

LANDSCAPE OF DIAGNOSTICS AGAINST  
ANTIBACTERIAL RESISTANCE,  
GAPS AND PRIORITIES



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Landscape of diagnostics against antibacterial resistance, gaps and priorities  
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# Glossary

ABR	antibacterial resistance	DST	drug susceptibility testing
AFB	acid-fast bacilli	DTR	discrete test region
AMR	antimicrobial resistance	DTS	direct tube sampling
ARI	acute respiratory infection	ECDC	European Centre for Disease Prevention and Control
ASO	antistreptolysin O	<i>E. coli</i>	<i>Escherichia coli</i>
AST	antimicrobial susceptibility testing	EDL	WHO Model List of Essential In Vitro Diagnostics
BC	blood culture	<i>E. faecalis</i>	<i>Enterococcus faecalis</i>
BCID	blood culture identification	EIEC	enteroinvasive <i>Escherichia coli</i>
BSI	bloodstream infection	ELISA	enzyme-linked immunosorbent assay
CAI	community-acquired infection	ESBL	extended spectrum beta-lactamase
CAP	community-acquired pneumonia	ESI	electron spray ionization
C <sub>2</sub> CA	circle-to-circle amplification	ET	fluorescent energy transfer
CDAD	<i>Clostridium difficile</i> -associated disease	ETEC	enterotoxigenic <i>Escherichia coli</i>
CDC	US Centers for Disease Control and Prevention	ETGA	Enzymatic Template Generation and Amplification
CDI	<i>Clostridium difficile</i> (or <i>C. diff</i> ) infection	EUCAST	European Committee on Antimicrobial Susceptibility Testing
<i>C. difficile</i>	<i>Clostridium difficile</i>	FDA	US Food and Drug Administration
cDNA	complementary DNA molecule	FFPE	formalin-fixed, paraffin-embedded
CE	Conformité Européenne	FISH	fluorescence in situ hybridization
CFU	culture-forming unit	FLLS	forward laser light scattering
cHDA	circulator helicase-dependent amplification	GARDP	Global Antibiotic Research and Development Partnership
CLIA	Clinical Laboratory Improvement Amendments	GBS	Group B <i>Streptococcus</i>
CLSI	Clinical and Laboratory Standards Institute	GC	<i>Neisseria gonorrhoeae</i> , also gas chromatography
CMV	cytomegalovirus	gDNA	genomic DNA
CNS	coagulase-negative staphylococci	GEL	gel electrofiltration
CO <sub>2</sub>	carbon dioxide	GES	GES-type beta-lactamase
CPE	carbapenemase-producing <i>Enterobacteriaceae</i>	GI	gastrointestinal
CPO	carbapenemase-producing organism	GLASS	Global Antimicrobial Surveillance System
CRE	carbapenem-resistant <i>Enterobacteriaceae</i>	HAI	hospital- and/or health-centre-acquired infections
CRP	C-reactive protein	HbA1c	glycated haemoglobin
CSF	cerebrospinal fluid	HBV	hepatitis B virus
CT	<i>Chlamydia trachomatis</i>	HCV	hepatitis C virus
CTX-M	CTX-M beta-lactamases	HDA	helicase-dependent amplification
DNA	deoxyribonucleic acid	HIC	high-income country
dPCR	digital PCR	HIV-1	human immunodeficiency virus-1

HPV	human papilloma virus	MLSb	macrolide-lincosamide-streptogramin B resistance
HSV 1	herpes simplex virus 1 (oral herpes)	MREJ	<i>mec</i> right extremity junction
HSV 2	herpes simplex virus 2 (genital herpes)	mRNA	messenger RNA
ICR	inducible clindamycin resistance	MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
ICU	intensive care unit	MS	mass spectrometry
ID	identification	MSSA	methicillin-susceptible <i>Staphylococcus aureus</i>
IgG	immunoglobulin G	MTB	<i>Mycobacterium tuberculosis</i>
IgM	immunoglobulin M	MTB complex	<i>Mycobacterium tuberculosis</i> complex
IL-6	interleukin-6	MxA	myxovirus resistance A
IMBI	Inhibition Magnetic Binding Immunoassay	m/z	mass-to-charge
IMDA	isothermal multiple displacement amplification	NAAT	nucleic acid amplification test
IMP	IMP-type metallo-beta-lactamase	NASBA	nucleic acid sequence-based amplification
iNAAT	isothermal nucleic acid amplification test	NDM	New Delhi metallo-beta-lactamase
IP-10	interferon gamma-induced protein-10	NEAR	nicking enzyme amplification reaction
IVDs	in vitro diagnostics	NG	<i>Neisseria gonorrhoeae</i> or <i>N. gonorrhoeae</i>
KPC	<i>Klebsiella pneumoniae</i> carbapenemase	NGS	next-generation sequencing
<i>K. pneumoniae</i>	<i>Klebsiella pneumoniae</i>	nm	nanometre
LAMP	loop-mediated isothermal amplification	nvCT	new variant of CT
LATE	late-linear-after-the-exponential	NTM	nontuberculous mycobacteria
LDT	laboratory-developed test	OXA	OXA-type carbapenem
LED	light-emitting diode	<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
LFIA	lateral flow immunoassay	PaLoc	pathogenicity locus
LIMS	laboratory information and management system	PBP	penicillin binding protein
LIS	laboratory Information system	PCR	polymerase chain reaction
LMICs	low- and middle-income countries	PCT	procalcitonin
LOD	limit of detection	PNA	peptide nucleic acid
LPA	line probe assay	POC	point of care
LPS	lipopolysaccharide	PT-INR	prothrombin time-international normalized ratio
LRTI	lower respiratory tract infection	qPCR	quantitative PCR
MAC	microfluidic agarose channel	RAA	residual antimicrobial activity
MALDI-TOF MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry	R&D	research and development
McF	McFarland unit	RALF	resuspension and lysis fluid
<i>mcr</i>	mobilized colistin resistance gene	RCA	rolling circle amplification
<i>mecA</i>	methicillin resistance <i>mecA</i> gene	RDT	rapid diagnostic test
<i>mecC</i>	methicillin resistance <i>mecC</i> gene	RIF	resistance to rifampicin
MEMS	microelectromechanical systems	RNA	ribonucleic acid
MG	<i>Mycoplasma genitalium</i>	RP	respiratory panel
MIC	minimum inhibitory concentration	RPA	recombinase polymerase amplification
mL	millilitre	rRNA	ribosomal RNA

<i>rpoB</i>	beta subunit of RNA polymerase	TAT	turnaround time
RSV	respiratory syncytial virus	TB	Tuberculosis
RT	reverse transcriptase	<i>tcdA</i>	<i>C. difficile</i> Toxin A gene
RT-PCR	reverse transcriptase PCR	<i>tcdB</i>	<i>C. difficile</i> Toxin B gene
RUO	research use only	<i>tcdC</i>	<i>C. difficile</i> Toxin C gene
RVFV	Rift Valley fever virus	TcdA	<i>C. difficile</i> Toxin A
SA or <i>S. aureus</i>	<i>Staphylococcus aureus</i>	TcdB	<i>C. difficile</i> Toxin B
SCMA	single-cell morphological analysis	TcdC	<i>C. difficile</i> Toxin C
SDA	strand displacement amplification	TMA	transcription-mediated amplification
SELDI	surface-enhanced laser desorption/ ionization	TNF	tumour necrosis factor
SFC	Sepsis Flow Chip	TPP	target product profile
SIRS	systemic inflammatory response syndrome	TRAIL	TNF-related apoptosis-inducing ligand
SMART	signal-mediated amplification of RNA technology	TV	<i>Trichomonas vaginalis</i>
SMS	small molecule sensor	UTI	urinary tract infection
SPC	sample processing control	<i>vanA</i>	vancomycin/teicoplanin A-type resistance protein <i>vanA</i>
SPIA	single primer isothermal amplification	<i>vanB</i>	vancomycin B-type resistance protein <i>vanB</i>
STAT	short turnaround time	VIM	Verona integron metallo-beta-lactamase
STEC	Shiga toxin-producing <i>Escherichia coli</i>	VOC	volatile organic compound
STI	sexually transmitted infection	VRE	vancomycin-resistant enterococci
<i>stx1</i>	Shiga toxin 1 gene	VZV	varicella-zoster virus
<i>stx2</i>	Shiga toxin 2 gene	WHO	World Health Organization
<i>S. typhi</i>	<i>Salmonella typhi</i>		

# Executive Summary

## Background

The increasing prevalence of antimicrobial resistance (AMR) is a serious threat to global public health and is especially burdensome in low- and middle-income countries (LMICs). The need for new diagnostics to combat AMR has been recognized (1, 2). Among the diagnostics needed are: (i) rapid tests that distinguish between bacterial and viral infections; (ii) tests for pathogen identification (ID); and (iii) tests for antimicrobial susceptibility patterns (3).

In order to address these needs, the World Health Organization (WHO) has undertaken in this report to map available and pipeline diagnostics against AMR, identify gaps in the availability of such diagnostics in LMICs, and establish a research and development (R&D) priority list of diagnostics against AMR for the next 3–5 years. In the second phase of the project, WHO will develop consensus target product profiles (TPPs) for the highest-priority diagnostics on the R&D priority list.

## Scope

While the increasing prevalence of AMR is a growing concern for viral, parasitic and fungal infections, antibacterial resistance (ABR) has now become a major global health issue that requires urgent solutions, including new diagnostics. Therefore, this report focuses on commercially available diagnostics to combat ABR and prioritizes the following key parameters: (i) diagnostics to improve clinical/syndromic management of patients to reduce the overprescription of antibiotics; (ii) antibiotics exhibiting the highest proportion of resistance as set forth in Annex I hereto; (iii) diagnostics that can be performed at primary and secondary care facilities in LMICs; (iv) diagnostics targeted at pathogens primarily related to community-acquired infections (CAIs) and secondarily to bacterial infections that are most frequently acquired in hospitals (HAIs); and (v) diagnostics to help distinguish bacterial from nonbacterial infections.

It should be noted that tuberculosis (TB) is a leading infectious disease that causes mortality worldwide, and drug-resistant *Mycobacterium tuberculosis* (MTB), the cause of TB in humans, is in the top 10 list of WHO priorities, as shown in Annex I. Extensive landscaping of diagnostics and drug-resistance testing

for MTB has already been performed, and a number of TPPs have been developed for priority diagnostic needs. Links to these materials are provided later in this report. Given the extensive work that has already been done with respect to diagnostics for MTB, these diagnostics are not a focus of this report. Nonetheless, because of the importance of TB, the report includes priorities for TB diagnostics R&D in addition to priorities for other targeted bacterial pathogens.

In addition, although the report does not cover issues related to uptake and implementation of new diagnostics in LMICs, to provide context for the diagnostic mapping, the report describes in general terms public health laboratory systems in LMICs. It maps typical facilities and capabilities from the lowest level of the system to the highest level – primary care facilities (Level I), secondary care/district hospital facilities (Level II), regional and provincial laboratories (Level III), and national and multicountry reference laboratories (Level IV). The laboratory structure and testing capabilities at each level of the public healthcare system have important implications for improving access to diagnostics to combat ABR. The needs for human capacity, infrastructure and quality systems in clinical microbiology laboratories mean that bacterial cultivation, antimicrobial susceptibility testing (AST) and even molecular testing are generally found only at Level III and Level IV facilities, limiting testing access to most patients.

## The question and the findings

The question that frames this diagnostics landscape report is, What are the gaps in diagnostics to combat ABR for prioritized, drug-resistant bacterial pathogens, with an emphasis on CAIs, at Level I and Level II of the public healthcare system in LMICs? In order to answer that question, the report maps phenotypic and nonphenotypic methods of specific bacterial ID as well as antimicrobial susceptibility and resistance testing methods available at all levels of the healthcare system in all settings. It also considers tests that detect the host response to bacterial pathogens, but do not specifically identify them.

The report details phenotypic methods of bacterial ID, including culture, both manual and automated, and biochemical testing. Although these methods are

still the backbone of diagnostic bacteriology, the test methods are slow and, when done manually, cumbersome. Given the lack of well trained, highly skilled microscopists in LMICs and the need for sophisticated infrastructure for automated methods of bacterial cultivation, such testing is generally limited to Level III and Level IV laboratories in LMICs. In general, simpler, faster methods of bacterial pathogen ID are needed, especially for low-resource settings.

The report details nonphenotypic methods of bacterial ID. These include rapid immunoassays, which have the advantage of being fast and often require no equipment other than a test cartridge. This makes them suitable for use at Level I in LMICs. Disadvantages include that the assays can generally only identify one bacterial pathogen at a time and the performance of some assays has been questioned.

There are also numerous commercial genotypic test systems, including molecular-based platforms (using hybridization or amplification methods), DNA microarrays and sequencing, as well as mass spectrometry (MS) methods for bacterial pathogen ID. All of these methods are more rapid than traditional phenotypic testing; however, like bacterial cultivation, these methods require sophisticated laboratories and well trained laboratory technicians. This means that many of these tests and test platforms are best placed in Level III and Level IV settings, again limiting access to testing in LMICs.

Following pathogen ID, it is also important to conduct AST or to detect resistance in individual bacterial isolates in order to guide treatment decisions. The report maps commercially available phenotypic and genotypic methods of combined bacterial ID and AST/resistance testing, as well as AST/resistance assays only.

Classical phenotypic AST methods can be done manually using various media, both solid and liquid, in which the growth of bacteria along with the organism's resistance or susceptibility to a select antimicrobial agent are measured. In addition to manual methods, automated instruments that combine bacterial pathogen ID and AST offer improved turnaround time (TAT). While widely used in high-income countries (HICs), these systems are not generally available in resource-limited settings, except at the highest levels of the system (4, 5).

Nonphenotypic methods, in particular molecular-based platforms and MS, are now being used routinely in clinical microbiology laboratories for both bacterial pathogen ID and resistance testing. Although all of the platforms offer faster results than phenotypic methods, most platforms, especially those for blood-stream infections (BSIs), which require culture samples, are systems best used in sophisticated laboratory settings with strong infrastructure and well trained

laboratory staff. Again, these systems are not widely available in LMICs.

There are also a number of phenotypic and non-phenotypic diagnostic systems in the development pipeline designed for use in LMICs. The systems are smaller and simpler to use than conventional systems designed for use in large laboratories. Some of these pipeline diagnostics provide pathogen ID as well as AST or resistance testing capabilities; some do not. Some perform monoplex testing only and some will only process swabs and urine, which limits the pathogens they are able to detect. Some systems will process complex matrices, including whole blood, which would offer the possibility of avoiding culture. However, detecting and identifying bacteria direct from whole blood with performance at least equivalent to blood culture has proven to be very difficult. It is a challenge that has not yet been met.

Finally, there are a number of assays that incorporate biomarkers that are host derived, including C-reactive protein (CRP) and procalcitonin (PCT), and that are capable of indicating host response to a pathogen. For CRP there are rapid diagnostic tests (RDTs) available for use in Level I settings in LMICs, while for PCT testing there are one or two instrument-based platforms available for use in Level II settings. Although not a complete solution to detecting bacterial pathogens, these tests could be used in primary healthcare settings for triage as indications of severity of infection and to determine whether an infection is more likely to be bacterial than nonbacterial, which could aid in antimicrobial stewardship.

## Diagnostic gaps

The answer to the question that frames this report is that although there are many commercially available diagnostic systems to identify and/or perform AST/resistance testing for prioritized bacterial pathogens, the tests are not well suited to primary and secondary healthcare facilities. Most systems are predicated on sophisticated, well equipped laboratories with well trained laboratory staff. In LMICs, this effectively limits access to these tests to Level III and Level IV.

In other words, what emerges from this report, as well as from additional work by WHO with respect to TB, is a series of significant gaps in tests and testing platforms for Level I and Level II facilities in LMICs, where most patients initially present when they are ill.

Gaps include:

- inadequate near-patient testing for (i) biomarker-based, non-sputum-based detection of TB; (ii) patient triage evaluation for TB; (iii) sputum-based replacement for acid-fast bacilli (AFB) smear microscopy; and (iv) TB drug susceptibility testing (DST) (see [https://www.who.int/tb/publications/tpp\\_report/en/](https://www.who.int/tb/publications/tpp_report/en/));

- little or no ability to perform simplified phenotypic bacterial ID and AST to enable definitive therapeutic decision-making at Level III, and potentially Level II, in LMICs, particularly in the context of BSIs, in particular sepsis;
  - inadequate near-patient testing options for ID and susceptibility testing for multidrug-resistant *Neisseria gonorrhoeae* (NG);
  - few RDTs or easy-to-use, robust diagnostic platforms for use in primary (or secondary) healthcare settings that can reliably distinguish between bacterial and nonbacterial infections from accessible, minimally invasive clinical specimens (e.g., whole blood, urine, stool and nasal swabs);
  - no multiplex platform suitable for Level II and/or Level I settings to detect bacterial pathogens, including BSIs, from whole blood (no culture required) with AST/resistance testing done on a separate platform or combined with AST/resistance testing on the same platform; and
  - no simple, easy-to-use test/platform suitable for use at Level II and/or Level I settings for AST from whole blood or other sample matrices (urine, stool, respiratory specimens) for which culture is not required.
- Host response tests: to provide additional tests to help distinguish between bacterial and non-bacterial infections at primary healthcare facilities. A consensus TPP for such tests has already been developed, but should be revisited to consider whether it should be refined/revised.
  - Multiplex diagnostic platform to identify bacterial pathogens and perform AST/resistance testing without culture: to provide a platform suitable for Level II facilities and higher that could identify a broad range of bacterial pathogens from whole blood as well as from other sample matrices – e.g., urine, stool, nasal swabs – and that optimally could perform AST/resistance testing on the same platform. A consensus TPP should be developed.
  - Simple, easy-to-use test/platform for AST only: to perform susceptibility testing at Level II or Level I settings from sample matrices such as urine, stool and nasal swabs, minimally, and optimally from whole blood, to be used following bacterial ID on a separate platform. A consensus TPP should be developed.

