRS: cotrimoxazole; CLI: clindamycin; VAN: vancomycin; TEI: teicoplanin; TIG: tigecycline; TET: tetracycline; ERY: erythromycin; FOS: fosfomycin

Table 45: Agreement between the results obtained on the Gram-positive AST panel (Beckman-Coulter) in Bicêtre for *Staphylococcus sp.* and the reference methods for antibiotics with major errors between

the Mini-Lab results and the reference method

Antibiotic	TRS	CLI	ERY	FOS
n tested	21	21	21	17
n Minor Error (S <> I, I <> R)	7			
n Major Error (R instead of S)		1		
n Very Major Error (S instead of R)	1	1	1	2
% Categorical Agreement	62	90	95	88
% Major Error	0	5	0	0
% Very Major Error	5	5	5	12

TRS: cotrimoxazole; CLI: clindamycin ; ERY: erythromycin ; FOS: fosfomycin

○ ***Enterococcus Genus***

Categorical agreement was excellent for all antibiotics interpreted for *Enterococci* on the Gram-positive AST plates. It should be noted however that only a very small number of strains were tested (Table 46).

Table 46: Agreement with reference methods of the results obtained on Gram-positive AST panel for Enterococcus sp. for antibiotics used for treatment

	AMP	VAN	TEI	TIG
n tested	4	4	4	4
n Minor Error (S <> I, I <> R)				
n Major Error (R instead of S)				
n Very Major Error (S instead of R)				
% Categorical Agreement	100	100	100	100
% Major Error	0	0	0	0
% Very Major Error	0	0	0	0

AMP: ampicillin; VAN: vancomycin; TEI: teicoplanin; TIG: tigecycline

- ***Fastidious Microorganism AST Panel (Streptococcus sp., Haemophilus sp.)***

It was difficult to evaluate the categorical agreement for this panel due to the small number of strains tested. However, the agreement was good, any errors were minor (Table 47).

Table 47: Agreement with reference methods of the results obtained on fastidious microorganism AST panel for *Streptococcus* sp and *Haemophilus* sp. for antibiotics used for treatment

	AMP	CRO*	CIP*	CLI [§]	VAN [§]	LEV [§]
n tested	8	3	3	5	5	5
n Minor Error (S <> I, I <> R)	2					1
n Major Error (R instead of S)	0				2	
n Very Major Error (S instead of R)	0					
% Categorical Agreement	75	100	100	100	60	80
% Major Error	0	0	0	0	40	0
% Very Major Error	0	0	0	0	0	0

AMP: ampicillin; CRO: ceftriaxone; CIP: ciprofloxacin; CLI: clindamycin; VAN: vancomycin; LEV: levofloxacin

*Pneumococcus only

[§] *Streptococcus* sp. only.

Resistance Mechanisms

Among all strains tested with the reference method, twelve Enterobacterales were detected by the Mini-Lab as multi-resistant organisms: two were Carbapenem Resistant *Acinetobacter baumannii* (CRAB) and ten were extended spectrum Beta-Lactamase (ESBL producing organisms). These resistance mechanisms were confirmed by reference methods at Bicêtre for the ten strains (disk synergy method between amoxicillin-clavulanate and 3rd generation Cephalosporins). The definition of extended spectrum Beta-Lactamase (ESBL) used by the Mini-Lab is outlined in Table 48 and is consistent with the EUCAST definition[115].

Table 48: . Criteria used for the definition of extended spectrum beta-lactamase (ESBL) from the minimal inhibitory concentration (MIC) results as per EUCAST and AST plate manufacturer (Beckman-Coulter) recommendations

Resistance Mechanism	Organisms	MIC Criteria (mg/dl)
ESBL	Enterobacterales <i>Pseudomonas aeruginosa</i>	and CTA 16 to > 16 and CTXAC <= 0.5/4 or CTZ 4 to > 16 and CCV <= 0.25/4 or CTA > 16 and CTXAC = 4/4

CTA: cefotaxime ; CTXAC: cefotaxime-clavulanate; CTZ: ceftazidime; CCV: ceftazidime-clavulanate

Antibiotics Used for Surveillance

The categorical agreement of the antibiotics used for surveillance rather than for treatments are shown Table 49 per type of MIC panel. Very good results for the Gram-negative AST panel was observed. Good results for Gram-positive AST Panel, except for ciprofloxacin (7/22 minor errors) and gentamicin (3/22 major errors) which may be due to the small number of strains tested. Good results were observed for the fastidious AST panel, except for cotrimoxazole and chloramphenicol which may be due to the very small number of strains tested.

Table 49: Agreement with reference methods of the results obtained on the various AST panels for antibiotics used for surveillance

	Gram-negative Panel				Gram-positive Panel (Staph + Enterococcus)				Fastidious panel				
	ERT	CHL	GEN	LIN	CIP	GEN	AMI	QUD	DAP	TRS	CHL	MEM	LIN
n tested	51	50	53	25	21	23	21	25	21	8	8	3	5
n Minor Error			2		7			2		3			
n Major Error	2	1	2			3	1	1	1	2	1		
n Very Major Error		2									1		
% Categorical Agreement	96	94	92	100	67	87	95	88	95	38	75	100	100
% Major Error	4	2	4	0	0	13	5	4	5	25	13	0	0
% Very Major Error	0	4	0	0	0	0	0	0	0	0	13	0	0

ERT: ertapenem; CHL: chloramphenicol; GEN: gentamicin (high concentration gentamicin for *Enterococcus* sp.); CFXS: cefotaxime; LIN: linezolid; CIP: ciprofloxacin; AMI: amikacin; FOS: fosfomycin; QUD: quinupristin-dalfopristin; DAP: daptomycin; TRS: cotrimoxazole; MEM: meropenem

3.3.3.4. Proportion of inconclusive results or difficult result interpretation in the Mini-Lab for pre-ID, ID and AST.

In total, 241 positive blood cultures bottles among 271 positive bottles (88.9%) were documented by the Mini-Lab team to record repeated or reread inconclusive tests.

For pre-ID testing, 26% of documented positive blood cultures led to the repetition of at least one test, mostly gram staining for 11%, AMNP for 9% and coagulase for 13% of the tested strains (Table 50). For ID testing, around 8% of ID plates processed had to be repeated, re-incubated or reread because of inconclusive or questionable results (Table 51). For AST testing, less than 1% of the plates processed had to be repeated (Table 52). Overall, depending on Gram status, the Mini-Lab team had to repeat more pre-ID tests (32.4%) and more ID tests (7.8%) for Gram positive bacteria than Gram negative bacteria (Table 51).

In the end, complete identification could not be done for four strains: two Gram-positive Cocci for which ID/AST plate with no growth or unreadable, one Gram-positive Cocci and one Gram-negative Cocci for which there was no growth in subculture.

Table 50: Proportion of repeated/ reread pre-ID tests and reasons

	Number of repetitions (N)		Total	%
	Gram+	Gram-		
Total pre-ID tests	142	97	239	
Total number of blood cultures with at least one repeated pre-ID test (N=239)	46	16	62	25.9
Gram staining (N=241)	18	8	26	10.8
Inconclusive/ discrepant results			16	
Questionable results			8	
Inconclusive AND questionable results			1	
Other			4 [£]	
Catalase (N=231)	12	3	15	6.5
Inconclusive/ discrepant results			11	
Questionable results			4	
Oxidase (N=231)	2	8	10	4.3
Inconclusive/ discrepant results			8	
Questionable results			2	
AMNP (N=129)	8	4	12	9.3
Inconclusive/ discrepant results			8	
Questionable results			5	
Colorex (N=225)	2	1	3	1.3
Inconclusive/ discrepant results			3	
Coagulase (N=150)	20	0	20	13.3
Inconclusive/ discrepant results			2	
Questionable results			15	
Other			3 [§]	

Note: denominators correspond to the samples which had a result (pos, neg, NA) documented in LIMS

[£] Repetition of a slide by isolate (3), rereading (1)

[§] For confirmation, reassurance.

Table 51: Proportion of repeated ID plates and reasons

	Number of repetitions (N)		Total	%
	Gram+	Gram-		
Total number of ID plates done (N=237)	141	96	237	
Number of repeated ID plates	11	7	18	7.6
Repetition of the same ID plate type	5	2	7	3.0
Inconclusive/ discrepant results	2		1	
Questionable results	2	1	3	
Other (no growth)	1		1	
Change of ID plate type used	1	1	2	0.8
Inconclusive/ discrepant results		1	1	
Questionable results	1		1	
Re-incubation	1	1	2	0.8
Specific visual rereading of test(s) on the same plate	4	3	7	3.0
Inconclusive/ discrepant results	1	1	2	
Questionable results	3 [£]	2 [*]	5	

*1 Rereading of the entire plate for *Hafnia alvei*, and rereading of ONPG for *Salmonella* sp.

[£] 1 Rereading of the entire plate for *Gemella* sp., 1 PHO for *S.epidermidis*, 1 PVR CoNS

Table 52: Proportion of repeated AST plates and reasons

	Number of repetitions (N)	Total	%
--	------------------------------	-------	---

	Gram+	Gram-		
Total number of AST plates done (N=231)	137	94	231	
Number of repeated AST plates	1	1	2	0.9
Repetition of the same ID plate type	1	1	2	0.9
Inconclusive/ discrepant results				
Questionable results	1	1		
Change of ID plate type used	0	0		
Re-incubation	0	0		

The two antibiograms requiring repetition due to questionable results were for *Acinetobacter lwoffii* and *E. faecium*.

Table 53: Proportion of inconclusive results or difficult result interpretation for pre-ID, ID and AST.

	N results	N inconclusive results requiring repetition/ rereading/ or re-incubation	% Inconclusive results or difficult result interpretation
Pre-ID (at least one test)			
Gram+	142	46	32.4
Gram-	97	16	16.5
ID			
Gram+	141	11	7.8
Gram-	96	7	7.3
AST			
Gram+	137	1	0.7
Gram-	94	1	1.1

3.3.4. Ease of Use and Usability of the Mini-Lab

3.3.4.1. Ease of use

At the end of the initial training, the 2 lab technicians considered all pre-analytical, analytical and post-analytical aspects of the Mini-Lab easy to use (score > 90%), except for (Table 54) (i) the reading of blood culture bottles which appeared more complicated for technician 1 after the first training, but not later on, (ii) the ID and AST plate inoculation system, but considered easy later on, (iii) the ID and AST plate reading and interpretation, but considered easy later on, and (iv) the use of the autoclave, but considered easy later on. A third technician came to the Mini-Lab in November 2021 and was trained by the microbiologist and the supervisor. The total ease of use score after the initial training was far lower when compared to his colleagues (score = 45%) but after 3 months of experience, the total score reached 83%, which was similar to his colleagues' scores (Figure 56). However, these result raises the question of training efficiency when the Mini-Lab is in use versus training at the time of implementation.

Table 54: Ease of use score (%) per section of the self-administered questionnaire for 3 laboratory technicians 3 times during the study period

	M+0 (After initial training)			M+2/3 (3 months after the end of training)			M+8 (8 months after the end of training)	
	Lab tech 1	Lab tech 2	Lab tech 3	Lab tech 1	Lab tech 2	Lab tech 3	Lab tech 1	Lab tech 2
	Mini-Lab Comfort and Workspace Layout	100	98	58	98	96	81	98
Signage	100	96	54	100	100	83	100	100
Sample Receipt	89	80	48	98	91	80	98	96
Oxidase Test	92	83	39	100	92	78	100	84
Gram Staining and Reading	91	87	47	96	93	79	93	93
Aminopeptidase Test	89	83	50	97	97	86	100	92
Blood Culture Bottle	81	92	42	94	90	81	96	96
Subculture System (InTray)	97	94	44	100	88	84	100	94
Plate Inoculation System (Renok+Prompt)	84	80	46	98	98	84	98	93
ID Plate Reading and Interpretation	79	77	35	96	85	79	92	94
AST Plate Reading and Interpretation	81	90	35	100	94	87	98	96
Waste Autoclaving	72	86	39	97	94	89	94	94
Final Score	88	87	45	98	93	83	97	94

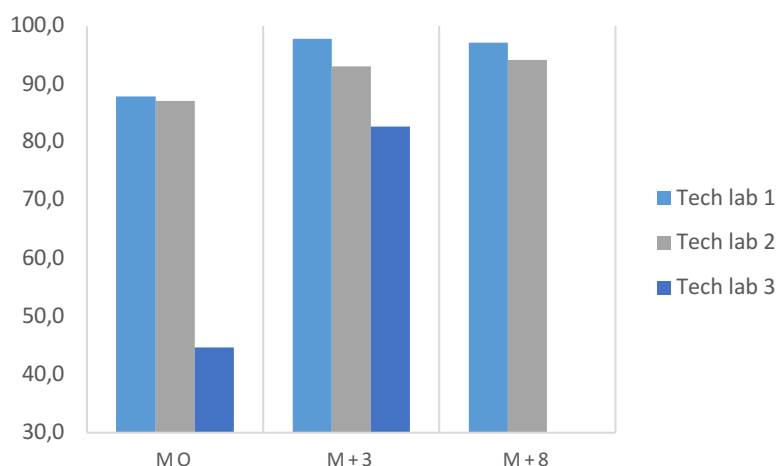


Figure 56: Final score (%) of the ease of use by a technician 3 times during the study period

3.3.4.2. Competency assessment

The analytical SOP compliance assessment (BCB, pre-ID, ID, AST reading) as assessed by the microbiologist, showed very high scores (> 90%) for most procedures (Table 5). The three technicians had difficulty preparing inocula for ID/AST plates and reading ID and AST plates after their first training, but there was a good improvement after the second training and 2 to 3 months of experience (Table 55, Figure 57).

Table 55: Analytical POS competency assessment score (%) evaluated by the microbiologist for 3 laboratory technicians, three times during the study period

	M+0 (after initial training)			M+3 (3 months after the end of training)			M+8 (8 months after the end of training)		
	Lab tech 1	Lab tech 2	Lab tech 3	Lab tech 1	Lab tech 2	Lab tech 3	Lab tech 1	Lab tech 2	
Sample Reception and Recording	100	100	100	100	100	100	100	100	
Blood Culture Bottle Incubation	100	100	100	100	100	100	100	100	
Sample Collection Kit	100	100	100	100	100	100	100	100	
Blood Culture Bottle Reading	90	100	100	100	100	100	100	100	
Subculture Inoculation	100	100	100	100	100	100	100	100	
Subculture Reading	100	75	80	100	100	100	100	100	
Gram	100	100	100	100	100	100	100	100	
Coagulase	100	100	100	100	100	100	100	100	
AMNP	83	100	100	100	100	100	100	100	
Oxidase	100	100	100	100	100	100	100	100	
Catalase	100	100	100	100	100	75	100	100	
Pre-ID Algorithm	100	100	100	100	100	50	100	100	
Inoculum Preparation for ID/AST Plates	100	100	69	100	100	100	100	100	
ID/AST Plate Inoculation	80	100	100	100	100	100	100	100	
ID/AST Plate Incubation	67	67	67	100	100	100	100	100	
ID Plate Reading	78	78	80	100	100	80	100	100	
MIC Plate Reading	67	78	64	100	100	91	100	100	
Equipment: Autoclave	100	100	88	100	100	100	100	100	
Equipment: Densitometer	100	100	100	100	100	100	100	100	
Equipment: Log Tag	100	100	100	100	100	100	100	100	
Task/ Maintenance/ Log Register	100	100	100	100	100	100	100	100	
Final Score	93	95	89	100	100	96	100	100	

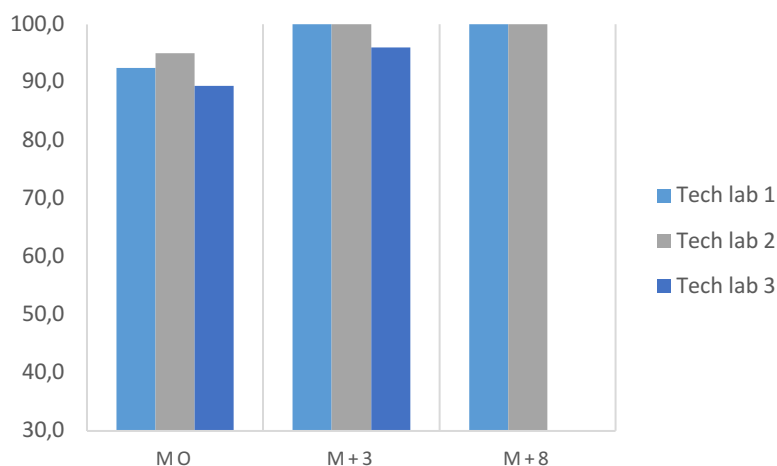


Figure 57: Final score (%) of competency assessment per technician 3 times during the study period

3.3.4.3. Incident reporting

Three major pre-analytical or post-analytical non-compliances were recorded; (i) a transcriptional error of identifiers into a final report (January 2022), (ii) a night sample was received without any identification on the order or on the bottle (December 2021), and (iii) a sample received with blood in the carrying bag (January 2022).

There were some malfunctions of the VBA-LIMS and the Mini-LIMS, but these were solved with updates to the Mini-LIMS and the new versions implemented, for example

- (i) “New Year’s Day Bug” for three days, prohibiting the recording of 22 samples, and
- (ii) several antibiograms were not visible in the VBA or Mini-LIMS sample log.

3.3.5. Robustness and Usability

Temperatures in the Mini-Lab rose above the normal ranges at times, due to air-conditioner breakdowns or the systems being switched off to reduce electricity consumption in the hospital when there were generator problems. The rises in temperature did not impact the functioning of the lab nor of equipment.

Regarding equipment, no major failure was noted which would have impacted the functioning of the Mini-Lab. A few problems were noted with:

- Autoclave: (i) Leaking out of the seal, (ii) error message about sterilisation because one of the 2 probes did not reach the required temperature, the problem was fixed by changing the probe’s positioning, (iii) cycles interrupted because of power cuts and (iv) the valve did not work during a monthly maintenance.
- Refrigerator: Too much condensation/ dampness, which required a maintenance visit from the biomedical supervisor
- Freezer: Rise in temperature following power cuts
- Several incubators: Falling below temperature target values (corrective action taken)

All these problems were recorded and taken into consideration by the Mini-Lab project team to improve training and follow-up for these equipment. Several problems were linked to the power supply in the Mini-Lab, which was sometimes unstable because it was dependent on the hospital generator. Power surges were noted, but no device was damaged thanks to voltage limiters included in the box benches modules.

3.3.6. Antibiotic prescription practice of clinicians related to patients with positive blood culture(s) with clinically significant pathogens(s)

This evaluation criterion highly depends on multiple factors: (i) Antibiotic Stewardship program, (ii) previous training, and (iii) the actual clinical prescription competencies of the clinicians.

Overall, 105/120 (87.5%) individuals with pathogen positive blood cultures were documented. Among them, 81 (77.1%) received empirical treatment before the reception of the first bacteriological results (Table 56). The empirical treatment most frequently administered was Ceftriaxone (29.6%), followed by Amoxicillin (18.5%) and the Cloxacillin/Ceftriaxone combination (12.3%) (Table 56).

Table 56: Empirical antibiotic treatment received for 105 participants with pathogen positive blood cultures

	Neonates	Child	Adult	Total	%
ceftriaxone	1	22	1	24	29.6
amoxicillin	0	11	4	15	18.5
cloxacillin + ceftriaxone	0	10	0	10	12.3
Ampicillin+ gentamicin	4	4	0	8	9.9
ampicillin	1	2	2	5	6.2
Amoxicillin+ ceftriaxone	0	3	1	4	4.9
amoxiclav	0	2	1	3	3.7
cloxacillin	0	2	0	2	2.5
Cloxacillin+ clindamycin+ceftriaxone	0	2	0	2	2.5
Amoxicillin+ azithomycin	0	1	0	1	1.2
Ampicillin+cefotaxime	1	0	0	1	1.2
Cloxacillin+cefotaxime	1	0	0	1	1.2
Cotrimoxazole+amoxiclav	0	0	1	1	1.2
cefixime	0	1	0	1	1.2
Ceftriaxone+ gentamycin	0	0	1	1	1.2
Ceftriaxone+ meropenem	0	1	0	1	1.2
Other	0	1	0	1	1.2
Total	9	82	14	105	100

Among the 81 patients having empirical antibiotic treatment before receiving any bacteriological result (Figure 58):

- Twenty-six individuals (32%) were already receiving empirical treatment adapted to the bacteriological results. The vast majority of these treatments (23/26, 88.5%) included ceftriaxone.
- Fifty-two individuals (64.2%) had no empirical treatment adapted to the bacteriological results. Fifteen individuals (27.8%) had amoxicillin, 15 patients (27.8%) had ceftriaxone (in combination or not) and 6 individuals had ampicillin-gentamicin (11.1%).
 - Forty-one individuals (50.6%) have had a change of treatment following the reception of the bacteriological results.
 - Eleven individuals (13.6%) have had no change of their empirical treatment following the reception of the bacteriological results.
 - Two individuals died and 1 individual was discharged before receiving the bacteriological results.

Among the 24 individuals having no empirical treatment before receiving the bacteriological result:

- Fourteen patients (58%) started antibiotic treatment after receiving the bacteriological results (12/14, 86% from the detection/ Gram results)
- Ten patients (9 children and 1 adult) did not start any antibiotic treatment in spite of a pathogen positive blood culture. Six individuals were reported as discharged before the reception of the results, 4 of whom were suffering from severe malaria.

Overall, 52% (55/105) of individuals with pathogen positive blood culture benefited from a change of antibiotics or the initiation of antibiotic treatment upon reception of bacteriological results. In addition, 25% (26/105) of individuals with pathogen positive blood culture were already receiving empirical treatment appropriate to bacteriological results, mainly with Ceftriaxone.

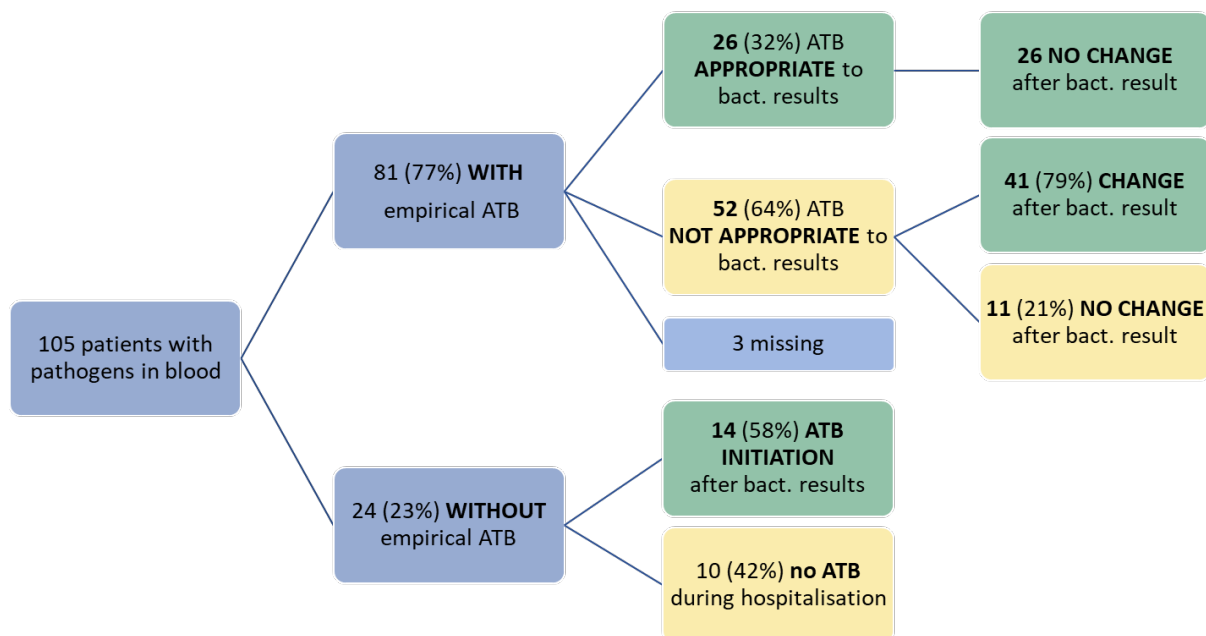


Figure 58: Change of antibiotic treatments depending on the bacteriological results

Significant results have also been noted in the AS internal consultant's monthly reports by the use of Point Prevalence Survey methodology used by the AS internal consultant, since the opening of the Mini-Lab and presented in Figure 59 and Figure 60 with (i) improvement of clinical record quality, (ii) better choice of antibiotics and dosage, (iii) increasing antibiotic treatment cessation when there are no signs of bacterial infection, (iv) improvement of antibiotic treatment de-escalation, (v) improvement in the practical attitude of clinicians in the choice of empirical ATB (drug, dose, duration), and (vi) more samples taken before starting antibiotics.

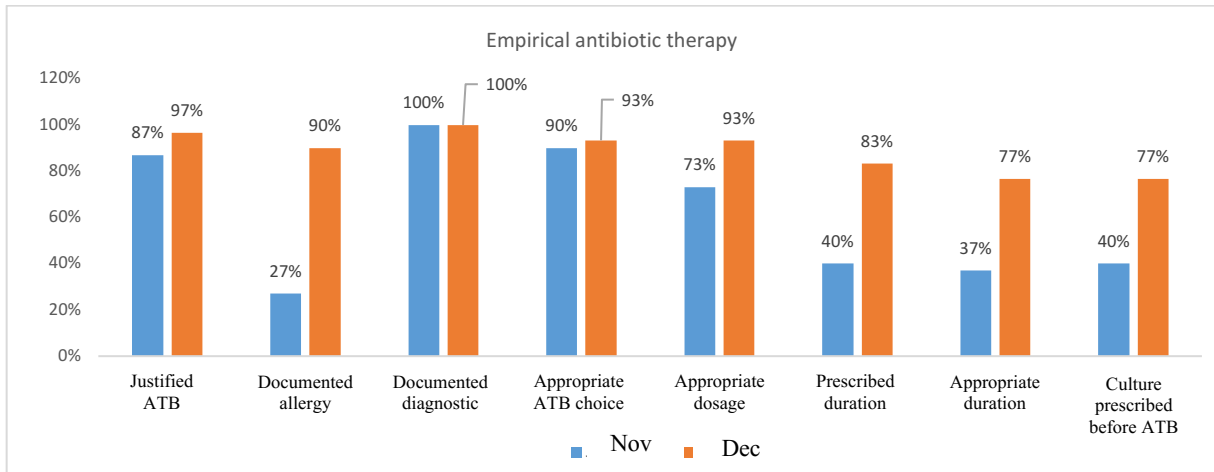


Figure 59: Result of PPS survey as per MSF protocol among 97 patients chart reviewed between November and December 2021 for empirical antibiotic therapy

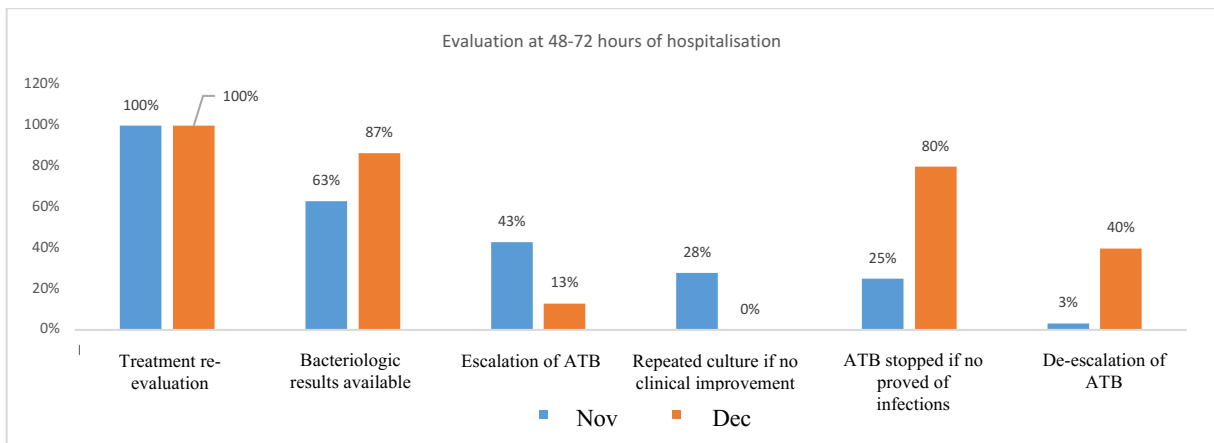


Figure 60: Result of PPS survey as per MSF protocol among 97 patients chart reviewed between November and December 2021 for patient therapeutic evaluation at 48h and 72 hours of hospitalisation

3.3.7. Microbiological Indicators in Carnot during the course of the study

3.3.7.1. Blood Culture Positivity Rate

The pathogen blood cultures positivity rate was higher among neonates (14.7%) and children between 6 months and 5 years of age (14.2%), unlike adults where it was lower (8.5%). The highest positivity rate was observed in the Intensive Care (15.4%) and TFC (14.6%) (Table 57).

Table 57: Proportion of pathogen positive blood cultures based on age groups and prescribers

	Number of blood cultures (n)	Number of pathogens positive blood cultures (n)	% Positivity
Age Range			
[0 – 30 days]	75	11	14.7
[1 month – 6 months]	96	12	12.5
[6 months – 5 years]	480	68	14.2
[5 years – 15 years]	127	14	11.0
> 15 years	177	15	8.5
Missing	5	0	0.0
Prescriber			
Neonatology	75	10	13.3
IC	143	22	15.4
Paediatrics A/B	466	60	12.9
Therapeutic Feeding Center	96	14	14.6
Internal Medicine	176	14	8.0
Other	1	0	0.0
Missing	3	0	0.0

3.3.7.2. Pathogen Allocation per Age Group

The most frequent strains observed among children aged between 1 month and 15 years were *Salmonella* non Typhi. Among neonates, several *Enterobacteriales* were mainly isolated, *Salmonella*, *Klebsiella* and *Enterobacter*. Among adults, *E. coli* was the most frequent, before Pneumococci (Table 58).

Table 58: Isolated Pathogen Allocation per Age Group(n=121)

Organism name	Age range									
	0-30 days		1 - 6 months		6 m - 5 years		5 - 15 years		> 15 years	
	n	%	n	%	n	%	n	%	n	%
<i>Salmonella sp</i>	2	18.2	6	50.0	37	53.6	7	50.0	2	13.3
<i>Escherichia coli</i>	0	0.0	1	8.3	3	4.3	1	7.1	7	46.7
<i>Streptococcus pneumoniae</i>	1	9.1	0	0.0	5	7.2	2	14.3	3	20.0
<i>Staphylococcus aureus</i>	0	0.0	0	0.0	5	7.2	2	14.3	0	0.0
<i>Klebsiella pneumoniae</i>	2	18.2	0	0.0	4	5.8	0	0.0	0	0.0
<i>Enterococcus faecium</i>	1	9.1	1	8.3	3	4.3	0	0.0	0	0.0
Gram-negative Cocci	1	9.1	0	0.0	2	2.9	1	7.1	0	0.0
<i>Haemophilus influenzae</i>	0	0.0	2	16.7	1	1.4	0	0.0	0	0.0
<i>Pseudomonas aeruginosa</i>	0	0.0	0	0.0	2	2.9	1	7.1	0	0.0
<i>Acinetobacter lwoffii</i>	0	0.0	0	0.0	2	2.9	0	0.0	0	0.0
<i>Enterobacter cloacae</i>	2	18.2	0	0.0	0	0.0	0	0.0	0	0.0
<i>Haemophilus sp.</i>	0	0.0	1	8.3	1	1.4	0	0.0	0	0.0
<i>Neisseria meningitidis</i>	0	0.0	0	0.0	2	2.9	0	0.0	0	0.0
<i>Acinetobacter baumannii</i>	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0
<i>Acinetobacter sp.</i>	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7
Gram-positive Bacilli	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7
<i>Comomonas testosteroni</i>	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7
<i>Gemella sp.</i>	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0
<i>Klebsiella sp.</i>	0	0.0	1	8.3	0	0.0	0	0.0	0	0.0
<i>Staphylococcus lugdunensis</i>	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0
<i>Staphylococcus paucimobilis</i>	0	0.0	0	0.0	1	1.4	0	0.0	0	0.0
Total	11	100.0	12	100.0	69	100.0	14	100.0	15	100.0

3.3.7.3. Antibiotic Resistance

Among multi-drug resistant isolates confirmed by the reference laboratory, the following were confirmed:

- 10/61 (16.4%) *Enterobacterales* ESBL (1 *Enterobacter sp.*, 4 *E. coli*, 4 *Klebsiella pneumoniae*, 1 *Salmonella sp.*)
- 1/2 (50%) Carbapenem resistant *Acinetobacter baumannii*
- 2/6 (33%) Ampicillin resistant *Haemophilus influenzae*
- 1/39 (2%) Fluoroquinolones resistant *Salmonella*
- 1/4 (25%) Penicillin resistant *Pneumococcus spp.*

Further analysis of these resistant strains using whole genome sequencing did not occur due to an inability to request the authorisation by the local ERB.

3.4. Discussion

After the study at Drouillard, Haiti, this was the second evaluation of the Mini-Lab but strictly with clinical samples at an MSF-supported hospital.

The study demonstrated that the key performance indicators of the Mini-Lab were very good as assessed during the study period. The one exception was the contamination rate which was 15%, which is higher than the recommended 3% threshold. Of note, the 3% contamination rate target is very strict and has not yet been reached in all MSF-supported bacteriological laboratories, even those that have been operating for years. In addition, some European hospital laboratories are also far from this target. A recent study on blood cultures quality indicators in a French hospital showed a 27.2% contamination rate over a one year period[149]. Thus, the improvement of sample quality and of asepsis measures is an actual challenge and requires targeted and sustained attention from the medical team.

Pre-ID

Compared to the previous version of the Mini-Lab, the use of the pre-ID automated algorithms helped to improve the final interpretation based on the different culture, pre-ID tests and subculture results. The introduction of Gram staining in the new version of the Mini-Lab was also very favourable. Few errors in Gram interpretation were noted (< 5%), these were mostly due to over-decolourisation, but this is acceptable when compared to published data from conventional laboratories in high-income countries. A large multicentre study showed that Gram staining results did not correspond to culture results for 5% of all samples[150]. If Aminopeptidase is kept in parallel with Gram status in pre-ID procedures, what to do in case of discrepancies between Aminopeptidase and Gram staining should be clear, and for which particular microorganisms. Other pre-ID tests remain crucial for referral diagnosis. Particular attention should be paid on Oxidase for Gram-negative Bacilli, as an erroneous interpretation of Oxidase results may lead to an erroneous referral diagnosis. As recommended following the 1st evaluation in Haiti, coagulase was implemented to discriminate *Staphylococcus aureus* from coagulase-negative Staphylococci. This was successful and even if some tests had to be repeated, there was no identification error for *Staphylococcus aureus* vs. Coagulase-negative *Staphylococci*.

ID

The comparison between the Mini-Lab results and the reference methods for ID generally shows very good correlation, as expected from previous analytical validations

of the different analytical components and the in the field evaluation study in Haiti. The agreement of pathogen identification to the genus and species of ID plates was 97%, and 90% respectively. For time saving and reliability purposes, an internal system to help with the reading and interpretation process of ID and AST panels was developed by the Mini-Lab team (assisted reading system ARS) and replaced the Biomic automated reader of the previous version of the Mini-Lab. Considering the poor performances of Gram-positive ID plates for Streptococci as highlighted in the assessment in Haiti, the Pastorex Meningitis (Bio-Rad®) agglutination test was introduced in the new version of the Mini-Lab to confirm *Streptococcus agalactiae* and *Streptococcus pneumoniae*. No *S. agalactiae* were isolated in Carnot, but among the 7 Pneumococci identified by the Mini-Lab and sent to the reference laboratory, 4 have been confirmed and 1 was shown to be a *Streptococcus oralis* (2 others had incorrectly been stored and could not be isolated). The discrepancy of *S. pneumoniae* vs *S. oralis* is not rare in any laboratory. These species are very close to the *Streptococcus mitis* group and the wrong identification of these species is due to a common evolutive origin and to horizontal gene transfer between *Streptococcus* species in the same ecological niche[151]. It could also be due to the loss of their inherent characteristics with successive seedings in the laboratory. However, the Streptococci grouping latex agglutination test (Pastorex)technique can sometimes be difficult to interpret, and false positive results have recently been observed with *Enterococcus* strains in another Mini-Lab site. It is therefore essential to reinforce training and the follow-up of these tests to ensure the most accurate possible interpretation.

