Blood Culture: Landscape of simplified and integrated systems for pathogen identification and antimicrobial susceptibility testing

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ABBREVIATIONS

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
BARDA	Biomedical Advanced Research and Development Authority
BSI	Bloodstream infection
CE-IVD	European conformity with the EU *In Vitro* Device directive
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
FDA	Food & Drug Administration
FIND	Foundation for Innovative New Diagnostics
FISH	Fluorescence in-situ hybridization
ID	Identification (pathogen)
LAMP	Loop-mediated isothermal amplification
LMICs	Low- and middle-income countries
NAAT	Nucleic acid amplification test
NIH	National Institutes of Health, USA
PCR	Polymerase chain reaction
R&D	Research and development
RLS	Resource limited settings
RNA	Ribonucleic acid
TBD	To be determined
TPP	Target product profile
UTI	Urinary tract infection
WHO	World Health Organization

Table of Contents

List of Tables & Figures

1. EXECUTIVE SUMMARY

Introduction

Antimicrobial resistance is a critical public health issue both in high and low resource areas. Accurate surveillance for antimicrobial resistance requires clinical microbiology laboratories capable of blood culture and antimicrobial susceptibility testing. The lack of laboratory capacity in low resource areas has hindered the understanding of the prevalence and dynamics of antimicrobial resistance. This also prevents both adequate patient care and knowledge of regional resistance patterns to inform empiric therapy guidelines that can be used to aid antimicrobial agent stewardship. There is a need in resource limited settings for simplified and accessible instrument systems for blood culture and antimicrobial susceptibility testing. In this report we describe the current landscape of technologies that address these needs.

Methods

We identified 26 technologies from commercial, academic, and technology institutes and these technologies were evaluated based on the published FIND template for technology partner selection guidelines. **Search and evaluation was completed in Q2 of 2018**. A full description of the FIND standard criteria can be found at: https://www.finddx.org/wp-content/uploads/2018/01/Tech-Partner-Selection-Guidelines_QP-02-08-02_V3.0.pdf. Technologies were also compared to the draft simplified blood culture TPP (See Appendix I), and top technologies were selected based on key features which satisfied the TPP requirements or could be adapted to do so. In subsequent work, the draft TPP was published that describes key requirements for a simplified blood culture system in LMICs (Dailey et al., 2019). Companies were given the opportunity to review and comment on the technology summaries. The technologies are sub grouped by those that perform ID and AST from blood culture and those that are developing systems capable of ID and AST directly from whole blood.

Results

Recent international “calls to action” (and funding) to address antimicrobial resistance have helped to spur new research in technologies to address blood stream infections. We are pleased to report that a significant amount of work and innovation is going on in this space. Most of these technologies are in early stages of development. Based on this assessment, we identified 11 technologies (Table 1 below) that satisfied our evaluation criteria, and which we describe in detail in this report. Eight of these technologies required conventional blood culture as an input to the system to perform ID and AST and four technologies were identified that involve ID and AST directly from whole blood.

Table 1: Summary of top technologies (alphabetical order by developer)

Technologies requiring culture		
	Platform	**Manufacturer**
1	Accelerate Pheno	Accelerate Diagnostics
2	216R™ Antibiotic Susceptibility Testing System	BacterioScan
3	TBD	MicrobeDX + ChipShop
4	TBD	Momentum Biosciences
5	ASTar™	Q-linea
6	Smarticles Technology	Roche (GeneWEAVE)
7	Reveal ID™ and Reveal AST™	Specific Diagnostics
Technologies for direct from whole blood detection		
	Platform	**Manufacturer**
1	BACFLOWDX	Fraunhofer CMI
2	Proteus / BSI Max	Genefluidics
3	ASTrID®	Q-linea
4	Inspector-01 & Inspector-02	Spectral Platforms, Inc.

Conclusions

We recommend FIND continue to monitor the companies and technologies described in this report, as well as new entrants, as they progress in development and commercialization efforts. A wide array of technological approaches for the diagnosis of BSIs and delivery of AST or AST-equivalent information are being employed by developers. There are government, regulatory, demographic, and market trends in high resource areas that may continue to encourage development of new technologies that may benefit resource limited settings. Further details and assessment insights are provided for each platform listed in Table 1 in this report.

2. PURPOSE AND SCOPE

2.1 Rationale

Bacterial blood stream infections are a common cause of mortality and morbidity around the world. As the causative agents and the resulting treatment decisions vary by country and region, simplified, automated and nearer-patient testing and surveillance tools are necessary to monitor the bacterial causes. A recent study suggested that about 534 lives per 100,000 patients could be saved with an incremental cost-effectiveness ratio of USD 4,739 (Penno et al., 2015) if surveillance for bloodstream infections to inform empiric management of suspected sepsis were implemented in resource limited settings (RLS). Blood cultures, although the accepted gold standard, are not widely available outside of reference or research centers in Africa and Asia. Blood culture, pathogen identification (ID), and antimicrobial susceptibility testing (AST) are critical laboratory techniques that provide the data for surveillance of antimicrobial resistance and enable antibiotic stewardship.

The 2017 WHO Global antimicrobial resistance surveillance system (GLASS) report was recently released (see Figure 1). “In the (Africa region), poor laboratory capacity, infrastructure, and data management hamper effective AMR surveillance. The surveillance structure, and diagnostic and laboratory quality assurance capacities are weak. As a result, there is limited information on the impact of antibacterial resistance on humans, animals, and the environment.” AMR data was only available from seven sub-Saharan African countries in this report. Even in these countries, data was compiled from four laboratories or fewer. Severe gaps also existed in South America as well as South, Southeast, and Central Asia.