## R&D priorities

These findings suggest the following R&D priority diagnostics against AMR for primary and secondary healthcare facilities over the next 3–5 years, for which consensus TPPs to stimulate product development are proposed:

- Improved near-patient testing for TB: to enable point-of-care (POC) assays capable of: (i) detecting all forms of TB by identifying characteristic biomarkers or biosignatures in specimen(s) other than sputum; (ii) low-cost patient triage by first-contact healthcare providers to identify those patients who need further testing; (iii) replacing AFB smear microscopy for detecting pulmonary TB; and (iv) determining first-line regimen-based therapy via DST that can be used at the microscopy-centre level of the healthcare system. These proposed TPPs have been developed. For details, see [https://www.who.int/tb/publications/tpp\\_report/en/](https://www.who.int/tb/publications/tpp_report/en/).
- Simplified phenotypic ID and AST: to enable the performance of culture and AST in key resistance categories, in particular sepsis, at Level II and higher facilities. Review published TPP and build on it as needed.
- Improved diagnostics and AST for NG: to provide a (i) rapid test to detect and distinguish NG and *Chlamydia trachomatis* (CT) for use in pri-

# Introduction

The increasing prevalence of AMR, which WHO defines as the “ability of a microorganism (like bacteria, viruses and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it” (6), is a serious threat to global public health and disproportionately burdens low-resource countries (1,2). The growing resistance to antibiotics of bacterial pathogens is recognized as the largest of these threats (7).

The urgency of the threat was highlighted when, in May 2015, the Sixty-eighth World Health Assembly endorsed a global action plan to combat AMR, including ABR (8). An extensive review of AMR published the previous year had concluded that by 2050 the lives of 10 million people per year would be at risk due to the increase in drug-resistant infections if solutions were not found (3). Among the recommendations made in that report was to promote new, rapid diagnostics to reduce the unnecessary use of antibiotics (3).

In recognition of the need for new diagnostics against AMR, in 2014 WHO had convened a consultation to facilitate dialogue among stakeholders with respect to creating a roadmap to stimulate the development of, and access to, appropriate rapid diagnostic tools for AST at all levels of the healthcare system in LMICs (9). The consultation also considered the need for diagnostics for pathogen ID and for discriminating between bacterial and viral infections in those settings and concluded that:

- the priority need is for rapid tests to distinguish between bacterial and viral infections;
- the next need is tests for pathogen ID; and then
- tests for susceptibility patterns (9).

In order to address the needs articulated in the 2014 consultation, including the finding that there was no authoritative list of commercially available diagnostics to combat AMR, WHO, with funding from Wellcome Trust, is undertaking the following initiative with respect to in vitro diagnostics (IVDs) against AMR, which is being carried out in two phases:

- In Phase I, WHO is:
  - mapping available and pipeline diagnostics against AMR;

- identifying gaps in the availability of such diagnostics; and
- establishing an R&D priority list of diagnostics against AMR for LMICs for the next 3–5 years.
- In Phase II, WHO will:
  - develop consensus target product profiles (TPPs) for the highest-priority diagnostics against AMR on the R&D policy priority list.

As required by Phase I of the work, this report maps available and pipeline in vitro diagnostics against ABR and identifies gaps in the availability of such diagnostics. The gaps on which this report focuses are those that are the result of the nonexistence or lack of technologies fit for purpose in LMICs as opposed to barriers to access to such diagnostics that exist for other reasons, including in-country policies or system failures. Detailed information on in vitro diagnostics and laboratories can be found at: <https://www.who.int/in-vitro-diagnostic/en/>.

Because resistance to antibiotics is the largest public health threat globally, the report focuses on IVDs that can play a role in limiting ABR. With respect to bacterial pathogens, such diagnostic tools comprise a spectrum of tests, including phenotypic, genotypic and immunologic test methods for identifying bacterial pathogens as well as various methods of AST, which measure bacterial growth in the presence of antimicrobial agents, and testing methods that identify bacterial resistance but do not measure susceptibility.

Within this framework, the following are the key parameters that define the mapping and scope of this report:

- Clinical/syndromic patient management. With respect to IVDs to address ABR, the primary focus is diagnostics to improve clinical/syndromic management of patients to reduce overprescribing of antibiotics, that is, the focus is on IVDs for antibiotic stewardship. A secondary focus is on IVDs for surveillance. The focus on stewardship implies an emphasis on faster/more accurate diagnostic testing, reducing the time to appropriate antibiotics, reducing their unnecessary

use and informing decisions regarding antibiotic de-escalation or discontinuation.<sup>1</sup> With appropriate tools, including diagnostic connectivity, rapid ID and susceptibility or resistance determination of bacterial pathogens will inform surveillance as well.

With respect to IVDs for national surveillance, the Global Antimicrobial Surveillance System (GLASS) has undertaken a landscape – *Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System* – which is available at: <https://www.who.int/glass/resources/publications/molecular-methods-for-amr-diagnostics/en/>. It is a resource that is complementary to this report.

- **High-priority bacterial pathogens.** The mapping focuses on IVDs to detect and categorize priority bacterial pathogens identified by WHO (11), the US Centers for Disease Control and Prevention (CDC) (12), and the European Centre for Disease Prevention and Control (ECDC) (13) that exhibit the highest proportion of resistance (i.e., those categorized as Critical/Serious or High/Serious, all of which are resistant to two or more classes of antibiotics, e.g., carbapenem-resistant, extended-spectrum beta-lactamase [ESBL]-producing) (14, 15), as well as bacterial pathogens that most affect LMICs (3). These are set out in Annex I.

TB is a leading infectious disease causing mortality worldwide, and drug-resistant MTB, the cause of TB in humans, is in the top 10 list of WHO priorities. Extensive landscaping of diagnostics and drug-resistance testing for MTB has already been performed, and a number of [TPPs](#) have been developed for priority diagnostic needs, which are highlighted in this report. In 2018, the WHO Global TB Programme issued a series of documents to improve drug susceptibility testing for TB in laboratories worldwide. The technical documents put together the latest knowledge on molecular mechanisms of drug resistance in MTB and describe state-of-the-art testing methods for determining drug resistance in the laboratory in order to design the most appropriate regimens for patients requiring treatment for drug-resistant TB. These documents include a [technical guide](#) for detecting mutations associated with drug resistance in MTB; a [technical report](#) that includes internationally

agreed critical concentrations for drug-susceptibility testing for detecting drug-resistant TB; and a [technical manual](#) of medicines used in treating TB. Given the extensive work that has been done with respect to diagnostics for MTB, such diagnostics are not a focus of this report. However, diagnostic platforms that include ID and/or resistance testing for MTB as well as other assays of relevance to this report are highlighted; the report also highlights priorities for TB diagnostics R&D. For a comprehensive landscape of diagnostics for MTB, see [https://unitaid.org/assets/TB-Dx-Landscape-5Ed\\_May2017\\_V2.pdf](https://unitaid.org/assets/TB-Dx-Landscape-5Ed_May2017_V2.pdf).

- **Primary care.** Given the WHO emphasis on universal healthcare (16) and essential diagnostics for healthcare systems articulated in the WHO Model List of Essential In Vitro Diagnostics (EDL) (17), the landscape focuses on IVDs that can be performed at primary and secondary care facilities in LMICs, which are generally referred to as Level I and Level II settings and are described more fully below.
- **Community-acquired infections.** Since Levels I and II are primarily outpatient facilities, the landscape focuses on bacterial pathogens that are most often community-acquired (CAIs), but also considers diagnostics for bacterial infections that are most frequently acquired in hospitals or in other in-patient healthcare facilities (HAIs) (also referred to as nosocomial infections), in the context of testing at higher-level facilities in LMICs. It should be noted that some pathogens are found commonly in both settings, e.g., MTB and *Staphylococcus aureus*). See Annex I. CAI pathogens considered are *Escherichia coli*, NG, *Helicobacter pylori*, *Campylobacter* spp., *Salmonellae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Shigella* spp. and MTB. HAI pathogens include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecium* and *Staphylococcus aureus* (18–20).
- **Host response tests.** The landscape also considers and maps other potential IVDs that may be useful in combating ABR. These include host response tests that incorporate host-derived biomarkers, including PCT and CRP, and novel biomarkers, which may be able to classify infections as bacterial or nonbacterial (21–23).

<sup>1</sup> WHO defines diagnostic stewardship as “coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen ID and accurate, timely reporting of results to guide patient treatment” (10).

# Methods

The material in this diagnostics landscape report has been gathered from the following sources:

- An extensive review of publicly available information, published and unpublished reports, and prospectuses. Databases may include Embase, the Cochrane Database of Systematic Reviews and PubMed, among others.
- With respect to individual commercial diagnostic platforms, company websites as well as the website of the US Food and Drug Administration ([www.fda.gov](http://www.fda.gov)).
- Relevant, publicly available diagnostic landscapes published by Unitaid, FIND, WHO and others, as well as additional sources, including, but not limited to, the Wellcome Trust, the Joint Programming Initiative on Antimicrobial Resistance, RAPP-ID, VALUE-Dx and the Longitude Prize.

# Organization of the landscape report

This diagnostics landscape report provides a description of the basic public healthcare delivery and laboratory system in LMICs, which sets the stage for understanding the types of IVDs that can be utilized at each level of the system.

The landscape then maps current diagnostic methods of identifying bacterial pathogens, including manual and automated methods primarily done at higher levels of the healthcare system in LMICs, with a primary focus on commercialized platforms. This includes light-touch mapping of current phenotypic methods of identifying bacterial pathogens, including classical phenotypic methods: morphologic characterization (microscopy and staining); growth in culture; and phenotypic and metabolic characterization by biochemical tests. The landscape also maps current molecular and other nonphenotypic methods of identifying bacterial pathogens (e.g., immunoassays, microarrays, sequencing, MS), describing, where available, commercial systems that have the potential to be used at primary and secondary healthcare facilities.

The landscape provides a mapping of current and emerging phenotypic methods combining bacterial pathogen ID and AST, including both manual and automated methods, most of which are best suited to higher levels of the healthcare system in LMICs. The landscape then maps commercially available nonphenotypic methods for simultaneous pathogen ID and detection of ABR or methods of identifying genes that directly confer antibiotic resistance only.

Throughout the mapping of diagnostics for ABR, emerging and pipeline diagnostics for bacterial pathogen ID are also considered. These include phenotypic and nonphenotypic methods of bacterial detection, AST and resistance testing. The focus is on commercial platforms that have the potential to be used at primary and secondary levels of the healthcare system in LMICs.

Finally, the landscape maps current and pipeline diagnostics that differentiate between bacterial and non-bacterial causes of infection. These are host response assays, including tests that incorporate host-derived biomarkers like CRP and PCT, as well as novel biomarkers, a combination of host biomarkers or combinations of protein biomarkers. The landscape considers the role such tests could play in combating ABR, particularly in primary and secondary healthcare settings in LMICs.

All of the commercial diagnostic platforms landscaped in detail in this report are summarized in Annex II.

For purposes of this report, of particular interest is the applicability of available and pipeline tests to the rapid ID and AST/resistance testing of multidrug-resistant bacteria found in community settings in primary and secondary care facilities in LMICs. The landscape also identifies primary gaps in the current diagnostics landscape and what IVDs are needed to improve clinical/syndromic management of patients in order to reduce overprescribing of antibiotics. The hypothesis is that the diagnostic needs going forward are faster, easier and less expensive testing at all levels of the healthcare system, and basic accessibility of appropriate testing at Level I and Level II, including syndromic testing.

# Laboratory systems in LMICs

In HICs, there are generally a large number of laboratories. For example, in the United States, there are approximately 5700 hospital-associated laboratories, 2000 independent laboratories and 10 000 physician-office labs/clinics (24). They offer a wide array of diagnostic testing from routine to the most targeted. Hospitals in HICs have access to nearly all requested testing with TAT of 24 hours or less. Some testing is performed in-house and some is sent out to large laboratory service companies. Tests for which results are needed immediately in order to manage medical emergencies will generally be performed in-house with TAT of an hour or less. In general, therefore, testing availability and access are not particularly problematic in HICs.

The same cannot be said for testing in LMICs. Over the last 10 years, access to treatment for people living with such priority diseases as HIV/AIDS, TB and malaria has substantially increased. However, the lack of laboratory and diagnostic capacity in resource-poor settings continues to be a barrier to achieving treatment targets outlined by countries and by international organizations. Simpler technology that is low cost and adapted to the needs of public laboratories in LMICs, in particular, is required in order to expand testing services to the communities that need them.

In order to understand laboratory testing in resource-limited settings, one needs to consider the typical public healthcare facilities and testing services available, which are usually characterized as a tiered system as follows (25).<sup>2</sup>

**Level I – Primary:**<sup>3</sup> Health post and health centre laboratories that primarily serve outpatients. Often, health posts have no laboratory capability, but are able to perform some POC testing. Generally, no clinicians are on-site at a health post. Health centres, however, usually have a simple laboratory where basic testing can be performed – e.g., POC assays and some microscopy (AFB smear by light microscopy), if a microscopist is available – and clinicians are generally on-site.

**Level II – District:** Laboratories in intermediate referral facilities, e.g., a district hospital. These facilities can perform all services provided at Level I and additionally provide a broader menu of tests, including Gram staining. They usually have automated equipment for tests such as CD4 count and blood chemistries. Physicians and other clinicians (e.g., nurses) are commonly available on-site.

**Level III – Regional and provincial:** Laboratories in a regional and provincial referral hospital that may be part of a regional or provincial health bureau. These facilities will have still more expansive test menus than those found at Level II facilities. In addition to performing all of the tests and services provided at Levels I/II, regional and provincial facilities can usually provide additional testing capabilities such as blood cultures, full chemistry testing and AFB smear (by fluorescent technique), as well as AFB culture, ID and susceptibility testing for first-line drugs. In addition, qualitative and quantitative nucleic acid amplification tests (NAATs) may also be available.

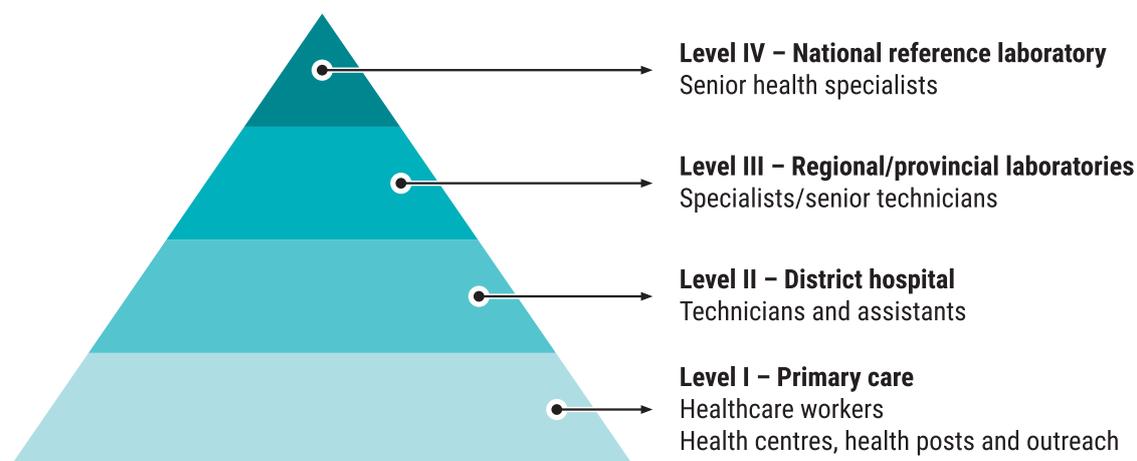
**Level IV – National and multicountry reference laboratory:** The national reference laboratories are specialized facilities charged with strengthening laboratory capacity for diseases of public health concern. They often provide linkages with research laboratories, academic institutions and other public health laboratories, forming integrated laboratory networks that can provide assistance in clinical trials, evaluation of new technologies and surveillance. In addition, national reference laboratories perform molecular and other sophisticated testing beyond the capabilities of Level III facilities – e.g., NAATs, HIV drug-resistance studies and AFB susceptibility testing for both first- and second-line drugs.

The laboratory system is often depicted as a pyramid (Fig. 1), which shows that there are generally a large number of Level I facilities and they serve the most patients directly. As one goes up the levels of the system, there are a smaller number of centralized facilities. In the case of national reference laboratories

<sup>2</sup> The Maputo meeting report notes that the tiered levels of a laboratory system and the testing performed at each level may vary depending on the population served (e.g., infants, adults), level of service available, physical infrastructure, electricity, water, road conditions and the availability of trained technical personnel in-country (25).

<sup>3</sup> The Maputo meeting report does not specifically place outreach services at Level I of the tiered laboratory system. Although some experts place outreach efforts at Level I, some consider patient outreach to be at a level below Level I and add a fifth tier to the system, referred to as sub-primary care or Level 0 (25).

Fig. 1. Diagram of tiered laboratory system in LMICs



and some provincial laboratories, they may not serve patients with a broad set of consultative services, but rather are referral centres for quality assurance and training or for conducting complex tests (either using samples drawn at facilities lower in the system and transported or by receiving patients referred directly from other facilities).