A few points of improvement were identified for organism identification;

- increase lab technician training on the necessity of culture purity before proceeding further with the investigation,
- resorting to subculturing to isolate colonies in case colonies are not usable must be a controlled process, routinely implemented, especially since sample contamination rates can be high at the beginning of the activity. Purity plates could be used along with ID and AST plates to check inoculum purity at the beginning, when the contamination rate is high and the technicians still have limited experience.
- Yeast recognition: reinforce training to help technicians identify yeast more easily on gram and culture.

Antibiogram

The agreement of Gram-positive and Gram-negative AST panel results compared with reference methods showed no systematic errors associated with an

organism/antibiotic combination. Some discrepancies can be explained by the variability of the inherent methods. Some variability in particular is known for amoxicillin-clavulanate [142] and colistin, even with automated reference methods. For example, for Colistin, Vitek 2 (bioMérieux®) showed < 90% categorical agreement with 36% of very major errors compared with microdilution methods [143]–[145]. ISO20776-2: 2007 standards on the assessment of antibiogram devices performances define method acceptability thresholds after advancing and standardised validation (categorical agreement $\geq 90\%$; major error rate $\leq 3\%$; very major error rate $\leq 3\%$). These criteria are not appropriate to evaluate the performance of the Mini-Lab AST method because as the procedures of a formal validation were not followed as this was not the goal of the field evaluation. However, the proportion of major errors (resistant instead of susceptible) or very major (susceptible instead of resistant) between the results given by the Mini-Lab and the reference methods was $\leq 5\%$ for most antibiotics used for treatment.

Significant discrepancies for **Gram-negative AST Panel** concerned colistin (3/20 major errors, 15%), among which 2 out of 3 concerned *Pseudomonas aeruginosa*. In spite of a small number of strains tested, this result had already been observed during the evaluation study in Haiti (11.2% major error). As resistance to this antibiotic is rare among Gram-negative Bacilli, it is recommended to add a “Rare Resistance to be controlled,” alert in the expert system for colistin-resistant non-fermentative Gram-negative Enterobacterales/Bacilli. It should be noted that colistin should only be used in combination with cases of Carbapenem highly resistant organism bacteraemia, as per MSF Treatment Guide.

Heterogeneous resistance was observed for *Salmonella* and other *Enterobacterales* for amoxicillin-clavulanate and for piperacillin-tazobactam on a solid medium in the reference laboratory (Chapter 4), questioning the diffusion method as a valid reference method and making it necessary to replace it with a microdilution method. This result had already been observed for amoxicillin-clavulanate but not for piperacillin-tazobactam in the initial Beckman-Coulter AST plate study[101] and in Haiti. However, heterogeneous resistance to piperacillin-tazobactam has been observed in several studies for *E. coli*, confirming our results[152], [153].

Significant discrepancies for **Gram-positive AST Panel** concerned cotrimoxazole (3/21 major errors, 14%), clindamycin (2/21 major errors, 10%), erythromycin (5/22 major errors, 23%) and fosfomicin (3/17 major errors, 18%). These major errors were not observed when comparing the results of Gram-positive AST plates done at Bicêtre and the reference method, confirming discrepancies between the AST plate interpretation of the Mini-Lab and the reference method. Cotrimoxazole has a specific MIC reading

procedure (well with 80% growth decrease) and is difficult to implement and required good experience. The technicians may have over-evaluated MIC in certain cases. A case of “skipped wells” on erythromycin was observed and falsely interpreted as resistance. It was recommended to retest of the plate if similar cases are observed in the future. Some cases of discrepancies on Fosfomycin were identified during the assessment in Haiti, and variability in MIC between observers had been noted during laboratory assessment without affecting categorical interpretation[101]. Additionally, the interpretation of the reference method for fosfomycin (agar dilution) is known to be difficult even for expert lab technicians at Bicêtre, and probably led to an erroneous MIC reading explaining some of the major and very major errors. However, there were only a small number of strains tested with the Gram-positive AST panel and the results have to be interpreted with caution. There is no questioning of the use of the Beckman-Coulter MicroScan method for Gram-positive organisms. Additionally, these discrepancies were mostly observed on contaminants not routinely tested and having no impact on treatment.

The AST panel for fastidious organisms was evaluated on a small number of isolates. It is therefore difficult to draw a conclusion on its performances. It was decided not to use this panel routinely in the newer version of the Mini-Lab because of the inoculation media short shelf life. Indeed, the vast majority of fastidious organisms are susceptible to empiric antibiotics, and the routine testing of the panel for therapeutic decision-making doesn't bring added value. However, this panel was used for antibiotic resistance monitoring by testing all fastidious organism (*Haemophilus* spp. and *Streptococcus* spp.) isolated in the Mini-Lab during the study (batch testing). Major errors were noted for vancomycin and it was recommended to add a “Rare Resistance” alert on Mini-LIMS to monitor these cases.

Mini-Lab Usability

The evaluation showed that the Mini-Lab was considered easy-to-use by laboratory technicians with no previous experience in microbiology and who attended a specific initial training for 6 weeks before the beginning of the study. At the end of this initial training, most pre-analytical, analytical and post-analytical procedures were considered as very easy to perform and competency assessment was very good. Quick progress was observed on inoculum preparation, ID/AST plate reading and blood culture visual reading, and excellent competency scores were obtained in all fields after 3 months into the study. However, some analytical components were found more complex by lab technician 3 who arrived in November 2021, after 3 months of routine functioning of the Mini-Lab and in a period of activity upsurge. The total ease of use score after the initial training was far lower than his colleagues' score at the same period (score = 45%)

but after 3 months of experience, the total score reached 83%, similar to his colleagues' scores. This result raises the question of training efficiency while the Mini-Lab is in service, compared to training efficiency at the time of the Mini-Lab implementation. The Mini-Lab showed good robustness to electrical problems impacting air-conditioning and cold chain systems.

Antibiotics Prescription Practices Following Bacteriological Results

Clinicians seem to have accepted the bacteriological results given by the Mini-Lab. Overall, 52% (55/105) individuals with pathogen positive blood culture benefited from a change of antibiotics or the initiation of antibiotic treatment upon reception of bacteriological results. In addition, 25% (26/105) individuals with pathogen positive blood culture were already receiving empirical treatment appropriate for on bacteriological results, mainly with ceftriaxone, and which was not changed upon reception of bacteriological results. Most cases where bacteriological results were not taken into consideration concerned children who were discharged or dead before the reception of the results.

3.5. Limitations

Eligibility assessment was not performed for about one hundred individuals during peak activities. The health assistant was the only one assessing eligibility following the coordinator's departure two weeks after the beginning of the study. Due to work overload, they may have forgotten to note on the eligibility register those individuals who had blood culture sampling but who were discharged before eligibility assessment. Even if eligible, these individuals could not have been included into the study because they were discharged before giving consent. Additionally, one hundred inclusions have been documented as not performed due to the discharge or the death of individuals before the health assistant could ask them to participate in the study. Thus, 20% of individuals having at least one blood culture during the study period were not included in the study, which restricts the representativeness of the study results.

Some strains sent to Bicêtre were contaminated, interchanged or non-viable upon arrival: re-isolating before storing had not always been done at the Mini-Lab due to work overload. However, blood cultures contamination rates were high. Thus, several strains sent to Bicêtre could not be used.

In total, 32 pathogens among 121 (26%) and 41 contaminants among 174 (24%) could not be sent to the reference laboratory due to shipment refusal at Bangui airport after several months of attempts. However, the distribution of these unshipped strains

was similar to that of the shipped strains processed in the reference laboratory. It therefore assumed that the results would not differ from those already obtained.

The transition between data entry systems in the Mini-Lab (VBA-LIMS, then Mini-LIMS) occurred with just a few problems and continuous improvement, led to data entry errors and losses, which could be identified and corrected.

3.6. Conclusion

The Mini-Lab, routinely implemented in a district hospital in Carnot, Central African Republic, showed good performances for key indicators as well as in the different analytical components. It also showed good usability and ease-of-use for inexperienced laboratory technicians after a one-month training. The major equipment, procedure and training adjustments and improvements suggested after the first Mini-Lab evaluation results at Drouillard, Haiti, were implemented in the new Mini-Lab version utilized in Carnot and have improved the Mini-Lab usability and results reliability. The study also showed that bacteriological results were appropriately used by the medical teams, with significant improvements in the prescription practice of empirical and targeted antibiotics, supported by the AS internal consultant. This improvement occurred in less than 6 months.

Discussion:

From the overall development process described in Chapter 1, the evaluation performed on a ready to use culture media in Chapter 2, the development and evaluation of an AST system in Chapter 3, to the different field evaluations of the prototypes iteration described in Chapter 4, this work has been done with a multidisciplinary approach in focus and field of expertise. It included technology scouting methods; microbiology analytical methods, biosafety measures, biomedical engineering and bioinformatics methods, instructional design engineering, identification, adaptation, development and testing, iteration of user centre-based approaches and public health approaches. Although this type of iterative development is long and tedious, it has provided solid scientific results by supporting clinicians in adapting patient treatment. The final validation of the Mini-Lab deployed in Carnot Public hospital (Central African Republic), together with the implementation of an antibiotic stewardship program and improvement of Infection Prevention Control methods, has shown that 52% (55/105) of patients with pathogen positive blood culture benefited from a change of antibiotics or the initiation of antibiotic treatment upon reception of bacteriological results. We demonstrated that the key performance indicators of the Mini-Lab were very good during the study period with pathogen positivity rate (12.5%) being in the range set within the scientific literature[146], except for the contamination rate which was higher (15%) than the recommended 3% threshold[146], [147], even if improvements were observed after retraining of the staff and involvement of the head nursing managers. The 3% contamination rate target is very strict and is challenging not only for MSF-supported wards but for any ward/laboratory in the sub-Saharan region[68] or even in high resource settings[149]. Thus, the improvement of sample quality and of asepsis measures is an actual challenge and requires targeted and sustained attention from the medical staff. Also, our results highlighted in Chapter 4 of the evaluation done in Carnot CPH hospital suggested that the aetiology of bacteria identified in positive blood cultures among the paediatric population is consistent with other evaluations done in the region, with non-typhi Salmonella being the top organism identified [35], [154], [155]. The rate of resistant organisms found among positive blood cultures was lower than what is described in several studies from Central African Republic. We observed 17% (10/57) of Enterobacterales being resistant to third-generation cephalosporins as opposed to the 26 and up to 84% of gram-negative bacteria resistant to third-generation cephalosporins described among neonatal sepsis at a maternity hospital in Bangui[156]. Also they did not report carbapenem-resistant *A. baumannii* as opposed to us, demonstrating

that paediatric populations in rural settings can be affected by such resistant organisms.

Furthermore, the Carnot study highlighted the necessity to avoid missing opportunities for prescription right from the beginning of any Mini-Lab activity. This should be linked to a reinforcement of the ASP and the IPC, physicians should be trained in blood culture prescription criteria long before the opening of the Mini-Lab and they should familiarize with these criteria through prescription simulations on admitted patients. To avoid missing opportunities for sampling and reducing BCB contamination rates, it is also important to plan nurse training to sampling in the daily busy activity planning, to train enough motivated nurses in a limited period and in good conditions. It may also be relevant, as was done in Carnot, to open the activities progressively, one unit after the other, so as not to overwhelm health care workers, the Mini-Lab staff and to reduce contamination rates.

The usability of the Mini-Lab concept was highlighted to be excellent by the first two laboratory technicians, during the evaluation of Carnot. Despite changes of box bench technology between the first prototype (plastic sheet boiling assembly) and the second prototype (fibre glass resin), laboratory technicians in Haiti ranked 95% and laboratory technician in Carnot ranked 97% for Mini-Lab Comfort and Workspace Layout, demonstrating that the overall concept, space provided by the bench was good to perform daily tasks. Also, rapid progress, after 3 months of practice, was observed on inoculum preparation, ID/AST plate reading (from 82% to 98%) and blood culture visual reading (from 86% to 92%), and excellent competency scores were obtained in all fields also after 3 months into the study. However, the total ease-of-use score after the initial training in Carnot of the third laboratory technician employed four months after others was far lower than of his colleagues' score at the same period (score = 45%) but after 3 months of experience, the total score reached 83%, similar to that of his colleagues' scores. After these results, it was decided to develop a specific process of hiring new laboratory technicians while the lab is functioning with the use of the e-learning module and dedicated twinning mechanisms described in the Quality Management System. Performance and usability results evaluate at different steps of the development, demonstrate the consistency of the iterative approach used to adapt the different components of the sample workflow management.

For instance, the initial evaluation of several blood cultures bottles by our colleagues at ITM, led to select the Autobio biphasic blood culture bottles[97] read twice a day for 48 hours then once a day until Day 7. Results of the evaluation in Haiti and in Carnot demonstrate that additional terminal sub-culturing is not necessary when we

subculture blindly negative BCB at Day 1 and when we use the BCB light box to improve visual reading of the BCB. Also the laboratory technician in Carnot mentioned that the colour atlas of different types of growth of added value.

The initial assumption to subculture BCB and obtain colonies to perform downstream analysis was supposed to be that biphasic media should allow recovery of colonies from the agar phase, unfortunately the experiment led by Ombelet et al. demonstrated otherwise[97]. We therefore demonstrated in Chapter 2 that ready to use sealed packed InTray cassettes composed of chocolate agar and chromogenic agar, initially developed for the food and veterinary industries related bacteriology control, were able to perform adequately on blood culture[99], allowing growth of most common bacteria of medical interest. Surprisingly, our results demonstrate that general bacteria thought to require CO₂ incubation, such as *S. pneumoniae*, were able to grow in the absence of CO₂ incubation [99]. However, despite the validation in laboratory of the InTray technology to be used for sub-culturing BCB and improvement made of the InTray preparation and subculturing techniques, the study set in Carnot demonstrated that it is still complex to obtain single colonies, especially when the BCB is contaminated. This finding let us to reinforce training so that technicians don't overload subculture plates to make it possible to observe a potential polymicrobial culture. Furthermore, we considered as well to incorporate in the workflow, a purity plate when an ID and AST inoculum is done, especially during first few months after Mini-Lab deployment when the contamination rate was high, and the technicians still had limited experience.

From the initial idea to the actual system, the pre-ID has been a process of particular attention and constant evolution to reach the performance described by the study done in Carnot. To improve the time of actionable results, it is current practice to report the Gram stain result (Gram-positive or Gram-negative as well as morphology (rods, cocci) and configuration or arrangement (chains, diplococci, etc.) to the clinician as soon as the blood culture bottle shows signs of growth [157]. As Gram stain can be error-prone in inexperienced hands[32], [158], the early concept was to combine single reagents commercially available (oxidase, catalase, aminopeptidase) in a customised cassette that would be directly used on colonies available from the biphasic BCB media with the addition of a single step staining process (methylene blue). With the previously described drawback on constant availability of a single colony at Day 1 of BCB positivity on agar slant [159] and the incapacity to use aminopeptidase as Gram indicators at Day 1 from the broth, the evaluation in Haiti revealed the necessity to incorporate the Gram staining in the process to provide gram orientation when the BCB turn positive but to

keep Aminopeptidase on the second day when colonies have grown on the InTray media, backing up Gram-staining results. New tests have slowly been added into the pre-ID system (coagulase, Colorex colour, etc.) that lead to the increased complexity of a paper-based algorithm. The decision to transform our approach in a more probabilistic approach, as used for the industries involved in phenotypic identification test development and incorporate into an expert system has shown during the study of Carnot to be successful providing adequate orientation results and was highlighted by the technicians to be easy-to-use. However, few discrepancies were identified, particularly on oxidase on Gram-negatives (82% agreement) resulting in non-fermenting Gram-negative results instead of Enterobacterales for a few results. Threshold in the algorithm was then changed and adapted to reduce the occurrence of such issue and provide a more conservative result of Gram-negative bacteria. It is difficult to compare the performance of the algorithm with the literature since we did not find any article that could be used to compare our finding. However, as an example, Diagnostic Microbiology Development Program (DMDP), a research group based in Cambodia with the support of Ellen Jo Baron, Johan Letchford and others [69], [146], [160] have tried a similar approach by only applying the probability to the generation of unpublished paper-based decision trees for test orientations, in use in several laboratories in Cambodia.

The use of dual ID Microscan panel developed by Beckman Coulter and the addition of Pastorex meningitis (agglutination test for identification of bacterial causing Meningitis) into the identification pathway has proven during the evaluation in Carnot to provide results of quality for the clinicians with an agreement to genera (refer to groups of clinical importance Annex 16) at 97% and with only 3% of discordance with an impact on clinical decision. The reduced performance shown by Ombelet et al. [100] on *Streptococcus* spp. (74% accuracy at species level) identification during the laboratory was then improved. Also, Ombelet et al. [100] raised several concerns in regard of the use of the dual ID panel by non-experts, such as difficulty to read colour or reactions, difficulties in calculating the biotypes and difficulties of use of the operating system provided by Beckman to interpret the results. As shown during the evaluation of Carnot, the development of adapted Colour Job aid (see Annex 3, 11, 13) to read and interpret the colour reaction, plus the addition of the Assisted Reading System, which guide the user to a proper reaction interpretation, has been appreciated by the laboratory technicians after 3 months of usage (average ease of use score of 90%) and might have benefited by increasing performance.

The AST system highlighted in Chapter 3, developed based on the Microscan MIC technology, has been described by the laboratory technician in Carnot to be user-

friendly in the preparation and inoculation of the MIC panels (score of 94%). The use of the PROMPT inoculation system and the Renok to transfer the inoculum preparation onto the MIC panel has always been appreciated in the different experiments we conducted [100], [101], however our experiment in laboratory suggest to use PROMPT only for MICNEG and Neg/Pos ID panels inoculation to avoid discrepancies on some molecules [101]. Standardisation of the AST system provided by the three panels developed with Beckman-Coulter should decrease inter-operator and inter-site result variability as opposed to standard disc diffusion method [161], [162]. However, reading of those panels has been described to be difficult for few molecules such as cotrimoxazole on the Gram-positive AST panel (MICPOS), where the MIC should be placed at 80% of the decrease growth compared to the growth control. Performance of visual reading results between Haiti and Carnot study highlighted this issue with a categorical agreement of: 84% and 81% respectively despite the use of the ARS to help the user in defining appropriate MIC. In the hand of expert users, results reach 95% of categorical agreement [101]. Also the agreement of Gram-positive and Gram-negative AST panel results compared with reference methods did not show systematic errors associated with an organism/antibiotic combination. However, some discrepancies highlighted on Clindamycin, Erythromycin and Fosfomycin for MICPOS2, on amoxicillin-clavulanate and colistin for MICNEG2 can be explained by the variability of the method inherent to any antibiogram method. Some variability in particular is known for amoxicillin-clavulanate [142] or colistin, even with automated reference methods. For example, for colistin, Vitek 2 (bioMérieux®) showed < 90% categorical agreement with 36% of very major errors compared with microdilution methods [143]–[145]. For surveillance purposes, MIC panels shown very good accuracy during the different experiments in detecting multi-drug-resistant organisms (ESBL, MRSA, CRAB, etc.).

The heterogeneous resistance phenomenon highlighted for *Salmonella* spp. tested with amoxicillin-clavulanate during the experiment in Bicêtre laboratories and during testing of the strain coming from Haiti and Carnot study sites, was a stable phenomenon across countries. However, another heterogeneous resistance phenomenon with Piperacillin-Tazobactam was described with the same bacteria coming from Carnot. We were not able to further push the experiments on this finding, but this should be addressed to understand the potential impact on disc diffusion AST testing results of this type of bacteria widely identified in the sub-Saharan region with an AST method widely used in those settings.

In contrast to the logistic supply chain required to perform disc diffusion in LRS [64], Microscan technology has several advantages such as lowering the number of items to

be managed, 12 months shelf life for MIC panels, storage at room temperature for the MICPOS2, MICNEG2, MICFAST2, inoculum reagent for MICPOS2, MICNEG2 as compared to the cold chain required for disc diffusion. However, inoculum broth for the MICFAST2, HTM and LHB, with short shelf life (6 months) and cold chain requirements forced us to change its usage. After a literature review and discussions with our scientific committee, we decided that for all fastidious organisms such as *Streptococcus* spp., *Haemophilus* spp., AST testing will be done as batch testing for surveillance purposes, upon arrival of fresh HTM and LHB broth or referred to the country public health referral laboratory. Indeed, the vast majority of fastidious organisms are susceptible to empiric antibiotics [163]–[166], and the routine testing of the panel for therapeutic decision-making doesn't bring great added value [167], [168]. However, for treatment purposes and for *Haemophilus* spp., Beta-lactamase detection test, a chromogenic cephalosporin test on a disc (disc of nitrocefin), was added.

The results presented in this thesis to simplify clinical bacteriology practices so that they meet LRS constraints demonstrate that: (i) the concept of clinical bacteriology laboratory in LRS should be patient-directed and guided by clinical reality [69]; (ii) it is designed to be operated by laboratory technicians that may be non-experts in microbiology; (iii) it is based on the idea that a CBLs for LRS should be well conceived, cost-effective and built-for-purpose, not an “entry-level” version of its counterpart in high resource settings [32]; (iv) it uses conventional simple and affordable culture-based technologies and quality-assured oriented AST, considered sufficient to improve patient care and to provide adequate surveillance data, while contributing to the control of hospital-acquired infections [64], [68]. In Annex 18, a comparison of the actual Mini-Lab specification to the original target product profile can be found.

The development of the Mini-Lab was possible with financial support and expert support as described throughout the document and in acknowledgement section. It took 6 years of work between selection of the analytical components and equipment (with adaptation of some equipment, process, validation in control environment, validation and adaptation of the procedures) through several field evaluations demonstrating positive results toward simplification of clinical bacteriology addressing LRS.

However, this work features as well that simplification of clinical bacteriology at field level implies an increased and shared burden of activity for the backup support, a vast majority of the difficulties inherent to clinical bacteriology, expert rules, SOP update, breakpoint yearly update, supply management, etc. being transferred outside of the laboratory in the field. This is achievable within an organisation such as MSF, where we can mobilised expert resources at the Head Quarter level and as well because MSF

projects are still much more controlled settings than an average district hospital in LMIC. Most of the time our projects are typically equipped with adequate infrastructure, efficient logistic chains with quality assurance and well-trained staff.

Therefore, it is not clear if the concept can be exported to other entities, despite the numerous numbers of organisations interested in the concept, if a strong back office, supply chain is not maintained either. Also, field studies in low-resource settings outside of MSF settings, are needed to evaluate and assure good performance in suboptimal conditions as well.

Along the different experiments, we highlighted the different limitations related to the studies. However, one limitation to the entire work done during this Ph.D is the cost-effectiveness part that we partly addressed. An important reason why culture laboratory is not widely implemented in low-resource settings outside of its complexity is the important cost associated with a high quality culture laboratory .

In addition, a thorough and comprehensive cost-effectiveness analysis of the implications of simplifying bacteriology diagnostic capacity has not been considered in this work but would be extremely beneficial to other organisations willing to tackle the simplification of clinical bacteriology for LRS. Nonetheless even without explicit figures, absence of adequate and timely diagnostics of bacterial or fungal infections have immediate consequences on patient care, misuse first-line treatments, increasing hospital stays and bed occupancy with the probability of encountering resistant strains, all events generating unnecessary added cost for health care.

As of today 13 January 2023, the cost of the Mini-Lab kit, composed of the equipment, and available from MSF catalogue (cost of transport not included) is around 37,998 € and was 34,264 € in May 2022. Also, the starter kit with all the necessary reagents and consumables to perform 800 blood cultures assuming a 10% positivity rate and 5% contamination rate was in January 2023 of 31,808 € and 34,903 € in May 2022. Since the COVID-19 crisis, we have seen a maximum of 35% inflation rate on prices that varies from an item to another. A comparison between the cost of the Mini-Lab analysis and the conventional microbiology laboratory that can be found in larger hospital of MSF is described in Table 59.

We demonstrated that the direct cost of the Mini-Lab is slightly higher than the conventional laboratory on MSF fields of activity, however indirect costs are not all integrated to this model, for example it takes only 6 months of presence of an experienced microbiologist to install and have the Mini-Lab running (training, etc.) as

opposed to the necessity today to have on the site for the conventional laboratory the same expert but for a minimum of a year, sometimes two years[64]

Table 59: Cost comparison between the Mini-Lab and MSF conventional clinical bacteriology laboratory as of January 2023, this cost does not include the price of transportation from MSF Logistique in Bordeaux (France) to another country

Items	Cost Mini-Lab	Cost Conventional MSF CBL
Equipment	35 et 42 k€	26 k€ some local purchase not included (benches, microwave, drainer, hotplate, CO2 jar)
Starter Kit 6 months (training + start of activity)	32k€	31k€
IT	1 700 €	Local desktop+ 1 laptop supervisor
HR Laboratory	Lab: Microbio Expat 6 months + 2 lab tech + 1 lab supervisor(can be shared with the general lab)+ Microbio referent visit after 1 year	Lab: Microbio Expat at least 1 year + 2 lab tech + 1 lab supervisor(can be shared with the general lab)+ Microbio referent visit after 1 year
HR Antibiotic Stewardship /Infection Prevention and Control:	HQ visit pre-deployment + AS and/or IPC consultant for training if needed + AS and IPC local supervisor/focal point	
Reorder	~ 25k€ / 4 months	~ 18k€ / 4 months
Cost per analysis		
Sampling BCB	5,46 €	9,38 €
NEG Sample	5,28 €	3,93 €
TOTAL NEG (including sampling)	10,74 €	13,31 €
Staph aureus	21,84 €	14,62 €
Staph Coag Neg	14,46 €	19,31 €
Enterococci	31,29 €	11,38 €
Streptococci (Colorex negative / Positive)	24,31 - 31,29€	11,56 €
<i>Salmonella</i> spp.	31,35 €	15,92 €
<i>E.coli</i>	19,32 €	
Non-Fermenting Bacilli Gram neg	31,22 €	16,52 €
<i>Haemophilus</i> spp.	22,48 €	17,55 €
<i>Neisseria</i> spp.	21,51 €	21,59 €
POS sample (without sampling) MIN	14,46 €	11,38 €
POS sample (without sampling) MAX	31,35 €	21,59 €

A recent micro-costing study for microbiology laboratories in Southeast Asia, including detection of AMR, described cost per specimen from 22 to 31 USD; cost per isolate ranged between 105 USD and 304 USD, depending on the volume of testing, the level of automation and consumable manufacturers [169]. This cost did not include the investment of starting up a microbiology laboratory. In contrast, an acceptable price for blood cultures in low-resource settings would be less than 10 USD, and ideally less than 5 USD[170].

Another development limitation of the Mini-Lab should be noted, the lack of capacity of this version to test pathogenic fungi causing bloodstream infections. Among the

fungal pathogens, *Candida auris* has recently emerged as a powerful public health threat. This yeast was first described in 2009 and has since been reported in over 30 countries reported to cause multiple outbreaks in health care settings[171]. Despite the rapid global spread, it is difficult to predict the actual burden of the infection. Clinical laboratories often do not identify *Candida* isolates to the species level, and because *C. auris* is misidentified by commonly available laboratory methods, *C. auris* may be present in other countries, but has not been detected or has not yet been reported. Infection with *C. auris* is associated with high mortality rates. The crude in-hospital mortality rate for *C. auris* candidemia among critically ill patients in ICUs is estimated to range from 30 to 72%[171]. WHO released in in 2022 a list of health-threatening fungi with the prioritization process focused on fungal pathogens that can cause invasive acute and subacute systemic fungal infections for which drug resistance or other treatment and management challenges exist[59]. A rapid Lateral flow immunoassay has recently been developed to detect *C. auris* directly on positive blood culture, such a test would be an ideal companion assay for the Mini-Lab (NG biotech, France), if tested using the same approach as done during our work. Therefore in future evolution of the Mini-Lab, inclusion of capacity to detect, identify and test susceptibility of fungi should be addressed.

Conclusion:

In this doctoral thesis manuscript, we demonstrate that adapting clinical bacteriology to LRS constraints, in an easy-to-use manner for laboratory technicians that are non-experts in microbiology with high quality results to clinicians in the absence of onsite experts is challenging yet feasible. To do so, we demonstrated the need of a multidisciplinary approach of development methodologies, some of which are taken from the field of expertise outside of In Vitro Diagnostic development. We also showed that ready to use sealed packed media with long shelf life, generally used in the food and veterinary industry for quality control, can be used to subculture blood culture bottles. Also, MIC panels in the form of dehydrated ready to use standardised panels was demonstrated to be an adapted system in the field of MSF intervention, to maintain quality of results in the hands of a non-expert and reduce the burden on the supply chain. Also, the iterative approach used to test the different versions of the prototype has proved to be valuable to integrate user feedback and improve usability and robustness. However, simplicity of use comes with a slight increase of cost per test and requires strong support services.

As of today, the Mini-Lab has been deployed with success in four hospitals supported by MSF (South Sudan, Iraq, Nigeria, Central African Republic) and other locations are planned in the coming years. Capacity to culture urines has been added to the workflow and integration of Multi Drug Resistant organism detection capacity through the use of rapid lateral flow immunoassay is in progress. Also, this development led to several collaborations with research centres or institutions to test or use the Mini-Lab outside of MSF settings, such as Congo Brazzaville with the French Research Institute on Development (IRD), Vietnam with the Oxford University Clinical Research Unit, Indonesia with the Global Antimicrobial Resistance and Use Surveillance System units from WHO. Also it led to a collaboration with institutions involved in the Tricycle surveillance project from WHO, a one health integrated trans-sectorial surveillance system focusing on Extended Spectrum Beta Lactamase producing *E. coli*, to adapt the Mini-Lab sample management to this surveillance protocol and test a Tricycle Mini-Lab version in Indonesia and Democratic Republic of Congo.

The work described in this thesis is unique and provides a versatile platform in need of continuous improvement to stay at the level of quality and ease-of-use. Also, new pieces of technologies adapted to LRS constraint, for the detection of resistance or identification of certain pathogens, are coming and should be added to improve the concept; for diagnostic or surveillance purposes and to address a wider range of microorganisms.

It will require significant investment to effectively include bacteriology diagnostic capacity as one of many essential functions of any diagnostic and laboratory systems. There is no easy approach to increase capacity in an era of limited resources and competing priorities. The laboratory capacity to detect, culture bacteria and fungi, identify and perform susceptibility testing, are essential laboratory activities in need of major improvement in many lower-income countries. Besides the simplification of clinical bacteriology as a quick alternative in some settings (areas with no AMR data generated, door opener for microbiology testing) and as part of a system approach, investment in local production of media, tele-microbiology, quality education, optimisation of sample network transportation, optimisation of CBL facility placements, prequalification mechanisms for quality assured bacteriology tests, supply chain optimisation, well-functioning public health reference laboratories and the establishment of laboratory networks in low-resource settings must be stimulated with political engagement to foster local capacity.