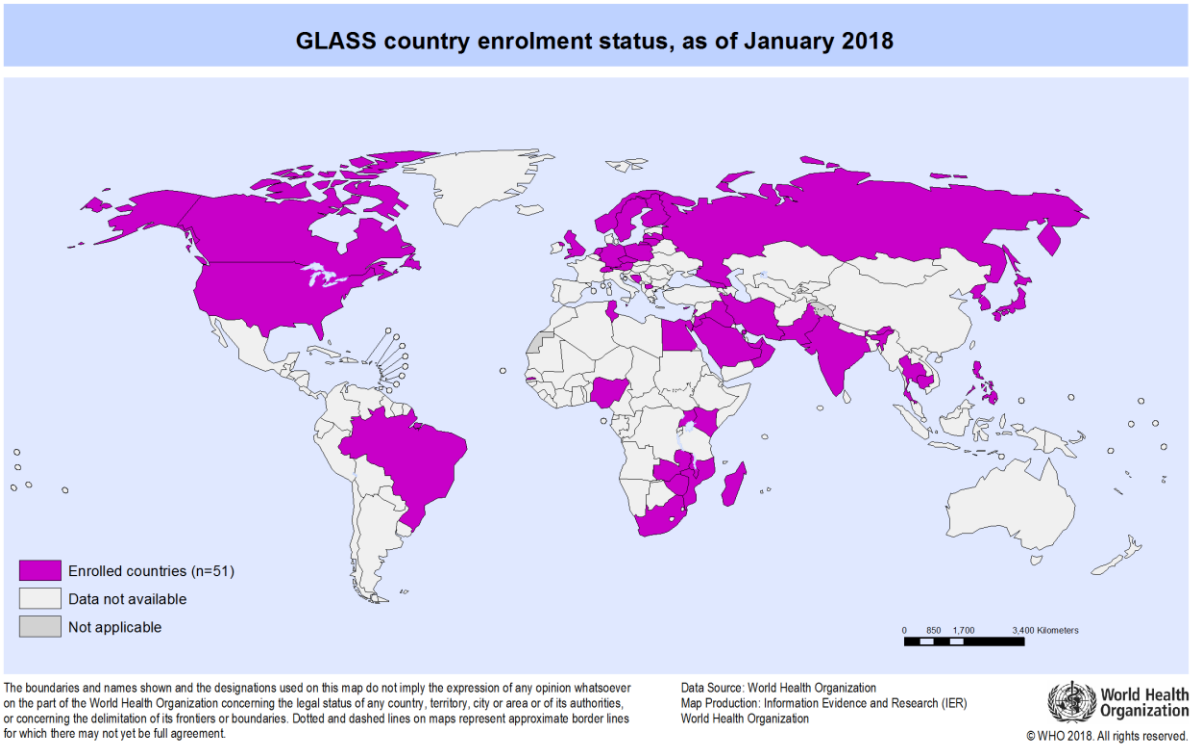


Figure 1: GLASS Country Enrollment Status (Source: WHO GLASS report, 2018)

It has been said that “without diagnostics, medicine is blind” (Dr Alain Mérieux). It is also true that without adequate microbiology laboratory capacity, AMR surveillance is blind. Public health surveillance is “information for action” (US Centers for Disease Control and Prevention). There can be no action on AMR without clinical microbiology capacity, especially blood culture, ID, and AST.

To extend the reach of these important diagnostic tools, it is crucial to engage product developers and academic/institute research partners to develop simple alternatives. Current culture methods as well as phenotypic ID and AST require many different steps and complex equipment, reagents, and skills. FIND has developed a draft target product profile (TPP) for a simplified blood culture system (Dailey et al. 2019).

2.2 Scope of work

This landscape addresses simplified blood culture systems with integrated identification and AST technologies that could potentially meet some or all of the criteria identified by stakeholders in the TPP. “The main objective of (AST) is to predict the outcome of treatment with the antimicrobial agent tested” (Manual of Clinical Microbiology, 11th edition, ASM Press, 2015). This is accomplished with a biological, phenotypic assay involving the growth of the pathogen in the presence of varying concentrations of an antimicrobial agent. The results reported are susceptibility categories (susceptible, intermediate, resistant) or a quantitative measure of anti-microorganism activity, the minimal inhibitory concentration (MICs). Phenotypic AST testing requires the growth of microorganisms. In this landscape, multiple technologies are described which simplify and accelerate the detection of growth of bacteria in the presence of antimicrobial agents.

Antimicrobial resistance (AMR) testing is different from AST and was specifically excluded from this landscape. AMR only tests for the presence of resistance genes and does not determine susceptibility. Also excluded from this landscape were technological approaches for blood culture identification and AMR testing involving next generation sequencing, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry and rapid multiplex PCR panels.

2.3 Aim of the work

The aim of this work was to compile a comprehensive list of technologies that can contribute to simplified and accelerated identification of blood stream infections (or other relevant infection) and antimicrobial susceptibility testing appropriate for use in resource limited settings (RLS). This information will be used to inform FIND regarding decisions on future investments and projects and will also help to ‘shape the agenda’ by informing the global health community about available technologies that could be deployed to support capacity building and infrastructure programs.

3. ASSESSMENT METHODOLOGY

An initial list of technologies to include in this landscape were compiled from multiple sources during a search period conducted from Q4 2017 to Q2 2018 and including the following:

1. Literature searches ("New, novel, automated" in combination with "blood culture, urinary tract infection, antimicrobial susceptibility testing", new technology review papers addressing blood culture, UTI and AST, reviews on the clinical microbiology laboratory of the future)
2. Agendas, abstracts, available online presentations from scientific meetings/conferences (ASM microbe, ECCMID, IDSA/ID Week)
3. Diagnostic industry conference agenda: JP Morgan, BioInvestor Forum, Molecular Medicine Tri-Conference, CHI Molecular Diagnostics Europe, Next Generation Dx Summit, BARDA Industry Day 2016, BIO-Europe 2017, and SELECTBIO 2017.
4. Attendance in-person at two scientific meetings: Southern California American Society for Microbiology (Fall 2017, San Diego, California, USA), Association of Molecular Pathology (Fall 2017, Salt Lake City, Utah, USA), AACC (Summer 2017, San Diego, California, USA); including discussions with exhibitors.
5. Discussions with key opinion leaders and experts.
6. Diagnostic industry online reports and news (e.g., Genomeweb and Fierce Biotech)
7. Internet searches to identify blood culture systems or other technologies with integrated phenotypic identification and integrated or separate/non-related simple identification and/or AST tools
8. Antimicrobial resistance grant programs and "challenges" including the US National Institutes of Health/BARDA "Antimicrobial Resistance Rapid, Point-of-Need Diagnostic Test Challenge", the United Kingdom "Longitude Prize", CARB-X, and the CDC Innovations to Slow Antimicrobial Resistance.

Several companies with new technologies have taken the strategy of addressing urinary tract infections first with their development efforts because urine is an easier sample to obtain, the clinical trials are less expensive, and there is lower development and regulatory risk. We have included these companies in our evaluations if they claim or we believe they would be potentially able to address blood stream infection diagnosis in the future.

This comprehensive search identified 23 potential technologies, three of which were excluded due to lack of publicly available information at the time of this landscape. To identify key technologies of interest for FIND, technologies were evaluated based on the published FIND template for technology partner selection guidelines. A full description of the FIND standard criteria can be found at: https://www.finddx.org/wp-content/uploads/2018/01/Tech-Partner-Selection-Guidelines_QP-02-08-02_V3.0.pdf. Technologies were also compared to the draft simplified blood culture TPP (See Appendix I), and top technologies were selected based on key features which satisfied the TPP requirements or could be adapted to do so. The technologies are sub grouped by those that perform ID and AST from blood culture and those that are developing systems capable of ID and AST directly from whole blood.

4. RESULTS

4.1 Overall results

The full list of technologies evaluated as part of this landscape can be found in the table below in alphabetical order by manufacturer.