This laboratory structure and the testing capabilities provided at each level of the system in LMICs have important implications for improving access to diagnostics to combat ABR. Bacterial pathogen ID, quantification and AST typically take place in clinical microbiology laboratories using culture-based techniques dating from the late 1800s (4). The majority of tests available today in LMICs were created for developed-country settings, where laboratory-based diagnostics are operated by highly trained technicians on sophisticated instrumentation that is costly, often run in standard 96-well formats (high patient loads), and require dedicated laboratory infrastructure and equipment, as well as strong quality assurance programmes (2). Also, these instruments rely on a complex medical infrastructure that uses extensive sample transport networks to collect samples from multiple hospitals and clinics and uses sophisticated patient-tracking mechanisms that enable doctors and hospitals to return results to patients over weeks. These systems are not easily adapted for use in most regions of LMICs, where access, cost, infrastructure and patient loss are significant barriers to increasing case detection rates.

These needs for human capacity, infrastructure and quality systems in clinical microbiology laboratories mean that cultures, susceptibility testing and even molecular testing are generally limited to Level III and Level IV laboratories in LMICs, which in turn means that such testing is often not available to most

patients. Indeed, the lack of equipment and assays for such testing has been called the Achilles heel of the containment of ABR in low-resource settings (26, 27). In order to improve access to diagnostics to combat ABR in LMICs, easier, faster and simpler methods of testing are needed.

# IVDs for identifying bacterial pathogens

## Phenotypic ID methods

Phenotypic methods of identifying bacterial pathogens are often referred to as traditional or classical methods. They rely on features of the organism beyond its genetic make-up, including cell and colony morphology and biochemical reactions. The basic methods of phenotypic ID of bacterial pathogens are discussed below.

### Bacterial cultivation

The lynchpin of phenotypic ID of bacteria is culture. The cause of infection is determined by isolating and culturing bacteria in artificial media. Cultivation of bacteria involves growing it in a culture medium that is suited to its metabolic needs; a pure culture (i.e., containing one strain of a single species) is required. Cultivation necessitates the use of optimal artificial media (liquid or solid) and incubation conditions (carbon dioxide [CO<sub>2</sub>] concentration and temperature) to isolate and identify the bacterial etiologies of an infection (28).

The cultivation of bacteria from various patient specimens is done by inoculating processed specimens directly onto the artificial media. Incubation conditions are selected for their ability to support the growth of the bacteria most likely to be involved in the infectious process. To enhance the growth, isolation and selection of etiologic agents, a small, measured amount of specimen inocula (less than 2–5 µL) is usually streaked over the surface of plates containing culture medium in a standard pattern, so that individual bacterial colonies are obtained and semi-quantitative analysis can be performed.

Culture media can be broth (liquid) or agar (solid). In the laboratory, nutrients are incorporated into culture media on or in which bacteria are grown, and because different bacterial pathogens require different nutrients, various types of culture media have been developed for use in diagnostic microbiology; these are widely commercially available (28). Some bacteria (fastidious) have complex nutrient needs, while most (nonfastidious) do not.

Culture media are generally one of four types: enrichment, nutritive, selective or differential.<sup>4</sup> As an

example, most bacteriology specimens are inoculated onto plates containing sheep blood agar (a selective medium), because this medium supports growth for all but the most fastidious clinically significant bacteria (28). On the other hand, MacConkey agar is both a selective and differential medium and is used to isolate and differentiate lactose-fermenting and non-lactose-fermenting enteric bacilli. The suspected pathogen as well as tissue type informs laboratories about the type of media on which to culture the sample.

In addition to the importance of nutrients required for growth, environmental factors that influence bacterial growth are also important to consider. One of the primary distinguishing characteristics of bacteria is whether it grows aerobically (in the presence of oxygen), anaerobically (in the absence of oxygen), facultatively (in the presence or absence of oxygen) or microaerobically (in the presence of a less than atmospheric partial pressure of oxygen) (29). Therefore, the oxygen/CO<sub>2</sub> levels of culture media are critical. Temperature, pH and moisture conditions are also important. Temperature is controlled by incubating the medium, usually at 35–37 °C. Moisture levels may be controlled by using humidified incubators, while the appropriate pH is generally provided in commercially available plates/tubes.

If appropriate conditions are met, after approximately 12–24 hours of incubation bacterial colonies will develop sufficiently to be seen by the naked eye; standard incubation times, however, are up to 5 days, which is sufficient to recover the majority of bacteria, including fastidious bacteria (30).

### Microscopic morphology

Microscopy confirms the presence of bacteria, allows detection of different organisms present in the same specimen and determines the organism's clinical significance (28). One of the initial steps in identifying bacteria is to determine their size and shape, which can be done quickly using direct microscopic examination of a specimen (from sterile and nonsterile body fluids, biopsies and positive cultures) on a wet mount slide; this will also determine whether the organism is a prokaryote, i.e., single celled.

<sup>4</sup> For a detailed discussion of culture media, see Tille (28).

Microscopy, enhanced by staining techniques, like Gram staining, is commonly used to differentiate among various types of bacteria based on the biochemical properties of their cell walls. It can detect nearly all clinically important bacteria and will indicate the Gram reaction of the organism, including its shape, whether it is acid fast, its motility and its cell arrangement. It will also indicate the bacteria's staining patterns, another important characteristic. Various types of microscopy, e.g., bright-field/light microscopy and fluorescence microscopy, may be utilized, and the method chosen will depend on the microorganisms to be detected (28).

Gram staining can also distinguish between gram-positive and gram-negative bacteria, the two major groups of bacteria, although some bacteria are morphologically indistinct. More specifically, Gram staining will generally allow the categorization of bacteria into one of four groups: gram-negative cocci, gram-positive cocci, gram-negative bacilli and gram-positive bacilli (28).

Because specimens often contain a low quantity of microbes, cultured specimens are generally used for bacterial ID and can be performed as soon as the culture is positive. For example, in suspected BSIs, blood specimens are collected and used to inoculate two standard bottles containing rich media (one aerobic bottle and one anaerobic bottle). As soon as the culture is positive, blood sample aliquots may be examined microscopically using a Gram stain.

Sometimes the Gram stain provides enough information to begin appropriate antimicrobial therapy while awaiting additional testing. For example, the Gram stain has been used effectively to diagnose urinary tract infections (UTIs), including those caused by *Escherichia coli* (31,32). But, in most cases, the Gram stain alone is not sufficient to definitively identify bacteria.

## Macroscopic morphology

The macroscopic (colony) morphology of bacteria can also be examined via culture. The properties of an individual bacterial colony, including its form, size, elevation, margin/border, surface, opacity (e.g., glistening, opaque, dry), colour (pigment) and in some cases odour, can be examined. These characteristics provide clues to the identity of the bacteria; for example, colonies of streptococci are generally fairly small relative to many other bacteria, such as staphylococci, and may be identified in this way (31).

## Biochemical tests

Testing of the nutritional and metabolic capabilities of a bacterial isolate is typically used to determine its genus and species (31). In other words, testing is done to determine the enzymatic capabilities of a given bacteria and to determine its ability to grow or survive in

the presence of inhibitors, e.g., salts, surfactants, toxins and antibiotics. Biochemical test-based bacterial ID systems generally consist of single-enzyme-based tests to measure the presence of a specific enzyme and/or tests to determine a complete metabolic pathway that may contain several different enzymes.

Single enzyme tests are easy to perform and can be used to determine what subsequent bacterial ID steps should be performed. Certain of these tests, including the catalase test and the oxidase test, are very commonly used. The catalase test is important in the ID scheme of many gram-positive bacteria, whereas the oxidase test plays a similar role for gram-negative bacteria. Other commonly used single-enzyme-based tests include the following: indole, urease, PYR and Hippurate hydrolysis.

Tests to determine the metabolic pathways of bacteria fall into three general categories: carbohydrate oxidation and fermentation (to determine whether bacteria substrate utilization is an oxidative or fermentative process), amino acid degradation (to assess the ability of bacteria to produce enzymes that deaminate, dehydrolyse or decarboxylate certain amino acids) and single substrate utilizations (to determine whether bacteria can grow in the presence of a single nutrient or carbon source). The detection methods used for determining the end products of different metabolic pathways use colorimetry, fluorescence or turbidity.

In general, a battery of tests will be run. The number and types of tests chosen will depend on the type of bacteria to be identified, the clinical significance of isolates and the availability of reliable methods. In the end, a metabolic profile of the bacteria will be determined based on the results of the test battery, and this profile will be compared with an extensive ID database to establish the identity of the specific isolate.

## Automated or semi-automated phenotypic testing methods

Essentially all of the steps involved in the phenotypic ID of bacteria described above can be done manually. There are, however, automated systems available for most elements of the process. These include commercially available systems for Gram staining specimens for microscopic examination, inoculation and specimen processing, bacterial culture and biochemical testing/metabolic profiling. Most of these systems are best suited to sophisticated, high-throughput laboratories. They are discussed below.

### Automated Gram staining

Several automated Gram staining systems are commercially available. These include, but are not limited to, the PREVI® COLOR GRAM (bioMérieux, France), MULTISTAINER® (ALL.DIAG - BIOSYNEX, S.A.,

France), Aerospray® Gram series 2 (ELITechGroup Solutions, France) and QuickSlide™ GramPRO 1™ automated Gram stain instrument (Hardy Diagnostics, USA), each of which is described briefly below.

### PREVI® COLOR GRAM

The PREVI® COLOR GRAM, pictured in Fig. 2, is a benchtop automated Gram staining system that uses a patented spray technology to provide rapid, standardized results for all specimen types; the system is closed and avoids cross-contamination. Standardized slides can be read in about 5 minutes. The system is high throughput, and at a minimum can process 12–120 slides per hour.

Fig. 2. PREVI® COLOR GRAM Gram staining platform



### MULTISTAINER®

The MULTISTAINER®, pictured in Fig. 3, is a benchtop system that can perform most cold staining – e.g., Gram stain and acid-fast staining – with a capacity of up to 20 slides simultaneously. A Gram staining cycle takes approximately 5 minutes.

Fig. 3. MULTISTAINER®



### Aerospray® Gram series 2

The Aerospray® Gram series 2, pictured in Fig. 4, is a benchtop staining system that can process more than 144 slides per hour. There is no cross-contamination

with the system as specimens contact only fresh stain, precisely metered from separate spray nozzles. The cycle time is as short as 5 minutes.

Fig. 4. Aerospray® Gram series 2



### QuickSlide™ GramPRO1™ automated Gram stain instrument

The QuickSlide™ GramPRO 1™ automated Gram stain instrument is one of several entirely automated instruments offered by Hardy Diagnostics. The GramPRO 1™ stains, decolorizes and counterstains each slide in 3.5–4.5 minutes. The system, pictured in Fig. 5, fits on a countertop. It has an easy user interface and operates on a push-button system with little hands-on time.

Fig. 5. QuickSlide™ GramPRO 1™ automated Gram stain instrument



Each of the automated gram staining instruments described above is easy to use, compact and could likely be used in Level II (and possibly Level I) laboratories in LMICs, but they are high-throughput instruments intended for use in laboratories where such capacity is required. In LMICs, this would most likely be in Level III settings and above.

## Automated specimen processing and inoculation of media

Although specimen preparation and processing may be done manually, there are also commercially available, semi-automated or automated instruments that provide standardized specimen processing and inoculation of media. The systems automate four main steps: (i) selecting the appropriate petri dish; (ii) inoculating the sample; (iii) spreading the inoculum on agar plates to obtain, upon incubation, well separated bacterial colonies; and (iv) accurate labelling and sorting of each inoculated medium.

These are large systems most appropriate for use in high-throughput, sophisticated laboratories at Level III and Level IV in LMICs. Systems include the BD™ Innova automated microbiology specimen processor (Becton Dickinson [BD], USA), BD Kiestra™ Inoqula+™ (BD, USA), PREVI® Isola (bioMérieux, France) and Copan WASP® DT: Walk-Away Specimen Processor (Beckman Coulter, a Danaher Corporation, USA).

### BD™ Innova automated microbiology specimen processor

The BD™ Innova automated microbiology specimen processor provides for complete automation of “front-end” processing of a variety of liquid samples. It can also streak various types of specimens without manual handling or changing components. The Innova, pictured in Fig. 6, is especially well suited to a high-volume laboratory due to its high capacity and extended walk-away time, although it can be used in smaller laboratories as well.

Fig. 6. BD™ Innova automated microbiology specimen processor



### BD Kiestra™ Inoqula+™

The BD Kiestra™ Inoqula+™ is a specimen processor that can handle both liquid and nonliquid bacteriology specimens – swabs, urine and other nonfluid samples.

The system’s rolling bead technology ensures discrete bacterial colonies and standardized streaking patterns. The system, pictured in Fig. 7, is high throughput, processing up to 250–400 inoculations per hour.

Fig.7. BD Kiestra™ Inoqula+™



### PREVI® Isola

The PREVI® Isola is an automated plate streaker that has five different-sized racks, one for each diameter of specimen tube. There are five input cassettes with a capacity of 30 plates in each stack; different agar plates can be loaded into each stack or each stack can hold the same type of agar. The PREVI® Isola, pictured in Fig. 8, processes a variety of specimens, including liquid specimens and swab systems with transport media such as liquid Amies medium to improve the diagnosis of aerobes, anaerobes and fastidious bacteria. The system offers consistent, automated and standardized inoculation/streaking, which optimizes bacterial colony isolation and eliminates risk of cross-contamination. The instrument is high throughput, processing 180 plates per hour, and as such is best suited to large laboratories.

Fig. 8. PREVI® Isola



## Copan WASP® DT: Walk-Away Specimen Processor

The Copan WASP® DT is an open-platform, modular instrument for specimen processing, including streaking as well as Gram slide preparation and enrichment broth inoculation. Pictured in Fig. 9, the system employs specimen load and unload conveyers with different-sized pallets for different-diameter tubes; it uses a universal decapper that decaps and recaps different-sized containers without any manual interface. The WASP® DT uses metal loops with 1, 10 and 30 µL sizes to inoculate plates rather than pipette tips. Samples can be loaded continually onto the instrument without batching, and the system accommodates most specimen types, including swabs, urine, faeces, sputum, body fluids and pre-enrichment broths. Like the other specimen systems described above, the WASP® DT is most appropriate for large, sophisticated labs that require high throughput.

Fig. 9. Copan WASP® DT



For a laboratory considering the selection of any microbiology specimen processing instrument, the following factors should be included in the selection: accuracy, capacity, manufacturer's technical support, modularity, flexibility (e.g., specimen types, loops, inoculation protocols, medium options, interface, if any, with laboratory information systems and cost) (33).

## Automated culture systems

Bacterial cultivation can be done manually, but a number of automated culture systems, which generally use blood or other sterile body fluids, are also available. The primary commercial culture incubation systems currently available are: BD BACTEC™ FX (BD, USA), BACT/ALERT® 3D (bioMérieux, France), BACT/ALERT® VIRTUO® (bioMérieux, France) and VersaTREK™ (Thermo Fisher Scientific, USA). These systems are discussed in more detail below.

Because in LMICs most bacterial culture is done in Level III and Level IV laboratories, the systems would

be most appropriate for use in those facilities. Further, most of the systems described below are high throughput, and their selection and implementation should be driven by testing needs.

## BD BACTEC™ FX

The BD BACTEC™ FX is a fully automated microbiology system designed to detect microbial growth from blood specimens. The system has a very sensitive fluorescent sensor of CO<sub>2</sub> production and a vial-activated workflow that allows for hands-off processing. It uses standard aerobic and anaerobic broth media specifically designed for small blood volume inoculation. The system has specific algorithms for fastidious organisms.

The most common configuration of the FX, pictured in Fig. 10, is a two-module system designed as a stack. The stack contains four drawers, each with a 100-vial capacity. Smaller-volume laboratories can choose a single, top-unit system. For high-volume capacity, multiple (up to 20) stack/top units can be seamlessly integrated into a single system using BD EpiCenter™.

Fig. 10. BD BACTEC™ FX



## BACT/ALERT® 3D

The BACT/ALERT® 3D platform is used for detecting the presence or absence of microorganisms in blood and sterile body fluids. It is a culture system that uses the colorimetric sensing of CO<sub>2</sub> production, which is designed to detect bacteria (as well as fungi and yeasts) early, even with delayed entry of 24 hours or more depending on culture bottle type.

The BACT/ALERT® 3D is modular, as illustrated in Fig. 11, which enables flexible, ergonomic configuration to meet space limitations. The control module manages all bottle inventory and data, while the incubator module performs the testing. Modules can be configured to accommodate volumes of 7000–84 000 bottles yearly. The system can be configured with or without laboratory information system (LIS) connectivity.

Fig. 11. BACT/ALERT® 3D



### BACT/ALERT® VIRTUO®

The BACT/ALERT® VIRTUO®, pictured in Fig. 12, is a more advanced and automated version of the BACT/ALERT® 3D blood culture system and uses the same detection principle – colorimetric detection by pH sensors of CO<sub>2</sub> produced by growing microorganisms (bacteria, fungi and yeasts). Improvements over the BACT/ALERT® 3D include incorporation of new instrument design to improve temperature stability, workflow improvement via automation of processes that are currently performed manually, an improved user interface and an enhanced proprietary algorithm to shorten time to detection of positive cultures.