References

- [1] R. P. Gaynes, "Alexander Fleming and the Discovery of Penicillin," *Germ Theory*, pp. 265–293, 2014, doi: 10.1128/9781555817220.ch13.
- [2] A. Cook, Michael and D. Wright, Gerard, "The past, present, and future of antibiotics," *Sci. Transl. Med.*, vol. 14, no. 657, 2022.
- [3] S. B. Levy, "The challenge of antibiotic resistance.," *Sci. Am.*, vol. 278, no. 3, pp. 46–53, 1998, doi: 10.1038/scientificamerican0398-46.
- [4] R. Laxminarayan, A. Duse, C. Wattal, A. K. M. Zaidi, H. F. L. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir *et al.*, "Antibiotic resistance-the need for global solutions.," *Lancet Infect. Dis.*, vol. 13, no. 12, pp. 1057–98, Dec. 2013, doi: 10.1016/S1473-3099(13)70318-9.
- [5] K. Iskandar, J. Murugaiyan, D. H. Halat, S. El Hage, V. Chibabhai, S. Adukkadukkam, C. Roques, L. Molinier, P. Salameh, and M. Van Dongen, "Antibiotic Discovery and Resistance: The Chase and the Race," *Antibiotics*, vol. 11, no. 2, 2022, doi: 10.3390/antibiotics11020182.
- [6] M. Nadimpalli, E. Delarocque-Astagneau, D. C. Love, L. B. Price, B. T. Huynh, J. M. Collard, K. S. Lay, L. Borand, A. Ndir, T. R. Walsh, D. Guillemot, A. De Lauzanne, A. Kerleguer, A. Tarantola, P. Piola, T. Chon, S. Lach, V. Ngo, S. Touch *et al.*, "Combating Global Antibiotic Resistance: Emerging One Health Concerns in Lower-and Middle-Income Countries," *Clin. Infect. Dis.*, vol. 66, no. 6, pp. 963–969, 2018, doi: 10.1093/cid/cix879.
- [7] K. Iskandar, L. Molinier, S. Hallit, M. Sartelli, F. Catena, F. Coccolini, T. C. Hardcastle, C. Roques, and P. Salameh, "Drivers of antibiotic resistance transmission in low-and middle-income countries from a 'one health' perspective—a review," *Antibiotics*, vol. 9, no. 7, pp. 1–23, 2020, doi: 10.3390/antibiotics9070372.
- [8] Z. I. Kimera, S. E. Mshana, M. M. Rweyemamu, L. E. G. Mboera, and M. I. N. Matee, "Antimicrobial use and resistance in food-producing animals and the environment: An African perspective," *Antimicrob. Resist. Infect. Control*, vol. 9, no. 1, pp. 1–12, 2020, doi:10.1186/s13756-020-0697-x.
- [9] S. Bandyopadhyay and I. Samanta, "Antimicrobial Resistance in Agri-Food Chain and Companion Animals as a Re-emerging Menace in Post-COVID Epoch: Low-and Middle-Income Countries Perspective and Mitigation Strategies," *Front. Vet. Sci.*, vol. 7, no. October, 2020, doi: 10.3389/fvets.2020.00620.
- [10] L. L. Founou, R. C. Founou, and S. Y. Essack, "Antibiotic resistance in the food chain: A developing country-perspective," *Front. Microbiol.*, vol. 7, no. NOV, pp. 1–19, 2016, doi: 10.3389/fmicb.2016.01881.
- [11] J. A. Ayukekbong, M. Ntemgwa, and A. N. Atabe, "The threat of antimicrobial resistance in developing countries: Causes and control strategies," *Antimicrob. Resist. Infect. Control*, vol. 6, no. 1, 2017, doi: 10.1186/s13756-017-0208-x.
- [12] S. Pokharel, S. Raut, and B. Adhikari, "Tackling antimicrobial resistance in low-

- income and middle-income countries," *BMJ Glob. Heal.*, vol. 4, no. 6, 2019, doi: 10.1136/bmjgh-2019-002104.
- [13] C. J. Murray, K. S. Ikuta, F. Sharara, L. Swetschinski, G. Robles Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S. C. Johnson, A. J. Browne, M. G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B. H. Kashef Hamadani, E. A. P. Kumaran, B. McManigal *et al.*, "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis," *Lancet*, vol. 6736, no. 21, 2022, doi: 10.1016/s0140-6736(21)02724-0.
- [14] World Health Organization, "The evolving threat of antimicrobial resistance: Options for action," 2014. Accessed: Nov. 21, 2013. [Online]. Available: <http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2014;volume=139;issue=1;spage=182;epage=183;aulast=Kapi>
- [15] K. H. Mayer, M. A. M. Huson, S. M. Stolp, T. Van Der Poll, and M. P. Grobusch, "Community-acquired bacterial bloodstream infections in HIV-infected patients: A systematic review," *Clin. Infect. Dis.*, vol. 58, no. 1, pp. 89–92, 2014, doi: 10.1093/cid/cit596.
- [16] J. B. Ndiokubwayo, A. A. Yahaya, A. T. Desta, G. Ki-Zerbo, E. A. Odei, B. Keita, A. P. Pana, and W. Nkhoma, "Antimicrobial resistance in the African Region: issues, challenges and actions proposed. Key Determinants for the African Region.," *WHO Off. African*, vol. 16, no. 16, pp. 27–30, 2013, [Online]. Available: <http://www.who.int/bulletin/>
- [17] J. O. 'Neill, "Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired," *Rev. Antimicrob. Resist.*, no. December, pp. 1–16, 2014.
- [18] WHO, "Global action plan on antimicrobial resistance," *World Heal. Organ.*, pp. 1–28, 2015, [Online]. Available: <https://www.who.int/publications/i/item/9789241509763>
- [19] J. O'Neill, "Review on Antimicrobial Resistance. Tackling a Global Health Crisis: Rapid Diagnostics : Stopping Unnecessary Use of Antibiotics," *Indep. Rev. AMR*, no. October, pp. 1–36, 2015.
- [20] J. I. M. O. Neill, "Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance.," no. May, 2016.
- [21] R. C. Founou, L. L. Founou, and S. Y. Essack, "Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis," *PLoS One*, vol. 12, no. 12, pp. 1–18, 2017, doi: 10.1371/journal.pone.0189621.
- [22] S. Gandra, G. Alvarez-Uria, P. Turner, J. Joshi, D. Limmathurotsakul, and H. R. van Doorn, "Antimicrobial resistance surveillance in low-and middle-income countries: Progress and challenges in eight South Asian and Southeast Asian countries," *Clin. Microbiol. Rev.*, vol. 33, no. 3, pp. 1–29, 2020, doi: 10.1128/CMR.00048-19.
- [23] World Health Organization, "Antimicrobial resistance: Global report on

- surveillance," Mar. 2014.
- [24] World Health Organization, *Global antimicrobial resistance and use surveillance system (GLASS) report 2021*. 2021. [Online]. Available: <http://www.who.int/glass/resources/publications/early-implementation-report-2020/en/>
- [25] B. T. Tadesse, E. A. Ashley, S. Ongarello, J. Havumaki, M. Wijegoonewardena, I. J. González, and S. Dittrich, "Antimicrobial resistance in Africa: A systematic review," *BMC Infect. Dis.*, vol. 17, no. 1, p. 616, 2017, doi: 10.1186/s12879-017-2713-1.
- [26] K. J. Bernabé, C. Langendorf, N. Ford, J. B. Ronat, and R. A. Murphy, "Antimicrobial resistance in West Africa: a systematic review and meta-analysis," *Int. J. Antimicrob. Agents*, vol. 50, no. 5, pp. 629–639, 2017, doi: 10.1016/j.ijantimicag.2017.07.002.
- [27] A. Chokshi, Z. Sifri, D. Cennimo, and H. Horng, "Global contributors to antibiotic resistance," *J. Glob. Infect. Dis.*, vol. 11, no. 1, pp. 36–42, 2019, doi: 10.4103/jgid.jgid_110_18.
- [28] L. M. Bebell and A. N. Muiuru, "Antibiotic use and emerging resistance: How can resource-limited countries turn the tide?," *Glob. Heart*, vol. 9, no. 3, pp. 347–358, Sep. 2014, doi: 10.1016/j.gheart.2014.08.009.
- [29] M. Alsan, L. Schoemaker, K. Eggleston, N. Kammili, P. Kolli, and J. Bhattacharya, "Out-of-pocket health expenditures and antimicrobial resistance in low-income and middle-income countries: an economic analysis," *Lancet Infect. Dis.*, vol. 15, no. 10, pp. 1203–1210, Oct. 2015, doi: 10.1016/S1473-3099(15)00149-8.
- [30] K. Tompkins, J. J. Juliano, and D. van Duin, "Antimicrobial Resistance in Enterobacteriales and Its Contribution to Sepsis in Sub-saharan Africa," *Front. Med.*, vol. 8, 2021, doi: 10.3389/fmed.2021.615649.
- [31] J. Jacobs, L. Hardy, M. Semret, O. Lunguya, T. Phe, D. Affolabi, C. Yansouni, and O. Vandenberg, "Diagnostic Bacteriology in District Hospitals in Sub-Saharan Africa: At the Forefront of the Containment of Antimicrobial Resistance," *Front. Med.*, vol. 6, no. September, 2019, doi: 10.3389/fmed.2019.00205.
- [32] S. Ombelet, J.-B. Ronat, T. Walsh, C. P. Yansouni, J. Cox, E. Vlieghe, D. Martiny, M. Semret, O. Vandenberg, and J. Jacobs, "Clinical bacteriology in low-resource settings: today's solutions," *Lancet Infect. Dis.*, vol. 18, no. 8, pp. e248–e258, Aug. 2018, doi: 10.1016/S1473-3099(18)30093-8.
- [33] World Health Organization, "Guidelines for the treatment of malaria," 2010.
- [34] V. D'Acremont, M. Kilowoko, E. Kyungu, S. Philipina, W. Sangu, J. Kahama-Marro, C. Lengeler, P. Cherpillod, L. Kaiser, and B. Genton, "Beyond malaria--causes of fever in outpatient Tanzanian children.," *N. Engl. J. Med.*, vol. 370, no. 9, pp. 809–17, Feb. 2014, doi: 10.1056/NEJMoa1214482.
- [35] P. Shrestha, P. Dahal, C. Ogbonnaa-Njoku, D. Das, K. Stepniewska, N. V. Thomas, H. Hopkins, J. A. Crump, D. Bell, P. N. Newton, E. A. Ashley, and P. J. Guérin, "Non-malarial febrile illness: A systematic review of published aetiological studies and case reports from Southern Asia and South-eastern Asia, 1980-2015," *BMC Med.*,

- vol. 18, no. 1, pp. 1–17, 2020, doi: 10.1186/s12916-020-01745-0.
- [36] M. W. Aregawi, A. S. Ali, A. W. Al-Mafazy, F. Molteni, S. Katikiti, M. Warsame, R. J. Njau, R. Komatsu, E. Korenromp, M. Hosseini, D. Low-Beer, A. Bjorkman, U. D'Alessandro, M. Coosemans, and M. Otten, "Reductions in malaria and anaemia case and death burden at hospitals following scale-up of malaria control in Zanzibar, 1999-2008," *Malar. J.*, vol. 10, pp. 1–9, 2011, doi: 10.1186/1475-2875-10-46.
- [37] V. D'Acromont, C. Lengeler, and B. Genton, "Reduction in the proportion of fevers associated with *Plasmodium falciparum* parasitaemia in Africa: A systematic review," *Malar. J.*, vol. 9, no. 1, pp. 1–11, 2010, doi: 10.1186/1475-2875-9-240.
- [38] M. K. Bouyou-Akotet, D. P. Mawili-Mboumba, E. Kendjo, M. Mabika-Mamfoumbi, E. B. Ngoungou, A. Dzeing-Ella, M. Pemba-Mihindou, E. Ibinga, E. E fame-Eya, T. Planche, P. G. Kremsner, and M. Kombila, "Evidence of decline of malaria in the general hospital of Libreville, Gabon from 2000 to 2008," *Malar. J.*, vol. 8, no. 1, 2009, doi: 10.1186/1475-2875-8-300.
- [39] M. V. Nielsen, N. Sarpong, R. Krumkamp, D. Dekker, W. Loag, S. Amemasor, A. Agyekum, F. Marks, F. Huenger, A. C. Krefis, R. M. Hagen, Y. Adu-Sarkodie, J. May, and N. G. Schwarz, "Incidence and Characteristics of Bacteremia among Children in Rural Ghana," *PLoS One*, vol. 7, no. 9, p. e44063, 2012, doi: 10.1371/journal.pone.0044063.
- [40] I. Guiraud, A. Post, S. N. Diallo, P. Lompo, J. Maltha, K. Thriemer, C. M. Tahita, B. Ley, K. Derra, E. Bottieau, A. Kazienga, C. Schurmans, R. Ravinetto, E. Rouamba, J. Van Griensven, S. Bertrand, H. Tinto, and J. Jacobs, "Population-based incidence, seasonality and serotype distribution of invasive salmonellosis among children in Nanoro, rural Burkina Faso," *PLoS One*, vol. 12, no. 7, pp. 1–17, 2017, doi: 10.1371/journal.pone.0178577.
- [41] J. M. Lewis, N. A. Feasey, and J. Rylance, "Aetiology and outcomes of sepsis in adults in sub-Saharan Africa: A systematic review and meta-analysis," *Crit. Care*, vol. 23, no. 1, pp. 1–11, 2019, doi: 10.1186/s13054-019-2501-y.
- [42] J. Valle, J. Vallés, J. Rello, A. Ochagavía, J. Garnacho, and M. A. Alcalá, "Community-acquired bloodstream infection in critically III adult patients: Impact of shock and inappropriate antibiotic therapy on survival," *Chest*, vol. 123, no. 5, pp. 1615–1624, 2003, doi: 10.1378/chest.123.5.1615.
- [43] M. W. Douglas, G. Lum, J. Roy, D. A. Fisher, N. M. Anstey, and B. J. Currie, "Epidemiology of community-acquired and nosocomial bloodstream infections in tropical Australia: a 12-month prospective study," *Trop. Med. Int. Heal.*, vol. 9, no. 7, pp. 795–804, Jul. 2004, doi: 10.1111/j.1365-3156.2004.01269.x.
- [44] S. M. Opal, G. D. Rubenfeld, T. Van Der Poll, J. L. Vincent, D. C. Angus, M. Singer, C. S. Deutschman, C. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G. R. Bernard, J. D. Chiche, C. M. Coopersmith, R. S. Hotchkiss, M. M. Levy, J. C. Marshall, G. S. Martin *et al.*, "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)," *JAMA - J. Am. Med. Assoc.*, vol. 315, no. 8,

- pp. 801–810, Feb. 2016, doi: 10.1001/jama.2016.0287.
- [45] M. J. Delano and P. A. Ward, "The immune system's role in sepsis progression, resolution, and long-term outcome.," *Immunol. Rev.*, vol. 274, no. 1, pp. 330–353, Nov. 2016, doi: 10.1111/imr.12499.
- [46] S. Fujishima, "Organ dysfunction as a new standard for defining sepsis," *Inflamm. Regen.*, vol. 36, no. 1, pp. 1–6, 2016, doi: 10.1186/s41232-016-0029-y.
- [47] M. Sanz Codina and M. Zeitlinger, "Biomarkers Predicting Tissue Pharmacokinetics of Antimicrobials in Sepsis: A Review," *Clin. Pharmacokinet.*, vol. 61, no. 5, pp. 593–617, 2022, doi: 10.1007/s40262-021-01102-1.
- [48] World Health Organisation, *Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions*. 2020. [Online]. Available:
<http://apps.who.int/bookorders.%0Ahttps://apps.who.int/iris/bitstream/handle/10665/334216/9789240010789-eng.pdf>
- [49] K. E. Rudd, S. C. Johnson, K. M. Agesa, K. A. Shackelford, D. Tsoi, D. R. Kievlan, D. V. Colombara, K. S. Ikuta, N. Kisson, S. Finfer, C. Fleischmann-Struzek, F. R. Machado, K. K. Reinhart, K. Rowan, C. W. Seymour, R. S. Watson, T. E. West, F. Marinho, S. I. Hay *et al.*, "Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study," *Lancet*, vol. 395, no. 10219, pp. 200–211, 2020, doi: 10.1016/S0140-6736(19)32989-7.
- [50] R. Markwart, H. Saito, T. Harder, S. Tomczyk, A. Cassini, C. Fleischmann-Struzek, F. Reichert, T. Eckmanns, and B. Allegranzi, "Epidemiology and burden of sepsis acquired in hospitals and intensive care units: a systematic review and meta-analysis," *Intensive Care Med.*, vol. 46, no. 8, pp. 1536–1551, 2020, doi: 10.1007/s00134-020-06106-2.
- [51] J. Church and K. Maitland, "Invasive bacterial co-infection in African children with Plasmodium falciparum malaria: A systematic review," *BMC Med.*, vol. 12, no. 1, pp. 1–16, 2014, doi: 10.1186/1741-7015-12-31.
- [52] L. M. Kalonji, A. Post, M. F. Phoba, D. Falay, D. Ngbonda, J. J. Muyembe, S. Bertrand, P. J. Ceysens, W. Mattheus, J. Verhaegen, B. Barbé, L. Kuijpers, C. Van Geet, O. Lunguya, and J. Jacobs, "Invasive salmonella infections at multiple surveillance sites in the Democratic Republic of the Congo, 2011-2014," *Clin. Infect. Dis.*, vol. 61, no. Suppl 4, pp. S346–S353, 2015, doi: 10.1093/cid/civ713.
- [53] E. a Reddy, A. V Shaw, and J. a Crump, "Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis.," *Lancet Infect. Dis.*, vol. 10, no. 6, pp. 417–32, Jun. 2010, doi: 10.1016/S1473-3099(10)70072-4.
- [54] J. Deen, L. von Seidlein, F. Andersen, N. Elle, N. J. White, and Y. Lubell, "Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: A systematic review," *Lancet Infect. Dis.*, vol. 12, no. 6, pp. 480–487, 2012, doi: 10.1016/S1473-3099(12)70028-2.
- [55] S. E. Park, G. D. Pak, P. Aaby, Y. Adu-Sarkodie, M. Ali, A. Aseffa, H. M. Biggs, M. Bjerregaard-Andersen, R. F. Breiman, J. A. Crump, L. M. Cruz Espinoza, M. A.

- Eltayeb, N. Gasmelseed, J. T. Hertz, J. Im, A. Jaeger, L. Parfait Kabore, V. von Kalckreuth, K. H. Keddy *et al.*, "The Relationship Between Invasive Nontyphoidal *Salmonella* Disease, Other Bacterial Bloodstream Infections, and Malaria in Sub-Saharan Africa," *Clin. Infect. Dis.*, vol. 62, no. suppl 1, pp. S23–S31, 2016, doi: 10.1093/cid/civ893.
- [56] J. A. Crump, H. O. Ramadhani, A. B. Morrissey, W. Saganda, M. S. Mwako, L. Y. Yang, S. C. Chow, S. C. Morpeth, H. Reyburn, B. N. Njau, A. V. Shaw, H. C. Diefenthal, J. F. Shao, J. A. Bartlett, and V. P. Maro, "Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania," *Clin. Infect. Dis.*, vol. 52, no. 3, pp. 341–348, 2011, doi: 10.1093/cid/ciq103.
- [57] World Health Organization., "WHO global strategy for containment of antimicrobial resistance," 2001. [Online]. Available: https://apps.who.int/iris/bitstream/handle/10665/66860/WHO_CDS_CSR_DRS_2_001.2.pdf
- [58] World Health Organization, "WHO bacterial priority pathogens list for R&D of new antibiotics," 2017. <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> (accessed Nov. 03, 2017).
- [59] World Health Organization, "WHO fungal priority pathogens list to guide research, development and public health action," 2022.
- [60] C. A. Petti, C. R. Polage, T. C. Quinn, A. R. Ronald, and M. A. Sande, "Laboratory medicine in Africa: A barrier to effective health care," *Clin. Infect. Dis.*, vol. 42, no. 3, pp. 377–382, Feb. 2006, doi: 10.1086/499363.
- [61] C. J. Tuijn, E. Msoka, D. L. Mushi, M. Sumari-de Boer, J. Chilongola, A. Van den Broek, "The interface between clinicians and laboratory staff: A field study in Northern Tanzania," *Afr. J. Lab. Med.*, vol. 3, no. 1, pp. 1–7, 2014, doi: 10.4102/ajlm.v3i1.126.
- [62] B. Spellberg, R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, and J. Edwards, "The epidemic of antibiotic-resistant infections: A call to action for the medical community from the infectious diseases society of America," *Clin. Infect. Dis.*, vol. 46, no. 2, pp. 155–64, Jan. 2008, doi: 10.1086/524891.
- [63] CDC, WHO, ASCP, PEPFAR, USAID, SCMS, BMGF, The Clinton Foundation, and The Global Fund, "Consultation on Technical and Clinical Laboratory Testing Harmonization and Standardization," 22-24 January 2008 Maputo, Mozambique, no. January, 2008, [Online]. Available: http://www.who.int/diagnostics_laboratory/3by5/Maputo_Meeting_Report_7_7_08.pdf
- [64] J. B. Ronat, A. Natale, T. Kesteman, A. Andremont, W. Elamin, L. Hardy, R. Kanapathipillai, J. Michel, C. Langendorf, O. Vandenberg, T. Naas, and F. Kouassi, "AMR in low-resource settings: Médecins Sans Frontières bridges surveillance

- gaps by developing a turnkey solution, the Mini-Lab," *Clin. Microbiol. Infect.*, vol. 27, no. 10, pp. 1414–1421, 2021, doi: 10.1016/j.cmi.2021.04.015.
- [65] Wellcome trust, "The Global Response to AMR: Momentum, success, and critical gaps," 2020. [Online]. Available: <http://gefans.org/speeches/speech122.html>
- [66] D. K. Saeed, R. Hasan, M. Naim, A. Zafar, E. Khan, K. Jabeen, S. Irfan, I. Ahmed, M. Zeeshan, Z. Wajidali, J. Farooqi, S. Shakoor, A. Chagla, and J. Rao, "Readiness for antimicrobial resistance (AMR) surveillance in Pakistan; a model for laboratory strengthening," *Antimicrob. Resist. Infect. Control*, vol. 6, no. 1, pp. 1–7, 2017, doi: 10.1186/s13756-017-0260-6.
- [67] C. Hazim, R. Abubeker Ibrahim, M. Westercamp, G. A. Belete, B. Amare Kibret, T. Kanter, G. Yimer, T. S. Adem, K. B. Stevenson, M. Urrego, K. N. Kale, M. W. Omondi, D. Vanderende, B. J. Park, M. M. B. Parsons, and K. M. Gallagher, "Establishment of a Sentinel Laboratory-Based Antimicrobial Resistance Surveillance Network in Ethiopia," *Heal. Secur.*, vol. 16, no. Suppl 1, pp. S30–S36, 2018, doi: 10.1089/hs.2018.0052.
- [68] S. Ombelet, B. Barbé, D. Affolabi, J. B. Ronat, P. Lompo, O. Lunguya, J. Jacobs, and L. Hardy, "Best Practices of Blood Cultures in Low- and Middle-Income Countries," *Front. Med.*, vol. 6, no. June, 2019, doi: 10.3389/fmed.2019.00131.
- [69] E. J. Baron, "Clinical Microbiology in Underresourced Settings," *Clin. Lab. Med.*, vol. 39, no. 3, pp. 359–369, Sep. 2019, doi: 10.1016/j.cll.2019.05.001.
- [70] A. C. Seale, C. Hutchison, S. Fernandes, N. Stoesser, H. Kelly, B. Lowe, P. Turner, K. Hanson, C. I. R. Chandler, C. Goodman, R. A. Stabler, and J. A. G. Scott, "Supporting surveillance capacity for antimicrobial resistance: Laboratory capacity strengthening for drug resistant infections in low and middle income countries," *Wellcome Open Res.*, vol. 2, no. 0, pp. 1–18, 2017, doi: 10.12688/wellcomeopenres.12523.1.
- [71] M. J. Ellington, O. Ekelund, F. M. Aarestrup, R. Canton, M. Doumith, C. Giske, H. Grundman, H. Hasman, M. Holden, K. L. Hopkins, J. Iredell, G. Kahlmeter, C. U. Köser, A. MacGowan, D. Mevius, M. Mulvey, T. Naas, T. Peto, J.-M. Rolain *et al.*, "The Role of Whole Genome Sequencing (WGS) in Antimicrobial Susceptibility Testing of Bacteria: Report from the EUCAST Subcommittee," *Clin. Microbiol. Infect.*, 2016, doi: 10.1016/j.cmi.2016.11.012.
- [72] J.-B. B. Ronat, J. Kakol, M. N. Houry, M. Berthelot, O. Yun, V. Brown, and R. A. Murphy, "Highly drug-resistant pathogens implicated in burn-associated bacteremia in an iraqi burn care unit.," *PLoS One*, vol. 9, no. 8, p. e101017, Jan. 2014, doi: 10.1371/journal.pone.0101017.
- [73] R. A. Murphy, O. Okoli, I. Essien, C. Teicher, G. Elder, J. Pena, J. B. Ronat, and K. J. Bernabé, "Multidrug-resistant surgical site infections in a humanitarian surgery project," *Epidemiol. Infect.*, vol. 144, no. 16, pp. 3520–3526, 2016, doi: 10.1017/S0950268816001758.
- [74] R. A. Murphy, L. Nisenbaum, A. S. Labar, R. L. Sheridan, J. B. Ronat, K. Dilworth, J. Pena, E. Kilborn, and C. Teicher, "Invasive Infection and Outcomes in a

- Humanitarian Surgical Burn Program in Haiti," *World J. Surg.*, vol. 40, no. 7, pp. 1550–1557, 2016, doi: 10.1007/s00268-016-3458-5.
- [75] R. A. Murphy, J.-B. B. Ronat, R. M. Fakhri, P. Herard, N. Blackwell, S. Abgrall, and D. J. Anderson, "Multidrug-resistant chronic osteomyelitis complicating war injury in iraqi civilians.," *J. Trauma*, vol. 71, no. 1, pp. 252–254, 2011, doi: 10.1097/TA.0b013e31821b8622.
- [76] C. L. Teicher, J. B. Ronat, R. M. Fakhri, M. Basel, A. S. Labar, P. Herard, and R. A. Murphy, "Antimicrobial drug-resistant bacteria isolated from Syrian war-injured patients, August 2011–march 2013," *Emerg. Infect. Dis.*, vol. 20, no. 11, pp. 1949–1951, 2014, doi: 10.3201/eid2011.140835.
- [77] F. Fily, J. Ronat, N. Malou, R. Kanapathipillai, C. Seguin, N. Hussein, R. M. Fakhri, and C. Langendorf, "Post-traumatic osteomyelitis in Middle East war-wounded civilians: resistance to first-line antibiotics in selected bacteria over the decade 2006–2016," *BMC Infect. Dis.*, vol. 19, no. 1, p. 103, Dec. 2019, doi: 10.1186/s12879-019-3741-9.
- [78] R. Kanapathipillai, N. Malou, J. Hopman, C. Bowman, N. Yousef, J. Michel, N. Hussein, P. Herard, J. Ousley, C. Mills, C. Seguin, and M. Saim, "Antibiotic resistance in conflict settings: lessons learned in the Middle East," *JAC-Antimicrobial Resist.*, vol. 1, no. 1, pp. 2–4, 2019, doi: 10.1093/jacamr/dlz002.
- [79] C. Langendorf, S. Le Hello, A. Moumouni, M. Gouali, A.-A. Mamaty, R. F. Grais, F.-X. Weill, and A.-L. Page, "Enteric Bacterial Pathogens in Children with Diarrhea in Niger: Diversity and Antimicrobial Resistance," *PLoS One*, vol. 10, p. e0120275, 2015, doi: 10.1371/journal.pone.0120275.
- [80] A.-L. Page, N. de Rekeneire, S. Sayadi, S. Aberrane, A.-C. Janssens, C. Rieux, A. Djibo, J.-C. Manuguerra, H. Ducou-le-Pointe, R. F. Grais, M. Schaefer, P. J. Guerin, and E. Baron, "Infections in children admitted with complicated severe acute malnutrition in Niger.," *PLoS One*, vol. 8, no. 7, p. e68699, 2013, doi: 10.1371/journal.pone.0068699.
- [81] R. Dellinger, M. Levy, J. Carlet, J. Bion, M. Parker, R. Jaeschke, K. Reinhart, D. Angus, C. Brun-Buisson, R. Beale, T. Calandra, J. Dhainaut, H. Gerlach, M. Harvey, J. Marini, J. Marshall, M. Ranieri, G. Ramsay, J. Sevransky *et al.*, *Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008.*, vol. 34, no. 1. 2008. doi: 10.1007/s00134-007-0934-2.
- [82] FDA, "Guidance for Industry and FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems Preface Public Comment: Additional Copies :," pp. 1–42, 2009.
- [83] ISO, "Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 2: Evaluation of performance of antimicrobial susceptibility t," *Test*, vol. 1, no. 20776–2, p. 5, 2007, doi: 10.5594/J09750.
- [84] H. Bachou, T. Tylleskär, D. H. Kaddu-Mulindwa, and J. K. Tumwine, "Bacteraemia

- among severely malnourished children infected and uninfected with the human immunodeficiency virus-1 in Kampala, Uganda.," *BMC Infect. Dis.*, vol. 6, p. 160, 2006, doi: 10.1186/1471-2334-6-160.
- [85] J. Maltha, I. Guiraud, B. Kabor??, P. Lompo, B. Ley, E. Bottieau, C. Van Geet, H. Tinto, and J. Jacobs, "Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso," *PLoS One*, vol. 9, no. 2, pp. 1–8, 2014, doi: 10.1371/journal.pone.0089103.
- [86] A. C. Seale, M. Mwaniki, C. R. Newton, and J. A. Berkley, "Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa," *Lancet Infect. Dis.*, vol. 9, no. 7, pp. 428–438, 2009, doi: 10.1016/S1473-3099(09)70172-0.
- [87] A. William and A. A. W. Dreyer, "Blood Culture Systems: From Patient to Result," *Sepsis - An Ongoing Signif. Chall.*, pp. 287–310, 2012, doi: 10.5772/50139.
- [88] A. P. K.Culbreath, "Balancing Enthusiasm for innovative technologies with Optimizing Value: An approach to adopt New Laboratory tests for infectious disease using Bloodstream Infections as Exemplar," pp. 1–19, 2013.
- [89] I. N. Miskin, R. Nir-Paz, C. Block, O. Merin, S. Burshtein, S. Pirogovsky, T. Halperin, D. Schwartz, and M. J. Schwaber, "Antimicrobial therapy for wound infections after catastrophic earthquakes.," *N. Engl. J. Med.*, vol. 363, no. 26, pp. 2571–2573, 2010, doi: 10.1056/NEJMc1005578.
- [90] J. M. Mansuy, "Mobile laboratories for Ebola and other pathogens," *The Lancet Infectious Diseases*, vol. 15, no. 10. p. 1135, 2015. doi: 10.1016/S1473-3099(15)00309-6.
- [91] S. Muhi, N. Tayler, T. Hoang, J. Prestedge, J. Y. H. Lee, S. A. Ballard, N. Isles, A. Wlodek, A. Greenhalgh, D. A. Williamson, B. P. Howden, and T. P. Stinear, "Feasibility of a refurbished shipping container as a transportable laboratory for rapid SARS-CoV-2 diagnostics," *Access Microbiol.*, vol. 4, no. 4, pp. 1–7, 2022, doi: 10.1099/acmi.0.000346.
- [92] C. Baraniuk, "A covid-19 laboratory for Jersey-in a shipping container," *BMJ*, vol. 370, pp. 3–4, 2020, doi: 10.1136/bmj.m3336.
- [93] D. J. Bridges, J. Colborn, A. S. T. Chan, A. M. Winters, D. Dengala, C. M. Fornadel, and B. Kosloff, "Modular laboratories-cost-effective and sustainable infrastructure for resource-limited settings.," *Am. J. Trop. Med. Hyg.*, vol. 91, no. 6, pp. 1074–8, Dec. 2014, doi: 10.4269/ajtmh.14-0054.
- [94] B.-M. Njanpop-Lafourcade, S. Hugonnet, H. Djogbe, A. Kodjo, A. K. N'douba, M.-K. Taha, P. Stoeckel, and B. D. Gessner, "Mobile microbiological laboratory support for evaluation of a meningitis epidemic in Northern Benin.," *PLoS One*, vol. 8, no. 7, p. e68401, Jan. 2013, doi: 10.1371/journal.pone.0068401.
- [95] D. Mondal, P. Ghosh, M. A. A. Khan, F. Hossain, S. Böhlken-Fascher, G. Matlashewski, A. Kroeger, P. Olliaro, and A. Abd El Wahed, "Mobile suitcase laboratory for rapid detection of *Leishmania donovani* using recombinase polymerase amplification assay," *Parasites and Vectors*, vol. 9, no. 1, pp. 1–8,

- 2016, doi: 10.1186/s13071-016-1572-8.
- [96] S. Ombelet, A. Natale, J. Ronat, O. Vandenberg, J. Jacobs, and L. Hardy, "Considerations in evaluating equipment-free blood culture bottles: A short protocol for use in low-resource settings," *PLoS One*, vol. 17, no. 4, p. e0267491, Apr. 2022, doi: 10.1371/journal.pone.0267491.
- [97] S. Ombelet, A. Natale, J.-B. Ronat, T. Kesteman, O. Vandenberg, J. Jacobs, and L. Hardy, "Biphasic versus monophasic manual blood culture bottles for low-resource settings: an in-vitro study," *The Lancet Microbe*, vol. 3, no. 2, pp. e124–e132, Feb. 2022, doi: 10.1016/S2666-5247(21)00241-X.
- [98] A. Natale, J.-B. Ronat, A. Mazoyer, A. Rochard, B. Boillot, J. Hubert, B. Baillet, M. Ducasse, F. Mantelet, S. Oueslati, S. Ombelet, C. Langendorf, T. Naas, O. Vandenberg, and J. Jacobs, "The Mini-Lab: accessible clinical bacteriology for low-resource settings," *The Lancet Microbe*, vol. 1, no. 2, pp. e56–e58, 2020, doi: 10.1016/s2666-5247(20)30012-4.
- [99] A. Natale, S. Oueslati, A. Rochard, S. Ombelet, D. Lopez-Baez, L. Hardy, J. Cunningham, C. Franquesa, O. Vandenberg, J. B. Ronat, and T. Naas, "Evaluation of InTray Cassettes Directly from Blood Cultures for the Diagnosis of Sepsis in Clinical Bacteriology Laboratories as an Alternative to Classic Culture Media," *Diagnostics*, vol. 13, no. 3, p. 523, Jan. 2023, doi: 10.3390/diagnostics13030523.
- [100] S. Ombelet, A. Natale, J.-B. Ronat, O. Vandenberg, L. Hardy, and J. Jacobs, "Evaluation of MicroScan Bacterial Identification Panels for Low-Resource Settings," *Diagnostics*, vol. 11, no. 2, p. 349, Feb. 2021, doi: 10.3390/diagnostics11020349.
- [101] J. B. Ronat, S. Oueslati, A. Natale, T. Kesteman, W. Elamin, C. Langendorf, L. Hardy, O. Vandenberg, and T. Naas, "Validation of Three MicroScan® Antimicrobial Susceptibility Testing Plates Designed for Low-Resource Settings," *Diagnostics*, vol. 12, no. 9, p. 2106, Aug. 2022, doi: 10.3390/diagnostics12092106.
- [102] S. Djibo, B. N. Lafourcade, P. Boisier, A. Moussa, G. Kobo, F. Sidikou, A. Hien, G. Bieboure, J. Aguilera, I. Parent, B. D. Gessner, and S. Chanteau, "Evaluation of the Pastorex® meningitis kit for the rapid identification of *Neisseria meningitidis* serogroups A and W135," 2006, doi: 10.1016/j.trstmh.2005.10.002.
- [103] P. Boisier, A. Elhaj Mahamane, A. Amadou Hamidou, F. Sidikou, S. Djibo, F. Nato, and S. Chanteau, "Field evaluation of rapid diagnostic tests for meningococcal meningitis in Niger," *Trop. Med. Int. Heal.*, vol. 14, no. 1, pp. 111–117, Jan. 2009, doi: 10.1111/j.1365-3156.2008.02192.x.
- [104] S. Chanteau, S. Darteville, A. E. Mahamane, S. Djibo, P. Boisier, and F. Nato, "New rapid diagnostic tests for *neisseria meningitidis* serogroups A, W135, C, and Y," *PLoS Med.*, vol. 3, no. 9, pp. 1579–1586, Sep. 2006, doi: 10.1371/journal.pmed.0030337.
- [105] World Health Organization, "Laboratory Quality Management System Handbook," 2011.
- [106] L. Smith and World Health Organization, *Laboratory Biosafety Manual*, 4th ed.,

- vol. 16, no. 4. Geneva: CDC, 2021. doi: 10.37774/9789275724170.
- [107] European Society for Clinical Microbiology and Infectious Diseases and Société Française de Microbiologie, *European Manual of Clinical Microbiology*. 2012.
- [108] Clinical and Standard Laboratory Institute, "Performance standards for antimicrobial susceptibility testing," *CLSI Doc.*, vol. 29th, no. M100-S-29, p. 136, 2020.
- [109] The European committee and Antimicrobial susceptibility testing, "Intrinsic Resistance and Unusual Phenotypes version 3.2 February 2020," https://Eucast.Org/Expert_Rules_and_Intrinsic_Resistance/, no. 3.2, pp. 1–12, 2020.
- [110] C. G. Giske, L. Martinez, R. Cantòn, S. Stefani, R. Skov, Y. Glupczynski, P. Nordmann, M. Wootton, V. Miriagou, G. Skov Simonsen, H. Zemlickova, J. Cohen-Stuart, and M. Gniadkowski, "EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and / or epidemiological importance," *Eur. Soc. Clin. Microbiol. Infect. Dis.*, no. December, pp. 1–40, 2013.
- [111] J. M. Stelling, "News and Notes WHONET : An Information System for Monitoring Recommendations for Preventing the Spread of Antimicrobial resistance," *Emerg. Infect. Dis.*, vol. 1, no. 2, pp. 66–67, 1995.
- [112] World Health Organization, "GLASS manual for early implementation," 2015.
- [113] P. Turner, P. Rupali, J. A. Opintan, W. Jaoko, N. A. Feasey, S. J. Peacock, and E. A. Ashley, "Laboratory informatics capacity for effective antimicrobial resistance surveillance in resource-limited settings," *Lancet Infect. Dis.*, vol. 3099, no. 20, pp. 1–5, 2021, doi: 10.1016/s1473-3099(20)30835-5.
- [114] D. D. Rhoads, V. Sintchenko, C. A. Rauch, and L. Pantanowitz, "Clinical microbiology informatics," *Clin. Microbiol. Rev.*, vol. 27, no. 4, pp. 1025–1047, 2014, doi: 10.1128/CMR.00049-14.
- [115] The European Committee on Antimicrobial Susceptibility Testing and The European committee and Antimicrobial susceptibility testing, "Breakpoint tables for interpretation of MICs and zone diameters, version 9.0," 2019. [Online]. Available: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf
- [116] D. Castillo, B. H. Harcourt, C. Hatcher, M. Jackson, L. Katz, R. Mair, L. W. Mayer, R. Novak, L. Rahalison, S. Schmink, M. Theodore, J. Thomas, J. Vuong, X. Wang, L. McGee, D. A. Caugant, and S. Chanteu, *Laboratory Manual for the Diagnosis of Meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae*, 2nd Editio. 2011. doi: 10.1007/SpringerReference_301104.
- [117] J. Orekan, B. Barbé, S. Oeng, J. B. Ronat, J. Letchford, J. Jacobs, D. Affolabi, and L. Hardy, "Culture media for clinical bacteriology in low- and middle-income countries: challenges, best practices for preparation and recommendations for improved access," *Clin. Microbiol. Infect.*, vol. 27, no. 10, pp. 1400–1408, 2021,

- doi: 10.1016/j.cmi.2021.05.016.
- [118] Clinical and Laboratory Standards Institute (CLSI), *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard — Third Edition*, vol. 24, no. 19. 2004.
- [119] F. M. Russell, S. S. N. Biribo, G. Selvaraj, F. Oppedisano, S. Warren, A. Seduadua, E. K. Mulholland, and J. R. Carapetis, "As a bacterial culture medium, citrated sheep blood agar is a practical alternative to citrated human blood agar in laboratories of developing countries," *J. Clin. Microbiol.*, vol. 44, no. 9, pp. 3346–3351, 2006, doi: 10.1128/JCM.02631-05.
- [120] V. C. Brenner and J. C. Sherris, "Influence of different media and bloods on results of diffusion antibiotic susceptibility tests.," *Antimicrob. Agents Chemother.*, vol. 1, no. 2, pp. 116–122, 1972, doi: 10.1128/AAC.1.2.116.
- [121] N. Niederstebruch, D. Sixt, B. I. Benda, and N. Banboye, "A suitable blood agar containing human blood especially for the use in laboratories of developing countries," *J. Infect. Dev. Ctries.*, vol. 11, no. 5, pp. 399–406, 2017, doi: 10.3855/jidc.8957.
- [122] C. Satzke, A. Seduadua, R. Chandra, J. R. Carapetis, E. K. Mulholland, and F. M. Russell, "Comparison of citrated human blood, citrated sheep blood, and defibrinated sheep blood Mueller-Hinton agar preparations for antimicrobial susceptibility testing of streptococcus pneumoniae isolates," *J. Clin. Microbiol.*, vol. 48, no. 10, pp. 3770–3772, Oct. 2010, doi: 10.1128/JCM.02357-09.
- [123] B. Sterzik and F. J. Fehrenbach, "Reaction components influencing CAMP factor induced lysis," *J. Gen. Microbiol.*, vol. 131, no. 4, pp. 817–820, Apr. 1985, doi: 10.1099/00221287-131-4-817.
- [124] I. N. Okeke, R. W. Peeling, H. Goossens, R. Auckenthaler, S. S. Olmsted, J. F. De Lavison, B. L. Zimmer, M. D. Perkins, and K. Nordqvist, "Diagnostics as essential tools for containing antibacterial resistance," *Drug Resist. Updat.*, vol. 14, no. 2, pp. 95–106, 2011, doi: 10.1016/j.drug.2011.02.002.
- [125] P.-E. Fournier, M. Drancourt, P. Colson, J.-M. Rolain, B. La Scola, and D. Raoult, "Modern clinical microbiology: new challenges and solutions," *Nat. Rev. Microbiol.*, vol. 11, no. 8, pp. 574–585, 2013, doi: 10.1038/nrmicro3068.
- [126] H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg, and J. Bartlett, "Bad bugs, no drugs: No ESCAPE! An update from the Infectious Diseases Society of America," *Clin. Infect. Dis.*, vol. 48, no. 1, pp. 1–12, 2009, doi: 10.1086/595011.
- [127] J. H. Jorgensen and M. J. Ferraro, "Antimicrobial susceptibility testing: a review of general principles and contemporary practices.," *Clin. Infect. Dis.*, vol. 49, no. 11, pp. 1749–55, Dec. 2009, doi: 10.1086/647952.
- [128] E. A. Ashley, J. Recht, A. Chua, D. Dance, M. Dhorda, N. V. Thomas, N. Ranganathan, P. Turner, P. J. Guerin, N. J. White, and N. P. Day, "An inventory of supranational antimicrobial resistance surveillance networks involving low- and middle-income countries since 2000," *J. Antimicrob. Chemother.*, vol. 73, no.