Table 2: Technologies identified in search phase

	Company	Platform
1	3iDx	Biospectrix
2	Accelerate Diagnostics	Accelerate Pheno
3	Bacterioscan	216R™ AST System
4	DNAe	LiDia
5	Fraunhofer CMI	BACFLOWDX
6	Genefluidics	Proteus/BSI Max
7	Gradientech	QuickMIC
8	HelixBind	Core Pathogen Identification (C/PID) platform
9	LifeScale (Affinity Biosensors)	LifeScale Sensor
10	MicrobeDX + ChipShop	TBD
11	Momentum Biosciences	TBD
12	OpGen	Acuitas Surveillance
13	PhAST diagnostics	TBD
14	Q-linea	ASTar™
15	Q-linea	ASTrID®
16	QuantaMatrix	QMAC dRAST System
17	Qvella	FAST^{a} analyzer
18	Roche (Geneweave)	Smarticles Technology
19	Specific Diagnostics	Reveal ID and Reveal AST
20	Spectral Platforms, Inc.	Inspector-01 & Inspector-02
21	T2 Biosystems	T2 Magnetic Resonance (T2MR)
22	Talis	dLAMP AST
23	Astrego*	qUTI

This search phase yielded roughly double the amount of technologies that were anticipated prior to conducting the landscape. We believe that the recent “calls to action” (and funding) to address antimicrobial resistance has helped to spur new research and innovation in this space. With all the emphasis on genotypic antimicrobial resistance, we were pleased to see funding and R&D directed at improved, simplified and rapid AST phenotypic (or equivalent) assays also. Because serious attention to funding product development to combat antimicrobial resistance is relatively recent, many of the technologies we evaluated are quite early stage.

Regulatory changes also underline the increasing interest in new AST technologies. The US FDA recently announced completion of its “FDA Breakpoints Initiative” to meet the provisions of the 21st Century Cures Act, signed into law in 2016. This initiative will enable diagnostic companies developing new and improved AST assays to obtain more streamlined and less burdensome regulatory clearance in the US for their products and support development of new technologies. This may have the effect of getting more AST tests on the market and encouraging development of new technologies.

4.2 Top technologies requiring blood culture

Technologies were divided into approaches that either required blood culture first, or that are developing systems capable of direct detection, ID, and AST from whole blood without the need for culture. The top technologies requiring blood culture are listed in Table 3 below in alphabetical order by manufacturer. The companies were given the option to review the content included in the public landscape.

Table 3: Top technologies requiring blood culture (alphabetical order)

Technologies requiring culture		
	Platform	**Manufacturer**
1	Accelerate Pheno	Accelerate Diagnostics*
2	216R™ Antibiotic Susceptibility Testing System	BacterioScan
3	TBD	MicrobeDX + ChipShop*
4	TBD	Momentum Biosciences*
5	ASTar™	Q-linea
6	Smarticles Technology	Roche (GeneWEAVE)
7	Reveal ID™ and Reveal AST™	Specific Diagnostics*

* Companies that did not provide revisions to the specific company sections for this landscape

a. Accelerate Pheno, Accelerate Diagnostics

Platform: Accelerate Pheno
Manufacturer: Accelerate

Location	Tucson, Arizona, USA
Website	http://acceleratediagnostics.com/
Stage of Development	FDA and CE-IVD cleared

Technology Overview
Multiple technologies are employed: • Morphokinetic cellular analysis • Gel electrofiltration • Electrokinetic concentration • Automated FISH • Dynamic dilution

Assay Specifications	
Turnaround Time	~ 7 hours
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	No
Identification	Integrated
AST	Integrated
Reports MICs	Yes

Company background:
Accelerate diagnostics is a small company in Tucson, Arizona, USA. They have developed, manufacture and market an instrument that performs rapid identification and AST on positive blood cultures. This test is both CE-IVD and US FDA cleared.

Technology overview:
Multiple complex technologies are involved in producing a result including morphokinetic optical cellular analysis, gel electrofiltration, electrokinetic concentration, automated FISH, dynamic dilution, and organism quantitation. There are many peer-reviewed publications evaluating this technology with external investigators including their clinical trials.

b. 216R Antibiotic Susceptibility Testing System, BacterioScan

Platform: 216R AST
Manufacturer: BacterioScan

Location	St. Louis, Missouri
Website	http://bacterioscan.com/
Stage of Development	Development, FDA clearance for UTI detection

Technology Overview
Measures microorganism growth in liquid specimens by optical density and forward light scattering.

Assay Specifications	
Turnaround Time	n/a
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	No
Identification	In development, integrated
AST	Integrated
Reports MICs	Yes

Company background:

BacterioScan is an *in vitro* diagnostic company located in St. Louis, Missouri, USA that is focusing on assays to improve patient care and support antibiotic stewardship. Their first product is the 216Dx system, which received FDA 510(k)-clearance in 2018 for the detection of bacterial UTIs directly from urine specimens. In development is the 216R ID/AST System that is an automated, walk-away platform that identifies infecting pathogens and phenotypically determines AST directly from 216Dx-positive urine samples within approximately two to four hours. BacterioScan is also adapting their current products to evaluate positive blood cultures and respiratory tract specimens for detection, identification, and AST with an expected time-to-result that is faster than relative to conventional methods. In January 2018 they received NIH funding to support protocol development for an improved bloodstream infection detection application for the 216Dx system.

Technology overview:

Their technology measures microorganism growth in liquid specimens by Optical Density and Forward Light Scattering.

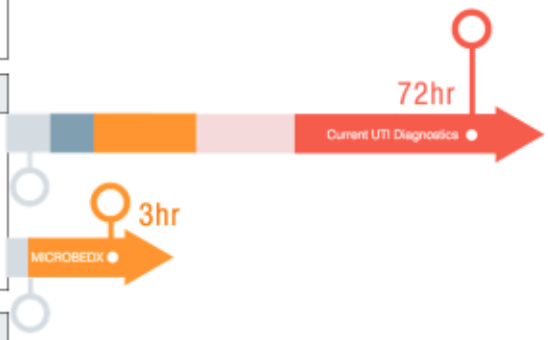
c. TBD, MicrobeDx / ChipShop

Platform: TBD

Manufacturer: MicrobeDx & Microfluidic ChipShop

Location	Los Angeles, CA, USA/ Jena, Germany
Website	www.microbedx.com/
Stage of Development	research, minimal data on website

Technology Overview
Automated hybridization and capture of ribosomal RNA with electronic amplification



Assay Specifications	
Turnaround Time	ID: 30-45 min./ AST: < 3 hrs.
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	No
Identification	Integrated
AST	Integrated
Reports MICs	n/a

Current focus of company is on UTI application

Company background:
MicrobeDx is an early stage start-up company based in the Los Angeles area, California, USA. They have applied for Longitude Prize funding. The founders are professors at the University of California, Los Angeles. They have partnered with Microfluidic ChipShop (MCS) of Jena, Germany for product development. MicrobeDx Europe GmbH is located on site at MCS.