Fig. 12. BACT/ALERT® VIRTUO® system



The VIRTUO® is a very high throughput system that holds 428 bottles; four instruments can be linked with a common loading area. The loading bay is motion activated with optical sensors to scan and automatically load BACT/ALERT® bottles via a conveyor belt and internal robotic arm. The scanning station rotates and images the entire BACT/ALERT® bottle for definitive ID from barcoded labels. Positive bottles are unloaded into an external chute automatically

or on demand, and negative bottles are unloaded into a removable waste container at the end of the culture period. The instrument's software has automated detector cell quality control and calibration and may be connected to an LIS or to Myla middleware (bioMérieux) (34).

### VersaTREK™

Thermo Fisher Scientific offers both manual and automated blood culture systems, including the only instrument that has four FDA-cleared tests on one platform for blood culture, sterile body fluids, mycobacteria detection and MTB susceptibility testing. VersaTREK™ is the only culture system capable of detecting any gas produced or consumed by organisms, not just CO<sub>2</sub>, which means that it is able to detect a wider range of both common and fastidious bacteria.

The VersaTREK™ system, pictured in Fig. 13, uses only two bottles and uses VersaTREK™ REDOX™ media, which have distinct characteristics, including specimen draws as low as 0.1 mL and true direct-draw bottles, both of which are FDA cleared. The VersaTREK™ system features scan functions, simple bottle removal and is generally designed for ease of use.

Fig. 13. VersaTREK™



### Biochemical testing

In addition to the commercial, automated or semi-automated culture systems described above, there are also commercially available manual and automated systems for biochemical testing to identify bacterial pathogens. In general, these systems simultaneously inoculate and incubate a series of miniaturized biochemical reactions based either on detecting bacterial enzymes or on cellular products that do not require bacterial growth. To identify bacteria, the enzymatic or biochemical results of the tests are combined and compared to the characteristics of bacterial organisms contained in a computer database. Some of these methods have reasonably rapid TAT, e.g., 2–4 hours,

compared to methods that require microbial growth, which require overnight incubation. But some of these assays still require 18–24 hours. Commercially available manual and automated systems for bacterial ID are described below. Note that diagnostic systems that both identify bacterial pathogens and perform AST are discussed later in this report.

Manual biochemical ID systems are typically easy to use and low cost relative to automated phenotypic and genotypic methods of pathogen ID. They do, however, require access to a computer database, usually via the Internet, for comparison of the characteristics of bacterial organisms found. In general, manual tests rely importantly on the skills of a well trained microscopist. Automated pathogen ID systems provide higher throughput as well as automatic analysis and comparison of pathogens, reducing the role of the microscopist. Results from either manual or automated systems are influenced by culture conditions. It is likely that automated biochemical ID systems, in particular, should not be implemented below Level III in the laboratory structure in LMICs for reasons of throughput, infrastructure requirements and human resource requirements.

### Manual bacterial ID systems

#### API® (bioMérieux, France)

BioMérieux offers a family of test strips (Fig. 14) for manually identifying bacteria to the species level from microorganisms isolated in an appropriate culture medium. The test kits, which consist of microvials on a plastic strip that contain dehydrated substrates to demonstrate enzymatic activity or carbohydrate fermentation, can identify gram-positive and gram-negative bacteria as well as yeast. The system offers a large database which can be accessed through the Internet via the company’s APIWEB™ service.

Fig. 14. API 20E test strips



The API® product line includes the following bacterial ID strips:

#### API gram-negative ID

- API 20E – 18–24-hour ID of *Enterobacteriaceae* and other nonfastidious gram-negative bacteria

- API Rapid 20E – 4-hour ID of *Enterobacteriaceae*
- API 20NE – 24–48-hour ID of gram-negative non-*Enterobacteriaceae*
- API NH – 2-hour ID of *Neisserial/Haemophilus*

#### API gram-positive ID

- API Staph – overnight ID of clinical staphylococci and micrococci
- RAPIDEC® Staph – 2-hour ID of commonly occurring staphylococci
- API 20 Strep – 4- or 24-hour ID of streptococci and enterococci

#### API anaerobe ID

- API 20A – 24-hour ID of anaerobes
- Rapid ID 32A – 4-hour ID of anaerobes.

#### BBL™ Crystal™ identification system (BD, USA)

The BD BBL™ Crystal™ identification system is a manual method of biochemical testing which is considered high complexity by the FDA. It utilizes miniaturized fluorogen- and/or chromogen-linked substrates to detect enzymes that bacteria use to metabolize a variety of substrates. The system, pictured in Fig. 15, consists of BBL Crystal panel lids, bases and inoculum fluid tubes. Only one step is required for inoculation, and the tubes provide a closed system when the bases and lids are snapped into place. Following the recommended incubation time, the vials are examined manually for colour changes or the presence of fluorescence. The resulting pattern of positive and negative test scores is used for bacterial ID with the BBL Crystal RGP (rapid gram-positive) database. Identification is derived from a comparative analysis of the reaction pattern of the test isolate to those contained in the database.

Fig. 15. BBL™ Crystal™ identification system



The BD™ BBL Crystal™ identification system is not intended for use directly with clinical specimens, but rather from culture isolates. It includes the following assays:

- Enteric/nonfermenter ID kit: an overnight ID method for identifying clinically significant aerobic gram-negative *Enterobacteriaceae* isolates and non-fermenting gram-negative rods;
- Neisseria/Haemophilus ID kit: a 4-hour ID method for identifying *Neisseria*, *Haemophilus* and other fastidious bacteria;
- Gram-positive ID kit: an 18-hour ID method for identifying both gram-positive cocci and bacilli;
- Rapid Gram-positive ID kit: a 4-hour ID method using conventional, fluorogenic and chromogenic substrates to identify gram-positive bacteria isolated from clinical specimens; and
- Anaerobe ID kit: a 4-hour ID method for identifying clinically significant anaerobic organisms.

### RapID™ systems (Thermo Fisher Scientific, USA)

Thermo Fisher Scientific's RapID™ systems (Fig. 16) comprise microbial ID systems based on enzyme technology. These are manual systems that simplify identifying bacteria and other microorganisms from cultured specimens with TAT of 4 hours.

Fig. 16. RapID™ system



Although the systems are manual, RapID™ requires no oil and no pipetting; the systems produce visible colour reactions. Time to result is 4 hours. The product line includes, but is not limited to:

- RapID™ ONE system: identifies over 70 medically important oxidase-negative, gram-negative bacilli;
- RapID™ ANA II system: identifies over 90 clinically important anaerobes;
- RapID™ NH system: identifies 30 taxa, including *Neisseria*, *Moraxella*, *Haemophilus* and related microorganisms;

- RapID™ NF PLUS system: identifies over 70 clinically important oxidase-positive, gram-negative bacilli, including *Vibrio* spp.;
- RapID™ STAPH PLUS system: identifies 40 different staphylococci and related genera;
- RapID™ STR system: identifies streptococci and related genera; and
- RapID™ SS/u system: identifies commonly isolated urinary tract pathogens in 2 hours.

RapID™ systems are paired with ERIC™ software and databases for faster ID of bacteria. The databases are updated regularly to provide better reporting coverage for thorough analysis.

### Oxoid™ Microbact™ biochemical systems (Thermo Fisher Scientific, USA)

Oxoid™ Microbact™ biochemical systems (Fig. 17) are manual systems currently available for identifying gram-negative organisms, *Staphylococcus aureus* and *Listeria* from pure culture isolates. ID is based on pH changes in various substrates and substrate utilization tests. The systems are designed for use with Microbact™ software, which features an expanded and regularly updated database compatible with 64-bit computer systems. Kits include:

- Microbact™ GNB kits: identify *Enterobacteriaceae* and common miscellaneous gram-negative bacilli. TAT is between 24 and 48 hours; and

Fig. 17. Microbact™ biochemical identification kit 24E for gram-negative bacteria



- Oxoid™ Microbact™ Staphylococcal 12S kit: identifies staphylococci, including *Staphylococcus aureus* and coagulase-negative staphylococci (CNS). TAT is 24–36 hours.

## Automated bacterial ID systems

### Biolog microbial identification systems (Biolog, Inc., USA)

Biolog offers three microbial ID systems: the OmniLog ID, a fully automated system (pictured in Fig. 18); the MicroStation ID, a semi-automated system; and the MicroLog M system, a manual ID system. Each system is capable of identifying bacteria, yeast and fungi, and all use the GENIII MicroPlate test panel, which is capable of identifying both gram-negative and gram-positive bacteria at the same time on a single panel. Gram stain and other pretests are not required on the systems.

Fig. 18. OmniLog ID system



Biolog systems use oxidation-reduction chemistry by which GENIII dissects and analyses the ability of a cell to metabolize all major classes of biochemicals,

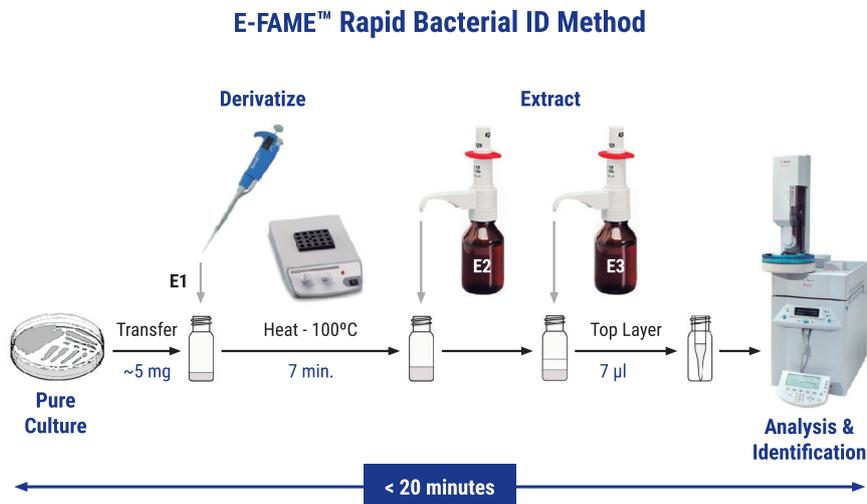
in addition to determining other important physiological properties such as pH, salt and lactic acid tolerance. Biolog systems produce a characteristic pattern or “metabolic fingerprint” from discrete test reactions performed in a 96-well microplate. Culture suspensions are tested with a panel of preselected assays, then incubated, read and compared to databases of human pathogens using the company’s RetroSpect 2.0 software tool. Depending on the panel run, TAT is 4–6 hours or 16–24 hours (35).

### Sherlock™ microbial identification system (MIDI, Inc., USA)

The Sherlock™ microbial identification system (MIS) analyses and identifies microorganisms isolated in pure culture or artificial media. The MIS uses a sample preparation procedure and gas chromatography (GC) analysis of extracted microbial fatty acid methyl esters (FAMES) to yield qualitatively and quantitatively reproducible fatty acid composition profiles. Fatty acids extracted from unknown bacteria are automatically quantified and identified by the Sherlock software to determine the fatty acid composition. The fatty acid profile is then compared to a library of profiles of reference strains stored in the computer to determine the identity of the bacteria.

The recently introduced Sherlock™ E-FAME™ assay, an acid-catalysed GC-FAME extraction and analysis method, is used to identify clinically important aerobic bacteria in less than 20 minutes. As illustrated in Fig. 19, the system is labour intensive and is designed for use in reference laboratories.

Fig. 19. E-FAME™ bacterial identification system workflow



## 216Dx UTI system (BacterioScan, USA)

BacterioScan has developed the 216Dx UTI system, which is a compact, semi-automated in vitro diagnostic system that uses forward laser light scattering (FLLS), analysing the angular variation in the intensity of the scattered light to measure bacterial growth directly from urine specimens incubated in trypticase soy broth. The BacterioScan 216Dx is for the qualitative determination (presumptive positive or presumptive negative) of bacteriuria at a defined cut-off, and is intended for use in conjunction with other clinical and laboratory findings to aid in diagnosing UTIs. The BacterioScan 216Dx has a limited scope as it is not intended to provide bacteriuria levels, bacterial ID or differentiation; presumptive positive urine samples must be cultured (as must presumptive negative urine samples if a low level of bacteriuria is suspected and is clinically relevant).

The BacterioScan 216Dx system, pictured in Fig. 20, consists of an instrument and handheld barcode scanner, a network appliance computer with preloaded software, required power and interconnecting Ethernet cable(s). BacterioScan provides single-use, disposable cartridges and multicuvettes for use in the system. One multicuvette contains four cartridge wells and can be used to test up to four samples; up to four multicuvettes can be processed by the 216Dx in a single run – or 16 samples total.

Fig. 20. BacterioScan 216Dx and multicuvettes



The 216Dx, which is FDA cleared, examines changes in optical signals which represent bacterial growth in the urine samples over a 3-hour period. Once the testing is complete in the multicuvette, the analysis is available.

It should be noted that despite advances with respect to automated specimen processing, plating and biochemical testing, nonautomated methods are often still needed for unusual pathogens or fastidious microorganisms that fail to grow. In addition, some clinical isolates of microorganisms may produce a biofilm or be too viscous for automated instruments, which will result in the inability to make an ID (28).

## Conclusion

Although the phenotypic methods of bacterial ID described above are still the backbone of diagnostic bacteriology, there are drawbacks to their use that affect LMICs significantly. The test methods are slow and, when done manually, cumbersome. The growth of bacteria in culture, for example, can take days to obtain results, especially for fastidious or slow-growing bacteria. In addition, there are operational challenges to performing bacterial culture in LMICs. These include, but are not limited to, inconsistent electricity, dust, lack of climate control and human resource constraints (36, 37).

As indicated above, bacterial cultivation for pathogen ID is generally limited to Level III and Level IV laboratories in LMICs. Even the use of manual phenotypic methods of bacterial ID requires well trained and highly skilled microscopists, who are often not available at Level II settings and below. Automated systems are often high throughput and require suitable infrastructure to accommodate the instrumentation, including appropriate space, consistent power supply, climate control, running water and access to transport. These capabilities and requirements also suggest the use of these phenotypic testing methods at Level III and above.

Recently, Ombelet and colleagues proposed a framework for implementing clinical bacteriology that would be suitable for use in basic diagnostic laboratories operated by technicians without expertise in microbiology in LMICs (37). This approach, together with using existing diagnostics (e.g., manual bacterial ID and biochemical test methods) and improved algorithms, could make clinical bacteriology possible in Level II facilities. In addition, however, simpler, faster methods of bacterial pathogen ID are needed. Immunoassay and molecular testing to identify bacterial pathogens are alternatives and are discussed next (3).

## Immunoassay methods of identifying bacterial pathogens

Rapid immunoassays, which use the binding of antibodies to antigens to identify and measure certain substances, are also available for detecting bacterial pathogens. The tests below, all of which are rapid diagnostic lateral flow assays which, unless otherwise indicated, require little or no ancillary equipment, are a selection of commercially available immunoassays relevant to ABR. While these tests can and are used in Level I settings in LMICs, it is important to consider the performance of each assay to determine whether its sensitivity and specificity are adequate for the intended use of the test and whether its ease of use is appropriate for the intended setting. Also, given

the frequent need to perform syndromic testing on patients presenting at Level I and Level II settings – e.g., for acute febrile illness, respiratory and enteric infections, among others – it is important to note that most lateral flow assays can only perform limited multiplexing. In addition to the physical limitations of such tests, there are also technical challenges, the most important of which is potential cross-reactivity (38, 39).

- Oxoid™ PBP2' latex agglutination test (Thermo Fisher Scientific, USA): a rapid latex slide agglutination test for detecting PBP2' from culture as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA) and in CNS. TAT is approximately 3 minutes after positive culture. The assay is FDA cleared.
- Clearview® Exact PBP2a test (Abbott, USA): a rapid immunochromatographic qualitative assay for detecting PBP2a direct from *Staphylococcus aureus* culture isolates as an aid in detecting MRSA. TAT is approximately 5 minutes after positive culture. The assay is FDA cleared.
- RAPID™ Hp StAR™ (Thermo Fisher Scientific, USA): an immunochromatographic assay for detecting *Helicobacter pylori* antigen in human stool samples. TAT is 15–20 minutes. The assay is CE marked.
- RAPIRUN® H. pylori antibody detection kit (Otsuka Pharmaceutical Co., Ltd., Japan): a rapid immunochromatographic assay intended for qualitatively detecting antibodies against *Helicobacter pylori* in urine to aid in diagnosing the infection. TAT is about 20 minutes. The assay is FDA cleared.
- ImmunoCard STAT!® CAMPY (Meridian Bioscience, Inc., USA): an immunochromatographic rapid test for qualitatively detecting specific *Campylobacter* antigens (*C. jejuni* and *C. coli*) in human stool, where stool may be either unpreserved or preserved in Cary-Blair-based transport media. TAT is 20 minutes. The assay is CE marked.
- C. DIFF QUIK CHEK COMPLETE® (Abbott, USA): a rapid membrane enzyme immunoassay for simultaneously detecting *Clostridium difficile* glutamate dehydrogenase antigen and *C. difficile* Toxin A (TcdA) and *C. difficile* Toxin B (TcdB) in a single reaction well. The test is to be used as an aid in diagnosing *C. difficile* infection (CDI). TAT is less than 30 minutes. The assay is FDA cleared.
- Xpect™ C. difficile Toxin A/B test (Thermo Fisher Scientific, USA): a rapid in vitro immunochromatographic assay for the direct, qualitative detection of TcdA and/or TcdB in human faecal specimens from patients suspected of having CDI. The test is intended as an aid in diagnosis. TAT is 20 minutes. The assay is FDA cleared.
- ImmunoCard® Toxins A&B (Meridian Bioscience, Inc., USA): a rapid, qualitative, horizontal-flow enzyme immunoassay for detecting TcdA and TcdB in human stool. The test is to be used as an aid in diagnosing CDI. TAT is about 15 minutes. The assay is CE marked.
- BioStar® OIA GC (Thermo Fisher Scientific/BioStar, USA): a rapid optical immunoassay test for qualitatively detecting gonococcal antigen in female endocervical swab and male urine specimens. The test is intended for use as an aid in identifying the presence of NG antigen. TAT is 25–40 minutes.
- BinaxNOW® S. pneumoniae Antigen Card (Abbott, USA): a rapid, qualitative in vitro immunochromatographic assay that detects the C-polysaccharide antigen of *Streptococcus pneumoniae* in 15 minutes from human urine. It is intended as an aid in diagnosing community-acquired pneumonia (CAP). The test can be read visually or with the use of the DIGIVAL reader. The assay is CE-IVD marked.
- BIOSYNEX S. pneumoniae (BIOSYNEX, S.A., France): a rapid immunochromatographic test for detecting *Streptococcus pneumoniae*-specific antigen in urine and cerebrospinal fluid (CSF). It is intended as an aid in diagnosing CAP. TAT is 15 minutes. The test can be read visually or with the use of the BIOSYNEX Flexireader®. The assay is CE-IVD marked.
- Typhidot® (Malaysian Biodiagnostic Research, Malaysia): a qualitative enzyme-linked immunosorbent assay (ELISA) in vitro diagnostic assay for detecting *Salmonella typhi* in serum. It detects either immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies against a specific antigen on the outer membrane protein of *S. typhi*. For specimens that are indeterminate (IgM negative and IgG positive), a confirmatory test, Typhidot-M, is recommended by the manufacturer. TAT is 60 minutes.
- TUBEX® (IDL Biotech, Sweden): a semi-quantitative in vitro diagnostic Inhibition Magnetic Binding Immunoassay (IMBI) assay that tests for antibodies against *Salmonella typhi* lipopolysaccharide (LPS) antigen in serum by quantifying the inhibition of binding between O9 monoclonal antibodies and LPS-coupled magnetic particles. A visible decolourization of the serum in the test reagent solution through magnetic particle separation indicates a positive result.