- April, pp. 1–13, 2018, doi: 10.1093/jac/dky026.
- [129] I. Gajic, J. Kabic, D. Kekic, M. Jovicevic, M. Milenkovic, D. Mitic Culafic, A. Trudic, L. Ranin, and N. Opavski, "Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods," *Antibiotics*, vol. 11, no. 4, p. 427, Mar. 2022, doi: 10.3390/antibiotics11040427.
- [130] T. Winstanley and P. Courvalin, "Expert systems in clinical microbiology," *Clin. Microbiol. Rev.*, vol. 24, no. 3, pp. 515–556, 2011, doi: 10.1128/CMR.00061-10.
- [131] G. Skiniotis, M. J. Boulanger, K. C. Garcia, and T. Walz, "Signaling conformations of the tall cytokine receptor gp130 when in complex with IL-6 and IL-6 receptor," *Nat. Struct. Mol. Biol.*, vol. 12, no. 6, pp. 545–51, Jun. 2005, doi: 10.1038/nsmb941.
- [132] S. Feng, D. Tseng, D. Di Carlo, O. B. Garner, and A. Ozcan, "High-throughput and automated diagnosis of antimicrobial resistance using a cost-effective cellphone-based micro-plate reader," *Nat. Publ. Gr.*, no. November, pp. 1–9, 2016, doi: 10.1038/srep39203.
- [133] B. Berg, B. Cortazar, D. Tseng, H. Ozkan, S. Feng, Q. Wei, R. Y. L. Chan, J. Burbano, Q. Farooqui, M. Lewinski, D. Di Carlo, O. B. Garner, and A. Ozcan, "Cellphone-Based Hand-Held Microplate Reader for Point-of-Care Testing of Enzyme-Linked Immunosorbent Assays," *ACS Nano*, vol. 9, no. 8, pp. 7857–7866, 2015, doi: 10.1021/acsnano.5b03203.
- [134] E. K. Korgenski and J. A. Daly, "Evaluation of the BIOMIC video reader system for determining interpretive categories of isolates on the basis of disk diffusion susceptibility results," *J. Clin. Microbiol.*, vol. 36, no. 1, pp. 302–304, 1998.
- [135] H. K. Geiss and U. E. Klar, "Evaluation of the BIOMIC video reader system for routine use in the clinical microbiology laboratory," *Diagn. Microbiol. Infect. Dis.*, vol. 37, no. 3, pp. 151–155, Jul. 2000, doi: 10.1016/S0732-8893(00)00132-2.
- [136] R. C. Fader, E. Weaver, R. Fossett, M. Toyras, J. Vanderlaan, D. Gibbs, A. Wang, and N. Thierjung, "Multilaboratory Study of the Biomic Automated Well-Reading Instrument versus MicroScan WalkAway for Reading MicroScan Antimicrobial Susceptibility and Identification Panels," *J. Clin. Microbiol.*, vol. 51, no. 5, pp. 1548–1554, May 2013, doi: 10.1128/JCM.03088-12.
- [137] I. Berke and P. M. Tierno, "Comparison of efficacy and cost-effectiveness of BIOMIC VIDEO and Vitek antimicrobial susceptibility test systems for use in the clinical microbiology laboratory," *J. Clin. Microbiol.*, vol. 34, no. 8, pp. 1980–1984, 1996, doi: 10.1128/jcm.34.8.1980-1984.1996.
- [138] E. J. Baron, H. D'Souza, A. Q. Wang, and D. L. Gibbs, "Evaluation of the biomic V3 microbiology system for identification of selected species on BBL CHROMagar orientation agar and CHROMagar MRSA medium," *J. Clin. Microbiol.*, vol. 46, no. 10, pp. 3488–3490, 2008, doi: 10.1128/JCM.02460-07.
- [139] WHO, *Laboratory Quality Standards and their Implementation*, no. 978 92 9022 397 9. World Health Organization, 2011.
- [140] A. P. Davies, M. Reid, S. J. Hadfield, S. Johnston, J. Mikhail, L. G. Harris, H. F. Jenkinson, N. Berry, A. M. Lewis, K. El-Bouri, and D. Mack, "Identification of clinical

- isolates of α -hemolytic streptococci by 16S rRNA gene sequencing, matrix-assisted laser desorption ionization-time of flight mass spectrometry using MALDI biotyper, and conventional phenotypic methods: A comparison," *J. Clin. Microbiol.*, vol. 50, no. 12, pp. 4087–4090, Dec. 2012, doi: 10.1128/JCM.02387-12.
- [141] P. P. Bosshard, S. Abels, M. Altwegg, E. C. Böttger, and R. Zbinden, "Comparison of Conventional and Molecular Methods for Identification of Aerobic Catalase-Negative Gram-Positive Cocci in the Clinical Laboratory," *J. Clin. Microbiol.*, vol. 42, no. 5, pp. 2065–2073, May 2004, doi: 10.1128/JCM.42.5.2065-2073.2004.
- [142] A. Soares, M. Pestel-Caron, F. Leysour de Rohello, G. Bourgoïn, S. Boyer, and F. Caron, "Area of technical uncertainty for susceptibility testing of amoxicillin/clavulanate against *Escherichia coli*: analysis of automated system, Etest and disk diffusion methods compared to the broth microdilution reference," *Clin. Microbiol. Infect.*, vol. 26, no. 12, pp. 1685.e1-1685.e6, Dec. 2020, doi: 10.1016/j.cmi.2020.02.038.
- [143] K. L. Chew, M. Van La, R. T. P. Lin, and J. W. P. Teo, "Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive enterobacteriaceae: Comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution," *J. Clin. Microbiol.*, vol. 55, no. 9, pp. 2609–2616, Sep. 2017, doi: 10.1128/JCM.00268-17.
- [144] J. R. Lo-Ten-Foe, A. M. G. A. de Smet, B. M. W. Diederén, J. A. J. W. Kluytmans, and P. H. J. van Keulen, "Comparative Evaluation of the VITEK 2, Disk Diffusion, Etest, Broth Microdilution, and Agar Dilution Susceptibility Testing Methods for Colistin in Clinical Isolates, Including Heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* Strains," *Antimicrob. Agents Chemother.*, vol. 51, no. 10, pp. 3726–3730, Oct. 2007, doi: 10.1128/AAC.01406-06.
- [145] N. Pfennigwerth, A. Kaminski, M. Korte-Berwanger, Y. Pfeifer, M. Simon, G. Werner, J. Jantsch, L. Marlinghaus, and S. G. Gatermann, "Evaluation of six commercial products for colistin susceptibility testing in Enterobacterales," *Clin. Microbiol. Infect.*, vol. 25, no. 11, pp. 1385–1389, Nov. 2019, doi: 10.1016/j.cmi.2019.03.017.
- [146] E. J. Baron, J. M. Miller, M. P. Weinstein, S. S. Richter, P. H. Gilligan, R. B. Thomson, P. Bourbeau, K. C. Carroll, S. C. Kehl, W. M. Dunne, B. Robinson-Dunn, J. D. Schwartzman, K. C. Chapin, J. W. Snyder, B. a Forbes, R. Patel, J. E. Rosenblatt, and B. S. Pritt, "A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)a.," *Clin. Infect. Dis.*, vol. 57, no. 4, pp. e22–e121, Aug. 2013, doi: 10.1093/cid/cit278.
- [147] A. L. Leber and R. Leber, *Clinical Microbiology Procedures Handbook*, 3rd ed. Washington, DC, USA: ASM press, 2016. doi: 10.1128/9781555818814.
- [148] CLSI, *Principles and Procedures for Blood Cultures, approved guideline*, vol. 26, no. 31. 2007.
- [149] C. Emeraud, S. Yilmaz, N. Fortineau, G. Cuzon, and L. Dortet, "Quality indicators

- for blood culture: 1 year of monitoring with BacT/Alert Virtuo at a French hospital," *J. Med. Microbiol.*, vol. 70, no. 3, 2021, doi: 10.1099/jmm.0.001300.
- [150] L. P. Samuel, J. M. Balada-Llasat, A. Harrington, and R. Cavagnolo, "Multicenter assessment of gram stain error rates," *J. Clin. Microbiol.*, vol. 54, no. 6, pp. 1442–1447, 2016, doi: 10.1128/JCM.03066-15.
- [151] C. Donati, N. L. Hiller, H. Tettelin, A. Muzzi, N. J. Croucher, S. V. Angiuoli, M. Oggioni, J. C. Dunning Hotopp, F. Z. Hu, D. R. Riley, A. Covacci, T. J. Mitchell, S. D. Bentley, M. Kilian, G. D. Ehrlich, R. Rappuoli, E. R. Moxon, and V. Masignani, "Structure and dynamics of the pan-genome of *Streptococcus pneumoniae* and closely related species," *Genome Biol.*, vol. 11, no. 10, Oct. 2010, doi: 10.1186/gb-2010-11-10-r107.
- [152] C. Shubert, J. Slaughter, D. Creely, A. Van Belkum, J. P. Gayral, W. M. Dunne, G. Zambardi, and D. Shortridge, "Population analysis of *Escherichia coli* isolates with discordant resistance levels by piperacillin-tazobactam broth microdilution and agar dilution testing," *Antimicrob. Agents Chemother.*, vol. 58, no. 3, pp. 1779–1781, 2014, doi: 10.1128/AAC.02181-13.
- [153] D. Creely, G. Zambardi, A. Van Belkum, W. M. Dunne, M. Peyret, J. P. Gayral, D. Shortridge, and C. Shubert, "International dissemination of *Escherichia coli* strains with discrepant behaviour in phenotypic antimicrobial susceptibility tests," *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 32, no. 8, pp. 997–1002, 2013, doi: 10.1007/s10096-013-1837-5.
- [154] R. P. H. Peters, E. E. Zijlstra, M. J. Schijffelen, A. L. Walsh, G. Joaki, J. J. Kumwenda, J. G. Kublin, M. E. Molyneux, and D. K. Lewis, "A prospective study of bloodstream infections as cause of fever in Malawi: Clinical predictors and implications for management," *Trop. Med. Int. Heal.*, vol. 9, no. 8, pp. 928–934, 2004, doi: 10.1111/j.1365-3156.2004.01288.x.
- [155] N. a Feasey, G. Dougan, R. a Kingsley, R. S. Heyderman, and M. a Gordon, "Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa.," *Lancet*, vol. 379, no. 9835, pp. 2489–99, Jun. 2012, doi: 10.1016/S0140-6736(11)61752-2.
- [156] A. Nebbioso, O. F. Ogundipe, E. C. Repetto, C. Mekiedje, H. Sanke-Waigana, G. Ngaya, B. Ingelbeen, and J. Gil-Cuesta, "When first line treatment of neonatal infection is not enough: blood culture and resistance patterns in neonates requiring second line antibiotic therapy in Bangui, Central African Republic," *BMC Pediatr.*, vol. 21, no. 1, p. 570, Dec. 2021, doi: 10.1186/s12887-021-02911-w.
- [157] T. J. Kirn and M. P. Weinstein, "Update on blood cultures: How to obtain, process, report, and interpret," *Clin. Microbiol. Infect.*, vol. 19, no. 6, pp. 513–520, 2013, doi: 10.1111/1469-0691.12180.
- [158] M. Søggaard, M. Nørgaard, and H. C. Schønheyder, "First notification of positive blood cultures and the high accuracy of the gram stain report," *J. Clin. Microbiol.*, vol. 45, no. 4, pp. 1113–1117, 2007, doi: 10.1128/JCM.02523-06.
- [159] S. Ombelet, A. Natale, J.-B. Ronat, T. Kesteman, O. Vandenberg, J. Jacobs, and L.

- Hardy, "Biphasic Versus Monophasic Manual Blood Culture Bottles for Low-Resource Settings: An In-Depth *in vitro* Evaluation Using Simulated Blood Cultures," *SSRN Electron. J.*, vol. 1, pp. 1–21, 2021, doi: 10.2139/ssrn.3781655.
- [160] E. Yeh, B. a Pinsky, N. Banaei, and E. J. Baron, "Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests.," *PLoS One*, vol. 4, no. 7, p. e6141, 2009, doi: 10.1371/journal.pone.0006141.
- [161] J. Åhman, E. Matuschek, and G. Kahlmeter, "The quality of antimicrobial discs from nine manufacturers—EUCAST evaluations in 2014 and 2017," *Clin. Microbiol. Infect.*, vol. 25, no. 3, pp. 346–352, 2019, doi: 10.1016/j.cmi.2018.05.021.
- [162] J. Åhman, E. Matuschek, and G. Kahlmeter, "EUCAST evaluation of 21 brands of Mueller–Hinton dehydrated media for disc diffusion testing," *Clin. Microbiol. Infect.*, vol. 26, no. 10, pp. 1412.e1–1412.e5, 2020, doi: 10.1016/j.cmi.2020.01.018.
- [163] A. S. Ginsburg, L. Tinkham, K. Riley, N. A. Kay, K. P. Klugman, and C. J. Gill, "Antibiotic non-susceptibility among *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates identified in African cohorts: A meta-analysis of three decades of published studies," *International Journal of Antimicrobial Agents*, vol. 42, no. 6, pp. 482–491, 2013, doi: 10.1016/j.ijantimicag.2013.08.012.
- [164] D. J. Hata, "Fastidious and Furious: Reporting Antimicrobial Susceptibility Testing for Fastidious or Infrequently Isolated Bacteria," *Clin. Microbiol. Newsl.*, vol. 39, no. 24, pp. 191–198, 2017, doi: 10.1016/j.clinmicnews.2017.11.003.
- [165] J. P. Lynch and G. G. Zhanel, "*Streptococcus pneumoniae*: Epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines," *Curr. Opin. Pulm. Med.*, vol. 16, no. 3, pp. 217–225, 2010, doi: 10.1097/MCP.0b013e3283385653.
- [166] U. Okomo, E. N. K. Akpalu, K. Le Doare, A. Roca, S. Cousens, A. Jarde, M. Sharland, B. Kampmann, and J. E. Lawn, "Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines," *Lancet Infect. Dis.*, vol. 19, no. 11, pp. 1219–1234, Nov. 2019, doi: 10.1016/S1473-3099(19)30414-1.
- [167] S. T. Shulman, A. L. Bisno, H. W. Clegg, M. a. Gerber, E. L. Kaplan, G. Lee, J. M. Martin, and C. Van Beneden, "Clinical practice guideline for the diagnosis and management of group a streptococcal pharyngitis: 2012 update by the infectious diseases society of America," *Clin. Infect. Dis.*, vol. 55, no. 10, pp. 1–17, 2012, doi: 10.1093/cid/cis629.
- [168] WHO, "World health Organization. Recommendations for management of common childhood conditions: newborn conditions, dysentery, pneumoniae, oxygen use and delivery, common causes of fever, severe acute malnutrition and supportive care.," 2012.
- [169] T. Roberts, N. Luangasanatip, C. L. Ling, J. Hopkins, R. Jaksuwan, Y. Lubell, M.

- Vongsouvath, H. R. van Doorn, E. A. Ashley, and P. Turner, "Antimicrobial resistance detection in Southeast Asian hospitals is critically important from both patient and societal perspectives, but what is its cost?," *PLOS Glob. Public Heal.*, vol. 1, no. 10, p. e0000018, 2021, doi: 10.1371/journal.pgph.0000018.
- [170] P. J. Dailey, J. Osborn, E. A. Ashley, E. J. Baron, D. A. B. Dance, D. Fusco, C. Fanello, Y. C. Manabe, M. Mokomane, P. N. Newton, B. Tessema, C. Isaacs, and S. Dittrich, "Defining system requirements for simplified blood culture to enable widespread use in resource-limited settings," *Diagnostics*, vol. 9, no. 1, 2019, doi: 10.3390/diagnostics9010010.
- [171] A. Cortegiani, G. Misseri, T. Fasciana, A. Giammanco, A. Giarratano, and A. Chowdhary, "Epidemiology, clinical characteristics, resistance, and treatment of infections by *Candida auris*," *J. Intensive Care*, vol. 6, no. 1, 2018, doi: 10.1186/s40560-018-0342-4.
- [172] World Health Organization, "Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA)," 2012. <https://www.who.int/tb/laboratory/afro-slipta-checklist-guidance.pdf> (accessed Oct. 12, 2016).
- [173] T. a M. Datema, L. Oskam, S. M. van Beers, and P. R. Klatser, "Critical review of the Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA): suggestions for harmonization, implementation and improvement.," *Trop. Med. Int. Health*, vol. 17, no. 3, pp. 361–7, Mar. 2012, doi: 10.1111/j.1365-3156.2011.02917.x.
- [174] J. Ronat, S. Oueslati, A. Natale, B. Baillet, A. Rochard, C. Langendorf, O. Vandenberg, J. Jacobs, and T. Naas, "Validation of three MicroScan® Antibiotic Susceptibility Testing microplates designed for Low Resource Settings Principal objective of this study was to verify the accuracy of the MicroScan Conclusions Our results indicate that performance of the 3," in *Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse*, 2020, p. 118.
- [175] J. Ronat, S. Oueslati, A. Natale, B. Baillet, A. Rochard, C. Langendorf, O. Vandenberg, J. Jacobs, and T. Naas, "Evaluation of the PROMPT inoculation system on the MicroScan® Antibiotic Susceptibility Testing (AST) microplate designed for Low Resource Settings (LRS)," in *Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse*, 2020, p. Unpublished poster.
- [176] M. Sharland, C. Pulcini, S. Harbarth, M. Zeng, S. Gandra, S. Mathur, and N. Magrini, "Classifying antibiotics in the WHO Essential Medicines List for optimal use—be AWaRe," *Lancet Infect. Dis.*, vol. 18, no. 1, pp. 18–20, Jan. 2018, doi: 10.1016/S1473-3099(17)30724-7.
- [177] Clinical and Laboratory Standards Institute (CLSI), *M100 Performance Standards for Antimicrobial Susceptibility Testing*, vol. M100-Ed29. 2019.
- [178] EUCAST, "EUCAST System for Antimicrobial Abbreviations," 2018.
- [179] S. Soeng, C. Ling, T.-P. Cusack, D. A. B. Dance, P. Hinfonthong, S. Lee, P. Newton, F. Nosten, T. Reed, T. Roberts, A. Sengduangphachanh, S. Sihalath, T. Wangrangsimakul, P. Turner, and E. Ashley, "Impact of delays to incubation and

storage temperature on blood culture results in tropical countries: A multi-centre study," *Int. J. Infect. Dis.*, vol. 101, p. 195, 2020, doi: 10.1016/j.ijid.2020.09.521.

Annexes

Annex 1 : Clinical bacteriology in low-resource settings: today's solutions

Clinical bacteriology in low-resource settings: today's solutions



Sien Ombelet*, Jean-Baptiste Ronat*, Timothy Walsh, Cedric P Yansouni, Janneke Cox, Erika Vlieghe, Delphine Martiny, Makeda Semret, Olivier Vandenberg, Jan Jacobs, on behalf of the Bacteriology in Low Resource Settings working group†

Low-resource settings are disproportionately burdened by infectious diseases and antimicrobial resistance. Good quality clinical bacteriology through a well functioning reference laboratory network is necessary for effective resistance control, but low-resource settings face infrastructural, technical, and behavioural challenges in the implementation of clinical bacteriology. In this Personal View, we explore what constitutes successful implementation of clinical bacteriology in low-resource settings and describe a framework for implementation that is suitable for general referral hospitals in low-income and middle-income countries with a moderate infrastructure. Most microbiological techniques and equipment are not developed for the specific needs of such settings. Pending the arrival of a new generation diagnostics for these settings, we suggest focus on improving, adapting, and implementing conventional, culture-based techniques. Priorities in low-resource settings include harmonised, quality assured, and tropicalised equipment, consumables, and techniques, and rationalised bacterial identification and testing for antimicrobial resistance. Diagnostics should be integrated into clinical care and patient management; clinically relevant specimens must be appropriately selected and prioritised. Open-access training materials and information management tools should be developed. Also important is the need for onsite validation and field adoption of diagnostics in low-resource settings, with considerable shortening of the time between development and implementation of diagnostics. We argue that the implementation of clinical bacteriology in low-resource settings improves patient management, provides valuable surveillance for local antibiotic treatment guidelines and national policies, and supports containment of antimicrobial resistance and the prevention and control of hospital-acquired infections.

Introduction

Bacterial sepsis is a leading cause of mortality and critical illness worldwide.^{1,2} Antimicrobial resistance is considered a major threat to global health and low-income and middle-income countries⁴ are disproportionately burdened.^{3,6} Contributing factors include patients' vulnerability to invasive bacterial illness, uncontrolled use of antibiotics, and poor laboratory support for clinical diagnosis resulting in overuse of antibiotics. Further, practices to prevent health care-acquired infections are generally absent in low-resource settings.⁵

Clinical bacteriology is the laboratory work-up needed for identification, quantification, and antibiotic susceptibility testing of bacteria found in clinical patient samples. Doing effective clinical bacteriology supports three of the five strategic objectives identified by WHO for containment of antimicrobial resistance: surveillance, appropriate use of antibiotics (antibiotic stewardship), and infection control in health-care settings.⁷ These objectives were reaffirmed at the UN General Assembly's high-level meeting on antimicrobial resistance in September, 2016.⁸ Clinical bacteriology contributes substantially to patients' diagnosis and guides antibiotic treatment. For severe sepsis, inappropriate antimicrobial therapy is a major contributor to mortality and sepsis guidelines emphasise the importance of culture-guided therapy.⁹ For tropical low-resource settings, inappropriate therapy is particularly problematic because life-threatening bacterial infections, such as non-typhoidal *Salmonella* spp bloodstream infections, are often clinically indistinguishable from severe *Mycobacterium tuberculosis* infection^{10,11} and are often

misdiagnosed.^{10,15} Identification of bacterial pathogens and antimicrobial susceptibility testing allow optimisation, de-escalation, or discontinuation of antibiotic treatment, resulting in improved patient outcomes, reduced costs, and reduced selection of antimicrobial resistance.¹⁶ Culture data from patient samples can be aggregated to identify common pathogens and determine their susceptibility patterns. Such local surveillance data will support validation of empirical guidelines for antibiotic treatment.¹⁷ In addition, daily reviews of clinical bacteriology data are vital for the detection of hospital-associated or community-associated outbreaks and to monitor emergence of resistance.^{18,19}

Diagnostic laboratories in low-income and middle-income countries face challenges of infrastructure, equipment, logistics, quality-assurance, and human resources.²⁰ In the past decade, considerable efforts have been made to improve laboratory systems in low-resource settings.^{21–23} Although diagnostics for tuberculosis, malaria, HIV, and Ebola virus have been successfully disseminated,^{5,24} clinical bacteriology covers a wide spectrum of pathogens and cannot be achieved by simple diagnostic and therapeutic algorithms or by a few rapid diagnostic tests or vertical control programmes. Furthermore, the design, development, and clinical validation of new diagnostics for clinical bacteriology can take 2–10 years.²⁵ The dissemination of diagnostics in low-resource settings comes with additional challenges, including procurement, distribution, and quality control issues.²⁶ In addition, manufacturers' production capacity and compliance with ISO standards (ISO 13485) are

Lancet Infect Dis 2018

Published Online
March 5, 2018
[http://dx.doi.org/10.1016/S1473-3099\(18\)30093-8](http://dx.doi.org/10.1016/S1473-3099(18)30093-8)

* Contributed equally

† Members listed in the contributors section

Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium (S Ombelet MD, J Cox PhD, E Vlieghe PhD, J Jacobs PhD); Médecins Sans Frontières, Operational Centre Paris, France (J-B Ronat MPH); Cardiff University, Cardiff, UK (T Walsh PhD); JD MacLean Centre for Tropical Diseases, McGill University Health Centre, Montreal, QC, Canada (C P Yansouni MD, M Semret MD); Department of General Internal Medicine, Infectious and Tropical Diseases, Antwerp University Hospital, Antwerp, Belgium (E Vlieghe); Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium (E Vlieghe); Department of Microbiology, LHUB-ULB, Pôle Hospitalier Universitaire de Bruxelles, Brussels, Belgium (D Martiny PhD, O Vandenberg PhD); St Mary's Hospital Centre, Montreal, QC, Canada (M Semret); Center for Environmental Health and Occupational Health, Public Health School, Université Libre de Bruxelles, Brussels, Belgium (O Vandenberg); and Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium (S Ombelet, J Jacobs)

Correspondence to: Dr Sien Ombelet, Institute of Tropical Medicine, Antwerp 2000, Belgium
sombelet@itg.be

often inadequate in such settings.²⁷ Further, with the advances in mass spectrometry technologies and automated bacterial identification and antimicrobial susceptibility testing in high-resource settings, the gap between these systems and practices of clinical bacteriology in low-resource settings has widened. Pending the implementation of new technologies, conventional culture-based techniques are still the best option for the application of clinical bacteriology in these settings; they are well studied, robust, universally accepted, and most have regulatory certification. Moreover, culture-based techniques are still essential for antimicrobial susceptibility testing, even in the presence of molecular or biomarker tests.

A framework towards the implementation of clinical bacteriology in low-resource settings, including common obstacles and global priorities, is urgently needed. In this Personal View we aim to describe a framework for implementation that is suitable for general referral hospitals in low-resource settings with a moderate infrastructure (ie, a basic diagnostic laboratory operated by laboratory staff without expertise in microbiology).²⁸ Although focused on operational and technical requirements, this framework will inform clinicians and health-care policy makers. We briefly discuss laboratory services in low-resource settings, such as supranational initiatives involved in funding, accreditation, diagnostic regulations, and manufacturing.^{29–32,33–35}

Clinical bacteriology in low-resource settings: six building blocks

Based on our collective expert opinion and review of the available literature, we propose that six essential building blocks have to be addressed for successful implementation of clinical bacteriology in low-resource settings.

Availability of equipment and consumables adapted for use in low-resource settings

Environmental conditions in low-resource settings affect electronic equipment and consumables, such as glassware and dehydrated culture media³¹ (table 1, figure 1). Availability of quality-assured diagnostics is further compromised by the absence of onsite production and inadequate supply chains, which are incompatible with the shelf life and cold storage requirements of many diagnostic kits.⁶ In addition, there is little commercial interest in the development of new diagnostics adapted for use in low-resource settings because of low profit margins.⁵

Equipment and consumables for diagnostics in low-resource settings need to be adapted so that they are resistant to harsh conditions, such as temperature, humidity, dust, sunlight, and transport. In addition to being robust, safe, and stable, equipment should consume little energy and be easy to maintain. Consumables (such as culture media or test reagents)

need to have a long shelf life and generate little waste. Where possible, internal quality controls should be included in reagent kits. Quality assurance in manufacturing, client support, post-marketing service, and maintenance should be guaranteed.

A first step towards adaptation of diagnostics equipment for use in low-income settings is to draft clear profiles of target products and technical specifications by regulatory agencies, manufacturers, and stakeholders. Some manufacturers have already launched research initiatives for low-cost innovation that target fever-related diagnostics in low-resource settings.³⁶ Furthermore, manufacturers in growing economies (such as China, Vietnam, and Thailand) are producing a wide range of diagnostics hitherto unknown outside their domestic markets. Some diagnostics used in clinical bacteriology (such as reagents used for phenotypical tests and serotyping) have not been extensively validated for stability and shelf life, which might have been arbitrarily set, for example, at 25°C and 6 months. However, actual use (and some guidelines) suggests a reliable performance outside these specifications³⁷ and identical products from different manufacturers have different storage and shelf life requirements. Therefore, in line with current practice for pharmaceutical products,³⁸ extended product stability testing (including temperature and storage stability) should be done to confirm the true limits in low-resource settings.

Other short-term goals are tropicalised packaging (eg, vacuum-sealed packaging to protect against humidity), clear labelling, and adaptation of instructions to an appropriate language and educational level.^{39,40} Furthermore, production of quality-assured consumables (such as culture media and phenotypical test reagents) can be outsourced to a centralised facility as has been done in Cambodia.⁴¹

Medium-term objectives include the development of low tech, low cost, and low maintenance equipment, such as electricity-free incubators,⁴² battery-operated centrifuges,⁴³ and autoclaves powered by solar energy.^{34,44} Alternatives for sheep blood, horse blood, and rabbit plasma should be explored; for example, goat, pig, and hair sheep (a breed of sheep adapted to tropical climates) blood are alternatives to sheep blood for homemade culture media,^{45,46} but lyophilised or synthetic media should also be considered. Likewise, agar (ie, solidifying substrate in culture media) is obtained from sea algae at a few harvesting sites; substrates such as cellulose produced by engineered bacteria⁴⁶ should be assessed as a commercial alternative.