Technology overview:
Their technology is based on automated hybridization and capture of ribosomal RNA with electronic amplification. Their first target market is urinary tract infections with a system they call rapid bacterial identification and antimicrobial susceptibility testing (RBID/AST). They claim that their technology can also be applied to other bodily fluids. If successful they could address detection, identification, and AST from positive blood culture bottles. On their website they claim that the technology would be useful in "resource poor environments."

As noted above, the detection and identification of microorganisms directly from blood with performance equivalent to blood culture (or better) is exceedingly difficult and has not been successfully accomplished yet. There are significant, sample preparation, assay development, clinical and manufacturing hurdles that stand in the way of development of a direct-from-blood assay and system.

d. TBD, Momentum Biosciences

Platform: TBD
Manufacturer: Momentum Biosciences

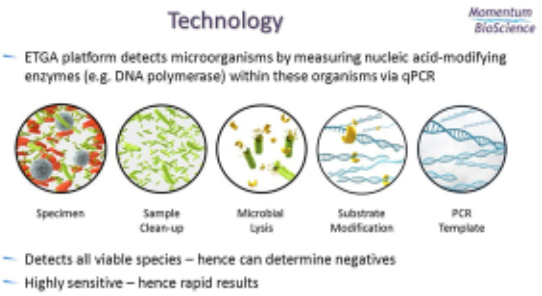
Location	Cardiff, UK
Website	www.momentumbio.co.uk/
Stage of Development	Early development for ID and AST

Technology Overview

- ETGA: enzyme template generation and amplification technique – detect presence and activity of bacterial, fungal DNA polymerases using PCR
- Quantitative PCR

Assay Specifications

Turnaround Time	n/a
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	Yes, detection
Identification	Integrated
AST	Integrated
Reports MICs	Yes



Initial product (Cognitor Minus) provides early, first day identification of negative blood cultures and is in CE-IVD clinical trials

Company background:
Momentum Biosciences has completed the development of their Initial product (Cognitor Minus) providing early, first day identification of negative blood cultures. They are located in Cardiff, United Kingdom.

Technology overview:
The Cognitor Minus product is based on ETGA – enzyme template generation and amplification technology. This technology detects the presence (or absence) of bacterial and fungal DNA polymerases after amplification with PCR. A follow-on product for the detection, identification and AST of positive blood cultures is planned. They have published preliminary evidence that they can use the same technology to do identification and AST also from blood cultures. CE-IVD trials with Cognitor Minus and a significant amount of data and publications are available. They are awaiting clearance.

e. ASTar, Q-linea

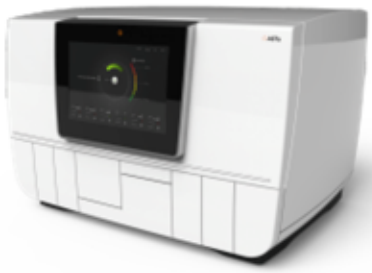
Platform: ASTar™
Manufacturer: Q- LINEA

Location	Uppsala, Sweden
Website	https://qlinea.com/
Stage of Development	Development

Technology Overview
• Fully automated phenotypic AST directly from positive blood cultures • True MIC results in 3 to 6 hours • Up to 48 antimicrobials in 5 to 11 two-fold dilutions • Up to 50 samples per day

Assay Specifications	
Turnaround Time	3 - 6 hours
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	Yes
Identification	No. External ID required
AST	Integrated in system
Reports MICs	Yes

Q-LINEA



Company background:

Q-linea AB is a diagnostic company located in Uppsala, Sweden. The company was founded in 2008 and its operations were based on technology developed by scientists from the Rudbeck Laboratory at Uppsala University, together with other companies such as Olink Bioscience AB and Uppsala University's holding company, UUAB. Q-linea is currently focused on developing and delivering solutions for healthcare providers. Their core product, ASTar™, is a system for rapid and automatic determination of the most effective antibiotic treatment against infectious diseases and is currently in product verification.

Technology overview:

The ASTar system is designed to deliver phenotypic AST results from positive blood cultures. Pathogen ID using another technology such as NAAT or MALDI-TOF is required for the final AST report. In the future, Q-linea intends to adapt this technology to other specimen types such as urine, respiratory, sterile aspirates and isolates. Q-linea's technology for the ASTar system includes automated time-lapse imaging of bacteria in wells containing dilutions of antimicrobial agents.

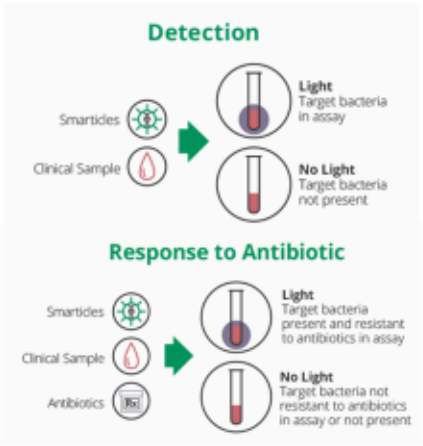
f. Smarticles Technology, Roche

Platform: Smarticles Technology
Manufacturer: Roche (GeneWEAVE)

Location	Pleasanton, CA, USA
Website	www.rochemicrobiologytests.com/healthcare-associated-infections/innovative-solutions.html
Stage of Development	Development

Technology Overview
• DNA-delivery non-replicative transduction particles consisting of a phage chassis with a custom DNA particle that cause viable bacteria to express luciferase.

Assay Specifications	
Turnaround Time	n/a
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	No
Identification	Integrated
AST	Integrated
Reports MICs	Yes



Company background:
The Smarticles technology was developed by a start-up company called GeneWEAVE BioSciences (GeneWEAVE), which was sold to Roche Molecular Systems (RMS) in 2015. RMS is located in Pleasanton, California, USA and development of the Smarticles technology is located in the former GeneWEAVE facility in Los Gatos, California, USA.

Technology overview:
Smarticles bioparticles are non-replicative transduction particles that bind specifically to bacteria and deliver DNA that contain a reporter luciferase gene that is expressed in the bacteria. This technology is used to both identify organisms and deliver AST-like results. Different Smarticles bioparticles are required for different organisms. In presentations at meetings, Roche has specifically identified blood culture as an application for this technology.