Samples are graded 0 to 10 according to the colour reaction; those with a grade greater than 2 are considered positive. TAT is 3 minutes. The assay is CE-IVD marked.

- Wellcogen™ Haemophilus influenzae b Rapid latex agglutination test (Thermo Fisher Scientific, USA): a rapid latex diagnostic test for the qualitative detection of antigen from *Haemophilus influenzae* type b from CSF, serum, urine or blood cultures. TAT is 3 minutes. The assay is CE-IVD marked.
- O157 Coli-Strip (Coris BioConcept, Belgium): a qualitative in vitro immunochromatographic assay for detecting *Escherichia coli* O157 bacteria in stool specimens (after broth enrichment). TAT is 15 minutes. The assay is CE-IVD marked.
- ImmunoCard STAT!® E. coli O157 Plus (Meridian Bioscience, Inc., USA): a rapid, qualitative, horizontal-flow enzyme immunoassay for detecting *Escherichia coli* O157:H7 in stool specimens or culture (broth enrichment or plate culture). TAT is less than 20 minutes. The assay is CE-IVD marked.

## Molecular methods of identifying bacterial pathogens

Molecular testing methods, which detect specific sequences of nucleic acids in DNA and RNA, have substantially changed the microbiological diagnosis of pathogens over the last decade. In particular, nucleic-acid-based test methods are widely used in clinical laboratory diagnostics. Molecular methods generally fall into one of three categories: (i) hybridization, (ii) amplification or (iii) sequencing (40).

### Hybridization methods

Hybridization assays use labelled oligonucleotide probes, which can confirm ID by culture or can directly detect microorganisms. For example, probe hybridization can be useful for identifying slow-growing bacteria after isolation in either liquid or solid media. Although this method displays high specificity, it requires a large number of target cells to achieve high sensitivity.

Fluorescence in situ hybridization (FISH) is a method that allows rapid detection and ID of the genus and species of bacteria (as well as yeasts and protozoa), combining the speed and ease of use of conventional bacterial staining methods with the specificity of molecular methods (41). The technique involves fluorescent-labelled probes that hybridize with target sequences of ribosomal RNA (rRNA); the probes fluoresce upon binding to the target and are detected by fluorescent microscopy (41).

In general, FISH methods have a short time to result (60–90 minutes) and hands-on time of about 15–20 minutes. FISH can be used to detect bacteria and other microorganisms in primary specimens (i.e., tissue samples), which also shortens the time to result, and it can be used to identify certain antimicrobial drug resistance, primarily in enterococci (41). FISH requires little equipment, but it does require experienced and well trained technicians, including a knowledgeable microscopist. In addition, detecting bacteria using FISH requires a targeted approach – meaning that the nature of the bacterial infection must be anticipated before probes are chosen.

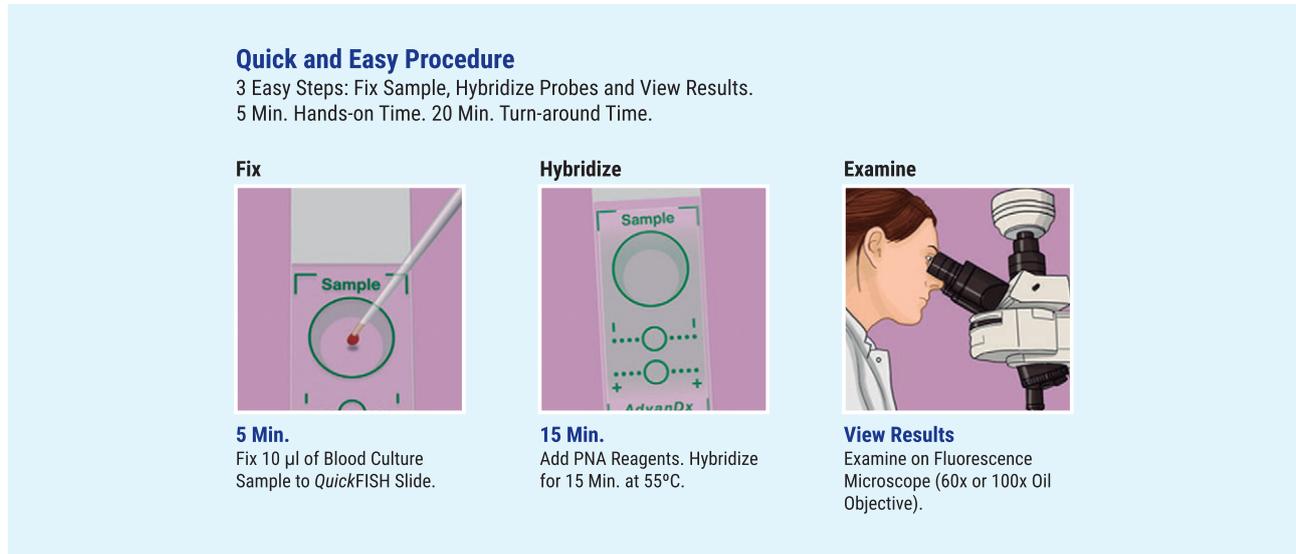
There have been a number of innovations and improvements on the basic FISH method since its introduction in 1980, one of which replaces standard DNA and RNA nucleic acids with a synthetic peptide nucleic acid (PNA) probe (41). So-called PNA-FISH reduces the number of steps in the test procedure and improves its specificity, although it is still laborious because individual probes have to be created for each bacterial species. PNA-FISH has also been standardized, and FDA-approved assays are available.

PNA-FISH requires only a microscope equipped with a fluorescent lamp and dual band filters for interpreting results. Therefore, the capital equipment cost of implementing the technology is low, and the technique is relatively easy to perform in a clinical laboratory. PNA-FISH does, however, require an accurate choice of PNA-FISH probe, which is contingent upon the correct interpretation of a Gram stain. As mentioned above, good performance of FISH methods generally requires an experienced and highly trained microscopist. In addition, the commercialized PNA-FISH methods described below require culture. Collectively, these factors suggest that PNA-FISH could be performed in LMICs, but likely only at Level III and above facilities.

### AdvanDx (OpGen, USA)

OpGen offers two AdvanDx FISH product lines, AdvanDx PNA FISH® and *QuickFISH*®, both of which are for the early pathogen ID of BSIs (bacteria and yeast) from positive blood cultures. The tests utilize FISH with PNA probes. Results are available about 48–72 hours earlier than using conventional phenotypic methods. AdvanDx PNA FISH® offers the following tests: *S. aureus*/CNS, *E. faecalis*/OE (other enterococci), gram-negative organisms and *Candida*. *QuickFISH*® offers tests for *Staphylococcus*, *Enterococcus*, gram-negative organisms and *Candida*. The *QuickFISH*® procedure is illustrated in Fig. 21. A number of the available tests are FDA cleared and CE-IVD marked.

Fig. 21. QuickFISH® procedure



## Amplification methods

This section of the landscape sets out the various molecular amplification methods to detect bacteria and other pathogens. Unless otherwise noted, there are commercially available platforms relevant to bacterial pathogen ID using each of the methods described. In this report, all of the test methods are described first; applicable commercial platforms are then described in some detail.

Although molecular hybridization methods are highly specific for bacterial detection and ID, their sensitivity is limited, particularly for fastidious organisms. Also, fluorescent imaging techniques require sufficient quantities of target sequences to generate adequate signal-to-noise ratios and reduce the probability of false positive results. In contrast, molecular methods that amplify target nucleic acid reaction in an organism can enhance bacterial pathogen detection and ID without sacrificing specificity (28). NAATs work on this principle.

Amplification methods are either designed to increase the number of target molecules (selected sections of DNA or RNA) to a level that permits detection (target amplification methods) or are aimed at increasing the signal generated by the method (signal amplification methods). The majority of commercially available NAAT platforms today are based on target amplification.

Regardless of whether a NAAT assay is based on target amplification or signal amplification, it will consist of the following three steps: (i) pre-amplification sample preparation and/or nucleic acid extraction; (ii) amplification of either the nucleic acid target or

detection signal; and (iii) post-amplification detection and/or quantification of the amplified nucleic acids.

*Pre-amplification.* Pre-amplification methods (sample preparation and/or nucleic acid extraction) are critical to the NAAT process. For each sample to be analysed correctly and to produce an accurate result, the nucleic acid must be both available for the reaction and purified. Protocols for pre-amplification steps include purifying the sample for specific organisms or cells, extracting nucleic acids and capturing target sequences in specimens. Molecular methods are sensitive to the extraction and processing steps prior to amplification; prompt processing of samples, rapid extraction methods and appropriate storage of specimens lead to improved sensitivity of the assays.

*Amplification.* There are several amplification methods used to detect RNA or DNA after preparing samples. In target amplification, the target sequence is identified and millions of copies of a portion of the nucleic acid are synthesized via an amplification reaction; in effect, this method enhances the ability to detect very low levels of nucleic acids that occur naturally in the specimen. In signal amplification methods, the reporter molecule generates a signal that is amplified. Some benefits to signal amplification include higher specificity and the ability to conduct isothermal reactions that do not require thermocycling.

*Post-amplification.* Finally, post-amplification methods require the detection and/or quantification of either the amplification products (in target amplification methods), called amplicons, or the signals

that have been generated (in signal amplification methods). Detection can be achieved using any one of a number of reagents, e.g., colorimetric, radioactive or fluorescence. Detection can either be done at the endpoint of the process (after a fixed number of amplification cycles) or in “real time” (after each amplification cycle).

Specific molecular methods of amplification and detection commonly used in commercial NAAT platforms are described below.

### *Polymerase chain reaction (PCR)*

Of the NAAT-based molecular methods, PCR, which was first introduced by Kary Mullis as a research tool in 1983, is the most common method used and “forms the backbone of molecular diagnostics in clinical microbiology” (42). PCR is an enzymatic process that exponentially amplifies a single copy of a nucleic acid target (selected sections of DNA), which may be undetectable by standard hybridization methods, to  $10^7$  or more copies in a relatively short time. There are three primary steps in conventional PCR: (i) denaturing double-stranded DNA at 95 °C; (ii) binding (annealing) PCR primers to the target sequence at 50–60 °C; and (iii) extending and polymerizing nucleic acids to the primer at 72 °C to generate amplicons of the target sequence (43). These steps are followed sequentially over multiple cycles (“thermocycling”); each cycle exponentially increases the amount of amplicon, as each amplicon serves as a template for additional amplification. Following amplification (i.e., a predetermined number of cycles), amplicons are available in sufficient quantities to be detected/visualized by fluorescence, hybridization or other methods. Detection requires using a labelled probe specific for the target sequence in the amplicon; this allows the amplicon to be visualized and ensures that the amplicon is the target sequence of interest (28).

Over the years, there have been variations and improvements on basic PCR-based NAAT. These methods include nested PCR, which can achieve greater specificity; quantitative PCR (qPCR), which quantitates the number of targets in a specimen; and digital PCR (dPCR), used to directly quantify and clonally amplify nucleic acid strands (DNA or RNA). Also of note, these methods include real-time PCR and multiplex PCR, which are discussed in more detail below.

### *Real-time PCR*

Real-time PCR arguably has had the greatest impact on detecting and identifying human pathogens in clinical microbiology laboratories (44). This method monitors the quantity of amplicons over time (after each cycle) rather than at the end-point

of the reaction; this real-time monitoring of the amplicon enables mathematical extraction of the starting concentration of target sequence (quantification). The method combines thermal cycling (repeated heating and cooling cycles) with fluorescent probes which bind to the amplicons as they are generated in the same reaction vessel; as such it is a closed system, which minimizes the risk of contamination. Since signal detection is monitored in real time, real-time PCR often leads to faster results than end-point PCR.

### *Multiplex PCR*

Also of note is the development of multiplex PCR testing, which combines a number of primer pairs into a single PCR for simultaneously detecting several targets. This allows for the inclusion of control primers as well as test primers that can be directed to a sequence specific to the particular organism or gene of interest. This approach is particularly useful in testing patients presenting with symptoms that could be attributable to a number of different pathogens – e.g., for use in patients with symptoms of an upper respiratory infection or enteritis.

A number of different PCR-based instruments and detection probe formats are available commercially (40). Many of these are real-time systems and some are capable of multiplexing.

### *Non-PCR-based molecular methods – isothermal amplification*

Non-PCR-based molecular methods include signal amplification (e.g., branched DNA) and some methods that use both target and signal amplification. But, in recent years, amplification techniques have turned to isothermal methods of nucleic acid amplification, or iNAATs, which eliminate the need for the rapid thermal cycling required by PCR-based techniques and can be more specific due to the non-temperature dependence of the reactions (45). They can also be combined with other detection technologies, e.g., fluorescent-probe-independent methods that eliminate the requirement of sophisticated optics. The most common iNAAT methods for which commercial platforms are available are described briefly below.

### *Transcription-mediated amplification (TMA) and nucleic acid sequence-based amplification (NASBA)*

Unlike PCR-based testing, TMA and NASBA amplify RNA rather than DNA. They use reverse transcriptase (RT) replication mechanisms to produce a modified complementary DNA molecule (cDNA) from an RNA template, which is then rapidly amplified into RNA amplicons; in other words, they effectively imitate *in vivo* retroviral replication

mechanisms to produce RNA amplicons from the RNA template (46). To date, most assays using NASBA or TMA target a single or a few analytes, using one or only a few oligonucleotide primer sets (46). Both methods can be used for identifying microorganisms.

#### *Loop-mediated isothermal amplification (LAMP) and helicase-dependent amplification (HDA)*

LAMP amplifies DNA under isothermal conditions. It generally uses four specifically designed primers to recognize six different areas of the DNA target combined with strand displacement activity. In brief, unlike PCR, which uses heat to denature and anneal primers to the target sequence, LAMP relies on complex binding kinetics and physical proximity of the target sequences and primers in a loop in order to generate a single-strand template without the need for heat denaturation (42, 46). Although high levels of amplicons can be generated at 60–65 °C and are generally achieved in less than an hour, sensitivity and specificity are reduced with LAMP.

HDA uses thermostable helicase enzymes to effect DNA strand separation. Once separated, single-stranded DNA binding proteins stabilize the single strands to allow binding of the PCR primers. DNA polymerase extends the primers, and the newly synthesized DNA duplexes serve as templates that are then hybridized by sequence-specific primers for further amplification cycles (28, 41, 42). Exponential amplification can be achieved at a single amplification temperature (60–65 °C), generally in 60–90 minutes (40).

#### *Strand displacement amplification (SDA)*

SDA uses bifunctional primers that incorporate both target recognition and endonuclease target regions. First, endonucleases make cuts or nicks at a specific site in the target sequence; then strand-displacing DNA polymerase, typically *Bst* DNA polymerase, Large Fragment (the original polymerase for LAMP), is used to initiate replication (41). The nicking site is then regenerated with each polymerase displacement step, and DNA is exponentially amplified. Amplification takes place at a temperature range of 55–59 °C.

In addition to the isothermal methods of nucleic acid amplification described above, there are additional methods described in the literature. These include the nicking enzyme amplification reaction (NEAR), recombinase polymerase amplification (RPA), signal-

mediated amplification of RNA technology (SMART), rolling circle amplification (RCA), isothermal multiple displacement amplification (IMDA), single primer isothermal amplification (SPIA) and circular helicase-dependent amplification (cHDA).<sup>5</sup> These methods are not described in detail in this report because currently no commercialized platforms utilize them.

## Commercially available platforms using molecular amplification technologies for detecting bacterial pathogens

Below are some of the major commercial IVD platforms that use molecular amplification techniques to identify bacterial and other pathogens. Generally, in the early years NAATs were focused on detecting viruses; real-time PCR has paved the way for their use in detecting bacterial pathogens (40). Most tests are qualitative, and until recently, they were monoparametric – one analyte per assay (40). Only in recent years have multiparametric NAATs been developed, in particular for detecting BSIs direct from whole blood specimens, as opposed to blood cultures.