Rationalised bacterial identification and antimicrobial susceptibility testing

Because of recent technological advances in identification techniques and continuous improvements to guidelines for antimicrobial susceptibility testing, state-of-the-art identification and testing in low-resource settings remains a major challenge (table 1).⁶ Although

	High-resource settings	Low-resource settings
Infrastructure	Infrastructure requirements covered by ISO 15189, Joint Commission International, CLSI QMS01-A4, Clinical Laboratory Improvement Amendments, or an equivalent quality management system ^{14, 15}	Basic requirements frequently not met: ^{1, 2} power, climate control and ventilation, dust reduction, water quality, light sources, biosafety requirements, and internet connectivity
Diagnostics	Increasing development of diagnostics ^{30, 31}	Sales are not lucrative enough for adequate return on investment to create rapid diagnostic solutions ³
Quality assurance	Quality-assured production and supply by ISO 13485 certified manufacturers ⁴⁰	Limited quality assurance of process and products; ⁴⁰ diagnostics manufacturers are often not certified by ISO 13485 ⁷
Consumables	Procurement and supply chain of diagnostics short and reliable	General absence of locally produced consumables; when locally produced, consumables are often substandard with poor quality assurance and irregular availability; ⁴⁰ some reagents are subject to dangerous goods regulations when shipped by air; ⁴¹ long delivery delays in combination with short shelf life and need for cold chain (2–8°C or as low as –20°C) for critical products; high stress conditions (heat, humidity) and limited environmental stability ³⁰
Logistics	Storage, stock management, and distribution systems in place	Stock management and inventory systems are not in place or inappropriate ³⁰
Standard operating procedures	Standards for standard operating procedures covered by ISO 15189 or equivalent quality management system ¹⁴	The creation of and updating of standard operating procedures and other documents are a significant obstacle; ³⁰ multiple language and cultural barriers to the understanding of such procedures ⁴⁴
Reference materials	Reference materials are easily accessible	Reference materials (such as culture type strains) are expensive, subject to strict shipment requirements ⁴⁰ and difficult to maintain
Documents	Guideline documents, standards and norms are affordable	Documents from the International Organization for Standardization and guidelines from CLSI are for sale but expensive. Recently, CLSI has made their key document, M100-S27, freely available online ⁴⁵
Culture-based growth	Automated blood culture systems with continuous growth monitoring and automated detection, ⁴⁶ identification with MALDI-TOF, and automated antimicrobial susceptibility testing have substantially decreased turnaround time ⁴⁶ and have increased simplicity	Automated equipment and MALDI-TOF are rarely used because of high cost and stringent requirements for infrastructure; ^{1, 47} service contracts are unavailable or unaffordable in many settings; ¹ manual blood culture systems require training and experience; many manual diagnostic products are no longer commercialised
Identification	Identification systems validated with bacterial collections obtained in high-resource settings	Identification methods not validated with bacterial collections from low-resource settings; ^{48, 49} some tropical bacteria cannot be reliably identified with commercial identification methods ⁵⁰
Antimicrobial susceptibility testing of bacteria	Harmonised criteria by international guidelines (CLSI, EUCAST) integrated in automated antimicrobial susceptibility testing methods assuring correct and updated use of breakpoints and expert rules ^{51, 52}	Guidelines are often temporary, not well disseminated, only available in English, and poorly followed; ⁴⁹ expert rules are too complicated to be mastered by staff without expertise in microbiology
Laboratory processes	Streamlined and automated trajectory with the help of Laboratory Information Management Systems contributes to shortening of preanalytical phase and post-analytical phase ^{53, 54}	Piecemeal sampling, paper-based systems, with hardcopy results collected too late or not at all; ⁵⁵ accuracy of results is seen as more important than speed and short turnaround time
Laboratory criteria	Clinically relevant criteria for sampling ⁵⁶	No laboratory guidance for selection, sampling, and transport of specimens results in inadequate sampling (eg, fistula and wound swabs, long transport delays); sampling biased to patients who are severely ill, failed initial treatment, or can afford testing
Communication	Proactive communication and reporting by the clinical microbiologist ⁵⁷	Poor communication between laboratory staff and clinicians ⁵⁸
Hours of operation	24/7 specimen processing, consultative role including out-of-hours call for a clinical microbiologist ⁵⁹	Laboratory activities mostly limited to office hours
Quality indicators	Quality indicators for sampling processing and reporting embedded in quality management systems	Quality indicators not systematically monitored (eg, blood culture contaminants)
Professional standards	Professional standards for clinical microbiologist and biomedical staff ⁶⁰ and professional societies at the international and national levels	No professional standards or profiles for clinical microbiologists; few professional societies and postgraduate activities
Laboratory staff	Dedicated, well trained, and experienced biomedical staff; clinical microbiology experts are involved in training of staff and management of laboratory	Frequent understaffing and poor staff retention; ^{1, 48, 61, 62} clinical microbiology experts are non-existent or scarcely involved; absent preservice training and education; ⁶³ few available training or teaching sites for clinical bacteriology
Patients	The patient is knowledgeable and has health insurance; clinical and nursing staff familiar with indications	Out-of-pocket payment, cultural differences, and insufficient knowledge from both patients and staff result in reluctance to take samples from patients ^{64, 65}
Clinicians	Clinicians are familiar with indications and interpretation of laboratory results; and trust and rely on the laboratory results as part of evidence-based clinical algorithms	Clinicians have a high reliance on clinical judgment, are reluctant to request laboratory tests, and tend to deny laboratory results ^{66, 67} because of negative perceptions of the laboratory, which include slow turnaround, ^{68, 69} poor accuracy of laboratory tests, ^{68, 69} inadequate laboratory capacity, and unavailability of consumables ⁶⁹
AMR	High awareness of AMR and infection control among clinicians, nursing staff, and hospital staff	AMR is considered mainly a worldwide or nationwide problem, but less of a problem within clinicians' own hospitals ⁷⁰
Antibiotics	Patients educated on appropriate and restrictive use of antibiotics	Pressure from patients to prescribe antibiotics; ^{71, 72} issues with self-medication because of widespread availability of antibiotics
Clinical decision making	Clinical microbiologist is an active member of the antibiotic stewardship committee ¹	Antibiotic stewardship committees or activities are mostly absent; few studies on non-use of antibiotics or de-escalation through the use of clinical bacteriology; ¹

(Table 1 continues on next page)

Personal View

	High-resource settings	Low-resource settings
(Continued from previous page)		
Antibiotic stewardship	AMR surveillance reports generated by the results of routinely submitted samples and aggregated in (supra)national or regional networks	AMR surveillance data based on poor data quality and representativeness, especially in Africa and the western Pacific. ^{174,175} AMR surveillance focused on intensive care units and vulnerable populations ¹⁷⁴
Infection control	Real-time alert function for infection control (community and hospital-based outbreaks) ¹⁷⁶	Infection control committees rarely work with laboratory data for outbreak investigation and management ¹⁷⁴
Surveillance	All laboratories take part in surveillance of (re-)emerging pathogens and vaccine-preventable diseases ¹⁷⁷	Public health surveillance is mostly confined to reference laboratories
Accreditation	Certification, accreditation, and regulation of clinical laboratories	WHO Regional Office for Africa provides an accreditation process as an interim pathway to meet international laboratory standards; ¹⁷⁸ other tools, such as Stepwise Laboratory Improvement Process Towards Accreditation and Laboratory Quality Stepwise Implementation tool, focus on malaria, tuberculosis, and HIV and have few applications to clinical bacteriology ¹⁷⁹
External quality control	External quality control programmes available	Existing external quality programmes for microbiology are scarce, expensive, and address mostly HIV, tuberculosis, and malaria ^{180,181}
Reference laboratories	Functional reference laboratories	Few reference laboratories, mainly oriented to research and outbreak management; referral of specimens for clinical bacteriology is more demanding (sampling, shipment) than for tuberculosis and HIV testing

AMR=antimicrobial resistance. CLSI=Clinical and Laboratory Standards Institute. EUCAST=European Committee for Antimicrobial Susceptibility Testing. MALDI-TOF=matrix assisted laser desorption ionisation-time of flight.

Table 1: Clinical bacteriology in high-resource settings compared with low-resource settings

For more on the International Organization for Standardization see <http://www.iso.org/iso/home.html>



Figure 1: Adapted consumables for use in low-income settings
At high relative humidity, glass slides for microscopy become opaque because of the development of condensation within the glass. Photograph taken in DR Congo.

some guidelines on antimicrobial susceptibility testing are now open access,^{45,200} building local capacity to implement annual revisions of such guidelines is a major challenge.

The relevance of the need to identify all bacteria to the species level is debatable;²⁰¹ grouping genera and species according to their clinical relevance might be more useful.⁶⁷ We propose the adoption of a two-tier approach consisting of first-line identification to the group level

with preliminary antimicrobial susceptibility testing, followed by a more advanced identification and testing at national reference laboratories. Practical guidelines for grouping bacteria according to clinical relevance, antimicrobial resistance profile, hospital epidemiology, and public health importance should be undertaken. Table 2 shows how Gram-negative enteric bacteria can be grouped according to clinical and infection control relevance.^{48,101,107,108} In most laboratories, the detail and level of identification of bacteria will be dependent also on the technical and economic feasibility of the identification system used.

The grouping of bacteria would encourage technologies for abbreviated identification through so-called spot tests, which are single substrate biochemical or enzymatic tests that can be read via colour, fluorescence, or turbidity. Spot tests, already available for key pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, are reliable and can contribute to clinical decision making.^{58,119,120} Valuable documents, such as the Clinical and Laboratory Standards Institute guidelines for the identification for bacteria and yeasts,¹¹⁹ should be updated, extended to tropical bacteria (eg, *Salmonella* spp, *Burkholderia pseudomallea*), validated with geographically representative strains, and made open access.

Testing panels for antimicrobial susceptibility should be adapted to the requirements of low-resource settings (eg, harmonised with the country's essential drug list). Disk diffusion testing has advantages of cost, flexibility, simplicity, and reliability. However, simplified tests for minimal inhibitory concentration—such as lyophilised microbroth dilution methods—could be less prone to error and are sometimes required (eg, ceftriaxone for *Streptococcus pneumoniae*). Simple and affordable reading apparatus would be helpful for accurate interpretations,

	Clinical relevance	Infection control relevance	Antimicrobial resistance profile	Public health importance
<i>Citrobacter</i> spp; <i>Cranobacter</i> spp; <i>Enterobacter</i> spp; <i>Hafnia alvei</i> spp; <i>Serratia</i> spp*	Pneumonia; post-operative site infections; urinary tract infections; wound colonisation or infection	Mostly health care-associated ²⁰²	Multidrug resistant; AmpC expression (cephalosporin resistance); ²⁰³ included on WHO's priority pathogen list† ²⁰⁴	No particular public health importance apart from health care-associated infections
<i>Klebsiella</i> spp; <i>Raoultella</i> spp	Bloodstream infections; pneumonia; pyogenic infections; urinary tract infections; wound colonisation or infection	Often health care-associated (hospital outbreaks); ²⁰² community-associated strains (pyogenic infections) ²⁰⁸	Multidrug resistant; ESBL and carbapenemase production; ²⁰⁶ included on WHO's priority pathogen list† ²⁰⁴	Global dissemination of antibiotic resistance genes; ^{204,208} clonal spread of multidrug resistant (sequence type 258) ²⁰⁹ hyperinfective community-associated strains ²⁰⁸
<i>Escherichia coli</i>	Bloodstream infections; enteritis; urinary tract infections	Mostly community-associated ²¹¹	Multidrug resistant; ESBL, carbapenemase production; ²⁰⁹ included on WHO's priority pathogen list† ²⁰⁴	Clonal spread of multidrug resistant sequence type 131 in community ^{209,208}
<i>Salmonella typhi</i> ; <i>Salmonella Paratyphi A</i> *	Enteric fever	Community-associated	Multidrug resistant; decreased ciprofloxacin resistance and occasionally ESBL; ²⁰⁶ included on WHO's priority pathogen list† ²⁰⁴	Human-to-human transmission; ²⁰⁶ clonal spread of multidrug-resistant H58 strain ²⁰⁸
Non-typhoidal <i>Salmonella</i> spp	Bloodstream infections; enteritis	Community-associated; hospital outbreaks	Multidrug resistant; decreased ciprofloxacin resistance, and occasionally ESBL; ²⁰⁶ included on WHO's priority pathogen list† ²⁰⁴	Major cause of bloodstream infection in children in sub-Saharan Africa; associated with more deaths than malaria; ^{212,213} clonal spread of invasive <i>Salmonella typhimurium</i> (sequence type 313) ²¹¹
<i>Morganella</i> spp; <i>Proteus</i> spp; <i>Providencia</i> spp	Post-operative site infections; urinary tract infections	Healthcare-associated	Antimicrobial susceptibility profile differs from other Enterobacteriaceae (eg. intrinsic colistin resistance) ²¹⁴	No particular public health importance apart from health care-associated infections
<i>Shigella</i> spp	Dysentery; enteritis; haemolytic-uremic syndrome	Community-associated	Included on WHO's priority pathogen list† ²⁰⁴	<i>Salmonella dysenteriae</i> serotype 1 is frequently involved in epidemics ²¹⁵

Detail and level of identification is dependent on technical and economic feasibility of the identification system used. We focused on isolates recovered from bloodstream pathogens. Multidrug resistance is defined as resistance to at least one agent in three or more antibiotic classes.²⁰⁶ ESBL=extended spectrum beta-lactamase. *If technically feasible and affordable, separate identification of this species would be desirable. †WHO's priority pathogen list is a global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.

Table 2: Proposed grouping of bacterial species by clinical and infection control relevance; example of Gram-negative enteric bacteria

particularly when coupled to an open-access expert system, such as the system available in WHONET software.

Communication between the laboratory and clinicians

The interface between clinicians and the diagnostic laboratory is poorly studied in low-resource settings.^{60,69,72} Guided by their reliance on clinical algorithms and syndromic approaches, clinicians are reluctant to request laboratory tests (table 1); a tendency that is aggravated by their perception that tests are expensive, slow, and often irrelevant.⁶⁵ In some regions, patients fear blood sampling and invasive procedures (figure 2).^{65,66} Additionally, senior managers in hospitals are typically academic clinicians, which increases the gap in communication between decision makers, clinicians, and laboratory staff. Laboratory staff often feel underappreciated and have limited professional opportunities.^{60,72}

In high-resource settings, clinical bacteriology is closely integrated with patient management and infection prevention and control.^{50,122} Such integration does not exist in low-resource settings, where trained clinical microbiologists are rare and the main focus of the laboratory work is on the analytical phase, with little professional collaboration and communication between laboratory staff and clinicians.^{20,60} Therefore, even when good quality diagnostic laboratories exist in low-resource settings, their impact is frequently compromised by

underuse, inadequate specimen collection, or post-analytical issues (such as ineffective reporting of results).^{53,165,168}

We advocate regular person-to-person interactions between clinicians and laboratory staff to address the communication gap and further highlight the importance of this interaction through pre-service and continuous training.^{123,124} The Strengthening Laboratory Management Toward Accreditation (SLMTA) programme provides useful strategies on how to improve communication between clinicians and laboratory staff.^{70,125} Likewise, the modules for specimen collection and test result reporting in the SLMTA toolkit could help to strengthen clinical bacteriology activities in low-resource settings.

Prioritisation of clinically relevant specimens

Although key to performance,⁷⁸ the minimal number of samples (critical volume) that should be processed to acquire expertise in clinical bacteriology is not defined. Clinical bacteriology relies on the competence and experience of laboratory staff; for example, the Gram stain, which provides crucial information during diagnosis of blood cultures,^{126,127} is notoriously prone to error in inexperienced hands.¹²⁸ Further, laboratories in low-resource settings tend to process few samples and often use inappropriately selected specimens (table 1).

For more on WHONET see <http://whonet.org>

Personal View



Figure 2: Lumbar puncture
This procedure requires sterile gloves, a specialised needle, and assistance from a nursing team. Photograph taken in DR Congo.

When resources are limited, we propose the prioritisation of key clinical specimens (table 3)—for example, blood cultures are highly relevant for patient management and for antimicrobial resistance surveillance.³⁸ By contrast, culture of cerebrospinal fluid, with its stringent culture requirements, might not have added clinical value (ie, after cell count and Gram stain testing) because standard treatment with third generation cephalosporins in the postneonatal period covers most bacterial pathogens.^{110,142}

Specimens from empyema, abscesses, and osteomyelitis, and surgical samples can guide clinical care, but procedures for these specimens are considerably more complex than laboratory work-up of blood culture samples or urine samples.¹³⁶ Clear sampling procedures and acceptance criteria need to be formalised, communicated, and implemented.¹²⁴

Provision of accessible and affordable training and reference materials

Training materials dedicated to clinical bacteriology in low-resource settings are scarce, seldom updated, and sometimes expensive. Training (both theoretical and practical) and reference materials should be specific to the setting, open access, available in local languages,

and be readable and comprehensible for non-expert users of various cultural backgrounds.⁴⁴ End-user feedback and validation is useful to refine and improve teaching materials.⁴⁴

Established guidance and toolkits should be linked to reference materials, including SLMTA toolkit modules, the Stepwise Laboratory Improvement Towards Accreditation checklist,²⁹ and WHO's Laboratory Quality Stepwise Implementation tool⁴⁸ can be complemented with real-life scenarios of clinical bacteriology. Clinical reference documents, such as WHO guidelines for hospital care of children and adults,^{30,42} should include recommendations for clinical bacteriology (indications, sampling, and transport) and use of antibiotics. New programmes adapted to low-resource settings, such as WHO's Laboratory Assessment Tool,⁴⁰ also offer guidance on how to assess laboratories and national laboratory systems.

Onsite training of clinicians, nurses, and laboratory staff will encourage the highest efficiency and retention. Experiences from the implementation of clinical medicine in low-resource settings highlight the value of onsite support through educational outreach and mentoring visits.¹⁴⁴ The role of bench-side coaching by experienced professionals cannot be underestimated as a way of teaching good clinical practice.¹⁴⁵ Remote learning with video is a valuable complement to bench-side exposure;¹⁴⁶ for example, the European Committee for Antimicrobial Susceptibility Testing has released instruction videos for disk diffusion.¹⁴⁷ Telemicrobiology, which is the transfer of images of cultures and microscopy, allows for real-time analysis, expert consultation, training, and quality assurance at an affordable cost.^{148,149}

Onsite validation and field adoption

As highlighted in the 2016 O'Neill report on antimicrobial resistance, more effort should be put into the development of new diagnostics for bacterial detection, identification, and antimicrobial susceptibility testing.¹⁵⁰ Promising new technologies are increasingly being used in clinical bacteriology (table 1), but when applied to low-resource settings there are budgetary, technical, human resource, and behavioural constraints and such technologies are rarely implemented outside of reference laboratories.²⁵ Furthermore, in low-resource settings, clinical performance studies are a major bottleneck to the development of diagnostics.¹⁵² Specifically, many diagnostics are not tested in low-resource settings because of financial or operational constraints; therefore, their implementation in these settings is delayed.

Some manufacturers are forming partnerships and investing in research exploring low-cost diagnostic innovation⁴⁶ and some of those technologies might develop into valuable diagnostics for low-resource settings. Rather than awaiting accreditation in resource-rich settings, new diagnostics could be evaluated in field settings in low-resource setting. Some of these

For an example of such partnerships see <http://www.finddx.org/partners>

	Prenatal and analytical feasibility	Relevance for individual patient management	Relevance for surveillance
Blood	+++ Technical requirements feasible, mostly one pathogen, top five pathogens account for most isolates ^{26,27}	+++ Unequivocal interpretation (except contaminants); high clinical impact, ²⁸ amenable to antibiotic stewardship ²	+++ Indications for sampling are standardised, quality indicators allow for inter-laboratory harmonisation and surveillance over time ⁶
Cerebro-spinal fluid	+++ Sampling requires skills and expertise; specific transport are required for cold-vulnerable pathogens	+++ Little value of culture over white blood cell count and Gram stain ²⁹	+++ Epidemics (<i>Neisseria meningitidis</i>) or serotype distribution (<i>Streptococcus pneumoniae</i>) ^{34,35}
Empyema, closed abscess, joint fluid	+++ Mixed, fastidious, and anaerobic flora possible; specific transport needs; selective media and considerable expertise required	+++ Gram stain and culture can guide diagnosis and treatment, particularly in severe infections and when complete drainage is not possible	+++ Variability in patient selection
Bone tissue	++ Surgically obtained samples; requires grinding of specimens, ³⁶ often polymicrobial infections ²⁷	++ Can guide treatment; some flora are difficult to interpret (eg. coagulase-negative staphylococci)	++ Variability in patient selection (eg. trauma, prosthesis material)
Respiratory tract (non-tuberculosis)	+ Useful for mixed flora and cold-vulnerable pathogens; contaminating oral flora; requires selective media and expertise	+ Difficult to determine difference between colonisation and infection	+ Variability in patient selection and contaminating or colonising flora
Urine	+ Long transport delay requires cold chain	++ Can alert to resistant bacteria; contaminating flora	++ Variability in patient selection (bias to antimicrobial resistance)
Stool	+ Challenging transport needs; selective culture media, microaerophilic incubation conditions (<i>Campylobacter</i> spp); considerable expertise required	+ Diarrhoea frequently of non-bacterial origin, ³⁸ long turnaround time, relatively low sensitivity for bacterial pathogens of greatest interest ³⁹	++ Suspected outbreaks of dysentery or cholera; confirmation of epidemic and antibiotic resistance patterns ^{40,41}

Grades of relevance and feasibility are shown as ++++ (high), +++ (moderate), ++ (low), or + (very low). Prenatal feasibility refers to indications, sampling, and transport; analytical feasibility refers to technical (eg. selective culture media or incubation conditions) and human (training or expertise) requirements.

Table 3: Relevance of different clinical specimens collected through routine patient care in low-resource settings

innovations might then diffuse to high-resource settings, a process called reverse innovation.^{252,253}

Well-functioning quality assured diagnostic laboratories could constitute reliable study sites to carry out clinical diagnostic studies in the target population. Beyond strict diagnostic performance, such studies should address the adoption of new diagnostics into practice, their integration into clinical care, and cost-effectiveness.⁷⁴

Beyond the six building blocks

Other factors can influence the implementation of clinical bacteriology in low-resource settings. Political commitment is essential to instal and equip clinical laboratories at all levels of health care and to strengthen health systems. It is therefore worth noting the recent resolution of the UN General Assembly, wherein Heads of State agreed to a broad, coordinated approach to tackle the root causes of antimicrobial resistance.⁸ The announcement by WHO of a forthcoming Essential Diagnostics List could also help to integrate diagnostic resources better adapted to low-income settings into national programmes.²⁵⁵

In addition, the professional, academic, and regulatory environment should facilitate implementation of clinical bacteriology in health-care organisation and biomedical curricula. Reference laboratories in low-resource settings should extend their capacity to support basic bacteriology

and antimicrobial resistance,²⁵⁶ rather than perpetuating existing silos through the restriction of their activities to HIV, tuberculosis, or malaria reference work.^{4,245} Further opportunities to support the implementation of clinical bacteriology are linkage to the WHO prequalification programme of in-vitro diagnostics²⁵⁷ and extension of the Maputo Declaration goals to include clinical bacteriology.²³

Furthermore, in response to the 2016 O'Neill report on antimicrobial resistance,²⁵⁸ wherein a Global Innovation Fund for non-commercial research was proposed, there have been calls for the development of a Global Antimicrobial Conservation Fund.²⁵⁹ We strongly endorse this initiative, which—in collaboration with the UK Fleming Fund—would further support the provision of basic bacteriology services in low-resource settings.²⁵⁹

Conclusions

Given the global attention given to antimicrobial resistance and several calls to action,^{7,246,261} it is time to address the issue of strengthening clinical bacteriology in low-resource settings. The benefits of clinical bacteriology are numerous, not only for individual patient care, but also for surveillance of outbreaks and emerging resistance, and also management of hospital infections and antimicrobial usage. The reference laboratories and networks, which were established in response to WHO's Maputo declaration on laboratory strengthening for the

Personal View

diagnosis of HIV, tuberculosis, and malaria, could be used to include clinical bacteriology—since little clinical bacteriology is currently being done in most of these reference laboratories.^{23,42,45}

We have outlined some challenges that might be encountered during the implementation of clinical bacteriology in low-resource settings and provide a framework as to how these difficulties could be overcome. The substantial progress made in the diagnosis and management of HIV, tuberculosis, and malaria has shown that non-expert staff can effectively deliver services that were previously considered too complicated and demanding.³² With similar concerted international efforts on an international scale, we believe such progress could be achieved for clinical bacteriology.

Contributors

JJ and J-BR had the rationale for this work. JJ did the literature review, wrote the initial draft, and supervised the manuscript revisions. SO contributed to manuscript preparation, writing of the draft, revision, data compilation, literature review, and project administration. J-BR contributed to manuscript preparation, literature review, revision, and project administration. TW, CPY, OV, DM, EV, JC, and MS wrote substantial paragraphs, attributed literature, and provided critical review and commentary. The members of the bacteriology in low-resource settings working group are: Octavie Lunguya (National Institute for Biomedical Research, DR Congo); Marie-France Phoba (National Institute for Biomedical Research, DR Congo); Palpouaguini Lompo (Institut de Recherche en Science de la Santé—Clinical Research Unit of Nanoro, Burkina Faso); Thong Phe (Sihanouk Hospital Center of Hope, Cambodia); Samuel Kariuki (Kenya Medical Research Institute, Kenya); Patal N Newton (Lao-Oxford-Mahosot Hospital, Wellcome Trust Research Unit, Laos); David A B Dance (Lao-Oxford-Mahosot Hospital, Wellcome Trust Research Unit, Laos); Clatade Muvuzayi (University of Rwanda, Rwanda); Sayda El Safi (University of Khartoum, Sudan); Barbara Barbe (Institute of Tropical Medicine, Belgium); Dadi Palay (University Hospital of Kisangani, DR Congo); Dissoua Afolabi (Centre National Hospitalier Universitaire Hubert Koutoucou Maga, Benin); Maurice Page (independent consultant); Céline Langendorf (Médecins Sans Frontières, France); Yves Cille (Biologie Sans Frontières, France); Tjalling Leenstra (National Institute for Public Health and the Environment, Netherlands); John Stelling (Brigham & Women's Hospital, MA, USA); Thierry Naas (Hôpital de Bicêtre, France); Thomas Kesteman (Fondation Mérieux, France); Daniel Seifu (Addis Ababa University, Ethiopia); Elisabeth Delarocque-Astagneau (Institut Pasteur, France); Constance Schultsz (Academic Medical Center of the University of Amsterdam, Netherlands); Heidi Schutt-Cerowitt (University of Cologne, Germany); Joanne Letchford (Diagnostic Microbiology Development Program, Cambodia); Heiman Wertheim (Department of Clinical Microbiology, Radboudumc, Netherlands); Gunnar Kahlmeter (EUCAST Development Laboratory, Sweden); and Awa Aidara Kane (World Health Organization, Switzerland).

Declaration of interests

MS reports non-financial support from bioMérieux. CPY reports non-financial support from bioMérieux, and BD Molecular Diagnostics. All other authors declare no competing interests.

Acknowledgments

This study was supported in part by the Baillet-Latour fund. We would like to thank Nada Malou and Rupa Kanapathipilai (Médecins Sans Frontières, New York, NY, USA) for their support.

References

- Vincent JL, Marshall JC, Namendys-Silva SA, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet Respir Med* 2014; 2: 380–86.
- Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med* 2016; 193: 259–72.



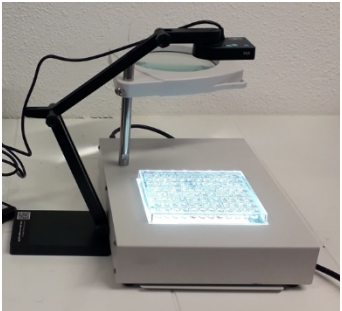


- Naghavi M, Wang H, Lozano R, et al. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2015; 385: 117–71.
- The World Bank. World Bank country and lending groups. <https://datahelpdesk.worldbank.org/knowledgebase/articles/906519> (accessed Jan 9, 2018).
- Bebell LM, Muiira AN. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? *Glob Heart* 2014; 9: 347–58.
- WHO. Global antimicrobial resistance surveillance system: manual for early implementation. 2015. http://apps.who.int/iris/bitstream/10665/188783/1/9789241549400_eng.pdf (accessed Jan 9, 2018).
- WHO. Global action plan on antimicrobial resistance. 2015. http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf (accessed Jan 19, 2018).
- WHO. At UN, global leaders commit to act on antimicrobial resistance. 2016. <http://www.who.int/mediacentre/news/releases/2016/commitment-antimicrobial-resistance/en> (accessed Jan 19, 2018).
- Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013; 41: 580–637.
- Nadim B, Mtwee G, Amos B, et al. Severe febrile illness in adult hospital admissions in Tanzania: a prospective study in an area of high malaria transmission. *Trans R Soc Trop Med Hyg* 2012; 106: 688–95.
- Crump JA, Ramadhani HO, Morrissey AB, et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. *Clin Infect Dis* 2011; 52: 341–48.
- Peters RP, Zijlstra EE, Schijffelen MJ, et al. A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management. *Trop Med Int Health* 2004; 9: 928–34.
- Evans JA, Adusei A, Timmann C, et al. High mortality of infant bacteraemia clinically indistinguishable from severe malaria. *QJM* 2004; 97: 591–92.
- Reyburn H, Mbatia R, Drakeley C, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ* 2004; 329: 1212.
- Phoba MF, Lunguya O, Mayimou DV, et al. Multidrug-resistant *Salmonella enterica*, Democratic Republic of the Congo. *Emerg Infect Dis* 2012; 18: 1692–94.
- Galar A, Leiva J, Espinosa M, Guillen-Grima F, Hernandez S, Yuste JR. Clinical and economic evaluation of the impact of rapid microbiological diagnostic testing. *J Infect* 2012; 65: 302–09.
- Vlieghe E, Sary S, Lim K, et al. First national workshop on antibiotic resistance in Cambodia: Phnom Penh, Cambodia, 16–18 November 2011. *J Glob Antimicrob Resist* 2013; 1: 31–34.
- Benbachir M. Role of the microbiology laboratory in infection control. In: Wenzel R, ed. *Guide to infection control in hospitals*. 4 edn. Boston: International Society for Infectious Diseases, 2008.
- Kalenic S. The role of the microbiology laboratory. In: Friedman C, ed. *IFIC basic concepts in infection control*. 2 edn. Portadown: International Federation of Infection Control, 2011.
- Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. *Clin Infect Dis* 2006; 42: 377–82.
- Nkengasong JN. Strengthening laboratory services and systems in resource-poor countries. *Am J Clin Pathol* 2009; 131: 774.
- Olmsted SS, Moore M, Meili RC, et al. Strengthening laboratory systems in resource-limited settings. *Am J Clin Pathol* 2010; 134: 374–80.
- WHO. The Maputo declaration on strengthening of laboratory systems. 2008. http://www.who.int/diagnostics_laboratory/Maputo-Declaration_2008.pdf (accessed Jan 19, 2018).
- Nouvellet P, Garske T, Mills HL, et al. The role of rapid diagnostics in managing Ebola epidemics. *Nature* 2015; 528: S109–16.
- Kettler HE, Marjanovic S. Engaging biotechnology companies in the development of innovative solutions for diseases of poverty. *Nat Rev Drug Discov* 2004; 3: 171–76.







- 26 Mukadi P, Gillet P, Barbe B, et al. SMS photograph-based external quality assessment of reading and interpretation of malaria rapid diagnostic tests in the Democratic Republic of the Congo. *Malar J* 2015; 14: 26.
- 27 Barbe B, Verdonck K, El-Safi S, et al. Rapid diagnostic tests for neglected infectious diseases: case study highlights need for customer awareness and postmarket surveillance. *PLoS Negl Trop Dis* 2016; 10: e0004655.
- 28 Girosi F, Olmsted SS, Keeler E, et al. Developing and interpreting models to improve diagnostics in developing countries. *Nature* 2006; 444 (suppl 1): S3–S8.
- 29 Okeke IN, Peeling RW, Goossens H, et al. Diagnostics as essential tools for containing antibacterial resistance. *Drug Resist Updat* 2011; 14: 95–106.
- 30 Fitagibbon JE, Wallis CL. Laboratory challenges conducting international clinical research in resource-limited settings. *J Acquir Immune Defic Syndr* 2014; 65 (suppl 1): S36–39.
- 31 Harbarth S BH, Goossens H, Jarlier V, et al. Antimicrobial resistance: one world, one fight! *Antimicrob Resist Infect Control* 2015; 4: 49.
- 32 Penno EC, Baird SJ, Crump JA. Cost-effectiveness of surveillance for bloodstream infections for sepsis management in low-resource settings. *Am J Trop Med Hyg* 2015; 93: 850–60.
- 33 Cheesbrough M. *District laboratory practice in tropical countries*. Cambridge: Cambridge University Press, 2006.
- 34 International Organization for Standardization. ISO 15189:2012. *Medical laboratories—requirements for quality and competence*. http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?csnumber=56115 (accessed Jan 19, 2018).
- 35 Clinical and Laboratory Standards Institute. *Quality management system: a model for laboratory services; approved guideline*. 2011. <https://clsi.org/standards/products/quality-management-systems/documents/qms01/> (accessed Jan 17, 2018).
- 36 Joint Commission International. *Accreditation standards for laboratories*. 3rd edn. 2017. https://www.jointcommissioninternational.org/assets/3/7/CL_Standards_for_Laboratories_STANDARDS-ONLY.pdf (accessed Jan 10, 2018).
- 37 Centers for Medicare and Medicaid Services. *Clinical laboratory improvement amendments*. <https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html#redirect=clia/> (accessed Jan 19, 2018).
- 38 Wolk DM, Dunne WM. New technologies in clinical microbiology. *J Clin Microbiol* 2011; 49 (suppl 9): S62–67.
- 39 van Belkum A, Dunne WM. Next-generation antimicrobial susceptibility testing. *J Clin Microbiol* 2013; 51: 2018–24.
- 40 International Organization for Standardization. ISO 13485:2016. *Medical devices—quality management systems—requirements for regulatory purposes*. http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?csnumber=59752 (accessed Jan 19, 2018).
- 41 Frean J, Perovic O, Fensham V, et al. External quality assessment of national public health laboratories in Africa, 2002–2009. *Bull World Health Organ* 2012; 90: 191–99.
- 42 Mukadi P, Gillet P, Lukwaka A, et al. External quality assessment of malaria microscopy in the Democratic Republic of the Congo. *Malar J* 2011; 10: 308.
- 43 International Air Transport Association. *Dangerous goods regulations*. 59th edn. Montreal: International Air Transport Association, 2018.
- 44 Barbe B, Verdonck K, Mukendi D, et al. The art of writing and implementing standard operating procedures (SOPs) for laboratories in low-resource settings: review of guidelines and best practices. *PLoS Negl Trop Dis* 2016; 10: e0005053.
- 45 Clinical and Laboratory Standards Institute. *CLSI M100 S27:2017. Performance standard for antimicrobial susceptibility testing*. 27th edn. Wayne, PA: Clinical and Laboratory Standards Institute, 2017.
- 46 Petti C, Weinstein M, Carroll K, Forman M, Valsamakis A. Systems for detection and identification of bacteria and yeasts. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warrack DW, eds. *Manual of Clinical Microbiology*, vol 1. Washington, DC: American Society for Microbiology, 2011: 15–26.
- 47 O'Hara CM. Manual and automated instrumentation for identification of Enterobacteriaceae and other aerobic gram-negative bacilli. *Clin Microbiol Rev* 2005; 18: 147–62.
- 48 Vitrat V, Hautefeuille S, Janssen C, Bougon D, Sirodot M, Pagani L. Optimizing antimicrobial therapy in critically ill patients. *Infect Drug Resist* 2014; 7: 261–71.
- 49 Fall B, Lo CI, Samb-Ia B, et al. The ongoing revolution of MALDI-TOF mass spectrometry for microbiology reaches tropical Africa. *Am J Trop Med Hyg* 2015; 92: 641–42.
- 50 Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol* 2015; 6: 791.
- 51 Murray PR. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology. *Clin Microbiol Infect* 2010; 16: 1626–30.
- 52 Murray PR. What is new in clinical microbiology—microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. *J Mol Diagn* 2012; 14: 419–23.
- 53 Leclercq R, Canton R, Brown DF, et al. EUCAST expert rules in antimicrobial susceptibility testing. *Clin Microbiol Infect* 2013; 19: 141–60.
- 54 Wayne P. *Performance standards for antimicrobial susceptibility testing*, 26th edn. Wayne, PA: Clinical and Laboratory Standards Institute, 2016.
- 55 Rhoads DD, Sintchenko V, Rauch CA, Pantanowitz L. Clinical microbiology informatics. *Clin Microbiol Rev* 2014; 27: 1025–47.
- 56 Kerremans JJ, van der Bij AK, Goossens W, Verbrugh HA, Vos MC. Needle-to-incubator transport time: logistic factors influencing transport time for blood culture specimens. *J Clin Microbiol* 2009; 47: 819–22.
- 57 Polage CR, Bedu-Addo G, Owusu-Ofori A, et al. Laboratory use in Ghana: physician perception and practice. *Am J Trop Med Hyg* 2006; 75: 526–31.
- 58 Baron EJ. Rapid identification of bacteria and yeast: summary of a National Committee for Clinical Laboratory Standards proposed guideline. *Clin Infect Dis* 2001; 33: 220–25.
- 59 Bruins MJ, Ruijs CJ, Wolffhagen MJ, Bloembergen P, Aarts JE. Does electronic clinical microbiology results reporting influence medical decision making: a pre- and post-interview study of medical specialists. *BMC Med Inform Decis Mak* 2011; 11: 19.
- 60 Tucker TJ, Manyike PT. Improving the clinic-laboratory-interface in the context of HIV diagnosis, treatment, and monitoring. *Curr Opin HIV AIDS* 2017; 12: 105–11.
- 61 Humphreys H. Where do out-of-hours calls to a consultant microbiologist come from? *J Clin Pathol* 2009; 62: 746–48.
- 62 Humphreys H, Nagy E, Kahlmeter G, Ruijs CJ. The need for European professional standards and the challenges facing clinical microbiology. *Eur J Clin Microbiol Infect Dis* 2010; 29: 617–21.
- 63 Zhang HL, Omondi MW, Mutyoka AM, et al. Challenges of maintaining good clinical laboratory practices in low-resource settings: a health program evaluation framework case study from East Africa. *Am J Clin Pathol* 2016; 146: 199–206.
- 64 Petrose LG, Fisher AM, Douglas GP, et al. Assessing perceived challenges to laboratory testing at a Malawian referral hospital. *Am J Trop Med Hyg* 2016; 94: 1426–32.
- 65 Thakur KT, Mateyo K, Hachaambwa L, et al. Lumbar puncture refusal in sub-Saharan Africa: a call for further understanding and intervention. *Neurology* 2015; 84: 1988–90.
- 66 Grietens KP, Ribera JM, Erhart A, et al. Doctors and vampires in sub-Saharan Africa: ethical challenges in clinical trial research. *Am J Trop Med Hyg* 2014; 91: 213–15.
- 67 English M, Esamai F, Wasunna A, et al. Delivery of paediatric care at the first-referral level in Kenya. *Lancet* 2004; 364: 1622–29.
- 68 Mbonye AK, Magnussen P, Chandler CI, et al. Introducing rapid diagnostic tests for malaria into drug shops in Uganda: design and implementation of a cluster randomized trial. *Trials* 2014; 15: 303.
- 69 Tuijn CJ, Msoka E, Mushi DI, Boer MS, Chilongola J, van den Broek A. The interface between clinicians and laboratory staff: a field study in northern Tanzania. *Afr J Lab Med* 2014; 3: 126.
- 70 Bates I, Maitland K. Are laboratory services coming of age in sub-Saharan Africa? *Clin Infect Dis* 2006; 42: 383–84.
- 71 Thriemer K, Katuala Y, Batoko B, et al. Antibiotic prescribing in DR Congo: a knowledge, attitude and practice survey among medical doctors and students. *PLoS One* 2013; 8: e55495.