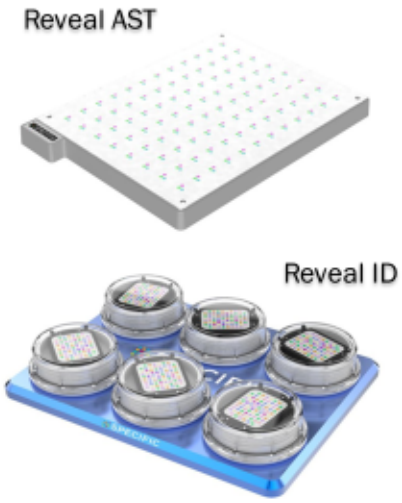
g. Reveal AST™ and Reveal ID™, Specific Diagnostics

Platform: Specific Diagnostics
Manufacturer: Reveal ID™ and Reveal AST™

Location	Sunnyvale, CA, USA
Website	www.specifictechnologies.net
Stage of Development	Development

Technology Overview

- Detection of volatile organic compounds (VOCs) produced during a culture incubation via a high-dimensional printed array of 70 color-active chemical indicators
- Current focus is on Reveal AST



Assay Specifications	
Turnaround Time	Reveal ID (hours), Reveal AST (4 hours)
Culture Required	Yes, sample is positive blood culture
Detection/Isolation	No
Identification	Reveal ID
AST	Yes, for Reveal AST from blood culture
Reports MICs	Yes

Company background:

Specific Diagnostics is a small start–up company located in Mountain View, California, USA. The company is focused on microbiology diagnostics, especially rapid diagnostics for detecting, identifying and delivering AST for BSIs. They are developing two instruments. Reveal ID™ provides the ID of microorganisms directly from blood culture in hours. Reveal AST™ will deliver rapid phenotypic AST in 4 hours, directly from positive blood culture.

Technology overview:

Their technology is based on the detection of volatile organic compounds (VOCs) produced during culture incubation via a high-dimensional printed array of 70 color-active chemical indicators.

4.3 Top technologies for direct from whole blood testing

Technologies that bypass the need for blood culture are ideal, as blood culture is time and labor intensive. However, detection and identification of microorganisms directly from blood with performance equivalent to blood culture (or better) is exceedingly difficult. There are significant, sample preparation, assay development, clinical and manufacturing hurdles that stand in the way of development of a direct-from-blood assay and system. Top technologies that were identified after evaluation are listed in alphabetical order by manufacturer in the table below. The companies were given the option to review the content included in the public landscape and all provided reviews

Table 4: Second Tier Technologies

Technologies for direct from whole blood detection		
	Platform	**Manufacturer**
1	BACFLOWDX	Fraunhofer CMI
2	Proteus / BSI Max	Genefluidics
3	ASTrID®	Q-linea
4	Inspector-01 & Inspector-02	Spectral Platforms, Inc.

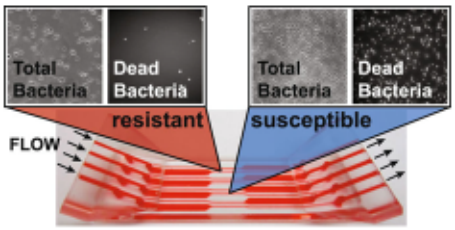
h. BACFLOWDX, Fraunhofer CMI

Platform: BACFLOWDX
Manufacturer: Fraunhofer CMI

Location	Brookline, Mass., USA
Website	www.cmi.fraunhofer.org/en/services/projects-our-work/biomedical/in-vitro-diagnostics.html#tabpanel-2
Stage of Development	Research: detailed manuscripts on technology published

Technology Overview

- Rapid sample preparation that isolates microorganisms ***directly from whole blood***
- Species-specific SERS Identification
- Microfluidic phenotypic assay utilizes shear stress – for AST



Rapid phenotypic stress-based microfluidic AST

Assay Specifications

Turnaround Time	n/a
Culture Required	No, direct from whole blood
Detection/Isolation	Yes
Identification	Yes
AST	Yes
Reports MICs	Yes

Company background:
The Fraunhofer Center for Manufacturing Innovation (CMI) is a non-profit applied research organization located in Brookline, Massachusetts, USA, affiliated with Boston University that has a research program in biomedical engineering with an emphasis on automation and manufacturability. The teams at Fraunhofer CMI have developed technologies for each aspect of the value chain of BSI diagnosis as academic projects and have published many peer-reviewed publications on prototype assays and instruments.

Dr Christine McBeth currently heads the biology division at Fraunhofer CMI and is expanding the center's work in microfluidic-based diagnostics. They are currently interested in identifying development partners for licensing and/or for targeted collaborations. The next steps for the project are to generate both the instrumentation and disposables required for high-throughput automated analysis.

Technology overview:
The key technologies involved include surface-enhanced Raman spectroscopy for identification, and automated microscopic identification of changes in sheer stress for AST in a microfluidic format. In addition, they have developed a method to isolate bacteria directly from whole blood. Development is at an early stage with "breadboard" instrument and prototype assays.

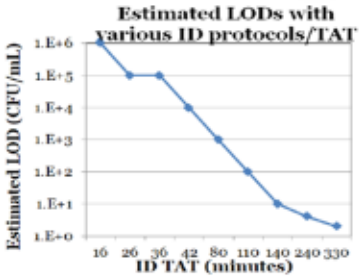
i. Proteus/Bsi Max, Genefluidics

Platform: Proteus
Manufacturer: Genefluidics

Location	Irwindale/Duarte, CA, USA
Website	http://genefluidics.com/
Stage of Development	Development BSI, FDA clinical testing and CE Mark (UTI)

Technology Overview
• Electrochemical biosensor measurement of bacterial 16S rRNA

Assay Specifications	
Turnaround Time	16-330 min ID, 70-130 min AST
Culture Required	No, directly from whole blood or urine
Detection/Isolation	Integrated
Identification	Integrated
AST	Integrated
Reports MICs	MIC or categorical reporting



UTI identification and AST product (UTI Max) has been CE-IVD listed

Company background:

Genefluidics is a small company in Irwindale and Duarte, California, USA. They are ISO 13485 certified and announced the CE marking of their UTI identification and AST product (UTI Max) in December 2017. The Company is now pursing FDA clinical testing on ProMax system followed by BsiMax (whole blood) and UtiMax (urine) systems. Currently they are in late stage product development and are working on preclinical validation on the BsiMax system with whole blood samples.

Technology overview:

Their technology is based on electrochemical measurement of bacterial 16S rRNA for the detection, identification, and AST of positive blood cultures. They have published several technology-oriented papers using whole blood and urine clinical specimens. They were initially developing a product for UTIs and then moved into validating for BSIs at a later time.

j. ASTrID, Q-linea

Platform: ASTrID™
Manufacturer: Q- LINEA

Location	Uppsala, Sweden
Website	https://qlinea.com/
Stage of Development	Early Development

Technology Overview

- Detects and identifies pathogens directly from blood samples without the need for blood culture. This instrument is in the early stage of development.
- Core technology of padlock probes and circle-to-circle isothermal amplification.
- The AST is based on the same technology as in ASTar

Q-LINEA

Assay Specifications	
Turnaround Time	ID in 4 hours/AST in additional 6 hours
Culture Required	No, direct from blood
Detection/Isolation	Integrated
Identification	Integrated
AST	Integrated
Reports MICs	Yes

Current company focus is on ASTar™ platform

Company background:
Q-linea AB is a diagnostic company located in Uppsala, Sweden. The company was founded in 2008 and its operations were based on technology developed by scientists from the Rudbeck Laboratory at Uppsala University, together with other companies such as Olink Bioscience AB and Uppsala University's holding company, UUAB. Q-linea's core product, ASTar™, is a system for rapid and automatic determination of the most effective antibiotic treatment against infectious diseases and is currently in product verification.