The commercial NAAT platforms described below are automated and have single and/or multiparametric assays for detecting bacterial pathogens; many of them have separate automated instruments to perform sample preparation and nucleic acid extraction. Except where indicated that the platform is available only for laboratory-developed tests (LDTs), the assays are standardized and available in quality-assured kits. For the most part, these platforms are designed for use in sophisticated laboratories with highly trained laboratorians where high throughput is needed. As such, the platforms are not well suited for use at or near POC; where platforms are potentially appropriate for near-patient testing, it is indicated. This is a nonexclusive list of NAAT platforms, which is limited to commercial platforms that can identify at least one bacterial pathogen on the prioritized list in Annex I.

### **Abbott *m2000* system (Abbott, USA)**

Abbott manufactures the Abbott RealTime CT/NG assay, which is a real-time PCR assay for direct, qualitative detection of the genomic DNA (gDNA) of NG and the plasma DNA of CT on its automated *m2000* and *m24* systems. The CT/NG assay is currently the only one on the *m2000* system targeted at detecting a WHO priority bacterial pathogen.

The assay may be used to test the following swabs from symptomatic patients: female endocervical

<sup>5</sup> For details of these methods, see Gill and Ghaemi (45).

swabs, clinician- or patient-collected vaginal swabs; male urethral swab specimens; and male and female urine swabs. The assay may also be used to test the following swabs from asymptomatic patients: clinician- or patient-collected vaginal swabs; and male and female urine swabs.

Additional RealTime assays offered by Abbott for the *m2000* system include cytomegalovirus (CMV), human immunodeficiency virus-1 (HIV-1 Quantitative), hepatitis B virus (HBV), hepatitis C virus (HCV), HCV Genotyping II and Zika virus (Emergency Use Authorization test).

The Abbott RealTime CT/NG assay (and the other assays listed above) can be automated using the Abbott *m2000rt* for amplification and detection and one of three methods for sample preparation: (i) manual (for laboratories with low-throughput requirements); (ii) the *m24sp* instrument (for laboratories with low-to medium-throughput requirements); or (iii) the *m2000sp* instrument (for laboratories with medium-to high-throughput requirements).

The *m24sp* pictured in Fig. 22 is a benchtop sample preparation and extraction device with a small footprint that is generally appropriate for facilities with medium-throughput requirements. It provides a variable extraction system (extraction output can be stored either in deepwell trays or 1.5 mL tubes) with ready-to-use and reusable reagents as well as flexible batch size capabilities.

Fig. 22. Abbott *m24sp* instrument



The *m2000sp* by Abbott (pictured on the left in Fig. 23) is a larger and more automated sample preparation device than the *m24sp*. With complete automation comes increased walk-away time for the operator. It is a high-throughput system with a maximum batch size of 96 samples per run; reagents and tips required for extraction are loaded manually by the operator. When combined with the Abbott *m2000rt*, the amplification and detection instrument, the system can provide automation from barcoded laboratory tube through to patient result.

Fig. 23. Abbott *m2000* system: *m2000sp* (left) and *m2000rt* (centre)



The Abbott *m2000rt* is the amplification and detection platform for use with manual extraction, the *m24sp* and the *m2000sp* instruments, as described above. It is a high-performance system, but is relatively compact, weighing just over 75 lbs. The *m2000rt* (pictured in Fig. 23, centre) can run 96 samples at a time in about 3 hours of cycling time (not including time for sample preparation). The system will run both quantitative and qualitative analyses.

### cobas® 6800/cobas® 8800 systems (Roche Molecular Diagnostics [Roche], USA)

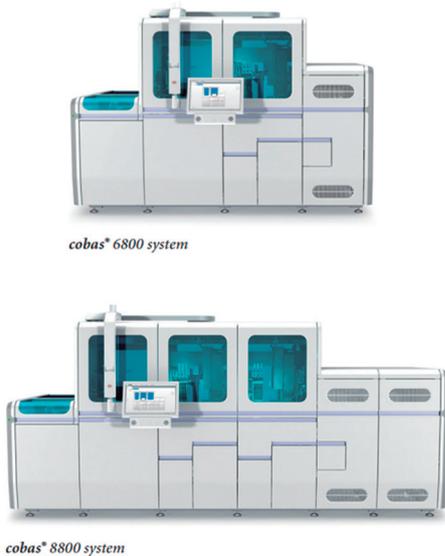
Roche offers the cobas® CT/NG assay for use on cobas® 6800/8800 systems. The assay is an automated, qualitative in vitro NAAT that utilizes real-time PCR for direct detection of CT and/or NG DNA in male and female urine, clinician-instructed self-collected vaginal swab specimens (collected in a clinical setting), clinician-collected vaginal swab specimens and endocervical swab specimens, all collected in cobas® PCR Media, and cervical specimens collected in PreservCyt® solution. The test is intended as an aid in diagnosing chlamydial and gonococcal disease in both symptomatic and asymptomatic individuals.

With respect to the cobas® CT/NG assay, target-specific primers and two probes are used to detect, but not discriminate between, the CT cryptic plasmid and the *ompA* gene. Additionally, target-specific primers and two probes are used to detect, but not discriminate between, two conserved sequences in the NG DR-9 region. A DNA internal control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing.

The cobas® 6800/8800 systems, pictured in Fig. 24, are CE-IVD marked, but not FDA cleared; they are therefore not currently available in the United States.

The systems offer the fastest time to results with the highest throughput available. The cobas® 6800/8800 systems are fully automated solutions designed for donor screening, viral load monitoring, women's health and microbiology testing.

Fig. 24. cobas® 6800 and 8800 systems



The 6800/8800 systems are available in medium- and high-throughput models. Each system provides results for the first 96 tests in less than 3.5 hours, with the 6800 system delivering up to 384 results in an 8-hour shift, and the 8800 system generating up to 960 results in the same amount of time. Both systems also allow for simultaneous processing of multiple assays and are designed to enable up to 8 hours (cobas 6800®) and 4 hours (cobas 8800®) of walk-away time with minimal user interaction.

In addition to the CT/NG assay, additional assays available for the 6800/8800 platforms include the quantitative cobas® HIV-1, HBV and HCV assays.

### cobas® 4800 system (Roche, USA)

Roche manufactures a qualitative multiplex NAAT, the cobas® 4800 CT/NG, which detects DR-9, a direct repeat region and target of the NG assay. It also simultaneously detects two CT independent DNA targets – one in the cryptic plasmid and the other on the CT genome. This design can detect infections caused by wild-type CT, the Swedish variant (nvCT) and other *Chlamydia* strains that may harbour deletions in the cryptic plasmid, or those that do not carry the cryptic plasmid. Approved samples include DNA in endocer-

vical swab specimens, clinician-collected vaginal swab specimens, clinician-instructed self-collected vaginal swab specimens, and male and female urine in cobas® PCR Media.

The cobas® 4800 CT/NG test utilizes amplification of target DNA by PCR and nucleic acid hybridization to detect these pathogens, and is intended to be used as a diagnostic as well as a screening tool in both symptomatic and asymptomatic individuals. These assays can only be run on the automated cobas® 4800 system.

Also available for the cobas® 4800 system is a diagnostic test for CDI. The cobas® Cdiff test selectively detects a specific *Clostridium difficile tcdB* gene directly from unformed (liquid or soft) stool specimens using real-time PCR technology. The test, which is not FDA cleared and therefore is not available in the United States, is intended for use as an aid in diagnosing CDI in humans in conjunction with clinical and epidemiological risk factors.

Additional assays from Roche available for use on the cobas® 4800 system include HPV, HSV 1 and 2, and MRSA/SA (*Staphylococcus aureus*) (which is not intended to diagnose, guide or monitor treatment for MRSA or *S. aureus* infections, or provide results of susceptibility to methicillin; it is for surveillance purposes).

The cobas® 4800 system, pictured in Fig. 25, integrates fully automated total nucleic acid isolation directly from primary and secondary tubes, automated PCR setup and real-time PCR. It is intended for laboratories with a medium workflow. The system comprises the cobas® x 480 instrument and the cobas® z 480 analyser and, per Roche, features minimal hands-on time (30 minutes for a run of 24–96 samples). It can take multiple sample types, can detect multiple test targets and has what the company describes as an intuitive workflow.

Fig. 25. Roche cobas® 4800 system



### cobas® Liat® system (Roche, USA)

Roche offers the cobas® Cdiff nucleic acid test for use on the cobas® Liat® system. The Cdiff test is an automated qualitative in vitro diagnostic test that utilizes real-time PCR to detect the *tcdB* gene of toxigenic *Clostridium difficile* in unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI. The cobas® Cdiff nucleic acid test for use on the cobas® Liat® system is intended as an aid in diagnosing CDI in humans in conjunction with clinical and epidemiological risk factors. The assay is CE-IVD marked and FDA cleared.

Additional, clinically validated assays for use on the cobas® Liat® system include Influenza A/B, Strep A and Influenza A/B & RSV (respiratory syncytial virus), all of which are CE-IVD marked and FDA cleared. These assays, together with the Cdiff assay, have received a Clinical Laboratory Improvement Amendments (CLIA) Waiver from the FDA. A CLIA Waiver determines that there is little risk of error due to the simple use of the test and that no special training is required.

All of the assays listed above are designed to be run on the cobas® Liat® system, pictured in Fig. 26, which is a compact, real-time PCR platform designed for on-demand short turnaround time (STAT) testing at POC or in the laboratory to support time-sensitive diagnoses and treatment decisions. Although the system is very easy to use, it does require a cold chain, which could impede its use in some settings in LMICs. All NAAT processes are fully automated, including sample preparation, amplification and real-time detection for qualitative and quantitative results, as well as results interpretation. Each cobas® Liat® assay tube contains all assay reagents for a single test.

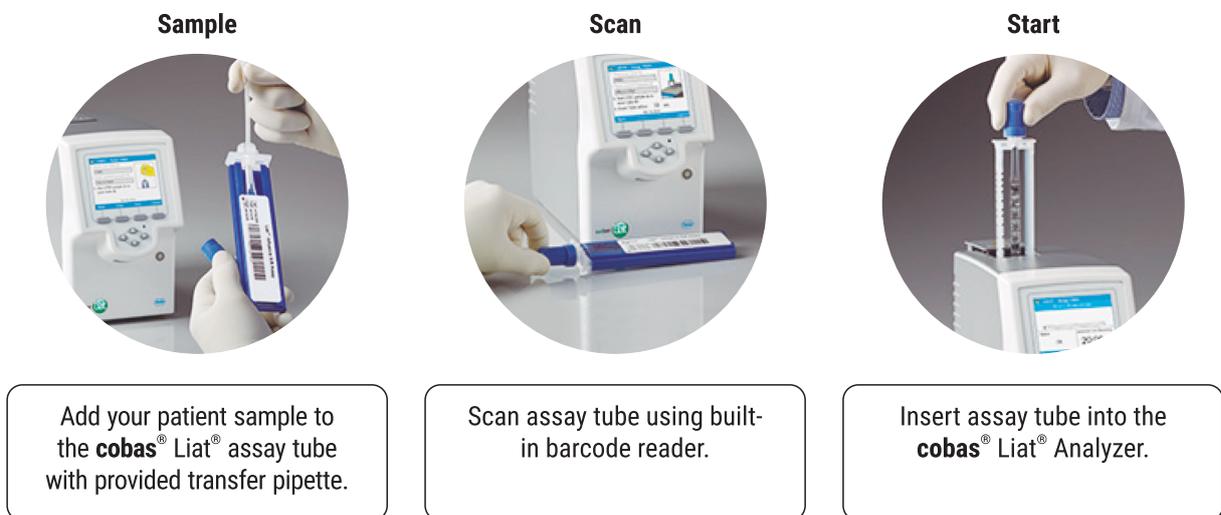
Fig. 26. cobas® Liat® system



To aid the operator and provide reliable results, the cobas® Liat® system incorporates a variety of intelligent and advanced features. The system self-checks at power on and has an error diagnostic system with comprehensive real-time monitoring, continuous self-calibrations and error message display. The graphical user interface provides on-screen prompts for easy-to-follow directions to guide the operator through sample loading and tube insertion. An onboard scanner supports a variety of barcode types for ease of use. Volume sensing ensures the appropriate amount of sample is used for the test, or delivers a warning if the sample volume is insufficient. A comprehensive set of sensors further monitors system operations in real time. Internal controls are pre-packed and process through every step, and quality-control reagents are used with each new assay tube lot.

As illustrated in Fig. 27, the cobas® Liat® test procedure is straightforward, with no sample manipulation or reagent loading steps, other than inputting

Fig. 27. cobas® Liat® test procedure



the sample directly into the cobas® Liat® assay tube. The cobas® Liat® system is a closed system, thus minimizing cross-contamination and biohazard risks, and allowing testing to be performed in nonlaboratory or near-patient facilities. The cobas® Liat® system is small and portable, weighing 8.3 lbs. It executes all required assay steps and reports a test result in about 20 minutes after loading a specimen on the system. The system runs one test at a time, and therefore can run a maximum of three tests per hour or approximately 24 tests in an 8-hour day.

The cobas® Liat® system has an internal optical system that provides independent optical detection channels, allowing for the detection of multiple targets in each test and providing future expandability for detection of multiple diseases. It is powered by AC mains.

### Hologic Panther® system (Hologic, USA)

Hologic manufactures the Aptima Combo 2® assay, which is a target amplification nucleic acid probe test that utilizes target capture for in vitro qualitative detection and differentiation of rRNA from CT and/or NG. The test is intended for use in the diagnosis of chlamydial and/or gonococcal urogenital diseases using the Panther® system or the Tigris® DTS® (direct tube sampling) automated analyser or semi-automated instrumentation, described below. The following specimens from both symptomatic and asymptomatic individuals may be used for the test: clinician-collected endocervical, vaginal and male urethral swab specimens, clinician-collected gynaecological specimens collected in PreservCyt® solution, patient-collected vaginal swab specimens, and female and male urine specimens.

Additional assays that can be run on the Panther® system include Aptima *Trichomonas vaginalis* (TV), HPV, HPV 16 18/45 genotyping assay and HIV-1 viral load. Assays in development for the system include HBV viral load, HCV viral load, *Mycoplasma genitalium*, HSV 1 and 2, bacterial vaginosis and *Candida*.

The Panther® system, pictured in Fig. 28, is a molecular diagnostic platform with random access testing capability on a fully integrated and automated NAAT system.

The Aptima Combo 2® assay and other assays run on the Panther® system involve three main steps, all of which take place in a single tube: target capture, target amplification by TMA, and detection of amplicons by the fluorescent-labelled probes (torches). Within the Panther®, all nucleic acid testing steps, from primary sample tube to results, are fully automated in one system with first reportable results within 3 hours after loading samples, and five results every 5 minutes thereafter. Samples can be continuously loaded with up to 120 samples at a time. Reagent controls and cali-

bration are valid for 24 hours. At least 275 samples can be run within an 8-hour shift, or 500 in a 12-hour period (an additional 225 samples can be run without operator attendance). Four reagent lanes allow up to four Aptima® test kits to be onboard and randomly accessed at any time: this could be four kits of the Combo 2® assay or any combination of the other molecular diagnostic assays available on the Panther®, including the HIV-1 Quant Dx, TV, HPV and HPV genotyping, HCV Quant Dx, HBV Quant and HSV 1 & 2 assays.

Fig. 28. The Panther® system



The Aptima Combo 2® assay as well as the Aptima® assay for *Neisseria gonorrhoeae* (GC assay) and the Aptima® assay for TV can also be run on the Tigris® DTS® (direct tube sampling) system (pictured in Fig. 29), which automates all phases of molecular diagnostics testing, from sample preparation, through amplification and detection, to results reporting. The Tigris® system uses target capture, which isolates the target nucleic acid from the sample and purifies it. TMA, using two enzymes (RT and RNA polymerase), amplifies the purified target RNA. The system hybridizes the amplicon to single-stranded nucleic acid probes and labels them.

Fig. 29. Tigris® DTS® system



The Tigris® system accommodates multiple assays on one system and has the ability to process approximately 450 samples in an 8-hour shift, and up to 1000 samples in approximately 13.5 hours. Because of the degree of automation of the system, it maximizes walk-away time during test processing.

### Hologic Panther Fusion® system (Hologic, USA)

In addition to the assays for its Panther® system previously described, Hologic offers a series of assays for its Panther Fusion® system. These currently include an assay for MRSA (CE-IVD marked) and the following additional assays: Flu A/B/RSV (CE marked and FDA cleared), Paraflu (CE marked and FDA cleared), AdV/hMPV/RV (adenovirus, human metapneumovirus and rhinovirus) (CE marked and FDA cleared), Group B streptococcal disease (GBS) (CE marked and FDA cleared) and *Bordetella* (CE marked). The company is also developing a gastrointestinal assay for the platform.

The Panther Fusion® system is a fully automated, high-throughput platform that combines the TMA capabilities of the Panther® with PCR-based testing capabilities on the Panther Fusion® instrument; the two instruments can be linked with one another as illustrated in Fig. 30. Together the instruments provide test menu consolidation, random access testing (any combination of sample types and assays may be performed at the same time) and continuous loading.