- 72 Quet F, Vlieghe E, Leyer C, et al. Antibiotic prescription behaviours in Lao People's Democratic Republic: a knowledge, attitude and practice survey. *Bull World Health Organ* 2015; 93: 219–27.
- 73 Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. *Clin Infect Dis* 2016; 62: e51–77.
- 74 Allegranzi B, Gayet-Ageron A, Damani N, et al. Global implementation of WHO's multimodal strategy for improvement of hand hygiene: a quasi-experimental study. *Lancet Infect Dis* 2013; 13: 843–51.
- 75 Burke NJ, Do HH, Talbot J, Sos C, Sny D, Taylor VM. Chumunguh thileum: understanding liver illness and hepatitis B among Cambodian immigrants. *J Community Health* 2011; 36: 27–34.
- 76 Wilson MP, Spencer RC. Laboratory role in the management of hospital acquired infections. *J Hosp Infect* 1999; 42: 1–6.
- 77 Canton R. Role of the microbiology laboratory in infectious disease surveillance, alert and response. *Clin Microbiol Infect* 2005; 11 (suppl 1): 3–8.
- 78 Gershy-Damet GM, Rotz P, Cross D, et al. The World Health Organization African region laboratory accreditation process: improving the quality of laboratory systems in the African region. *Am J Clin Pathol* 2010; 134: 393–400.
- 79 WHO. WHO guide for the stepwise laboratory improvement process towards accreditation in the African region (checklist). Geneva: World Health Organization, 2010: 1–60.
- 80 WHO. Laboratory quality stepwise implementation tool. <https://extranet.who.int/lqsi/> (accessed Jan 19, 2018).
- 81 Cham F, Maleka M, Masango M, et al. The World Health Organization African region external quality assessment scheme for anti-HIV serology. *Afr J Lab Med* 2012; 1: 39.
- 82 Chongo P, Siteo N, Viegasa S, et al. Quality assurance for point-of-care testing in Mozambique's National Health Service. *Afr J Lab Med* 2016; 5: 445.
- 83 Meyers AFA, Sandstrom P, Denny TN, et al. Quality assurance for HIV point-of-care testing and treatment monitoring assays. *Afr J Lab Med* 2016; 5: 552.
- 84 Boeras DI, Peeling RW, Onyebujoh P, Yahaya AA, Gumedde-Moetsi HN, Ndihekubwayo JB. The WHO AFRO external quality assessment programme (EQAP): linking laboratory networks through EQA programmes. *Afr J Lab Med* 2016; 5: 560.
- 85 Audu RA, Onubogga CC, Okoye RN, et al. Proficiency testing for HIV, tuberculosis and malaria diagnosis in clinical laboratories in Nigeria. *Afr J Lab Med* 2014; 3: 102.
- 86 Biomérieux. Annual report 2014. http://www.biomerieux-finance.com/sites/investord7/files/rapport_annuel-gb-web.pdf (accessed Jan 19, 2018).
- 87 The Canadian Association for Laboratory Accreditation. PO7—CALA application of requirements in ISO/IEC 17025:2005. 2014. http://www.cala.ca/PO7-CALA_Application.pdf (accessed Jan 19, 2018).
- 88 Khan SR, Kona R, Faustino PJ, et al. United States Food and Drug Administration and Department of Defense shelf-life extension program of pharmaceutical products: progress and promise. *J Pharm Sci* 2014; 103: 1331–36.
- 89 Jacobs J, Barbe B, Gillet P, et al. Harmonization of malaria rapid diagnostic tests: best practices in labelling including instructions for use. *Malar J* 2014; 13: 505.
- 90 WHO. Harmonization of rapid diagnostic tests for malaria and implications for procurement. 26–27 February 2015 Geneva, Switzerland. Meeting report. 2015. http://www.who.int/malaria/publications/atoz/9789241509978_eng.pdf?ua=1 (accessed Jan 19, 2018).
- 91 Diagnostic Microbiology Development Program. Centralized media making facility. <http://dmdp.org/media-making-lab/> (accessed Jan 19, 2018).
- 92 Andrews JR, Prajapati KC, Bypper E, et al. Evaluation of an electricity-free, culture-based approach for detecting typhoidal *Salmonella* bacteremia during enteric fever in a high burden, resource-limited setting. *PLoS Negl Trop Dis* 2013; 7: e2292.
- 93 Science with a mission. Centrifuge info. <http://www2.sciencewithamission.org/centrifuge-info> (accessed Jan 18, 2018).
- 94 Nathawitharana R, Coronel J, Moore DA. Solar disinfection of MODS mycobacterial cultures in resource-poor settings. *PLoS One* 2007; 2: e1100.
- 95 Chitnis V, Chitnis S, Patil S, Chitnis D. Solar disinfection of infectious biomedical waste: a new approach for developing countries. *Lancet* 2003; 362: 1285–86.
- 96 WHO. Distributed surgical instrument sterilization using solar powered autoclaves in low resource settings. http://www.who.int/medical_devices/poster_a18.pdf (accessed Jan 19, 2018).
- 97 Yeh E, Pinsky BA, Banaei N, Baron EJ. Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood agar for diagnostic microbiology laboratory tests. *PLoS One* 2009; 4: e6141.
- 98 Anand C, Gordon R, Shaw H, Fonseca K, Olsen M. Pig and goat blood as substitutes for sheep blood in blood-supplemented agar media. *J Clin Microbiol* 2000; 38: 591–94.
- 99 Yin N, Santos TM, Auer GK, Crooks JA, Oliver PM, Weibel DB. Bacterial cellulose as a substrate for microbial cell culture. *Appl Environ Microbiol* 2014; 80: 1926–32.
- 100 European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. version 21. 2017. http://www.eucast.org/filesadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_21_Breakpoint_Tables.pdf (accessed Jan 19, 2018).
- 101 Janda JM. Taxonomic update on proposed nomenclature and classification changes for bacteria of medical importance, 2013–2014. *Diagn Microbiol Infect Dis* 2015; 83: 82–88.
- 102 Tacconelli E, Cataldo MA, Dancer SJ, et al. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clin Microbiol Infect* 2014; 20 (suppl 1): 1–55.
- 103 Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev* 2009; 22: 161–82.
- 104 Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2017; published online Dec 21. DOI:10.1016/S1473-3099(17)30753-3.
- 105 Rossi B, Gasperini MI, Leflon-Guibout V, et al. Hypervirulent *Klebsiella pneumoniae* in cytogenetic liver abscesses, Paris, France. *Emerg Infect Dis* 2018; 24: 221–29.
- 106 Navon-Venezia S, Komdratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev* 2017; 41: 252–75.
- 107 Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clin Microbiol Rev* 2015; 28: 565–91.
- 108 Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014; 27: 543–74.
- 109 Crump JA, Sjolund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive salmonella infections. *Clin Microbiol Rev* 2015; 28: 901–32.
- 110 Wong VK, Baker S, Pickard DJ, et al. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of *Salmonella* Typhi identifies inter- and intracontinental transmission events. *Nat Genet* 2015; 47: 632–39.
- 111 Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. Global burden of invasive nontyphoidal *Salmonella* disease, 2010. *Emerg Infect Dis* 2015; 21: 941–49.
- 112 WHO. World malaria report 2017. <http://apps.who.int/iris/bitstream/10665/259492/1/9789241565523-eng.pdf?ua=1> (accessed Jan 24, 2018).
- 113 Kariuki S, Onsare RS. Epidemiology and genomics of invasive nontyphoidal salmonella infections in Kenya. *Clin Infect Dis* 2015; 61 (suppl 4): S317–24.
- 114 Olaitan AO, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front Microbiol* 2014; 5: 643.
- 115 WHO. Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. 2005. <http://apps.who.int/iris/bitstream/10665/43252/1/924159330X.pdf> (accessed Jan 24, 2018).

- 116 Drees M, Pineles I, Harris AD, Morgan DJ. Variation in definitions and isolation procedures for multidrug-resistant Gram-negative bacteria: a survey of the Society for Healthcare Epidemiology of America Research Network. *Infect Control Hosp Epidemiol* 2014; 35: 362–66.
- 117 Janda JM, Abbott SL. Bacterial identification for publication: when is enough enough? *J Clin Microbiol* 2002; 40: 1887–91.
- 118 Oren A, Garrity GM. Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. *Antonie Van Leeuwenhoek* 2014; 106: 43–56.
- 119 Clinical and Laboratory Standards Institute. Abbreviated identification of bacteria and yeast: approved guidelines, 2 edn. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
- 120 York MK. Highlights of CLSI document—abbreviated identification of bacteria and yeast-M35-A2. *Clin Microbiol News* 2010; 32: 65–72.
- 121 Van den Broek A, Tuijn CJ, van't Klooster L, et al. Understanding the interface between clinical and laboratory staff. *Afr J Lab Med* 2014; 3: 127.
- 122 Bonten MJ. Medical microbiology laboratories in Dutch hospitals: essential for safe patient care. *Ned Tijdschr Geneesk* 2008; 152: 2650–52.
- 123 Bouza E, Sousa D, Munoz P, Rodriguez-Creixems M, Fron C, Lechuz JG. Bloodstream infections: a trial of the impact of different methods of reporting positive blood culture results. *Clin Infect Dis* 2004; 39: 1161–69.
- 124 WHO. Good clinical diagnostic practice: a guide for clinicians in developing countries to the clinical diagnosis of disease and to making proper use of clinical diagnostic services. 2005. <http://apps.who.int/iris/bitstream/10665/119735/1/dsa236.pdf> (accessed Jan 19, 2018).
- 125 Strengthening Laboratory Management Toward Accreditation. SLM TA training materials (2015 version). 2015. <https://www.slm-ta.org/tool-kit/english> (accessed Jan 24, 2018).
- 126 Uehara Y, Yagoshi M, Tanimichi Y, et al. Impact of reporting gram stain results from blood culture bottles on the selection of antimicrobial agents. *Am J Clin Pathol* 2009; 132: 18–25.
- 127 Munson EL, Diekema DJ, Beekmann SE, Chapin KC, Doern CV. Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. *J Clin Microbiol* 2003; 41: 495–97.
- 128 Chandler L. Challenges in clinical microbiology testing. In: Dasgupta A, Sepulveda JL, eds. *Accurate results in the clinical laboratory*. London: Elsevier, 2013: 315–26.
- 129 Vlieghe ER, Phe T, De Smet B, et al. Azithromycin and ciprofloxacin resistance in *Salmonella* bloodstream infections in Cambodian adults. *PLoS Negl Trop Dis* 2012; 6: e1933.
- 130 Lunguya O, Phoba MF, Mundeke SA, et al. The diagnosis of typhoid fever in the Democratic Republic of the Congo. *Trans R Soc Trop Med Hyg* 2012; 106: 348–55.
- 131 Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; 10: 417–32.
- 132 Maltha J, Guiraud I, Kabore B, et al. Frequency of severe malaria and invasive bacterial infections among children admitted to a rural hospital in Burkina Faso. *PLoS One* 2014; 9: e89103.
- 133 Wu HM, Cordeiro SM, Harcourt BH, et al. Accuracy of real-time PCR, Gram stain and culture for *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* meningitis diagnosis. *BMC Infect Dis* 2013; 13: 26.
- 134 Teysou R, Muros-Le Rouzic E. Meningitis epidemics in Africa: a brief overview. *Vaccine* 2007; 25 (suppl 1): A3–7.
- 135 Elshafie S, Taj-Aldeen SJ. Emerging resistant serotypes of invasive *Streptococcus pneumoniae*. *Infect Drug Resist* 2016; 9: 153–60.
- 136 Desplaces N. Diagnostic microbiologique des infections ostéo-articulaires: les pièges à éviter. *Faillites de Biologie* 2014; 55: 5–13.
- 137 Fritz JM, McDonald JR. Osteomyelitis: approach to diagnosis and treatment. *Phys Sportsmed* 2008; 36: 50–54.
- 138 Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet* 2016; 388: 1291–301.
- 139 Perilla M, Ajello C, Bopp C, Elliott J, Facklam R. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Geneva: World Health Organization, 2003.
- 140 WHO. Foodborne disease outbreaks: guidelines for investigation and control. 2008. http://www.who.int/foodsafety/publications/foodborne_disease/outbreak_guidelines.pdf (accessed Jan 28, 2018).
- 141 WHO. Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. 2005. <http://apps.who.int/iris/bitstream/10665/43206/1/9241546700.pdf> (accessed Jan 17, 2018).
- 142 WHO. IMAI district clinician manual: hospital care for adolescents and adults. Guidelines for the management of illnesses with limited resources. 2011. <http://www.who.int/hiv/pub/imai/imai2011/en/> (accessed Jan 17, 2018).
- 143 WHO. Laboratory Assessment Tool (LAT). 2012. http://www.who.int/ihp/publications/laboratory_tool/en/ (accessed Jan 28, 2018).
- 144 Imani P, Jakech B, Kirunda I, Mbonye MK, Naikoba S, Weaver MR. Effect of integrated infectious disease training and on-site support on the management of childhood illnesses in Uganda: a cluster randomized trial. *BMC Pediatr* 2015; 15: 103.
- 145 Barbe B, Yansoumi CP, Affolabi D, Jacobs J. Implementation of quality management for clinical bacteriology in low-resource settings. *Clin Microbiol Infect* 2017; 23: 426–33.
- 146 Guarner J, Nimo SM. Microbiology learning and education online. *J Clin Microbiol* 2016; 54: 1203–08.
- 147 Kahlmeter G. EUCAST disk diffusion antimicrobial susceptibility testing method. Preparation of inoculum. 2016. https://www.youtube.com/watch?v=M6KpdQjsgdI&list=PLQU_kWRWBld4DhwITIKOR5bKUUTJ2v6W (accessed Jan 17, 2018).
- 148 Mochon AB, Santa Cruz M. Telemicrobiology: focusing on quality in an era of laboratory consolidation. *Clin Microbiol News* 2016; 38: 19–24.
- 149 Schultz C, Lan NP, Van Dung N, et al. Network building and knowledge exchange with telemicrobiology. *Lancet Glob Health* 2014; 2: e78.
- 150 O'Neill J. Review on antimicrobial resistance. Tackling drug-resistant infections globally: final report and recommendations. 2016. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf (accessed Jan 21, 2018).
- 151 Mabey D, Peeling RW, Ustianowski A, Perkins MD. Tropical infectious diseases: diagnostics for the developing world. *Nat Rev Microbiol* 2004; 2: 231–40.
- 152 Depasse JW, Lee PT. A model for 'reverse innovation' in health care. *Global Health* 2013; 9: 40.
- 153 GE Healthcare. Market-relevant design: making ECGs available across India. <http://newsroom.gehealthcare.com/ecgs-india-reverse-innovation/> (accessed Jan 21, 2018).
- 154 Culbreath K, Petti CA. Balancing enthusiasm for innovative technologies with optimizing value: an approach to adopt new laboratory tests for infectious diseases using bloodstream infections as exemplar. *Open Forum Infect Dis* 2015; 2: o6075.
- 155 WHO. WHO to develop Essential Diagnostics List. 2017. http://www.who.int/medicines/news/2017/WHO_develop_essential_diagnostics_list/en/ (accessed Jan 19, 2018).
- 156 Best M, Sakande J. Practical recommendations for strengthening national and regional laboratory networks in Africa in the Global Health Security era. *Afr J Lab Med* 2016; 5: 471.
- 157 WHO. Prequalification of in vitro diagnostics. http://www.who.int/diagnostics_laboratory/evaluations/en/ (accessed Jan 18, 2018).
- 158 Mendelson M, Dar OA, Hoffman SJ, Laxminarayan R, Mpundu MM, Rottingen JA. A global antimicrobial conservation fund for low- and middle-income countries. *Int J Infect Dis* 2016; 51: 70–72.
- 159 Wellcome Trust. Fleming Fund launched to tackle global problem of drug-resistant infection. <https://wellcome.ac.uk/press-releases/fleming-fund-launched-tackle-global-problem-drug-resistant-infection> (accessed Jan 21, 2018).
- 160 The Interacademy Panel–The Interacademy Medical Panel. Antimicrobial resistance: a call for action. 2013. http://www.interacademies.net/IAP_IAMP_Nov2013.aspx (accessed Jan 18, 2018).
- 161 Carlet J, Jarlier V, Harbarth S, et al. Ready for a world without antibiotics? The Penicillins Antibiotic Resistance Call to Action. *Antimicrob Resist Infect Control* 2012; 1: 11.
- 162 Callaghan M, Ford N, Schneider H. A systematic review of task-shifting for HIV treatment and care in Africa. *Hum Resour Health* 2010; 8: 8.

Annex 2 : Mini-Lab equipment Atlas

<p>Mini-LIMS: Mini-Lab laboratory information and management system</p>	
<p>Incubators: Equipment to maintain an appropriate temperature (35 +/-2°C) for the culture of bacteria. Kit is composed of :</p> <ul style="list-style-type: none"> • two VWR IL 56 liter capacity (UK) to accommodate 8 racks of 10 blood culture bottles.. • one INCUDIGIT SV 30L (JP Selecta, Spain) an incubator of 30 liter capacity, can be left unpowered for 12 hours due to an insulation system using phase change materials, can accommodate 60 Intray cassettes and 30 ID or AST 	
<p>ARS (Assisted Reading System): System used for reading identification and antibiotic susceptibility plates. Composed of (i)</p> <ul style="list-style-type: none"> • microplate viewer box (JP Selecta, Spain), on which the plate to be read is placed, and • the stand-mounted camera that takes a photo of the microplate (for archiving or for use in the Mini-LIMS). 	
<p>Microscope: Used for observation of bacteria after Gram staining and at 1000x magnification (eyepiece lens x 10 and objective lens x 100) and including a camera connected to the Mini-LIMS, MOTIC, Panthera series (China)</p>	
<p>Autoclave: Apparatus for sterilising waste in the Mini-Lab, Model 2840 ELC (Tuttnauer, Holland) capacity of 28L with failsafe mode</p> <p>Distiller: Apparatus for making distilled water for running the autoclave (JP Selecta, Spain)</p>	

<p>Refrigerator: Allows certain reagents to be stored at a temperature between 2°C and 8°C, Ice linen (Vestfrost) 90Liter of capacity with 24 hours capacity of functioning without electricity</p> <p>Freezer: Allows strains and reagents to be stored at temperatures below -20°C, Ice linen (Vestfrost) 90Liter of capacity with 24 hours capacity of functioning without electricity</p>	
<p>Densitometer: Apparatus used for the production of calibrated bacterial suspensions (BioSan)</p>	
<p>LogTag: Recording thermometer for temperature monitoring (incubators, refrigerator, freezer, room temperature)</p>	
<p>RENOK: System used for inoculation of ID and AST plates (Beckman Coulter)</p>	
<p>GENbag CO2: CO2 generator to facilitate the growth of certain bacteria</p>	
<p>Scale: Equipment used to weigh blood culture bottles before and after collection</p>	


<p>Barcode reader: Reads sample barcodes</p>	 A black handheld barcode reader mounted on a black stand with a circular base. The device has a green light and a cable extending from the back.
<p>Slide warmer: Mug Warmer used to fix smears before Gram staining,</p>	 A circular black slide warmer with a white base. The brand name 'Zimory' is visible on the black top surface. It is connected to a power cord.
<p>Printer: Used for printing barcode labels, results and various documents</p>	 A black multifunction printer with a control panel on the right side and a paper tray at the bottom.
<p>Bottle viewer: LED lamp for easy reading of blood culture bottles developed base on Rhesuscope lamp technology</p>	 A white rectangular LED lamp with three green-capped bottles of blood culture media placed on top. The bottles contain liquids of different colors (yellow, orange, red). The lamp has a label on the front.

Annex 3: Bench aid describing the sampling kit

BENCH AID

05

Médicins Sans Frontières
Mini-Lab



Collection

Sampling kit

This document is a summary of the various SOPs « 9.3. Kit de prélèvement ».
Please read the POS before using this BA.

- This kit is intended to take blood cultures and urine samples, it includes all the equipment for disinfection and sampling.
- The kit is sized to collect two to three samples: **Caution => 1 kit = 1 patient.**
- Frequently used equipment and PPE (gloves, mask, etc.) are not provided in the kit, and must be made available in the service.
- **Blood culture vials awaiting transfer to the Mini-Lab should be stored at room temperature. Never refrigerate!!**
- **Urine vials awaiting transfer to the Mini-Lab should be stored between 2 and 8°C. Never put in an incubator!!**
- The material provided must be returned to the kit after use and returned to the laboratory.
- The Mini-Lab takes care of the cleaning and replenishment of each kit.

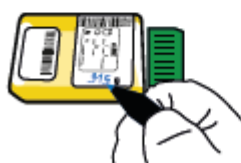
1

 Cleaning the kit


- 01- Put Surfanios on paper towel.
- 02- Clean the outside of the sampling case.
- 03- Open the case, and inspect the inside.
- 04- If the briefcase is soiled, take all the material out of the briefcase.

- 05- Clean the inside with absorbent paper impregnated with diluted Surfanios.
- 06- Make an inventory of the equipment present in the kit.
- 07- Add the missing material according to the quantities indicated below:

ARTICLES	QTY/KIT	ARTICLES	QTY/KIT
Blood culture bottle	3	Isopropyl alcohol 70% (wipe)	10
Chlorhexidine aqueous solution (new born)	1	Alcoholic chlorhexidine in wipe	10
Finned luer needles (23G)	3	Medical adhesive tape	1
Sterile luer needle 21G (green)	3	Permanent marker	1
Sterile compresses	10	Toumiquet	1
Non-sterile compresses	10	Bench aid	1
Plastic bag for transport of samples	3	Sterile syringe 5 ml and 10 ml	3 + 3
Sterile container for urine collection	3	Hydroalcoholic solution 100 ml	1



- 01- Weigh new bottles of blood culture.
- 02- Note the weight on the bottle.

Effective date : 10/11/2020

Version : V1

Code : FM-5-KITPRE

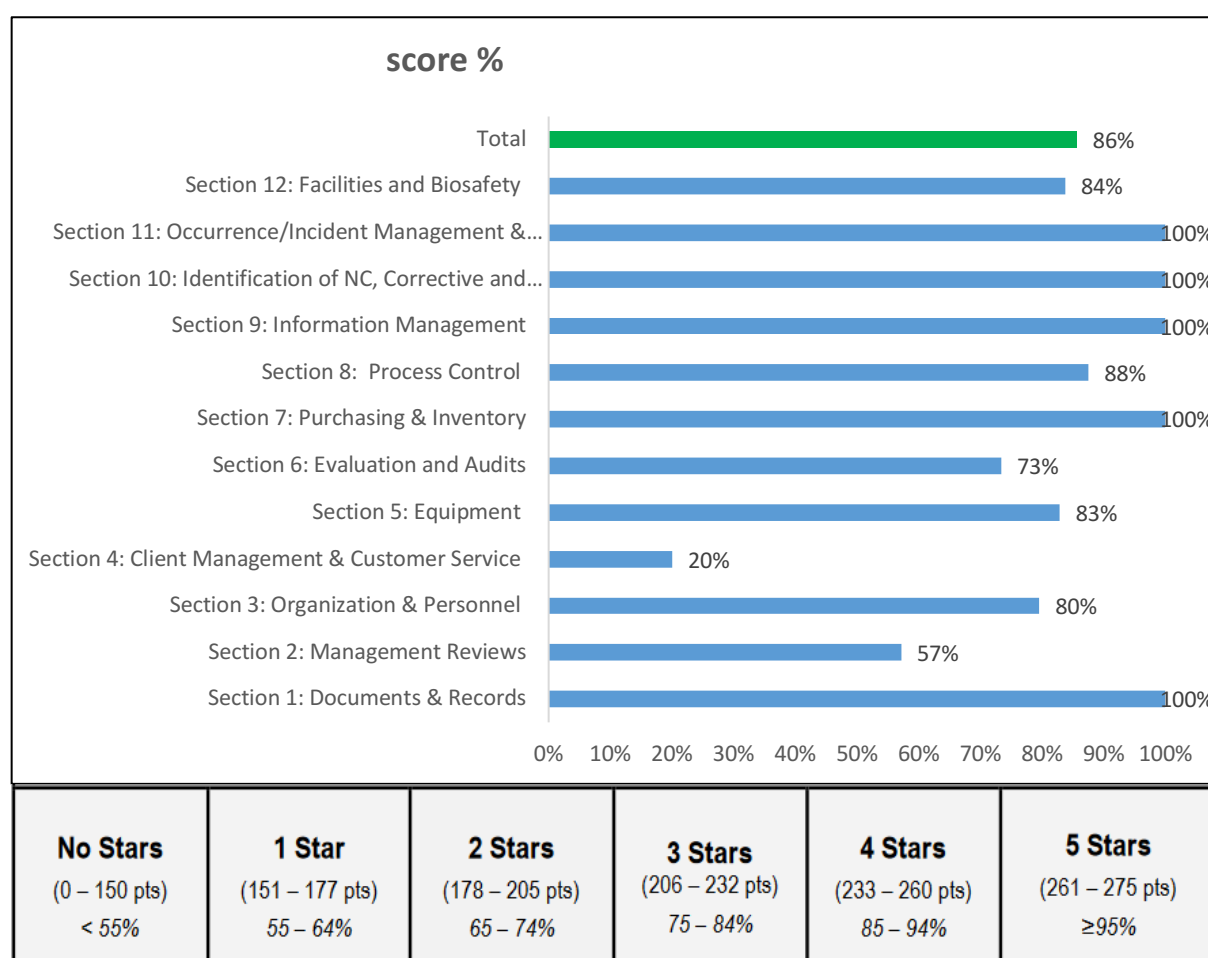
19



Annex 4: Evaluation of the Mini-Lab Carnot using the SLIPTA checklist

The evaluation was done during the month of November 2021 using the SLIPTA methodology[172], [173]. Name of the evaluator: JB Ronat

	Score obtain	Total max	score %
Section 1: Documents & Records	28	28	100%
Section 2: Management Reviews	8	14	57%
Section 3: Organization & Personnel	17,5	22	80%
Section 4: Client Management & Customer Service	2	10	20%
Section 5: Equipment	29	35	83%
Section 6: Evaluation and Audits	11	15	73%
Section 7: Purchasing & Inventory	24	24	100%
Section 8: Process Control	28	32	88%
Section 9: Information Management	21	21	100%
Section 10: Identification of NC, Corrective/Preventive Actions	19	19	100%
Section 11: Incident Management & Process Improvement	12	12	100%
Section 12: Facilities and Biosafety	36	43	84%
Total	235,5	275	86%



Annex 5: QC strains identification sheet

8.1.1 QC Strain Identification Sheet


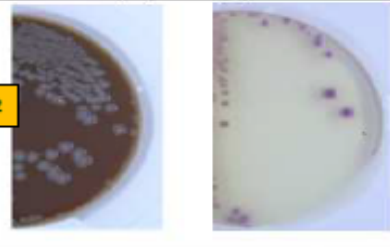
The highlighted in grey correspond to the modifications made on this version

How to use the QC strain identification sheets

These sheets were established by performing numerous repetitions of the tests in the laboratory in order to identify the expected ranges of the reactions, particularly for AST.

The sheets are divided into 5 parts:

1. The name and ATCC (American Type Culture Collection) identifier and in brackets whether the strain is wild type or has a particular resistance mechanism.
2. Morphology under the microscope and on InTray Chocolate and Colorex agar.
3. Reactions in pre-identification tests and identification.
4. Information on MIC targets or expected ranges and interpretation of clinical categories using **EUCAST version 11**, where applicable.
5. Information on reactions for certain resistance mechanisms.

Escherichia coli ATCC 25922 (Wild)					
Morphology under the microscope			Colony morphology (Chocolate - Colorex)		
Short plump bacilli			Circular, regular margin, smooth		
					
Expected pre-ID	Gram	Negative	Identification	Genus	Escherichia
	AMNP	Positive		Species	Escherichia coli
	Oxidase	Negative			
	Catalase	NA			
Expected AST				Resistance mechanism	
Antibiotics	Target MIC (mg/L)	MIC Interval (mg/L)	S/lyr ²	Mechanism Name	Target Outcome
Ampicillin	28	-	5	ESBL	Negative
Amoxicillin-clavulanic acid	8	2-8	5	CRE	Negative
Piperacillin-tazobactam	58	-	5	FQRS	-
Cefotaxime	22	-	-	PARK	-
Cefotaxime/Clavulanic Acid	20.5	-	-	IRPA	-
Cefazidime	51	-	5	CRAB	-
Ceftazidime/Clavulanic Acid	20.25	-	-		
Ceftroxone	21	-	5		
Ciprofloxacin	20.05	-	5		
Amikacin	58	-	5		
Gentamicin	22	-	5		
Tigecycline	-	-	-		
Chloramphenicol	24	24-8	5		
Gallitin	22	-	5		
Fosfomicin	-	-	-		
Trimethoprim-sulfamethoxazole	2.2	-	5		
Meropenem	20.22	-	5		
Imipenem	20.5	-	5		
Ertapenem	20.22	-	5		
				- : No defined MIC interval or interpretation	
				* Interpretation made from version V 11. of EUCAST	

Annex 6: List of bacteria identified by Mini-Lab Systems:

This below bacteria list can be identified using the Microscan MSF Neg/Pos ID panels

Gram-negative Bacteria			Gram-positive Bacteria		
Bacterial group	Examples of species in group	ID Possibility	Bacterial group	Examples of species in group	ID Possibility
Enterobacter/Citrobacter/Serratia	Citrobacter (<i>freundii</i> , <i>koseri</i> , <i>braakii</i> ...) Cronobacter (<i>sakazakii</i> , <i>malonaticus</i> ...) Enterobacter (<i>cloacae</i> , <i>aérogènes</i> ...) Hafnia <i>alvei</i> Serratia (<i>marcescens</i> , <i>liquefaciens</i>)*	Yes Yes Yes Yes Yes	Streptococcus <i>hémolytiques</i>	Streptococcus <i>pyogenes</i> Streptococcus <i>agalactiae</i> Streptococcus <i>equi</i> Streptococcus <i>dysgalactiae</i>	Yes Yes Yes Yes
		Yes Yes		Streptococcus <i>pneumoniae</i>	Streptococcus <i>pneumoniae</i>
Klebsiella	Klebsiella (<i>pneumoniae</i> , <i>ocytoca</i> ...) Raoultella spp.	Yes Yes	Anginosus <i>group</i> Streptococcus	Streptococcus <i>anginosus</i>	Yes
Escherichia coli	Escherichia coli	Yes		Streptococcus <i>constellatus</i>	Yes
			Streptococcus <i>intermedius</i>	Yes	
Salmonella Typhi Salmonella Paratyphi A	Salmonella Typhi Salmonella Paratyphi A*	Yes Yes	Groupe <i>viridans</i> Streptococcus	Streptococcus <i>oralis</i> Streptococcus <i>mitis</i> Streptococcus <i>sanguinis</i> Streptococcus <i>bovis</i> * Streptococcus <i>suis</i> *	Yes Yes Yes Yes Yes
Salmonella spp non typhoidal.	Non-Typhoidal Samonella	Yes	Enterococcus spp.	Enterococcus <i>faecalis</i> Enterococcus <i>faecium</i>	Yes Yes
Proteus/Providencia /Morganella	Morganella <i>morganii</i> Proteus (<i>mirabilis</i> , <i>vulgaris</i> , ...) Providencia spp.	Yes Yes Yes	Staphylococcus aureus	Staphylococcus aureus	Yes
Shigella spp.	Shigella (<i>boydii</i> , <i>dysenterie</i> , <i>flexneri</i> ...)	Yes	Staphylocoques <i>négatifs de coagulase</i>	Staphylococcus <i>epidermidis</i> Staphylococcus <i>homini</i> Staphylococcus <i>warneri</i> ...	Yes Yes Yes
Aeromonas/Vibrio/ Plesiomonas	Plesiomonas <i>shigelloides</i> Aeromonas spp. Vibrio <i>non-cholerae</i>	Yes Yes No	Bacillus (<i>non-anthrax</i>)	Bacillus.spp	Yes
Non fermenter Gram-negative Bacilli	Pseudomonas <i>aeruginosa</i> Complexe <i>Burkholderia cepacia</i> Stenotrophomonas <i>maltophilie</i> * Acinetobacter <i>baumannii</i> .	Yes Yes Yes Yes	Corynebacterium (<i>non-diphthérie</i>)	Corynebacterium.spp	Yes
Burkholderia pseudomallei	Burkholderia pseudomallei	Yes	Listeria	Listeria <i>monocytogenes</i>	Yes
Brucella	Brucella spp.	No			
Haemophilus spp.	Haemophilus <i>influenzae</i>	Yes			
Neisseria meningitidis	Neisseria meningitidis	Yes			

Annex 7: Details of the MSF MicroScan AST panels:

Gram Pos panels (C32698) for *Staphylococcus spp.* and *Enterococcus spp.* species, MSF MicroScan Gram Neg panels (C32699) for Gram-negative bacilli isolates and MSF MicroScan Fastidious microplate (C32700) for *Streptococcus* and *Haemophilus* species, with antibiotics molecules classified as per the WHO 2019 Access, Watch, Reserve (“AWaRe”) classification of antibiotics and 2019 Essential Medicine List

Antibiotic	Class	AWaRe Category	Listed on EML 2019	MIC POS Panel		MIC NEG Panel			MIC FAST Panel*	
				Staphylococci	Enterococci	Enterobacteriales	Non-Fermenting bacilli	Streptococci	Haemophilus	
Ampicillin	Penicillins	Access	Yes	X	X	X			X	
Benzylpenicillin	Penicillins	Access	Yes	X					X	
Oxacillin	Penicillins	Access	No	X						
Amoxicillin/clavulanic Acid	Beta lactam - beta lactamase inhibitor	Access	Yes		X	X			X	X
Chloramphenicol	Amphenicols	Access	Yes		X	X				
Gentamicin	Aminoglycosides	Access	Yes	X			X			
Gentamicin (high level)	Aminoglycosides	Access	Yes		X					
Amikacin	Aminoglycosides	Access	Yes	X			X			
Clindamycin	Lincosamides	Access	Yes	X						
Inducible clindamycin resistance	Lincosamides	Access	Yes	X						
Tetracycline	Tetracyclines	Access	No	X						
Trimethoprim	Trimethoprim	Access	No	X			X		X	X
Cefoxitin screen	Second-generation cephalosporins	Watch	No	X						
Ceftazidime	Third-generation cephalosporins	Watch	Yes			X	X			
Ceftriaxone	Third-generation cephalosporins	Watch	Yes			X	X		X	X
ESBL test	Third-generation cephalosporins	Watch	No			X	X			
Piperacillin/tazobactam	Beta lactam - beta lactamase inhibitor	Watch	Yes			X	X			
Ertapenem	Carbapenems	Watch	No			X	X			
Imipenem/cilastatin	Carbapenems	Watch	No			X	X			
Meropenem	Carbapenems	Watch	Yes			X	X		X	X
Ciprofloxacin	Fluoroquinolones	Watch	Yes	X	X					
Levofloxacin	Fluoroquinolones	Watch	No						X	X
Teicoplanin	Glycopeptides	Watch	No	X	X					
Vancomycin (IV)	Glycopeptides	Watch	Yes	X	X				X	X
Erythromycin	Macrolides	Watch	No	X						
Tigecycline	Glycylcyclines	Reserve	No	X						
Daptomycin	Lipopeptides	Reserve	No	X						
Linezolid	Oxazolidinones	Reserve	Yes	X					X	
Fosfomycin (IV)	Phosphonics	Reserve	Yes	X			X			
Colistin	Polymyxins	Reserve	Yes	X			X			X
Dalfofrisin-quinupristin	Streptogramins	Reserve	No	X	X					

*MIC FAST Panel may be provided as an option for surveillance purpose

Annex 8: Summary of validation studies results, in controlled environment, of the Mini-Lab analytical components.