Technology overview:
The ASTrID platform detects and identifies pathogens **directly** from blood samples without the need for blood culture. This instrument is in the early stage of development. This system is based on Q-linea's core technology of padlock probes and circle-to-circle isothermal amplification, combined with the same AST technology that is used in the ASTar platform.

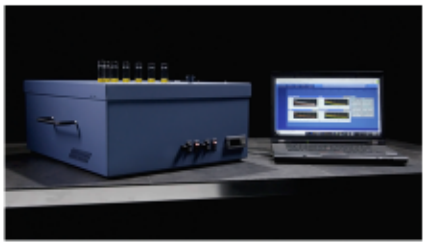
k. Inspector-01/02/03, Spectral Platforms, Inc.

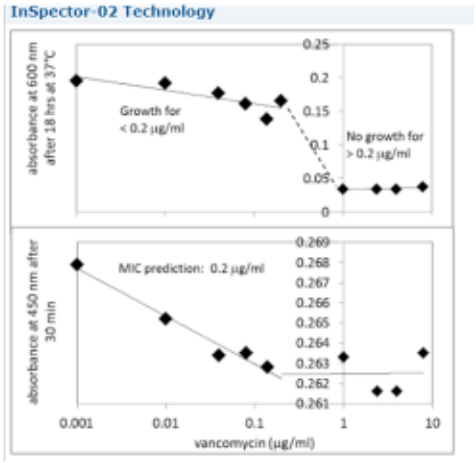
Platform: Inspector-01, 02, & 03
Manufacturer: Spectral Platforms, Inc.

Location	Monrovia, CA, USA
Website	www.spectralplatforms.com/research
Stage of Development	Research

Technology Overview
Resonance Raman technology to characterize an optical tag that is preferentially concentrated on the surface of any pathogenic microorganism

Assay Specifications	
Turnaround Time	~ 4 hours
Culture Required	Direct from blood
Detection/Isolation	Yes
Identification	Yes
AST	Yes
Reports MICs	Predicts MIC





Company background:
Spectral Platforms is a small, very early stage company located in Southern California, USA near the California Institute of Technology. They were a semifinalist in the NIH Antimicrobial Resistance Diagnostic Challenge.

Technology overview:
Spectral Platforms is developing three instrument platforms based on resonance Raman spectroscopy: Inspector-01, Inspector-02, and Inspector-03. These instruments would address detection, identification, and functional AST directly from blood. The technology used is resonance Raman spectroscopy coupled with an optical tag on the surface of microorganisms. According to the company website, they have made a preliminary submission to the FDA in January of 2018. The company claims to have performed pre-clinical studies at independent sites. No data is available.

4.4 New technologies since completion of the search phase

Important note as of March, 2019. Readers should be aware that this work, including the search and evaluation of new technologies in development, ***was completed in Q2 of 2018****. Progress on the platforms that we evaluated has occurred since then. In addition, since that time, we have become aware of the following additional platforms:*

Klaris Diagnostics

http://klarisdx.com/

Klaris Diagnostics is a privately held company located in Austin, Texas, USA. They are developing a platform that delivers rapid pathogen identification and AST results from "a variety of samples, including sputum, blood, and urine" according to the company. Their technology is based on single-cell biometric analysis.

Selux Diagnostics, Inc. (Next Generation Phenotyping platform)

https://seluxdx.com/

Selux Diagnostics is a privately held company located in Charlestown, Massachusetts, USA that is developing technology and a platform that provides rapid, high-throughput, automated AST "results from positive blood cultures as well as isolated bacterial colonies" according to the company. In addition, they have been awarded BARDA funding to support development of a second-generation rapid sepsis diagnostic system. Their technology evaluates bacterial morphologies and is able to detect bacterial growth using a surface-binding fluorescent amplifier.

5. SUMMARY

The need for automated and simplified blood culture, ID, and AST has drawn a significant amount of investment in new technology due to international concerns with the threat of antimicrobial resistance. In this report we identified 23 efforts by industry, academia and technology institutes to address this need. Because funding opportunities in this area have arisen recently, a concerted effort to "dig deep" and include organizations in early stages of development was made. In describing new technology, we paid particular attention to:

- The extent and integration of their efforts to address all points in the blood stream infection diagnostic value chain;
- Demonstration of performance through commercializing the product, peer reviewed publications, poster presentations, or data on the company website;
- Comparison to a draft target product profile for a simplified blood culture system

Based on these characteristics, we focused on 11 technologies from 10 companies. There were seven technology systems that required previous blood culture, and four that were "direct from blood" systems. The figure below summarizes the stage of development for the technologies included. As Figure 2 demonstrates, the majority of the technologies are in early stages of development, greater than 5 years to a commercially launched product. Only one technology is currently commercially available (Accelerate Pheno).