Fig. 30. Hologic Panther Fusion® system



### BD ProbeTec™ ET system (BD, USA)

BD Diagnostics offers the BD ProbeTec ET amplified DNA assays, which allow direct, qualitative detection of CT and GC DNA in endocervical swabs, male urethral swabs, and male and female urine specimens (with and without preservative) that are collected from either symptomatic or asymptomatic patients. The assays may be run on the BD ProbeTec™ ET system, pictured in Fig. 31, which uses SDA technology as the

amplification method and fluorescent energy transfer (ET) as the detection method to test for the presence of CT and GC in the clinical specimens indicated.

Fig. 31. BD ProbeTec™ ET system



While the BD ProbeTec™ is relatively compact and requires no special room, in addition to the ET instrument, pictured above centre, the system requires separate priming and amplification microwells, a pipettor, and a lysing rack and heater. Nonetheless, the system can generate CT/GC results for up to 46 patient samples in 3 hours – or 276 CT/GC results in one 8-hour shift. Total hands-on time is less than 2 minutes per sample.

### BD Viper™ LT system (BD, USA)

BD offers the GC Q<sup>x</sup> and CT Q<sup>x</sup> amplified DNA assays, which use SDA technology for the direct, qualitative detection of NG DNA or CT DNA, respectively, in clinician-collected female endocervical and male urethral swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and male and female urine specimens. The assays are indicated for use with asymptomatic and symptomatic female individuals and symptomatic male individuals to aid in diagnosing gonococcal urogenital disease. The assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescently labelled detector probe.

The GC and CT Q<sup>x</sup> amplified DNA assays can be performed using the BD Viper™ LT system, pictured in Fig. 32, which uses homogeneous SDA as the amplification method and ET as the detection method to test for the presence of pathogens by their genetic content in clinical specimens. The system uses four fluorescent channels with advanced optics and LED light sources for detection, and a thermocycler with 24 control zones designed to ensure uniform temperature control. The BD Viper™ LT system provides up to 30 CT/GC sample results in 3.5 hours, and 120 results per day. As such, the system is designed for a medium-throughput laboratory.

Fig. 32. BD Viper™ LT system



All of the assays for use on the BD Viper™ LT system are for sexually transmitted infections (STIs). In addition to the GC and CT assays, assays for detecting HSV 1 and 2, TV and HPV are also available.

### BD MAX™ system (BD, USA)

BD offers several bacterial detection assays to be run on its BD MAX™ system. One of these is a CT/GC/TV assay. The BD MAX™ system incorporates automated DNA extraction and PCR for the direct, qualitative detection of DNA from CT, GC and/or TV. The assay may be used to detect CT and/or GC DNA in male urine specimens, and CT, GC and/or TV DNA in female urine specimens, clinician-collected female endocervical swab specimens and patient-collected vaginal swab specimens (in clinical settings). The assay is indicated for use to aid in diagnosing chlamydial urogenital disease, gonococcal urogenital disease and/or trichomoniasis in asymptomatic and symptomatic individuals.

In addition to the CT/GC/TV assay, there is also the BD MAX™ Cdiff assay. As performed on the BD MAX™ system, the assay is an automated in vitro diagnostic test for the direct qualitative detection of *Clostridium difficile tcdB* in liquid or soft stool specimens from patients suspected of having CDI. The test is performed directly on the specimen and utilizes real-time PCR to amplify *tcdB* DNA and fluorogenic target-specific hybridization probes to detect the amplified DNA. The Cdiff assay is intended to aid in diagnosing CDI.

A third assay, the BD MAX enteric bacterial panel, also performed on the BD MAX™ system, is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. The panel detects nucleic acids from *Salmonella* spp.; *Campylobacter* spp. (*C. jejuni* and *C. coli*); *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC);

and Shiga toxin 1 (*stx1*)/Shiga toxin 2 (*stx2*) genes (found in Shiga toxin-producing *E. coli* [STEC]).

The BD MAX™ enteric bacterial panel is performed on unpreserved soft to liquid stool specimens or Cary-Blair preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The test is performed directly on the specimen, utilizing real-time PCR to amplify *SpaO*, a *Campylobacter*-specific *tuf* gene sequence, *ipaH* and *stx1/stx2*. The test utilizes fluorogenic sequence-specific hybridization probes to detect the amplified DNA.

BD indicates that the test is intended for use, in conjunction with clinical presentation, laboratory findings and epidemiological information, as an aid in the differential diagnosis of *Salmonella*, *Shigella*/EIEC, *Campylobacter* and STEC infections. Results of this test should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

BD also offers the BD MAX™ extended enteric bacterial panel. Performed on the BD MAX™ system, it is an automated in vitro diagnostic test for the direct qualitative detection and differentiation of enteric bacterial pathogens. It is used in conjunction with the BD MAX™ enteric bacterial panel as an optional master mix. The BD MAX™ extended enteric bacterial panel detects nucleic acids from *Plesiomonas shigelloides*; *Vibrio* (*V. vulnificus*, *V. parahaemolyticus* and *V. cholerae*); enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin (LT)/heat-stable enterotoxin (ST) genes; and *Yersinia enterocolitica*.

Additional assays available for use on the BD MAX™ system that are not discussed in detail in this report include the BD MAX™ vaginal panel, BD Max™ GBS, BD MAX™ enteric parasite panel and the BD MAX™ enteric viral panel. BD also offers two additional assays to combat transmission prevention and infection control for HAIs: BD MAX™ MRSA XT (as an aid in preventing and controlling MRSA infections in healthcare settings and not for in vitro use) and BD MAX™ StaphSR (for surveillance only).<sup>6</sup>

All of these assays are run on the BD MAX™ platform, pictured in Fig. 33, which automates sample preparation, including target lysis, DNA extraction and concentration, reagent rehydration and target nucleic acid amplification using real-time PCR. The amplified DNA targets are detected using hydrolysis (TaqMan®) probes, labelled at one end with a fluorescent reporter dye (fluorophore) and at the other end with a quencher moiety. The system software automatically interprets test results. The BD MAX™ platform is capable of batch processing and analysing up to 24 specimens simultaneously. Test results

<sup>6</sup> For more detail on BD MAX™ MRSA XT and StaphSR, which are not covered in detail in this report as they are not assays for in vitro use, see WHO Global AMR Surveillance System (GLASS) (10).

generally take about 2.5–3 hours with an additional 15–20 minutes of hands-on time for completing 24 specimens.

Fig. 33. BD MAX™ instrument



### Great Basin analyser system (Vela Diagnostics, Singapore)

Vela Diagnostics recently acquired Great Basin Scientific, Inc., and now offers several FDA-cleared assays for detecting bacterial pathogens. These are the Great Basin Stool Bacterial Pathogens panel, Great Basin Staph ID/R Blood Culture panel and Great Basin Toxigenic *C. difficile* test. The assays are configured for use on the Great Basin analyser, the PA 500 Portrait analyser.

The Great Basin Stool Bacterial Pathogens panel is a multiplexed, qualitative in vitro diagnostic for detecting and identifying DNA targets of enteric bacterial pathogens directly from Cary-Blair or C&S medium preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. The panel detects *Campylobacter* (*C. coli* and *C. jejuni*), *Salmonella*, *stx1*, *stx2*, *Escherichia coli* serotype 0157 and *Shigella*. The test is intended for use as an aid in diagnosing specific agents of gastrointestinal illness in conjunction with clinical and epidemiological information, but is not intended for use in monitoring these infections. TAT is less than 2 hours.

The Great Basin Staph ID/R Blood Culture panel is a qualitative, multiplex, in vitro diagnostic assay intended for simultaneously identifying nucleic acid from *Staphylococcus aureus*, *Staphylococcus lugdunensis* and various *Staphylococcus* spp. to the genus level, and detection of the *mecA* gene directly from patient positive blood culture specimens. The assay is intended for use in conjunction with other clinical or laboratory findings to aid in diagnosing BSIs, but is not intended for monitoring these infections. TAT is less than 2 hours.

The Great Basin Toxigenic *C. difficile* test is a qualitative in vitro diagnostic test that detects the

*tcdB* gene in human stool samples collected from patients suspected of having CDI. It is intended as an aid in diagnosing CDI. The test is not intended for use in near-patient care settings. TAT is approximately 90 minutes.

The Great Basin analyser system, pictured in Fig. 34, is a fully automated system that comprises two major components: the control platform (touch-screen) and the PA500 Portrait analyser, below it, which is a molecular in vitro diagnostic device. Resident software in the device is used to control, analyse and determine test results.

Fig. 34. Great Basin analyser system



The Great Basin analyser system utilizes automated, hot-start PCR amplification technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface, in a single-use, self-contained disposable test cartridge. The system contains linear actuators that open small reagent containers, linear actuators that open and close valves, linear motors that depress reagent blisters to move fluid through the cartridge, pictured in Fig. 35, a motor to facilitate mixing processes and analog sensors that detect the presence of fluid.

Fig. 35. Great Basin test cartridge



The system takes unprocessed samples, and the cartridge contains, either in blister packs or lyophilized, all of the reagents required to run the test. The analytical steps of the assay, including sample prep, amplification and detection, are performed in chambers present on the cartridge.

Additional assays available for the platform and not described here in detail are the Great Basin Shiga Toxin Direct test, the Great Basin *Bordetella* Direct test and the Great Basin Group B *Streptococcus* test.

### illumigene™ molecular diagnostic system (Meridian Bioscience, Inc., USA)

Meridian Bioscience offers an FDA-cleared *Clostridium difficile* assay, the illumigene™ *C. difficile* DNA amplification assay, for use on its Alethia™ platform which, it should be noted, the company indicates is only for use in hospital, reference or state laboratory settings and not for use at POC. The assay utilizes LAMP technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile* strains from stool specimens. The illumigene™ *Clostridium difficile* assay detects the PaLoc by targeting a partial DNA fragment on the *C. difficile* Toxin A gene (*tcdA*). The *tcdA* target region was selected as an intact region remaining in all known A<sup>+</sup>B<sup>+</sup> and A<sup>-</sup>B<sup>+</sup> toxinotypes.

Meridian Bioscience also offers assays for respiratory infections – Group A *Streptococcus*, *Mycoplasma* Direct and Pertussis – as well as two assays for sexual health – Group B *Streptococcus*, and HSV 1&2.

These assays are intended to be performed on the Alethia™ platform, pictured in Fig. 36, which is a compact, automated isothermal amplification and detection system. There is a separate sample preparation device. The Alethia™ is a menu-driven laboratory instrument with two independent sample-processing blocks, identified as Block A and Block B. Sample heating and optical detection is carried out for up to five two-chambered illumigene™ devices per block. Each two-chambered Alethia™ device contains a sample chamber and a control chamber. Amplification of target DNA occurs during the heat cycle and results in the formation of precipitate detected by the Alethia™ optics system. The precipitate generated by the presence of amplified target DNA leads to a turbid sample/control reaction solution which is then measured by absorbance.

One to 10 qualitative results are available in less than an hour on the Alethia™ instrument.

Fig. 36. Alethia™ instrument



### Solana® platform (Quidel Corporation, USA)

Quidel corporation offers the Solana® *C. difficile* assay, which is an in vitro diagnostic test for the direct, qualitative detection of *tcdA* in unformed stool specimens of patients suspected of having CDI. The assay is intended for use as an aid in diagnosing CDI. The assay utilizes HDA to amplify a highly conserved fragment of the *tcdA* sequence. The Solana® *C. difficile* assay is intended for use only with the Solana® instrument platform.

The Solana® platform, pictured in Fig. 37, uses a helicase enzyme to unwind double-stranded DNA into single strands, eliminating the need for a thermocycler. The company emphasizes that unlike other isothermal amplification methods, HDA uses a probe-based detection method, thereby resulting in greater specificity. In addition, because HDA only detects amplicons, rather than turbidity caused by amplification as with LAMP, it provides assurance that amplification of only the intended target will be identified as positive. HDA can also multiplex in a single tube.

Fig. 37. Solana® platform



Solana® is a compact benchtop instrument measuring 9.4 × 9.4 × 5.9 inches that allows rapid detection of targets. TAT for the tests is as short as 35 minutes. In addition, the platform permits operators to batch up to 12 samples in a single run, allowing for testing scale-up as needed.

The company believes the platform is ideal for small- to medium-sized microbiology labs where the low total cost of the instrument and disposables enables molecular testing at the volumes seen in these settings. In resource-limited settings, this would likely translate into use at Level II settings and above.

Additional assays that can be run on the Solana® platform include Influenza A+B, Group A *Streptococcus*, Group B *Streptococcus*, HSV 1+2/VZV (varicella-zoster virus) and TV, all of which are FDA approved. Quidel is also developing additional assays for the Solana platform.

### AmpliVue® platform (Quidel Corporation, USA)

Quidel offers the AmpliVue® *C. difficile* assay, which is an in vitro diagnostic test for the direct, qualitative detection of *tcdA* in unformed stool specimens of patients suspected of having *C. difficile*-associated disease (CDAD). The AmpliVue® *C. difficile* assay is intended for use as an aid in diagnosing CDAD. The assay utilizes HDA to amplify a highly conserved fragment of the *tcdA* sequence, and a self-contained disposable amplicon detection device that allows for manual evaluation of assay results.

The AmpliVue® is an “instrument-free” molecular diagnostic platform. Like the Solana® platform, AmpliVue® combines simple specimen processing and HDA technology, but has qualitative lateral-flow de-

tection housed in a cassette. The platform is low cost, as it only requires a heat block for amplification.

The typical workflow for the *C. difficile* assay on the AmpliVue® platform is illustrated in Fig. 38.

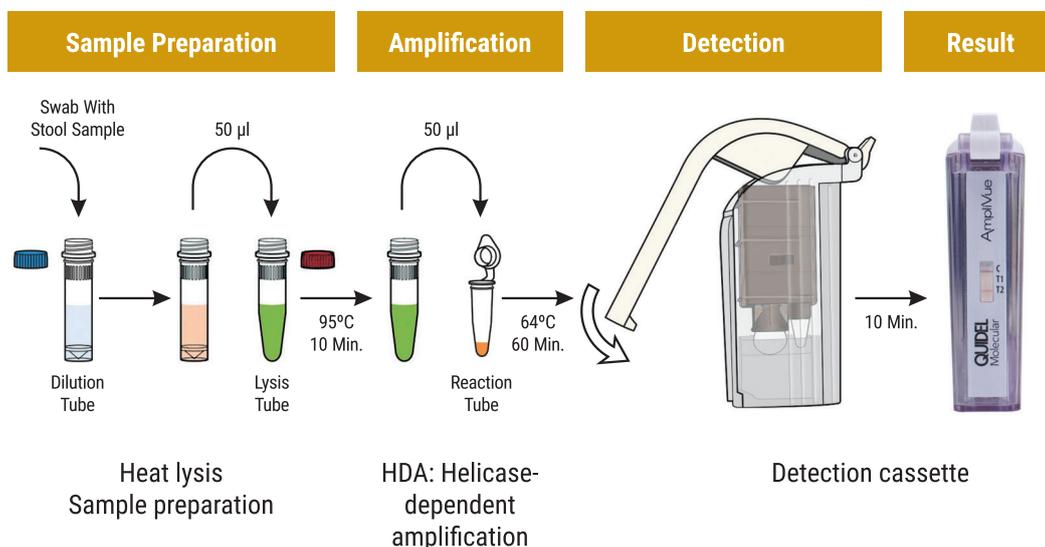
The testing process uses manual specimen preparation (dilution of sample and pipetting of same followed by heat lysis), with the entire process, including heat lysis, taking about 60–90 minutes (depending on the assay).

The first assay developed for the AmpliVue® platform was the *C. difficile* assay, which is both CE marked and FDA cleared. Similarly, five additional assays are CE marked and FDA cleared – assays for herpes simplex (cutaneous and mucocutaneous) lesion specimens (HSV 1+2), Group B *Streptococcus*, Group A *Streptococcus*, TV and *Bordetella pertussis*. The assay for detecting TV in women (using vaginal specimens) was launched in 2015 and is FDA cleared.

### VERSANT® kPCR molecular system (Siemens, USA)

The Siemens VERSANT® kPCR system is an automated system which combines extraction of nucleic acids from 96 samples with subsequent real-time PCR. The VERSANT® CT/GC DNA 1.0 kinetic PCR (kPCR) assay detects CT and GC in a multiplex real-time PCR on this automated system, including the recently described new variant of CT (nvCT). The assay is designed for the qualitative detection of CT and NG in symptomatic and asymptomatic individuals from urine specimens from males and females, male urethral and female endocervical swab samples. The assay is CE-IVD marked, but is not available in the United States.

Fig. 38. Typical test workflow on AmpliVue® platform



The VERSANT® CT/GC DNA 1.0 assay is an automated amplification method based on reverse transcription and real-time PCR technology. The system, pictured in Fig. 39, consists of two modules: a sample preparation module used to extract both RNA and DNA from plasma as well as a wide variety of other samples, and an amplification detection module, along with VERSANT® MiPLX software. For the CT/GC assays, the sample preparation is universal for either urine or swabs. The system is flexible and allows for either a “one-room” technology with no need for clean-room operations due to closed-tube processing and other physical and chemical contamination controls or two separate rooms, depending on the individual laboratory’s setting.

The VERSANT® kPCR sample preparation module pipettes purified RNA to a PCR plate containing appropriate primer/probe mix and enzymes. The wells are then sealed and transferred to the amplification detection module, where the CT/GC and internal control RNA molecules are reverse transcribed to make cDNA and then simultaneously amplified and detected using the kPCR technique. The system can produce 188 patient results per shift.

Fig. 39. VERSANT® kPCR molecular system



Additional assays available for the VERSANT® kPCR molecular system include the VERSANT® HIV-1 RNA 1.5 assay (kPCR) and HCV RNA 1.0 assay (kPCR).