Analytical component	Component type	Manufacturer	Study site	Reference method used in the study	N of samples analysed	Performance results
Blood Cultures [97]	Bi-phasic blood culture bottle	Autobio Diagnostics, China	Institute of Tropical Medicine, Antwerp, Be	BacT/ALERT (bioMérieux, France) manual and automated	N= 1057	Overall yield: 95.9% Positivity at D1: 90.7% Positivity at D2: 100%
Sub-culturing system [99]	MH Chocolate agar	Biomed Diagnostics Inc, OR, United States	Le Kremlin-Bicêtre Hospital, Hygiene Unit, Fr	PolyViteX, bioMérieux	N= 70	Mono-microbial culture Yield MH chocolate: 97% Positivity at D1: 97%
	Colorex Screen			UriSelect™4, Bio-Rad	N=33	Multi-microbial cultures Yield Colorex Screen: 94.4% Yield MH Chocolate agar: 88.9%
Pre- ID system	Gram staining	Panreac Química S.L.U.	Institute of Tropical Medicine, Antwerp, Be	Expected results of known organisms	N=186	Morphology: 86% agreement Gram classification: 96.8%
	Aminopeptidase	Liofilchem, IT			N=143	Gram classification: 85%
	Catalase	Liofilchem, IT			N=115	Accuracy: 84% agreement
	Oxidase	Hardy Diagnostics, US			N=185	Accuracy: 96% agreement
	Coagulase	Liofilchem, IT	Mini-Lab Drouillard, Haiti		N=40	In broth: Se 45%, Sp 100% On colonies: Se 92%, Sp 90%
ID (Identification) system [100]	MSF Neg/Pos ID Panel Type 2	Beckman Coulter, US	Institute of Tropical Medicine, Antwerp, Be	MALDI-ToF	N=195	Accuracy Gram Neg at species level: 94.9%
	MSF Neg/Pos ID Panel Type 2				N=128	Accuracy Gram Pos at species level: 85.9%
					N=84	Accuracy Enterococcus/Streptococcus sp: 74%
AST system [101], [174], [175]	MicroScan MSF Gram Positive panel	Beckman Coulter, US	Le Kremlin-Bicêtre Hospital, Hygiene Unit, Fr	Disk diffusion, EUCAST breakpoints, ISO 20776-2:2007	N=123	Categorical agreement: > 90% VMJ <3% (except for 2 Ab/org comb)
	MicroScan MSF Gram-negative panel				N=157	Categorical agreement: > 90% VMJ <3%
	MicroScan MSF Fastidious panel				N=107	Categorical agreement: > 90% VMJ <3%

Yield : percentage of positive over total inoculated; Se : Sensitivity; Sp : Specificity; Ab: antibiotic; org: organism; VMJ: Very Major error.

Annex 9: Summary of the validation studies results of the Mini-Lab pre-ID test

This evaluation took place in early 2019 during the validation study of the ID**[100] and during a specific proof of concept of the pre-ID*. Same protocol as described in the article[100] was used for testing the strains presented in below table

Species tested	PID*	ID**
<i>Acinetobacter baumannii</i>	11	8
<i>Bacillus</i> species	6	
<i>Burkholderia cepacia</i>	6	9
<i>Citrobacter freundii</i> complex		9
<i>Corynebacterium</i> species	3	
<i>Enterobacter cloacae</i> complex		11
<i>Enterococcus faecalis</i>	6	
<i>Enterococcus faecium</i>	7	
<i>Escherichia coli</i>		11
<i>Escherichia paracoli</i>		9
<i>Klebsiella oxytoca</i>		3
<i>Klebsiella pneumoniae</i>		9
<i>Kluyvera ascorbate</i>		1
<i>Listeria monocytogenes</i>	2	
<i>Pseudomonas aeruginosa</i>	3	9
<i>Salmonella Choleraesuis</i>		9
<i>Salmonella Paratyphi A</i>		8
<i>Salmonella Typhi</i>		8
<i>Salmonella Typhimurium</i>		10
<i>Shigella</i> species		10
<i>Stenotrophomonas maltophilia</i>	3	6
<i>Streptococcus agalactiae</i>	5	
<i>Streptococcus pneumoniae</i>	2	
<i>Streptococcus pyogenes</i>	5	
Total n = 189	59	130

Table below show the results of the two studies on pre-ID test. Accuracy of the different pre-ID tests (all bacterial strains combined). For oxidase and catalase, tests were included only when correct/incorrect responses were easy to determine (*e.g.* for oxidase only Gram-negative organisms were included, for catalase Enterobacterales were not included)

	Correct results (%)	Incorrect results (%)	Inconclusive results (%)	Total
Gram stain (morphology + reaction)	157 (84.4)	3 (1.6)	26 (14.0)	186
Gram morphology	160 (86.0)	2 (1.1)	24 (12.9)	186
Gram reaction	180 (96.8)	1 (0.5)	5 (2.7)	186
Methylene blue (morphology)	161 (86.6)	2 (1.1)	21 (12.3)	186
Oxidase (Hardy)	172 (93.0)	13 (7.0)	0	185
Catalase (Liofilchem)	97 (84.3)	17 (14.8)	1 (0.1)	115
Gram Test (Liofilchem)	121 (84.6)	10 (7.0)	12 (8.4)	143
Lanagram™ (Hardy)	120 (88.9)	6 (4.4)	9 (6.6)	135
Vancomycin	59 (100)	0	0	59

Annex 10: Extract of the pre-ID biotype database

Class	Group	Organism	Morphotype			Gram		Coccobacille		Cocci		Cocci chain		Cocci cluster		Coagulase	
			Valeur	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Gram negative bacilli	Enterobacterales	Escherichia coli	Bacille	Maj	0.98	Moy	0.015	Moy	0.05	NC	0	NC	0	NC	0	NC	0
Gram negative bacilli	Enterobacterales	Klebsiella spp or/ou Enterobacter spp or/ou Serratia spp or/ou Citrobacter spp	Bacille	Maj	0.98	Moy	0.015	Moy	0.05	NC	0	NC	0	NC	0	NC	0
Gram negative bacilli	Non fermenting Gram negative bacilli	Pseudomonas spp	Bacille	Maj	0.98	Maj	0.035	Moy	0.1	NC	0	NC	0	NC	0	NC	0
Gram positive cocci	Micrococcus spp or/ou Staphylococcus spp	Staphylococcus aureus	Cocci	Maj	0.96	Maj	0.99	Moy	0.01	Moy	0.33	Moy	0.1	Moy	0.56	Maj	0.88
Gram positive cocci	Micrococcus spp or/ou Staphylococcus spp	Staphylococcus coagulase negative or/ou Micrococcus spp	Cocci	Maj	0.96	Maj	0.99	Moy	0.01	Moy	0.33	Moy	0.1	Moy	0.56	Maj	0.08
Gram negative bacilli	Enterobacterales	Salmonella spp	Bacille	Maj	0.98	Moy	0.015	Moy	0.05	NC	0	NC	0	NC	0	NC	0
Gram negative bacilli	Non fermenting Gram negative bacilli	Stenotrophomonas spp	Bacille	Maj	0.98	Maj	0.035	Moy	0.1	NC	0	NC	0	NC	0	NC	0
Gram negative bacilli	Enterobacterales	Proteus spp or/ou Morganella spp or/ou Providencia spp	Bacille	Maj	0.98	Moy	0.015	Moy	0.05	NC	0	NC	0	NC	0	NC	0
Gram positive bacilli	Gram positive bacilli	Gram positive bacilli	Bacille	Maj	0.98	Moy	0.835	Maj	0.17	NC	0	NC	0	NC	0	NC	0
Gram positive cocci	Streptococcus spp or/ou Enterococcus spp	Enterococcus spp	Cocci	Maj	0.96	Maj	0.985	Moy	0.03	Moy	0.48	Moy	0.45	Moy	0.04	Maj	0.1
Gram positive cocci	Streptococcus spp or/ou Enterococcus spp	Streptococcus spp	Cocci	Maj	0.96	Maj	0.985	Moy	0.03	Moy	0.48	Moy	0.45	Moy	0.04	Maj	0.01
Gram negative bacilli	Haemophilus spp	Haemophilus spp	Bacille	Maj	0.98	Maj	0.01	Maj	0.35	NC	0	NC	0	NC	0	NC	0
Gram negative cocci	Neisseria spp or/ou Moraxella spp	Neisseria spp or/ou Moraxella spp	Cocci	Maj	0.96	Maj	0.01	Maj	0.01	Moy	0.32	Moy	0.66	Moy	0.01	NC	0
Yeast	Yeast	Yeast	Levure	Maj	0.67	Maj	0.835	NC	0	NC	0	NC	0	NC	0	NC	0
Polymorphic flora	Polymorphic flora	Polymorphic flora	Levure	Maj	0.7	Maj	0.4	NC	0	NC	0	NC	0	NC	0	NC	0
Gram positive bacilli	Gram positive bacilli	Listeria spp	Bacille	Maj	0.98	Moy	0.835	Maj	0.17	NC	0	NC	0	NC	0	NC	0
Gram negative bacilli	Non fermenting Gram negative bacilli	Acinetobacter spp	Bacille	Maj	0.98	Maj	0.035	Moy	0.1	NC	0	NC	0	NC	0	NC	0
Undefined morphotype	Undefined morphotype	Undefined morphotype	Levure	Maj	0.7	Maj	0.4	NC	0	NC	0	NC	0	NC	0	NC	0
Gram positive cocci	Streptococcus spp or/ou Enterococcus spp	Streptococcus spp (fastidious)	Cocci	Maj	0.96	Maj	0.985	Moy	0.03	Moy	0.48	Moy	0.45	Moy	0.04	Maj	0.01

Annex 11: Extract of plates reading ID NEG bench aids

BENCH AID

17

Médecins Sans Frontières
Mini-Lab



Plates reading ID NEG

This document is a summary of the various SOPs « 10.14 Plates reading ». Please read the SOPs before using this FM.

REAGENTS AND CONSUMABLES

- TDA reagent
- VP2 reagent
- IND reagent
- NIT1 reagent
- VP1 reagent
- NIT2 reagent

EQUIPMENT

- ARS
- Incubator
- Plate reader

Bacterial growth reading (cloudy) on black background / Chromogenic test reading (colour) on white background

	Correct control	Incorrect control
Well « C » Negative control	Clear no cloudiness present	Cloudy well
Well « G » Positive control	<ul style="list-style-type: none"> • White clouding throughout the well • White spot in the centre of the well • Fine grain growth 	Clear well



**Caution: Read the plate only if wells C and G are correct
=> see procedure if not**

01- Read the wells: GLU, SUC, SOR, RAF, RHA, ARA, INO, ADO, MEL, P4, K4, C14, Fd64, C18, To4 **02- Record the results => Reading sheet or ARS**

03- Check the criteria :

If the **oxidase is negative** and **OF/G, 64Fd** and **MAL** are positives



Or if any of these tests are positive: **GLU, SUC, SOR, LYS, ORN**



Or ARG and OF/G



Or ARG and CET



Continue reading.

If the criteria are not met, return the plate to the incubator **overnight**.
The next day continue reading (**do not reread the tests you have already read**).

Effective date : 10/11/2021

Version : V1

Code : FM-17-LECIDNEG

49

Annex 12: Extract of plates reading aids ID NEG bench aids

BENCH AID

18

Medicine Sans Frontières
Mini-Lab

ID/AST

Plates reading aids ID NEG

If in doubt, refer to the procedure « POS-10.14-LECID »

POSITIVE TESTS

GLU (b) Yellow	RAF Yellow to orange	INO Yellow to orange	URE Magenta to pink	LYS (c)	TDA (a) Any shade of brown	CIT Blue to blue-green any trace of blue = POS	TAR Blue to blue-green any trace of blue = POS	OF/G (d)
SUC Yellow to orange	RHA Yellow to orange	ADO Yellow to orange	H2S Black precipitate or blackening	ARG (e)	ESC Clear to brown to black	MAL Blue to blue-green any trace of blue = POS	ACE Blue to blue-green any trace of blue = POS	OF/B
SOR Yellow to orange	ARA Yellow to orange	MEL Yellow to orange	IND (b) Pink to red	ORN (c)	VP (a) Red	ONPG (a) Pale yellow	CET	DCB

NEGATIVE TESTS

GLU (b) Orange to red	RAF Orange to red	INO Orange to red	URE Yellow, orange, light pink	LYS (c)	TDA (a) Yellow to orange	CIT Green to yellow	TAR Green to yellow	OF/G Green to blue
SUC Orange to red	RHA Orange to red	ADO Orange to red	H2S No blackening	ARG (e)	ESC Beige to colorless	MAL Green to yellow	ACE Green to yellow	OF/B Green to blue
SOR Orange to red	ARA Orange to red	MEL Orange to red	IND (b) Pale yellow to orange	ORN (c)	VP (a) Colorless to pale pink	ONPG (a) Colorless	CET	DCB

Read on black background:
Cloudy if positive (growth)

(a) Add reagent before reading
(b) Golden yellow for some non-fermenting bacteria = NEG

(c) Compare LYS, ARG and ORN to DCB:
Purple is considered positive when compared with a grey DCB well. Grey is considered positive when compared with a yellow DCB well. For non-fermenters, a positive test should be significantly more purple than the baseline control. If the baseline is purple, the baseline medium has been alkalised and LYS, ARG and ORN should be considered negative

(d) Compare with OF/B:
OF/B green => POS if yellow / OF/B blue to green-blue => POS if yellow to green

(e) ONPG:
Compare any ONPG wells that appear clear with the control well. If the ONPG well looks yellow in comparison with the control well, record the result as positive.

Effective date : 10/11/2021

Version : V1

Code : FM-18-AIDIDNEG

Annex 13: Supplementary material of the InTray proof of concept

Table 60: table describing the formulation of the different InTray agar evaluated

InTray cassette	Agar media description	Formulation per 1 liter
Tryptic Soy Agar (TSA)	Soybean-Casein-Digest Agar Medium	Agar..... 15.0 g Pancreatic Digest of Casein..... 15.0 g Papaic Digest of Soybean..... 5.0 g Sodium Chloride..... 5.0 g Purified water..... 1 L
TSA w/ 5% blood	Preferred medium for haemolytic bacteria	Agar..... 15.0 g Pancreatic Digest of Casein..... 15.0 g Papaic Digest of Soybean..... 5.0 g Sodium Chloride..... 5.0 g Defibrinated Sheep's Blood..... 50 mL Purified water..... 1 L
Mueller Hinton (MH) Chocolate Agar	MH medium supplemented with 1% hemoglobin	Beef Extract Powder..... 2.0 g Acid Digest of Casein..... 17.5 g Starch..... 1.5 g Agar..... 17.0 g Dehydrated Hemoglobin..... 10.0 g IsoVitaleX Supplement..... 10 mL Purified water..... 1 L
Colorex Screen	Chromogenic medium	Agar..... 15.0 g Peptone and yeast extract..... 17.0 g Chromogenic mix..... 1.0 g Purified water..... 1 L

Table 61: table describing the formulation of reference agars used during the different studies

Biomerieux Petri Dish	Agar media description	Formulation per 1 liter
Blood Agar	Columbia agar + 5% sheep blood (COS)	Meat and casein peptone 10 g Hydrolyzed animal proteins 10 g Heart peptone..... 3 g Corn starch..... 1 g Sodium chloride..... 5 g Agar..... 13.5 g blood (sheep)..... 50 mL Purified water 1 L
Chocolate Agar	Chocolate agar PolyViteX (PVX)	Bovine casein peptone..... 7,5 g Meat peptone..... 7,5 g Starch..... 1 g Potassium phosphate..... 4 g Sodium chlorid..... 5 g Hemoglobin..... 10 g Agar..... 10 g PolyViteX..... 10 mL Purified water..... 1 L
UriSelect™ 4	Non selective chromogenic medium for the isolation, differentiation and enumeration of urinary tract pathogens	Peptones mix 21g Silica 20g Chromogenic mix <1g Tryptophan 1g Agar 16g

Annex 14: Supplementary materials of the InTray evaluation study

Table 62: percentage and number of subcultures done that show growth on InTray and BD chocolate agar, with comparison between CO2 incubator and normal incubator and at different times of incubation.

	Nr. of subcultures done	CO2 incubator						Ambient air incubator					
		% growth on InTray (nr. of grown subcultures)			% growth on BD (nr. of grown subcultures)			% growth on InTray (nr. of grown subcultures)			% growth on BD (nr. of grown subcultures)		
		16h	24h	48h	16h	24h	48h	16h	24h	48h	16h	24h	48h
Total													
<i>S. pyogenes</i>	58	83% (48)	83% (48)	83% (48)	100% (58)	100% (58)	100% (58)	93% (54)	93% (54)	93% (54)	100% (58)	100% (58)	100% (58)
<i>S. pneumoniae</i>	65	89% (58)	92% (60)	94% (61)	98% (64)	98% (64)	100% (65)	95% (62)	97% (63)	100% (65)	95% (62)	100% (65)	100% (65)
BacT/ALERT bottles													
<i>S. pyogenes</i>	30	80% (24)	80% (24)	80% (24)	100% (30)	100% (30)	100% (30)	90% (27)	90% (27)	90% (27)	100% (30)	100% (30)	100% (30)
<i>S. pneumoniae</i>	33	97% (32)	97% (32)	97% (32)	97% (32)	97% (32)	100% (33)	97% (32)	97% (32)	100% (33)	94% (31)	100% (33)	100% (33)
Autobio bottles													
<i>S. pyogenes</i>	28	86% (24)	86% (24)	86% (24)	100% (28)	100% (28)	100% (28)	96% (27)	96% (27)	96% (27)	100% (28)	100% (28)	100% (28)
<i>S. pneumoniae</i>	32	81% (26)	88% (28)	91% (29)	100% (32)	100% (32)	100% (32)	94% (30)	97% (31)	100% (32)	97% (31)	100% (32)	100% (32)

Table 63: percentage of strains growing consistently on InTray and BD chocolate agar (growth on all replicate subcultures), with comparison between CO2 incubator and normal incubator and at different times of incubation

	Nr. of subcultures done	CO2 incubator						Ambient air incubator					
		% consistent growth on InTray			% consistent growth on BD			% consistent growth on InTray			% consistent growth on BD		
		16h	24h	48h	16h	24h	48h	16h	24h	48h	16h	24h	48h
Total													
<i>S. pyogenes</i>	10	80%	80%	80%	100%	100%	100%	90%	90%	90%	100%	100%	100%
<i>S. pneumoniae</i>	11	55%	73%	82%	91%	91%	100%	82%	82%	100%	82%	100%	100%
BacT/ALERT bottles													
<i>S. pyogenes</i>	10	80%	80%	80%	100%	100%	100%	90%	90%	90%	100%	100%	100%
<i>S. pneumoniae</i>	11	91%	91%	91%	91%	91%	100%	91%	91%	100%	91%	100%	100%
Autobio bottles													
<i>S. pyogenes</i>	10	80%	80%	80%	100%	100%	100%	90%	90%	90%	100%	100%	100%
<i>S. pneumoniae</i>	11	64%	82%	91%	100%	100%	100%	91%	91%	100%	91%	100%	100%

Annex 15: MSF prescription criterias for blood culture collection

Annex 2 : MSF guidelines for blood culture - 2021

Introduction and Scope

This document provides guidance for staff who are responsible for ordering laboratory tests for patients. In some contexts, this will predominantly be MDs, in others this responsibility could be delegated to other staff such as clinical officers and some nursing staff. It is the responsibility of the project/mission to ensure relevant staff understand and follow these guidelines.

Background

Blood stream infections (BSI) are common and cause significant morbidity and mortality. Typically, patients present with clinical signs of significant or overwhelming infection, but in some groups, in particular children and neonates, signs of sepsis may be less clear. Currently the only way to diagnose BSIs is by taking blood for blood cultures which are processed by an MSF validated laboratory capable of culture, Gram stain and sensitivity testing. Initial Gram stain results should be provided as soon as they are available and can be helpful in indicating likely pathogen. Blood culture results can be very helpful in both understanding the local ecology and in directing specific antibiotic therapy.

Culture and sensitivity testing is resource intensive and in order to ensure the best use of the laboratory services the following guidance has been developed. Blood culture results from a facility or district can be collated over time to provide cumulative surveillance information which helps inform best practice for empiric antibiotics.

Recommendation	Explanation
<p>1 Take blood cultures before antibiotics are given where possible. Blood cultures can be taken if on antibiotics and there are signs of sepsis.</p>	<p>Taking blood cultures after antibiotics have been given will significantly reduce the chance of a positive culture. For patients already on antibiotics, sepsis may indicate ongoing blood stream infection despite antibiotics.</p>
<p>2 If a patient is failing treatment (ongoing symptoms, new fever or hemodynamic instability after 48-72 hours of empiric treatment, consider taking or repeating blood cultures even if the patient is on antibiotics.</p>	<p>If a patient is failing treatment, bacteremia may be present despite antibiotics. Antibiotics may need to be changed and BCs should be done prior to new antibiotics being started.</p>
<p>3 Do not delay commencing antibiotics in severely ill patients.</p>	<p>For patients with sepsis or septic shock, for every hour delay in antibiotics, mortality increases. Patients who are severely ill with sepsis need immediate intravenous access, ideally blood cultures taken, then antibiotics given within one hour of presentation or diagnosis of sepsis.</p>
<p>4 Blood cultures must be taken using aseptic technique. Do not take blood cultures from existing intravenous lines. For details on technique, skin disinfection and sampling see here: Nursing Manual of Procedures - how to collect blood cultures</p>	<p>Blood culture contamination is usually as a result of not adhering to aseptic technique. Blood culture contamination requires laboratory resources, provides a result which may not be helpful and potentially delays a diagnosis. In high income countries the acceptable rate of blood culture contamination is less than 3%.</p>
<p>5 Use the correct sized blood culture bottles and fill them to the recommended level. Adult, paediatric and neonatal bottles are available.</p>	<p>Blood culture bottles are designed to maximise a positive result but rely on using the right bottles and filling them adequately. If bottles are underfilled this reduces the chance of a positive result.</p>
<p>• Ensure the blood culture is reported in a timely fashion (a preliminary result within 48 hours) and this and the final result documented in the medical records</p>	<p>Blood cultures should be processed and reported, including Gram stain in a timely fashion because effective antimicrobial therapy critically influences the outcomes of patients with sepsis. Any procedure or significant test needs to be documented in the medical records. If a result is telephoned, document the time and date of the call, the result and the name of the person who received the call.</p>

When should blood cultures be performed?

Blood cultures are designed to identify pathogenic microorganisms in blood. In many cases these infections will be associated with signs of sepsis or septic shock, but BSI may also be associated with focal infections such as pneumonia, meningitis or skin infection. Please see below for the clinical settings in which blood cultures should be performed⁶

Adults Presenting to the Emergency Room

Signs of Sepsis with/without focal signs (e.g., pneumonia, meningitis)

Fever (axillary T° ≥ 38°C) OR Hypothermia (axillary T° ≤ 36°C) AND at least one of the following signs

Hypotension (systolic blood pressure =< 100 mmHg)

Confusion or altered conscious level (Glasgow coma scale < 15)

Increased respiratory rate (>= 22 per minute)

OR suspicion of other severe infection e.g. Severe malaria, typhoid

Adults in hospital for > 48 hours.

On treatment with antibiotics and are failing treatment (have signs of sepsis⁷ or septic shock)

New onset of sepsis whether on antibiotics or an alternate diagnosis has already been made

Children – either on presentation or while an inpatient

Sepsis presentations in children may be similar to those of adults but may include poor feeding and irritability. Optimising blood cultures are covered here⁸.

Do blood cultures in children with the following.

Fever (axillary T° ≥ 38°C) OR history of fever (last 48 h) OR hypothermia (axillary T° ≤ 36°C)

OR at least one of the following signs of sepsis

Signs of circulatory failure (poor pulse volume, severe tachycardia or bradycardia, capillary refill time >3 seconds) or poor urine output)

Confusion, irritability or drowsiness

Severe respiratory distress or O2 sat ≤ 92%

Non-blanching rash or petechiae

OR

Suspicion of severe localised infection (e.g. pneumonia, meningitis, intrabdominal infection, osteomyelitis)

OR

Suspicion of other severe infection (e.g. malaria, typhoid)

OR (if an inpatient)

New onset signs of sepsis⁹ or septic shock, or no improvement if already on antibiotics

⁶ Ombelet S, Barbé B, Affolabi D, Ronat J-B, Lompo P, Lunguya O, Jacobs J and Hardy L (2019) Best Practices of Blood Cultures in Low and Middle-Income Countries. *Front. Med.* 6:131. doi: 10.3389/fmed.2019.00131

⁷ Hypotension, tachycardia, altered conscious level, hypoxia and/or fever

⁸ Buttery JP. Blood cultures in newborns and children: optimising an everyday test. *Arch Dis Child Fetal Neonatal Ed* 2002;87:F25-F28

⁹ Hypotension, tachycardia, altered conscious level, hypoxia and/or fever

Neonates

Sepsis in neonates is usually accompanied by non-specific signs. Blood cultures are recommended for all unwell neonates and should be considered in any neonate who has significant maternal risk factors for infection¹⁰.

Some of the signs of sepsis in neonates are here:

Fever (axillary T $\geq 37.5^{\circ}\text{C}$) OR history of fever (in the past 48H) OR hypothermia (axillary T ≤ 35.5).

Shock - Signs of circulatory failure (poor pulse volume, severe tachycardia or bradycardia, capillary refill time >3 seconds) or poor urine output)

drowsiness, irritability or loss of consciousness

seizures including subtle or abnormal movements

cyanosis, apnoea, severe respiratory distress or Saturations $< 90\%$

more than one episode of hypoglycaemia (BGL $< 45\text{mg/dL}$ or 2.5mmol/L)

OR

Neonates with suspicion of severe localised infection (e.g. meningitis, pneumonia, meconium aspiration syndrome, necrotising enterocolitis, omphalitis, neonatal tetanus or any other severe localised infection)

OR (if an inpatient)

New onset signs of sepsis¹¹ or septic shock, or no improvement if already on antibiotics

¹⁰ Neonatal Care: Clinical and Therapeutic Guidelines. MSF 2018 internal document.

¹¹ Hypotension, tachycardia, altered conscious level, hypoxia and/or fever

Annex 16: Proposal of grouping of bacterial species according to clinical and infection control relevance [32]

Table 64: Proposal of grouping for Gram negative bacteria: enteric bacteria

Bacterial group	Examples of species in group	Clinical relevance	Infection control relevance/Notes
<i>Enterobacter/Citrobacter/ Serratia</i>	<i>Citrobacter (freundii, koseri, braakii...)</i> <i>Cronobacter (sakazakii, malonaticus)</i> <i>Enterobacter (cloacae, aerogenes...)</i> <i>Hafnia alvei</i> <i>Serratia (marcescens, liquefaciens) *</i>	Pneumonia Post-operative site infections Urinary tract infections Wound colonization/infections	Mostly healthcare-associated Multidrug resistant
<i>Klebsiella</i>	<i>Klebsiella (pneumoniae, oxytoca...)</i> <i>Raoultella spp.</i>	Bloodstream infections Pneumonia Pyogenic infections (liver abscess) Urinary tract infections Wound colonization/infection	Mostly healthcare-associated Multidrug resistant Hospital outbreaks (neonatal care) Hypervirulent community-associated strains (pyogenic infections)
<i>Escherichia coli</i>	<i>Escherichia coli</i>	Bloodstream infections Enteritis [◇] Urinary tract infections	Mostly community-associated
<i>Salmonella</i> Typhi <i>Salmonella</i> Paratyphi A	<i>Salmonella</i> Typhi <i>Salmonella</i> Paratyphi A*	Enteric fever	Note: <i>Salmonella</i> Paratyphi A very rare in Africa
Non-Typhoidal <i>Salmonella</i> spp.	Non-Typhoidal <i>Salmonella</i>	Bloodstream infections (< 5 years old – malaria and malnutrition; HIV-infection)	Community-associated Hospital outbreaks
<i>Proteus/Providencia/Morganella</i>	<i>Morganella morganii</i> <i>Proteus (mirabilis, vulgaris, ...)</i> <i>Providencia spp.</i>	Post-operative site infections Urinary tract infections	Healthcare-associated
<i>Shigella</i> spp.	<i>Shigella (boydii, dysenteriae, flexneri...)</i>	Dysentery Enteritis Hemolytic-Uremic Syndrome (HUS) [◇]	Community-associated Note 1: rarely isolated from other specimens than stool Note 2: <i>S. dysenteriae</i> serotype 1 has public health relevance (epidemics)

* “nice to have”: if technically feasible and affordable, separate identification of this species is desirable

◇ In case of hemolytic-uremic syndrome, the strain of *E. coli* or *Shigella* should be sent to a referral laboratory for further testing

Table 65: Proposal of grouping for Gram negative bacteria: others

Bacterial group	Examples of species in group	Clinical relevance	Infection control relevance/Notes
<i>Aeromonas/Vibrio/ Plesiomonas</i>	<i>Plesiomonas shigelloides</i> <i>Aeromonas</i> spp. <i>Vibrio</i> non-cholerae	Gastroenteritis Wound infections	Water-related source Community-associated
Non-fermenting Gram-negative bacilli	<i>Pseudomonas aeruginosa</i> <i>Burkholderia cepacia complex</i> <i>Stenotrophomonas maltophilia</i> * <i>Acinetobacter</i> spp.	Bloodstream infections Pneumonia (ventilation) Urinary tract infections Wound infections	Healthcare-associated Immunocompromised patient Bacteria present in environment (soil, water) Frequently multi-drug resistant Hospital-related outbreaks
<i>Burkholderia pseudomallei</i>	<i>Burkholderia pseudomallei</i>	Melioidosis bloodstream infection, abscesses, ...)	Community-associated potential aerosol production <u>Note:</u> BSL-3 pathogen Very rare outside South-East Asia
<i>Brucella</i>	<i>Brucella</i> spp.	Brucellosis	Community-associated <u>Note:</u> BSL-3 pathogen
<i>Haemophilus</i> spp.	<i>Haemophilus influenzae</i>	Bloodstream infection Meningitis	Community-associated
<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i>	Bloodstream infection Meningitis	Community-associated Potential for epidemics <u>Note:</u> BSL-3 pathogen

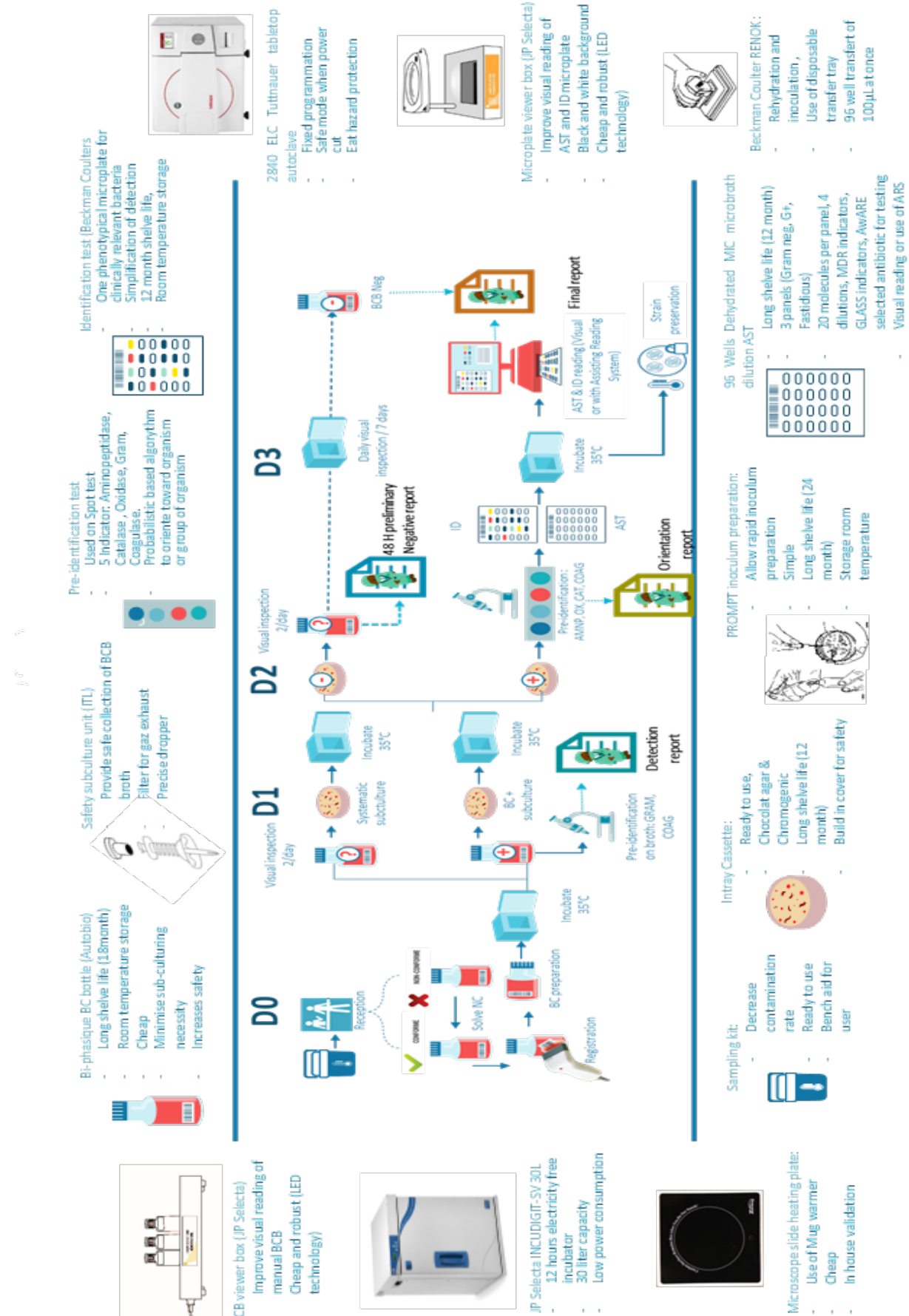
Table 66: Proposal of grouping for Gram positive bacteria: streptococci

Bacterial group	Examples of species in group	Clinical relevance	Infection control relevance/Notes
Large colony hemolytic Streptococci	<i>Streptococcus pyogenes</i> <i>Streptococcus agalactiae</i> <i>Streptococcus equi</i> <i>Streptococcus dysgalactiae</i>	Bloodstream infections Neonatal sepsis (<i>S. agalactiae</i>) Skin and soft tissue infections Urinary tract infections	Mostly community-associated <u>Note:</u> <i>S. agalactiae</i> = group B hemolytic streptococcus less frequent a cause of neonatal sepsis in tropical settings compared to Europe/U.S
<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	Bloodstream infections Meningitis Otitis media Pneumonia	Mostly community-associated
Anginosus group	<i>Streptococcus anginosus</i> <i>Streptococcus constellatus</i> <i>Streptococcus intermedius</i>	Deep tissue abscesses Pleuro-pulmonary infections Soft-tissue infections	Community-associated <u>Note:</u> previously “ <i>Streptococcus milleri</i> ” group
Viridans group	<i>Streptococcus oralis</i> <i>Streptococcus mitis</i> <i>Streptococcus sanguinis</i> <i>Streptococcus bovis</i> * <i>Streptococcus suis</i> *	Mostly contaminants (oral flora) Bloodstream infection Endocarditis Meningitis (<i>S. suis</i>)	Community-associated <u>Note:</u> <ul style="list-style-type: none"> <i>S. suis</i> very rare outside (South-East) Asia <i>S. bovis</i> bloodstream infection is associated with colonic cancer; overall rare finding
<i>Enterococcus spp.</i>	<i>Enterococcus faecalis</i> <i>Enterococcus faecium</i>	Bloodstream infection Endocarditis Urinary tract infections Wound infections	Frequently healthcare-associated Outbreaks of vancomycin-resistant enterococci

Table 67: Proposal of grouping for Gram positive bacteria: others

Bacterial group	Examples of species in group	Clinical relevance	Infection control relevance/Notes
<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	Bloodstream infections Endocarditis Intravascular device infections Severe pneumonia Skin and soft tissue infections	Community-associated Methicillin-resistant <i>S. aureus</i> (MRSA) is a healthcare-associated pathogen, can cause hospital outbreaks
Coagulase negative staphylococci	<i>Staphylococcus epidermidis</i> <i>Staphylococcus homini</i> <i>Staphylococcus warneri</i> ...	Contaminant Foreign body infection Intravascular device related infections	Healthcare-associated
<i>Bacillus</i> (non-anthrax)	<i>Bacillus cereus</i>	Contaminant Wound infection (in tropical settings)	
<i>Corynebacterium diphtheriae</i> (non-)	<i>Corynebacterium jeikeum</i>	Contaminant Intravascular-device related infections	Healthcare-associated
<i>Listeria</i>	<i>Listeria monocytogenes</i>	Bloodstream infection Meningitis Neonatal sepsis (rare in tropical settings) Pregnancy (miscarriage)	Community-associated Note: most at risk are immunocompromised patients and pregnant women

Annex 17: General schematic overview of the main Mini-Lab components developed or adapted

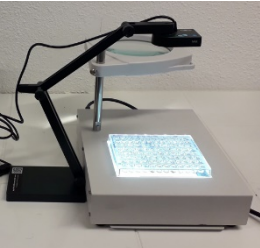


Annex 18: Comparison of the Mini-Lab final specifications with the initial target product profile

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
INTENDED USE			
Target disease	Bloodstream infection, osteomyelitis ⁷	Bloodstream infection	Bloodstream infection, Urine tract infections
Technical and strategy purpose	<ul style="list-style-type: none"> It aims to perform Bacterial identification and antibiotic susceptibility testing focusing on a minimal but clinically relevant level of identification and susceptibility testing tailored to the required and available antibiotics in the field. This concept must follow a modular approach in order to permit usage flexibility by field workers /team targeting three different strategies such as for patient care, surveillance and for operational field studies: Patient care: Support the diagnostic of above-mentioned disease and help target / adapt antibiotic therapy Surveillance: Support clinical based surveillance of antibiotic resistance pattern on the field to capture relevant data to permit the update of empirical treatments guidelines. Operational studies: Support field studies on diagnostic performance and on Infection, prevention strategies 		<ul style="list-style-type: none"> Allow to identify bacteria causing infections (urinary tract infection and sepsis) and determine their antibiotic susceptibility patterns “All in one kit” with 6 modules, transportable, fully equipped and modular Detect hospital outbreaks and support improvement of infection prevention & control strategy Provide epidemiology indicators for local AMR surveillance based on GLASS priority pathogen list and the possibility to calculate a hospital based antibiogram (pattern of resistance / molecule /bug / site) to adapt locally empiric therapeutic
Target population	<ul style="list-style-type: none"> Any hospitalised patient with suspected sepsis, immunocompromised or not. Hospitalised patients with infections related to war, trauma or prior surgery. 	Hospitalised patients: Malnourished children, neonates, malaria co-infected children, severely ill children, patients with burn wounds with suspected sepsis, patients living with HIV and admitted with fever.	<ul style="list-style-type: none"> Any hospitalised patient with suspected sepsis, urinary tract infections, immunocompromised or not.
Target use setting	MSF supported second level of care structure with or without laboratory facilities on site		MSF supported second level of care structure with or without laboratory facilities on site
Identification target pathogens	<ul style="list-style-type: none"> Minimum and most clinically relevant bacteria causing bloodstream infection and osteomyelitis in LRS with minimal identification to the genus or the family if of clinical relevance and growing in standard broth (To Exclude 		<ul style="list-style-type: none"> A customised panel for MSF, called “MSF Neg/Pos ID Panel Type 2” (Beckman Coulter, US, ref C38213) was specially designed using manufacturer MicroScan technology, combining the commercially available identification

⁷ Any bacterial infections, outside of extrapulmonary Tuberculosis, that could be diagnosed through collection of sterile body fluids might be done using Mini-lab as well.