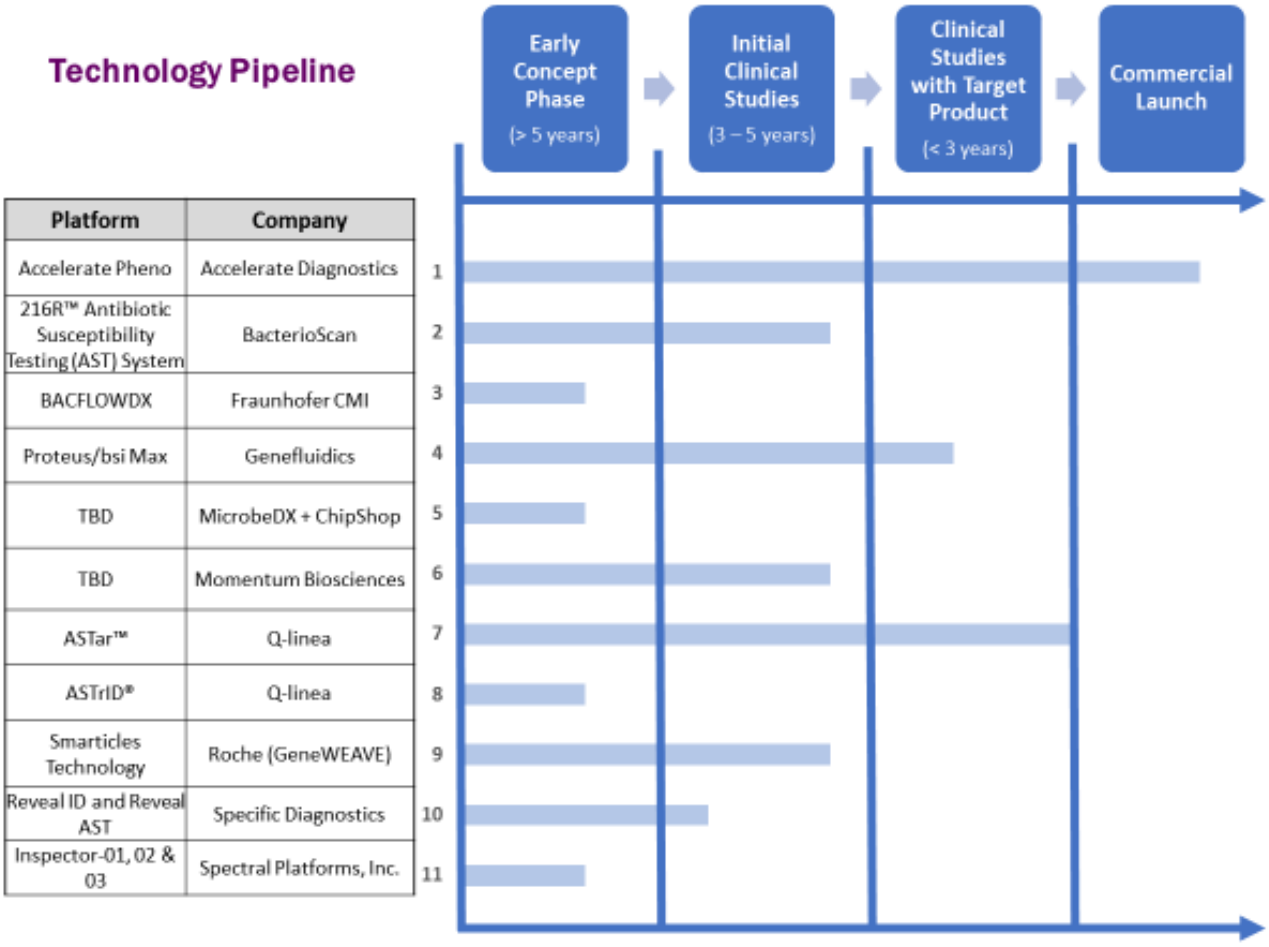


Figure 2: Pipeline of top technologies

5.1 Comparison of key platforms and key attributes

We recommend FIND continue to monitor the companies and technologies described in this report, as well as new entrants, as they progress in development and commercialization efforts. A wide array of technological approaches for the diagnosis of BSIs and delivery of AST or AST-equivalent information are being employed by developers. Several companies are first developing systems to diagnose and provide AST for UTIs with BSI diagnosis as a next step. This strategy makes sense as the technical risk and clinical trial cost is lower and time to market likely shorter. Examples include BacterioScan and Genefluidics. Three companies expressed strong or moderate interest on their websites or interviews in addressing needs in resource limited areas including Specific Diagnostics, Q-linea, and BacterioScan.

Q-linea is a startup company in Uppsala, Sweden. They are developing two instruments that address the complete BSI diagnostic chain in an integrated fashion. Specific Diagnostics is targeting ID and AST of positive blood culture bottles and have an interest in RLS. Accelerate Diagnostics is a young company that has successfully commercialized a CE-IVD and FDA cleared instrument and assay for the ID and AST of positive blood cultures. They are in the lead in developing a new diagnostic paradigm for the clinical microbiology laboratory by incorporating rapid AST. Clinical trials for assays to detect, ID, and determine AST for bacteria causing lower respiratory tract infection are in progress. The Roche Molecular Systems' "Smarticles" technology holds the promise to deliver integrated ID and AST of positive blood cultures. However, this is a complex technology (using engineered phage), and is early in development. Two other companies, Momentum Biosciences and BacterioScan either have or are nearing regulatory approval of instruments and assays. However, they currently are addressing UTIs (BacterioScan) or only part of the BSI diagnostic value chain (Momentum). Genefluidics has plans to develop an integrated system for direct detection, identification, and AST of BSIs and currently have and ID and AST system for UTIs that is CE-IVD registered.

In conclusion, there are government, regulatory, demographic, and market trends in high resource areas that may continue to encourage development of new technologies that may benefit resource limited settings. Until the last 10 years, clinical microbiology has been dominated by manual, labor-intensive techniques requiring a skilled laboratorian. An aging work-force of clinical microbiology technologists, grudging acceptance of near patient testing, increased automation, continued AMR funding, and recognition of the need for less burdensome regulatory guidelines will combine to encourage further research and development of simplified and automated testing.

6. LIMITATIONS

Evaluation of technologies was limited to publically available information in most cases. One of the challenges of this assessment is that we reviewed both established technologies with significant pipelines and very early stage companies with little or no data. This can bias assessments towards the new companies, since it is much easier to "promise" than to deliver a product. In this report we have tried to make clear the difference between claims and products. There are very early stage companies with new technologies entering the blood culture diagnostic arena that are not covered in this landscape. In most cases, these technologies are not at the stage that they have any data or even complete descriptions of their platform. In most cases, these technologies were not evaluated in detail in this report because of the aim and scope of this landscape. We recommend continued monitoring of new entrants to blood culture diagnostics.

All landscapes are a snapshot in time and are not living documents. The content provided in this report reflects publicly available information collected during Q4 2017 to Q2 2018. Furthermore, companies were given the opportunity to review the content of the technology summaries for accuracy during Q1 2019 and in some instances, updated figures and text were provided.

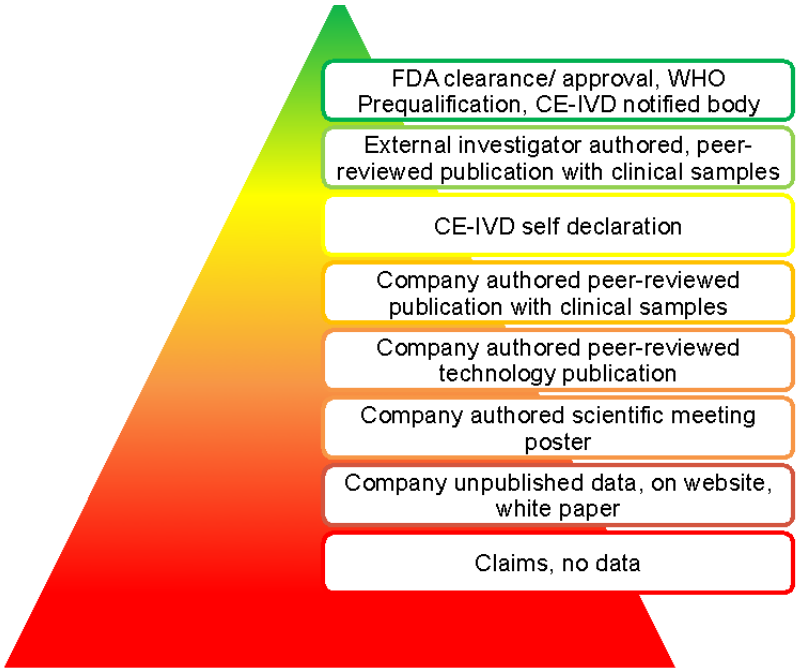


Figure 3: Hierarchy of scientific evidence, from strongest (top of pyramid) to weakest (bottom)

7. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

It should be noted that FIND established and completed a research collaboration with Specific Diagnostics in 2017 to evaluate their technology and develop plans for a product concept to be used in RLS. This did bias their assessment in this evaluation for the following reasons: 1) we knew more about their technology and, 2) they have reviewed and commented on an early draft of the TPP, FIND did fund Specific Diagnostics for product feasibility development.