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
	<p>Bartonella, Leptospira, etc). To adapt quantity of ID System to geographical epidemiology</p> <ul style="list-style-type: none"> • Possibility to have a sub-list of pathogens depending on the geographical zone/site (core pathogens + site depending pathogens). The system should permit the storage and conservation of strains as well for a minimum period of 6 month and safe shipment to referral laboratory in the country or overseas to perform further investigation, confirmation, or quality control. 		<p>panels of Gram-negative and Gram-positive organisms on one single microplate [94].</p> <ul style="list-style-type: none"> • The list of all identifiable bacteria can be found in Annex 6 • The combination of the MSF Neg/ Pos ID panel Type 2 and the Pastorex meningitis provide a performance agreement of 97% at genus level.
Antibiotic List to be tested	<ul style="list-style-type: none"> • Reflect the MSF-WHO essential drug list including and adapted to local spectrum of pathogens and antibiotic resistance patterns list with "must have" and "nice to have" including last resources ATB. • Proxy indicator of other resistance. List must be adapted according to the above strategy (patient care, surveillance, etc.). • The system should permit the storage and conservation of strains as well for a minimum period of 6 months and safe shipment to referral laboratories in the country or overseas to perform further investigation, confirmation, or quality control 		<ul style="list-style-type: none"> • The selection of antibiotics was based on (i) the list of antibiotics available as CE-IVD from Beckman Coulter, (ii) the list of antibiotics used in MSF facilities, and (iii) the WHO's essential drug lists. Those pan-els were tailored to the needs of the patients, the local epidemiology, and expected antibi-otic resistance (ABR) patterns (See annex 7). • Special attention was given to commonly-used antibiotics, antibiotics of last resort, and proxy indicators of resistance mechanisms as per GLASS requirements [112]and AWaRe classifications [176]. Drug dilutions were chosen to match both CLSI and EUCAST breakpoints in 2019 [115], [177] and were embedded by Beckman Coulter on the MicroScan panels; antibiotics abbreviations are defined as per EUCAST recommendations [178]
Diagnostic Performances	<ul style="list-style-type: none"> • Yield of pathogens must be comparable with current state of the art, "blood culture "system (50 to 60% Se if enough blood volume sampled and culture) [81] • System to allow good quality indicators (fewer than 3 % contamination rate and between 10 – 15% positivity (pathogen) rate > 80% appropriate filling rate of blood cultures bottles. Antimicrobial susceptibility test (AST) devices that are used to determine minimum inhibitory concentrations (MIC) and/or interpretive category determinations of susceptible, intermediate and resistant should note permit "very major" errors to occur [82] and should follow FDA guidance on discrepancies agreements[82] or ISO 20776-2:2007[83]. 		<ul style="list-style-type: none"> • Yield of pathogens is be comparable with current state of the art, "blood culture "system (50 to 60% Se if enough blood volume sampled and culture) [81] Overall yield: 95.9%[97]. • Contamination rates is dependent on the adherence of nurses to good sample collection practices and positivity rate depend on the adherence to the clinician to the adherence to the collection criteria's[68]. Evaluation in Carnot shown a positivity rate of 12.5%. Contamination rate during sample collection = 15.7% (151/960)* • All MIC Microscan panel provide Categorical agreement: > 90% with VMJ <3% for molecule of clinical use*. • Lyophilized MIC AST micro-broth dilution systems provide first-rate information, such as MIC, which can be read manually or with an automatic reader, and produces high-reproducibility and standardized results thanks to its pre-prepared panels[101].
Clinical Specificity	Same as the conventional culture		Same as the conventional culture
Type of Analysis	Analysis based on existing growth-based methods and non-culture-based tests available on the market, with adaptations and improvements when appropriate		The Mini-Lab focuses on diagnostics of bloodstream infections and urine tract infection using manual (i.e. automatic equipment-free) blood cultures and providing

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
	and feasible. Manual system is preferred – but “objective” growth indicator needed (agar paddle, agar slant). Screw cap and wide mouth preferable		preliminary results by pathogen group classification, followed by full pathogen identification by combined biochemical testing and AST by micro-broth dilution method both read manually or with the support of an Assisted Reading System. A Laboratory Information and Management System composed of experts systems allow to interpret results and warn technician in case of errors.
Reading system	Visual & semi-automated, with dedicated equipment	Visual	Visual & semi-automated reading of ID and AST plate using an assisting Reading system (ARS) composed of a Microplate viewer box and a camera 
Throughput	30 new tests/days; pick loads of 200 max per 5 days.	10 new tests / days; pick loads of 75 max per 5 days.	Standard Mini-Lab kit design for an average of 10 new tests/days, possibility of pick load up to 30 new bottles per days for a short period (less than 3 months)
Sample type	Blood and bone / Tissues / Pus-aspirate	Blood	Blood, Urine, CSF
TEST PROCEDURE			
Number of steps	< 6	<10	8 steps: Sampling, registration, preparation, reading, sub-culturing, Pre-ID, ID, AST
Biosafety	No need for biosafety cabinet at any step of performing the laboratory tests. No Bunsen burner, no gas required	If a biosafety cabinet or other measures are needed, the materials need to fit the below described specifications.	No need of biosafety cabinet, biological risk is mitigated by work organization measures, by the choice of techniques (sealed transfer system from identified hazardous steps), upstream equipment, by wearing of PPE in adequation of the risk, by the information and training of staff as well as by the maintenance /cleaning of premises and equipment and good waste management.
Time to result	<ul style="list-style-type: none"> Total incubation time not longer than 5 days. 85% must turn “growth positive” < 48 h and AST results should be available 24 hours after “growth positive” 		<ul style="list-style-type: none"> Total incubation time of 7 days Positivity at D1: 90.7%, Positivity at D2: 100%[97] Median time interval from sampling to start of incubation, (n=234), 24 min (IQR 14; 45)* Median time to positivity for blood cultures with pathogens, (n=122), 1 day (IQR= 0; 1; min = 0, max = 5)*. Median turnaround time for blood cultures with pathogens from sample recordings in the laboratory to final report transmission to the clinician (n=101), 2 days (IQR 2; 4, min = 1, max = 9)*.

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
Volume and type of samples required	<ul style="list-style-type: none"> Should be adequate volume regarding field, patient, and technics constraints in a manner that it does not affect much the diagnostic performance From 1ml to 10 ml of whole blood sampled by phlebotomy. Disinfection and sampling procedures / materials should allow sampling of blood in a way to limit skin contamination of samples and to facilitate the collection of the required volume of blood. Should permit sample collection of patients under antibiotic treatment. Sample kit should include all materials for sample taking (antiseptic pads, needles, gloves, sterile pads, etc.) 	<ul style="list-style-type: none"> < 10 ml of whole blood Disinfection and sampling procedures / materials should allow sampling of blood in a way to limit skin contamination of samples and to facilitate the collection of the required volume of blood. 	<p>As shown by Ombelet et al during the laboratory validation, the Biphasic Autobio bottles can accommodate up to 10 ml of blood[97].</p> <ul style="list-style-type: none"> The protocol used by MSF for skin disinfection recommend double disinfection with first the use of alcohol wipe and a second disinfection with Chlorhexidine gluconate alcohol wipe as advise by Ombelet et al in a recent review on good blood culture sampling practices[68]. However, the Biphasic Autobio bottles does not contain resin beads that allow the neutralisation of antibiotic A Mini-Lab sampling kit, composed of regular phlebotomy materials, The sample collection kit comes in a carrier case. The case is organised into separate compartments, which may or may not be detachable; (See Annex 3) This kit includes all the material required to disinfect the skin before and after collecting the sample and for injecting the blood in the blood culture bottle; The collection is via syringe and needle, the Autobio bottle does not allow vacutainer like collection
Sample transport and Sample preparation	<ul style="list-style-type: none"> Blood culture system needs to withstand environmental temperature and delay pending incubation at 35°C. Should withstand a delay of 4 hours to incubation. No Preparation Requirement 	<ul style="list-style-type: none"> Blood culture system needs to withstand environmental temperature and delay pending incubation at 35°C. Target fewer than 4 hours at a temperature between 20°C and 35°C No preparation requirement 	<ul style="list-style-type: none"> Prolonged time from collection to incubation have not been tested, however according to a recent multi centric study done by Ling et al [179] on the impact of delays to incubation and storage temperature on blood culture, no significant loss in yield when blood cultures were stored < 24 h at 25 °C, however, storage for 24 h at 40 °C decreased yields and longer storage times increased times to detection. As it is a biphasic media with the agar media within the bottle, blood culture bottles upon arrival need to lay down for 15 min to allow broth containing blood to flood the agar.
Internal Control	<ul style="list-style-type: none"> All necessary internal quality control strains/material should be available within the kit. It usage, storage and interpretation should be made as easy as possible for non-expert but trained personnel. The minimum of control strains should be aimed and validated. 		<ul style="list-style-type: none"> Three kits of ATCC strains where developed with Microbiologics, Inc (St. Cloud, Minnesota, USA) based on the KWIK-STIK™ technology. The first two kits are composed of 12 ATCC strains advised by EUCAST and the third kit is composed of few others ATCC strains for training purposes (contaminant mainly). All QC strains have been validated with 20 repetitions on each of the reagent to calculate the standard deviations for the quantitative methods (MIC) and standard growth behaviour.

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
			<ul style="list-style-type: none"> All information's made available by strain on a QC Strain Identification sheet (see 8.1.1 QC Strain Identification Sheet EUCAST V11-DOC-V2.2 or Annex 5) and in the database of the QC follow up tool. A SOP (8.1 Internal Quality Control-SOP-V1.1) describe all the steps and the QC program and a resolution plan is available to support the technician in the root cause analysis if any discrepancy arise(8.3 Resolution Plan-DOC-V1.1). An excel based tool is available for QC follow-up, user enter results, the tool warn the user if the value is out of the defined range and provide QC follow up indicators.
OPERATIONAL CHARACTERISTICS			
Operating conditions	<ul style="list-style-type: none"> 15 – 40°C 25-90% RH 	<ul style="list-style-type: none"> 15 – 35°C 25-80% RH 	<ul style="list-style-type: none"> Performance of the laboratory have been tested in tropical environment but the laboratory was equipped with air conditioning system for staff comfort.
Shelve life (stability)	<ul style="list-style-type: none"> Upon arrival on the site in the country the product should be stable 18 months Reagents / consumables should withstand environmental temperatures below -10 °C and 40°C for a period of at least 48 hours. 	<ul style="list-style-type: none"> Upon arrival on the site in the country, the product should be stable 12 months Reagents / consumables should withstand environmental temperatures below -10 °C and 40°C for a period of at least 24 hours. 	<ul style="list-style-type: none"> Mini-Lab starter kit was designed to accommodate 800 BCB samples (given 15% positivity rate and 10 % contamination rates). It is composed of 110 items, of which 45 are consumables, 40 are reagents and test, 13 are administrative furniture's and 12 are small laboratory equipment's. Average shelve life of reagent is of 18 month. Among the reagents, 45% (18/40) have a shelve life > 18month, 50% (20/40) have a shelve life of 12 months and only 5% (2/40) have a shelve life of 6 months.
Storage conditions	Can be stored between 2°C and 40°C. A maximum of 45 L (Net storage capacity) volume of reagents/consumables for one week of activity may require 2 - 8°C storage.	Can be stored between 2°C to 30°C. A maximum of 108 L (Net storage capacity) volume of reagents/consumables for one week of activity may require 2 - 8°C storage.	<ul style="list-style-type: none"> Among the reagents 50%(20/40) can be stored between 2°C to 30°C, 33% (13/40) between 2°C to 8°C and 18% (7/40) have no temperature restrictions. Pharmacy storage space required to accommodate the starter kit: <ul style="list-style-type: none"> Controlled temperature 1.5 m3 Cold chain 0.3m3 = 2 refrigerators (Vestfrost VLS404A AC) 145l net, 230V (PCOLREFEVI4) only for Mini-Lab items. Dangerous goods: 0.016 m3
In use stability (under tropical conditions)	Minimum of 4 weeks below 40 °C if pouch sealed (ziplock) after use.	Minimum of 4 weeks at 2 to 8 °C if pouch sealed (ziplock) after use.	<ul style="list-style-type: none"> Once opened the Pastorex meningitis, kit have a stability of 4 weeks at 2 to 8°C Coagulase test are small vials of 2 ml of dehydrated rabbit plasma to aliquot and preserved at -20°C for a period of 1 months VP 2 reagent; 15 days shelve life once reconstituted at room temperature.


KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
Reagents reconstitution	<ul style="list-style-type: none"> All reagents and media ready for use except for lyophilised / dehydrated reagents/consumables (in that case appropriate volumes and easy transfer required). This step should not have biosafety requirements such as a biosafety cabinet or hood. 	<ul style="list-style-type: none"> Reconstitution acceptable if very simple to do. All reconstitution fluids (including water) already provided in the kit. 	<ul style="list-style-type: none"> All reagent but except Rabbit plasma for coagulase test are ready to use Coagulase test are small vials of 2 ml of dehydrated rabbit plasma to aliquot and preserved at -20°C InTray Cassettes need to be prepared one hour I advance to remove the moisture present at the surface of the agar. However this step does not necessitate biosafety cabinet but just the use of aseptic pipette and an incubator to dry it out.
End user profile	<p>Trained laboratory technician in health care facilities:</p> <ul style="list-style-type: none"> Personnel using the Mini-Lab should have at least diploma in laboratory technician science and should be able to read and understand the technical language of the Mini-Lab either English/French/Arabic 		All the laboratory technician that have worked in Haiti or in Carnot for the first and the second field pilot where all having a standard diploma in laboratory technician sciences
Biosafety requirement	<p>Biosafety requirement not higher than level 2:</p> <ul style="list-style-type: none"> Biosafety requirements should take in consideration till risk 3 class pathogens. All material, reagent should comprise safely practice or should mitigate the risk of contamination to the technician. Appropriate containment levels (anti splash practices, personal protective equipment) to be provided in case of (suspected) pathogens such as <i>Salmonella</i> Typhi, <i>Brucella</i> spp., <i>Shigella dysenteriae</i> and <i>Burkholderia pseudomallei</i>. 		<ul style="list-style-type: none"> Biological risk is mitigated by work organization measures, by the choice of techniques (sealed transfer system from identified hazardous steps), upstream equipment, by wearing of PPE in adequation of the risk, by the information and training of staff as well as by the maintenance /cleaning of premises and equipment and good waste management. Spillage containment measure are available with spillage kits, googles, mask and laboratory technicians are train on the use. Ergonomic risk of accident and risk of spillage is reduced through identification of specific furniture's and collective protective equipment's (rack to transport slides, removal of sharp edge of equipment, etc.). Specific module of 1 day on site training and a e-learning have been developed on Hygiene, Security and Safety, with case studies, simulation of the used of safety equipment's (extinguisher, spillage kit, etc.).
Training / Communication	<ul style="list-style-type: none"> All training materials must be provided to allow training by an experienced staff (laboratory technician / microbiologist/ MD) as well as by self-study. Training material should consist of easy understandable standard operating procedures, video, bench aids/job aids and should be easily accessible on soft and hard version. <p>Laboratory personal training aim: Being able to use properly the Mini-Lab and provide results according to specifications set</p> <ul style="list-style-type: none"> Laboratory personal should have a minimum of Laboratory certificate diploma level after high school (1-2 year diploma). No experience in bacteriology required 		<ul style="list-style-type: none"> Specific onsite training has been developed for the lab technicians, the lab supervisor, practitioners and nurses collecting blood on the Mini-Lab procedures (from test prescriptions to test results), with theoretical, interactive and practical modules and teacher guides to help the microbiologist facilitate the onsite training. A e-learning version of the training for the laboratory technician has been developed for continuous education purpose. Become a Mini-Lab Technicians (pBMLT) training, 135 hours, 20 days. Target Laboratory technicians and supervisors recruited for set-up. Learning objectives: To enable learners to give quality results to clinicians, to use the

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
	<ul style="list-style-type: none"> Minimum training should consist of 5 days of theoretical training and 5 days of practical exercise. Mentorship of one month minimum from an experienced laboratory staff with minimum supervision visit every 6-9 month <p>Prescriber training aim /Diagnostic Stewardship: Practitioners should be able to understand the added value of the use of Mini-Lab, when to ask for analysis and how to interpret the results and best use of them for patient treatment.</p> <ul style="list-style-type: none"> Medical Doctor or Medical officer with General Practitioner level. No experience in clinical bacteriology laboratory required Minimum training should consist of 2.5 days of theoretical training and 2.5 days of practical exercise. Mentorship of one month minimum from an experienced MD with minimum supervision visit every 6-9 month <p>Nurses' training aim: Nurses should be able to collect samples properly in order to mitigate the risk of contamination and provide the best possible quality and quantity of samples accordingly.</p> <ul style="list-style-type: none"> Certified nurse or nurse assistants. Experienced in standard phlebotomy Minimum training should consist of 0.5 days of theoretical training and 1 days of practical exercise. Mentorship of one week minimum from an experienced Nurse with minimum close supervision first 2 months and visit every 6 month <p>A standard certificate will be provided to all mentees/trainees that have been going through the process of Mini-Lab and specific register should be available to keep a record of trained staff.</p>		<p>various technical equipment that is in the Mini-Lab, to develop a critical sense of their work and to manage the laboratory optimally.</p> <ul style="list-style-type: none"> Become a Mini-Lab Supervisor (BMLS) training, 30 hours, 20 days. Target: Mini-Lab supervisor. Learning objective: To enable learners to solve problems or find help to resolve non-conformity in analyses or in the operation of the laboratory, validate and communicate test results to clinicians, collect and analyse activity data, quality monitoring and epidemiological surveillance indicators, manage the stock of consumables and reagents in the laboratory, support the pharmacist in pharmacy inventory management and international ordering, assess the competence of technicians and provide adequate training to new-comers. Become a Mini-Lab Sampler (BMLC) training, 4 h or 2 times 2 h. Target: Nurses or medical staff authorised to take samples. Learning objectives: To enable learners to understand the challenges of quality samples and the impacts on the patient of a wrong sample, identify the analyses in line with the indications (justify the purpose of the prescription), master and carry out the different procedures for collecting and routing samples. Mini-Lab Diagnostic tools Stewardship (MLDS) training, 3 hours. Target: clinicians. Learning objective: To allow learners to understand the challenges of the proper use of the diagnostic tools made available to them, by understanding the test prescription criteria, to acquire an understanding and a critical sense of the results provided by the Mini-Lab. Become Mini-Lab Technician (Distance learning) (eBMLT), 20 -25 h, 32 Modules. Target: Laboratory technicians in the field, prerequisites for future implementers. Learning objective: To support Mini-Lab users with different e-learning modules. Before the face-to-face training for the first time and in their daily use of the Mini-Lab for the second time and allow them to understand more quickly the use of the different techniques and equipment used at the Mini-Lab, to develop a critical sense of their work and to manage the laboratory optimally. Become a Mini-Lab implementer (BMLI), 70 hours, 10-day. Target: future implementers (microbiologist or experienced lab tech). Learning objectives: To be able to deploy, implement the Mini-Lab and its activities, train the lab tech, supervisor, and nurses to use properly all the available procedures. To Integrate the activities of the Mini-Lab into the processes of the mission

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
Equipment	<ul style="list-style-type: none"> To be carried by hand without need for a specific lifting machine All equipment of the Mini-Lab will need to be easy transportable from the supply centre to the end- user and from one project site to another without a need for a big truck. All the boxes of the Mini-Lab should be fit to be transported in a standard Toyota pick-up (2m length x 0.98m width x 1.5m height) or van car. Total weight < 800 kg; maximum of weight per case should be 100 kg. Volume at transport < 4 m³ List of potential minimum equipment: Requiring electricity: Charger/ invertor/ UPS, Incubator, computer, light, safety cabinet Class 2 Not requiring electricity: Should incorporate bench tops and all the material for administrative work, stool, extinguishers, eye wash station, first aid kit, fire blanket, etc. 		<ul style="list-style-type: none"> 2 days to install, 2 days to repack All-in-one kit: <ul style="list-style-type: none"> The Mini-Lab can be easily shipped, deployed and used thanks to its box-bench, an innovative furniture designed for the Mini-Lab. The laboratory equipment is packed in the boxes which dimensions are fitted for air and sea shipment. Each box can be lifted, carried and opened by 2 to 4 people. Once in place, the box is deployed and settled as a workstation bench where laboratory operations can be performed.
Power Requirements	<ul style="list-style-type: none"> Material requiring electricity should permit a flexible and robust connection to available energy supply and should be able to last up to 8 hours without external power supply (rechargeable batteries). The Mini-Lab should be able to be connected to fluctuant city supply / generators or solar energy Electrical Requirements: 100-240V 50/60Hz Wattage: Min 150 W, max 500 W Consumption per 24 H: min 1000 Wh, max 5000 Wh 		<ul style="list-style-type: none"> Power requirements: 100-240V 50/60Hz , 49 kWh per day. Peak value up to 4.85 kW. Possibility to connect to a fluctuant energy system (UPS and surge protectors included). 8 wall outlets (placed at 1.7m high) recommended (4 acceptable) and 6 ceiling lighting points



Figure 61: Box-Bench ready to be assembled in MSF Logistic warehouse, Bordeaux, France

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
Waste management	Can require a specific waste management material using easy and accessible system fitting in MSF waste management scheme.	Should not require any specific waste management outside of the existing one present in MSF hospital.	 <p>Figure 62: Module 6, waste management installed in Carnot Hospital, Central African Republic</p> <ul style="list-style-type: none"> • Several types of waste are produced daily and segregated in the lab, a horizontal loading autoclave is included to inactivate soft biological waste, all procedures are provided to have a safe and eco-friendly final disposal by type of waste: <ul style="list-style-type: none"> - "Soft" decontaminate biological waste: 2,5kg /day volume produced, to be incinerated by MTIBL: Medium temperature incineration with batch loading system or higher version. - "Sharp cutting" biological waste: 5 Liters sharp box / 3 weeks, needle pit or MTIBL. - Uncontaminated liquid chemical waste (diluted Gram stain, etc.): 2 liters /week, MTIBL, encapsulation or dilution process. - Administrative waste: 1 bag of 20 liters / day, MTIBL • Expired reagents: the quantity produced depends on supply chain efficiency, expired reagent should be processed by batch with the use of HTI: High temperature incineration, continuous loading system or MTI: Medium temperature incineration, continuous loading system or HTIBL: High temperature incineration with batch loading system or MTIBL <p>More details are provided in document 6.10 External waste management-DOC-V1</p>
Reporting	<ul style="list-style-type: none"> • The system must be compatible with the existing MSF HIS system requirements with the possibility to capture bacteriology data, provide expert system based information, provide result to practitioner and support bacteriology data analysis for the trend of resistance • "Expert" system guiding interpretation and directing the report to the clinician (e.g. AST selective reporting). The software should be a decision tool and data entry allowing working without access to the internet but with an automatic connection to the Internet when it is possible in order to upload information on a data server in a cloud accessible by specific persons. The system should be able to work on PC (windows) environment as well as on the Android system. 		<ul style="list-style-type: none"> • Laboratory Information and Management System included (Mini-LIMS) • Integrates management of, samples, workflow, result edition, expert system, database and incident report. • A Microbiology Decision Support System (MDSS) is a component (expert system) of the LIMS and ensures results robustness in the absence of an experienced microbiologist in the lab. • European General Data Protection Regulation compliant, data privacy, restoration / Backup procedures • Web app (Node JS/Mongo DB), work offline, open source • Data extraction feature and connectivity with WHONET (WHO laboratory software for AMR data analysis and surveillance) for standard reporting to GLASS WHO surveillance network and MSF medical management team. • Includes a dedicated server for Offline working, connected with remote administration servers for up-date and activity follow-up

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
			Note: Internet: Access to internet daily is recommended via WIFI (30 Go monthly), if random network outages, server may be updated at night connecting it at the office.
Need for additional equipment in addition	None, all equipment, consumables, etc. included	Simple equipment acceptable	Equipment to be provided on the site: 2 cupboard, metal, ± 200x100x40, AFUR-CUPBM2010
Need for maintenance/spare parts	<ul style="list-style-type: none"> Only preventive maintenance required and within the competence of a trained laboratory technician (no requirement for a bioengineer technician. All spare parts needed for maintenance and basic repairs (fuse, sockets, etc.) as much harmonised as possible, available on site, well coded, easy to order and deliverable in a short delay (to be defined according to equipment). 		<ul style="list-style-type: none"> Preventive maintenance conducted by lab technicians Autoclave annual maintenance conducted by trained Biomedical technician Corrective maintenance conducted by field logisticians Spare part: kit of principal spare part provided (fuses, etc.) Distance support. Access to Microbiologist advisor by mail for direct support (standard form generated by Mini-LIMS); Mini-Lab administrator for Mini-LIMS questions
Specimen collection and transfer device	<p>Regular phlebotomy materials, complemented by specimen collection containers (wide mouth, screw caps), transport media/materials and transfer devices (loops, transfer pipettes, etc.). All materials complying with standard biosafety precautions and in case anti splash practices (e.g. disposable loops rather than metal wired loops and Bunsen burner).</p> <p>Personal protective equipment including gloves, masks, eye protection and gowns.</p>	<p>Use of dedicated / specific non-MSF phlebotomy material specifically fitting with blood cultures collection devices, complemented by specimen collection containers (wide mouth, screw caps), transport media/materials and transfer devices (loops, transfer pipettes, etc.). All materials complying with standard biosafety precautions and in case anti splash practices (e.g. disposable loops rather than metal wired loops and Bunsen burner).</p> <p>Personal protective equipment including gloves, masks, eye protection and gowns.</p>	<ul style="list-style-type: none"> A Mini-Lab sampling kit, composed of regular phlebotomy materials, The sample collection kit comes in a carrier case. The case is organised into separate compartments, which may or may not be detachable; This kit includes all the material required to disinfect the skin before and after collecting the sample and for putting the blood in the blood culture bottle; The kit is designed to take at least two samples. The kit also includes the material required to collect an additional sample, if anything unexpected occurs; Equipment frequently used on site is not provided in the kit, and must be made available on the ward; If disinfectants are not provided in the kit, use those in use on the ward;
Development / adaptability capabilities	The minilab will be able to accept over time new methods that will increase its effectiveness.		The Mini-Lab is able to accept over time new methods that will increase its effectiveness. All changes to be made need to be incorporated into the QMS document, proper document track record allow to see the evolution of the Mini-Lab versioning.
Infrastructure requirement	<ul style="list-style-type: none"> The Mini-Lab must be able to be set up in non-laboratory specific facility environment. Should just have access to clean water, storage facilities in compliance with the above specification and separate administration/staff room. Examples: set up under a tent or in an existing building with no specific requirements the one found below. 		<ul style="list-style-type: none"> Space: Clean dust-proof with washable floor stable and walls, ~20m², Air conditioning desired Structure: Long-term structure (no plastic sheeting), Container (40 F²) or Tent Equipment to be provided on the site: 2 cupboard, metal, ± 200x100x40, AFUR-CUPBM2010

KEY FEATURES	DESIRED	ACCEPTABLE	Mini -Lab final version (January 2023)
	<ul style="list-style-type: none"> Space requirement of a minimum of 10 m² and maximum 20m² Clean dust-proof room with concrete or washable floor and walls Access to clean and chlorinated water (10 Litre per day) Minimal of 4 Linear meters of work bench or to place 4 linear meters of work bench Electricity connection (see above specifications) Internet connection through Wireless, LAN or USB 3G/4G key (Upload: min 128 Kbs; download: min 256Kbs) 		<ul style="list-style-type: none"> Water: Access to clean and chlorinated water (average consumption of 10L per day). To install by local team, a standard sink in earthenware or stainless steel, with a degreaser tank on the exit of the gray water collection. Internet: Access to internet daily is recommended via WIFI or with provision of Internet Box, ok if random network outages (30 Go monthly) Pharmacy storage space required: <ul style="list-style-type: none"> Controlled temperature 1.5 m3 Cold chain 0.3m3 = 2 refrigerators (Vestfrost VLS404A AC) 1451 net, 230V (PCOLREFEVI4) only for Mini-Lab items. Dangerous goods: 0.016 m3 <p>More details are provided in document 2.1 Installation of the Mini-Lab-structure layout-DOC-V1</p>
COST			
Cost per consumables (e.g. cartridges, strips,) (for procurement)	<p>Cost expectation regarding the response scenario for Patient care: Fewer than 7 €/specimens (on positive sample), average of 5 euros (including negative if 20% of positive).</p> <p>Other cost scenarios cost should be a minimal as possible</p>	<p>Cost expectation regarding the response scenario for Patient care: Fewer than 10 €/specimens (on positive sample), average of 7 euros (including negative if 20% of positive).</p> <p>Other cost scenario cost should be a minimal as possible</p>	<ul style="list-style-type: none"> Consumables: 36k€ for starter kit including 4 months consumables (incl. quantities for 800 samples) + 1 month back-up; then 25k€/4 months Transport: depending on the country and transport mode Analysis cost (included in consumables cost): <ul style="list-style-type: none"> Average cost: 15 euros per samples (Given 15% positivity rate and 10% contamination rate and average BSI bacterial aetiology from sub Saharan paediatric population)[155], [166] Analysis cost breakdown: blood collection (5.46€), negative Blood culture processing (5.28 €), positive blood culture processing (14-31 €) HR cost: 2 lab technicians and 1 supervisor to be determined locally, 1 expatriate to train and support technicians/supervisor for 4-6 months (~15 - 22 k€) Participation to MSF External Quality Control Scheme: 1500 €, 4 strains, 4 times/ year, including shipment price. Maintenance: estimated < 500€ / year
Cost per equipment (for procurement)	<p>Fewer than 10000 €</p> <p>Maintenance cost of < 50 € per trimester, should include preventive weekly, monthly and bi yearly grand service</p>	<p>< 15 000 €</p> <p>Maintenance cost of < 200 € per trimester</p>	Mini-Lab Kit: between 30k€ to 40k€ according to the modularity requested

Funding And Conflict Of Interest

Jean-Baptiste Ronat was funded by MSF as part of its Long Term Contract arrangement. He did not received any research grant or travel grants for research stays in Haiti or in Central African Republic. All laboratory validation studies for the Mini-Lab project (Chapter 1 to 3), all validations studies on the field (Chapter 4) and all development pertaining any component, except one, of the Mini-Lab (Chapter 1) were funded by Médecins Sans Frontières. However Beckman-Coulter (West Sacramento, USA) provided a specific discount to MSF for the development cost of the MSF POS MIC PANEL type 2 (C32699), MSF NEG MIC PANEL type 2 (C32698), MSF FAST MIC PANEL (C32700) and MSF NEG/POS ID PANEL type 2 (C38213). The only equipment that was developed and fully funded by a third party was the Incudigit JPS 30, 12 hours Electricity free incubator, manufactured by JP Selecta (Barcelona, Spain) under licence of Global Goods Inc (Seattle, USA) who funded the development and evaluation of the first prototype in Laos. Jean-Baptiste Ronat, nor any of the co-authors mentioned in this work, have a conflict of interest to report.