8. APPENDIX

8.1 Proposed target product profile for a simplified BC system based on stakeholder input and international guidelines.

Characteristic	Minimal	Optimal	References
SCOPE			
Goal	A simplified blood culture system suitable for resource limited settings to support patient management and surveillance activities		Stakeholder interviews
Target population	Total population (including neonates and immunocompromised individuals) presenting with fever		
Target level of health system	Level 3 (Regional/Provincial Hospital) and above	Appropriate for use in level 2 (District Hospital)	(Ghani et al., 2015)
Target user	Moderately trained lab technicians (e.g., 1-2 year certificates)	Lab technicians with limited training (e.g. 3-6 months, able to operate an integrated test with minimal additional steps)	(Petti et al., 2006)
Platform cost	< US $20,000	< US $5,000	Stakeholder interviews
Price of individual test (2 culture bottles)	< US $10 per test	< US $5 per test	Stakeholder interviews
TEST PERFORMANCE			
System detection capabilities	- Culture positivity, Gram status - Antimicrobial susceptibility can be determined with additional methodologies	Pathogen identification and antimicrobial susceptibility are automated outputs of the system	Stakeholder interviews
Pathogen detection	>95% sensitivity for detection of positive BC for either monomicrobial or polymicrobial		Stakeholder interviews
Pathogen identification	Identifies 90% of isolates to species level, 95% genus level	Identifies 95% to species level, 99% to genus level	Standard BC and identification by MALDI-TOF is the reference (Faron et al., 2017, Lagace-Wiens et al., 2012)
Ability to determine the presence of mixed BCs	Not able to determine monomicrobial from polymicrobial infections	Able to determine monomicrobial from polymicrobial infections	Stakeholder interviews

Interfering substances	Able to provide an accurate result in the presence of malaria infection (Aesif et al., 2014, de Vries et al., 2007)	Able to provide an accurate result in the presence of malaria infection and/or antibiotics (Khennavong et al., 2011)	Stakeholder interviews
TEST PROCEDURE			
Ease of use / test complexity	The entire test procedure for system operation after sample collection to result should require a maximum of 2 steps by the user	The entire test procedure for system operation after sample collection to result should require a maximum of 1 step by the user and no additional steps required by user after the sample has been placed into the instrument	(Denkinger et al., 2015)
Sample volume	- Test consumable (culture bottle) should support smaller volumes (5mL or less) for pediatric samples and low volume draws. - Separate culture bottles for pediatric samples are acceptable	The same test consumable (culture bottle) should support smaller volumes (5mL or less) for pediatric samples and low volume draws	International and local guidelines regarding blood volumes and number of samples collected should be followed
Delayed entry	- Allows for room temperature storage of BC bottles post collection for < 4 hours prior to culture - If sample transport takes longer than 2 hours, incubation at 35°C is preferred		(CLSI, 2011)
QC testing of BC bottles	Same as standard BC		M22-A3: Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard - 3rd Edition
TEST RESULTS			
Preliminary result	Test reports positive or negative culture results	Test reports culture positive and >95% Gram status and morphology information	Stakeholder interviews
Final result	Provides pathogen identification	Provides pathogens identification with resistance categories of interest (MRSA vs MSSA, ESBL producing Enterobacteriaceae) and CRE	http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

Antimicrobial susceptibility testing	Antimicrobial susceptibility determination requires separate methodologies	Antimicrobial susceptibility is included as an automated output of the test result and therapy recommendations based on local treatment guidelines	Stakeholder interviews
Data interpretation and output	- Alert for preliminary and final report - Capable of paper-based and electronic results to not only laboratory, but physician and ward of patient	Minimal requirements in addition to therapy recommendations based on local treatment guidelines	Stakeholder interviews
CONSUMABLES			
Sample collection components	None provided	All components required for sample collection are included in the kit	Stakeholder interviews
BC bottles	Only compatible with BC media bottles from the test manufacturer	Compatible with local manufacture of BC media bottles with a specified media formulation	Stakeholder interviews
Sample tracking / Patient ID	Compatible with 2D barcodes and labels	Stakeholder interviews	
Storage conditions of BC bottles	6 months at +5°C to 35°C, 70% humidity, including transport stress (48 hours at 50°C); no cold chain required	12 months at +5°C to +40°C at 90% humidity & transport stress (72 hours at 50°C); no cold chain required	High environmental temperatures and high humidity is often a problem in many countries. High environmental temperatures and high humidity is often a problem in many countries. https://www.finddx.org/wp-content/uploads/2016/01/HCV-TPP-Report_17July2015_final.pdf

Shipping conditions of consumables & kit	No cold chain required; tolerance of transport stress for a minimum of 48 hours at 5°C to +40°C	No cold chain required; tolerance of transport stress for a minimum of 72 hours at 5°C to +40°C	Refrigerated transport is costly and often cannot be guaranteed during the entire transportation process. Frequent delays in transport are commonplace. https://www.finddx.org/wp-content/uploads/2016/01/HCV-TPP-Report_17July2015_final.pdf
Waste disposal	Consumables should be able to be disposed of as biohazardous waste as specified by WHO guidelines according to the safe management of waste from health-care activities or per country regulations		WHO, 2014. Safe managment of wastes from health-care activities.
OPERATIONAL CHARACTERISTICS			
Biosafety	Same as standard BC in a closed system Biosafety alert is provided when a pathogen identified is on a predefined biosafety list	- No need for a biosafety cabinet; basic safety procedures need to be followed (standard PPE) - Alarms present for organisms that pose a biosafety risk for laboratory acquired infections	Stakeholder interviews
Operating conditions	- Between +10°C to +35°C at 70% humidity and at a max altitude of 2000 meters above mean sea level - Ability to function in a high dust environment, with manual cleaning via standard lab consumable clean wipes or cleaning tool provided with the instrument	- Between +5°C to +40°C at 90% humidity and at a max altitude of 3000 meters above mean sea level - Ability to function in a high dust environment with minimal manual cleaning required by user	High environmental temperatures and high humidity and dust are often an issue in LMICs. High environmental temperatures and high humidity and dust are often an issue in LMICs.

MALDI-OFTOF: Matrix-assisted laser desorption/ionization-time of flight; OC: Quality control; ESBL: Extended spectrum beta-lactamases; MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*; CRE: *carbapenem-resistant Enterobacteriaceae; WHO: World Health Organization; PPE: Personal protective equipment*