#### ARIES® and ARIES® M1 systems (Luminex, USA)

Luminex offers the ARIES® *C. difficile* assay, which is a real-time PCR-based qualitative in vitro diagnostic test for the direct detection of toxigenic *Clostridium difficile* nucleic acid in unpreserved, unformed (liquid or soft) stool specimens obtained from patients suspected of having CDI, i.e., symptomatic patients. The test targets the *Clostridium difficile* *tcdA* and *tcdB* and is indicated for use as an aid in diagnosing indi-

viduals suspected of having CDI. The assay is FDA cleared and CE-IVD marked.

The ARIES® *C. difficile* assay is designed for use on the Luminex ARIES® system, a two-module instrument, or ARIES® M1 system, a single module instrument, pictured in Fig. 40, each of which consists of the associated ARIES software, a stool resuspension kit, an assay-specific test cassette and an assay-specific protocol file. It is a sample-to-result system. The ARIES® *C. difficile* assay cassette is a disposable, single-use device that contains nucleic acid purification reagents, an internal sample processing control (SPC), and an assay-specific master mix for detecting *tcdA* and *tcdB*. Cassettes can be stored at room temperature.

The systems require a universal assay protocol (i.e., identical sample preparation, amplification reagents and conditions) that may enable multiple sample types and up to 12 different IVD assays to be run together in a random batch, which is not the same as random access. TAT for the *C. difficile* assay is approximately 2 hours. The systems are appropriate for moderate-sized laboratories.

Additional FDA-cleared and CE-marked assays for the ARIES® systems include *Bordetella*, Flu A/B & RSV, Group A Strep, Group B Strep and HSV 1&2.

Fig. 40. ARIES® (left) and ARIES® M1 (right) systems



#### Novodiag® (Mobidiag, Finland)

Mobidiag offers two assays for its Novodiag® platform – the Novodiag® *C. difficile* assay and the Novodiag® Bacterial GE+ assay. The *C. difficile* assay screens for *tcdB* from unformed stool samples, while the Bacterial GE+ assay screens for the most relevant bacteria responsible for diarrhoea, including *Campylobacter coli*, *Campylobacter jejuni*, *Clostridium difficile* *tcdB*, *Salmonella* spp. and *Shigella* spp. Results are available in just under an hour.

The Novodiag® platform, pictured in Fig. 41, is a four-bay, benchtop, automated sample-in, result-out system for detecting infectious diseases. The platform

combines real-time qPCR and microarray technologies appropriate for screening one or multiple pathogens. The system offers random access for on-demand testing together with automated data analysis and reporting with laboratory information and management system (LIMS) connectivity.

Fig. 41. Novodiag® system



In addition to the Novodiag® *C. difficile* and Bacterial GE+ assays, the company is developing assays for antibiotic resistance, meningitis and parasites.

### Respiratory Multiplex Array II/Vivalytic analyser (Randox Laboratories, Ltd., UK/Bosch Healthcare Solutions, Germany)

Bosch Healthcare Solutions has developed the Vivalytic analyser, a universal cartridge-based platform for sample-to-answer molecular diagnostics (pictured in Fig. 42), with the first tests available being the Respiratory Multiplex Assay and the Randox STI Multiplex Array.

Fig. 42. Bosch Vivalytic analyser



The Vivalytic platform accommodates a wide variety of samples and allows for different methods of

analysis to run in a fully automated way in a short timeframe, with results from 30 minutes. Single or multiple pathogens can be detected simultaneously in the patient sample. In addition, the Vivalytic platform is an open system that can process molecular diagnostic tests from various assay manufacturers.

The Vivalytic analyser is a small-footprint, fully automated device with no peripherals, capable of quantitative and qualitative PCR procedures with three stable isothermal zones, where rapid microfluidic transfer between these zones achieves fast heating and cooling cycles. The analyser has a universal optical evaluation unit, which enables microarrays, qualitative or quantitative PCR, as well as melting curve analyses to be read out in one system. Four standard colour channels can be evaluated per PCR strand. This corresponds to a degree of multiplexing of up to eight for qualitative or quantitative PCR, or up to 16 in multichannel melting curve analysis. Via geometrical multiplexing with the help of microarrays, a much higher number can be achieved. Up to 100 properties can be examined.

The Vivalytic system has built-in connectivity and can be easily integrated with popular standard Internet technology systems. Further, an analyser device can be networked and combined with many other devices, so that several series of tests can be carried out at the same time.

Randox Laboratories has developed a number of infection arrays that have been adapted for use with the Vivalytic analyser. Of particular interest for this report is the Respiratory Multiplex Array (pictured in Fig. 43), a qualitative assay that simultaneously detects 21 bacterial and viral pathogens from the upper and lower respiratory tract in nucleic acid extracted from a single sputum, lavage or nasopharyngeal sample.

Fig. 43. Randox respiratory tract infection and STI panels



The Respiratory Multiplex Array assay is based on a combination of multiplex PCR and biochip array hybridization. PCR priming technology permits high discrimination between multiple targets. A unique

primer set is designed for each target that will hybridize to a complementary oligonucleotide probe spotted on a biochip discrete test region (DTR). This combination of PCR priming and spatially organized biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template nucleic acid, through PCR, to data readout in about 6 hours.

The STI array, a qualitative assay that is CE-IVD marked, detects 10 of the most important bacterial, viral and protozoan sexually transmitted infections (STIs), providing a comprehensive infection profile from a single swab sample. The test panel includes CT, NG and TV, as well as MG, *Ureaplasma urealyticum*, *Haemophilus ducreyi*, *Mycoplasma hominis*, and HSV 1 and 2.

Each cartridge contains internal controls that indicate successful extraction, amplification, hybridization and detection; all of these must pass acceptance criteria in order for the Vivalytic analyser to return patient results. Further, test results do not require interpretation; positive or negative results are indicated for each target without ambiguity.

#### STAT-Dx (subsidiary of QIAGEN N.V., Germany)

QIAGEN's STAT-Dx subsidiary offers two CE-IVD-marked panels for syndromic testing: the QIAstat-Dx™ Respiratory Panel V2 and QIAstat-Dx™ Gastrointestinal Panel V2. Each of these is a sample-to-result solution that can be performed in less than an hour on the QIAstat-Dx™ Analyzer 1.0, pictured in Fig. 44, which consists of an analytical module and an operational module. It is appropriate for use at or near POC.

Fig. 44. QIAstat-Dx™ Analyzer 1.0



The QIAstat-Dx™ Analyzer 1.0 utilizes real-time qPCR that is fully integrated and can process up to 48 targets from a variety of sample types, including swabs, tissue and liquids, but not yet whole blood. The system has built-in connectivity capability. The instrument, which is modular and scalable, has a

six-wavelength optical sensor. Each cassette has eight possible reaction chambers. The system utilizes a single-use assay cartridge, pictured in Fig. 45, that contains all required sample reagents and has the ability to process samples onboard. It is appropriate for near-patient testing.

Fig. 45. QIAstat-Dx™ Analyzer 1.0



The QIAstat-Dx™ (DiagCORE®) Respiratory Panel V2 simultaneously detects 18 viral and three bacterial pathogens (*Mycoplasma pneumoniae*, *Legionella pneumophila* and *Bordetella pertussis*) from a 300 µL nasopharyngeal sample. The QIAstat-Dx™ (DiagCORE®) Gastrointestinal Panel V2 detects six viruses, 14 bacteria, including diarrhoeagenic *Escherichia coli*, Shiga-like toxin-producing *E. coli*, *Shigella*, EIEC, pathogenic *Campylobacter* spp., *Clostridium difficile* (Toxin A/B) and *Salmonella*, as well as four parasites from 200 µL stool resuspension. Additional syndromic panels in the pipeline include a meningitis panel, a sepsis panel, panel(s) for STIs and a traveller's fever panel.

#### revogene® (GenePOC™, Canada)

GenePOC, which was recently acquired by Meridian Bioscience (USA), has developed a fully automated platform for NAAT-based testing at POC. The system combines a compact benchtop instrument, the revogene®, pictured in Fig. 46, with a single-use microfluidic cartridge (PIE) that can perform sample homogenization, microorganism lysis, dilution, amplification and detection of target nucleic acid sequences from multiple specimens using fluorescence-based real-time PCR. Manual sample preparation steps are required, however; specimens must be transferred with a disposable transfer loop into a sample buffer tube and subsequently transferred into the PIE using a disposable transfer tool. The PIE must also be manually loaded into, and unloaded from, the revogene® carousel. TAT is approximately 70 minutes. The complexity of the system suggests it would best be used in Level III settings or higher.

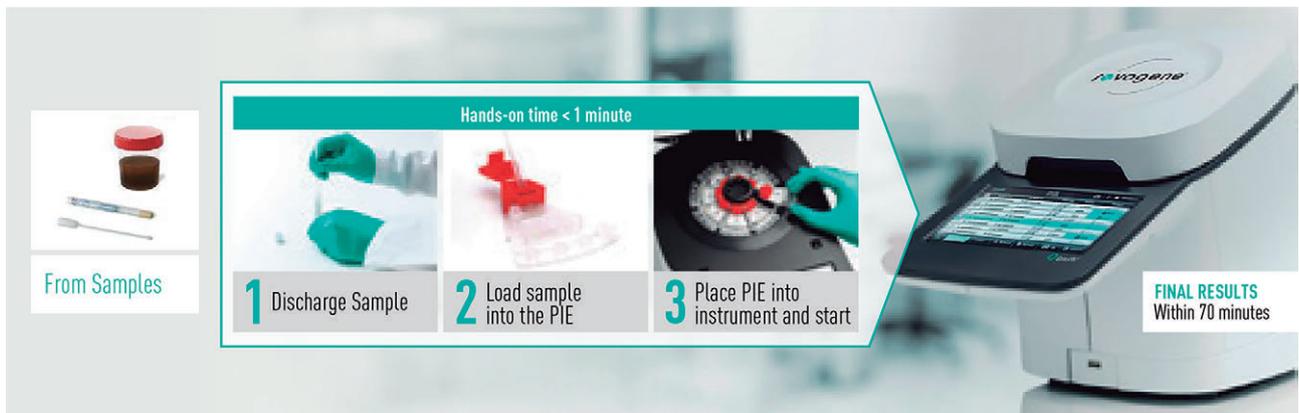
Fig. 46. revogene® instrument



The revogene® instrument can process up to eight clinical samples simultaneously, detecting up to 12 genetic targets per sample, but it does not offer random access. Different assays can be run at the same time on the instrument only if sample prep and the programmes for amplification and detection are the same. It should also be noted that the within each PIE, the processed sample is divided into three parts, allowing for unique reactions to be run; however, this “geographic” or “geometric” multiplexing can reduce test sensitivity. The system does not support direct Wi-Fi connectivity, but does support bidirectional LIS connection.

To date, the company has several commercially available assays. Of interest with respect to this report is the GenePOC CDiff assay, which is a qualitative in vitro diagnostic test to detect the *tcdB* gene of toxigenic *Clostridium difficile* direct from unformed (liquid or soft) stool specimens. The assay is intended to aid in diagnosing CDI and is FDA cleared and CE-IVD marked. The workflow for the test is illustrated in Fig. 47.

Fig. 47. GenePOC CDiff assay workflow on the revogene® platform



Additional assays available for the platform include two tests for Group B *Streptococcus*: (i) the GenePOC GBS DS test direct from vaginal/rectal swabs, and (ii) the GenePOC GBS LB test from Lim Broth samples. These assays are CE-IVD marked.

GenePOC plans to develop assay panels for gastrointestinal infections and respiratory infections and also plans to include AMR applications.

### T2Dx® instrument (T2 Biosystems, USA)

T2 Biosystems offers two multiplex panels for identifying infectious pathogens: the T2Bacteria panel and the T2Candida panel. These assays are performed on the T2Dx® instrument, pictured in Fig. 48.

Fig. 48. T2Dx® instrument



The T2Bacteria panel is a qualitative test using T2 magnetic resonance (T2MR®) to directly detect bacterial species in K2-EDTA human whole blood specimens from individuals suspected of bacteraemia. The T2Bacteria panel identifies five species of bacteria: *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylo-*

*coccus aureus*. The T2Bacteria panel is to be used as an aid in diagnosing bacteraemia; blood cultures are required to recover organisms for AST or further ID. It is both CE-IVD marked and FDA cleared.

Similarly, the T2Candida panel is a qualitative T2MR<sup>®</sup> assay for directly detecting *Candida* spp. in K2-EDTA human whole blood specimens from patients with symptoms of, or medical conditions predisposing the patient to, invasive fungal infections. The panel identifies five species of *Candida*. The panel is performed independent of blood culture, although blood cultures are required to recover organisms for AST or further ID. The T2Candida panel is FDA cleared.

Both the T2Bacteria panel and the T2Candida panel are performed on the T2Dx<sup>®</sup> instrument, which is automated and executes all steps after specimen loading (approximately 4 mL). On the instrument, bacterial DNA is amplified with target-specific primers and amplicons are hybridized to target-specific probes. The hybridization that occurs in individual tubes is analysed in the MR reader, and a signal for each target is generated and detected by the T2MR<sup>®</sup>, indicating the presence of the target organism. TAT from loading the first specimen on the instrument is approximately 3–5 hours. The results are interpreted by software on the device.

The T2 system is suitable for use in centralized laboratories and is not intended for near-patient testing.

### GenomEra<sup>®</sup> CDX system (Abacus Diagnostica, Finland)

Abacus Diagnostica offers several CE-marked assays for its GenomEra<sup>®</sup> CDX platform. Of these, the *C. difficile*, MRSA/SA AC (with *mecA* and *mecC*), MRSA/SA Multi Swab and *Streptococcus pneumoniae* assays are of relevance to this report.

- The GenomEra<sup>™</sup> CDX *C. difficile* test is a rapid and simple molecular assay for diagnosing toxigenic *Clostridium difficile* directly from unformed stool samples.
- The GenomEra<sup>™</sup> CDX MRSA SA AC test is an assay for detecting *Staphylococcus aureus* and MRSA as well as both resistance genes, *mecA* and *mecC*, from a droplet of blood culture or plate samples using the same test kit.
- The GenomEra<sup>™</sup> CDX MRSA Multi Swab test simultaneously screens multiple body sites (nose, throat and groin/perineum) for MRSA colonization using swab specimens pooled in liquid medium (eSwab<sup>™</sup> MRSA Collection System [Copan]). The same medium can be used for confirmation by culture, thus eliminating the need for collecting additional samples.

- The GenomEra<sup>™</sup> CDX *S. pneumoniae* test enables the detection of pneumococcus from positive blood cultures or equivalent liquid bacterial cultures.

All of the above assays are designed for use on the GenomEra<sup>®</sup> CDX system, pictured in Fig. 49, which comprises (i) a proprietary test chip, (ii) an automated PCR analyser and (iii) a PC-controlled graphical interface with built-in results interpretation technology.

Fig. 49. GenomEra<sup>®</sup> CDX system: test chips (left and centre), and analyser and PC for test interpretation (right)



Per the company, the system combines highly fluorescent proprietary lanthanide labels with PCR for high detection sensitivity with no signal interference from the clinical samples. The system uses patented multiblock thermal cycling technology that enables rapid PCR and RT-PCR amplification with 45 thermal cycles. The PCR or RT-PCR reagents are lyophilized and preloaded into the GenomEra<sup>®</sup> test chips. The software loaded on the PC incorporates clear and simple results interpretation technology.

TAT for four patient samples is 50 minutes. Sample preparation of four samples takes about 5–10 minutes depending on the assay kit.

### CLART<sup>®</sup> technology (GENOMICA S.A.U., Spain)

GENOMICA offers a number of CE-IVD-marked assays for infectious disease targets for use with its CLART<sup>®</sup> technology, which includes genetic amplification and visualization in low-density microarrays. For purposes of this report, the most interesting of these are the CLART<sup>®</sup> EnteroBac and the CLART<sup>®</sup> SeptiBac assay panels. CLART<sup>®</sup> EnteroBac detects the presence in stool samples of the main types of bacteria that produce endotoxins causing diarrhoea; these include *Salmonella* spp., *Shigella* spp., enteropathogenic *Escherichia coli*, *Campylobacter* spp. and *Clostridium difficile* B. Similarly, the CLART<sup>®</sup> SeptiBac assay detects and types gram-positive and gram-negative bacteria and fungi that cause septicaemia from positive blood culture. These include *Staphylococcus* spp., *Streptococcus* spp. (including

the methicillin-resistance marker *mecA*), *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Salmonella enterica*, *Enterobacter* spp., *Haemophilus* spp., *Acinetobacter baumannii* and *Pseudomonas*.

The testing process begins with sample preparation, DNA extraction and DNA amplification/labelling of targeted molecules using standard laboratory equipment not provided by GENOMICA. This is followed by specific hybridization (labelled sample incubation) and visualization (conjugation and precipitation of the staining). Finally, colorimetric detection and analysis is done using the CAR (Clinical Array Reader from GENOMICA), pictured in Fig. 50. GENOMICA also offers two instruments that can be used to automate the visualization step: *autoclar*<sup>®</sup> and *autoclar*<sup>®</sup> plus.

Fig. 50. CAR<sup>®</sup> (Clinical Array Reader)



Additional assays available for use in association with the CLART<sup>®</sup> technology include CLART<sup>®</sup> HPV2, CLART<sup>®</sup> ENTHERPEX, CLART<sup>®</sup> PneumoVir, CLART<sup>®</sup> STIsA, CLART<sup>®</sup> STIsB, CLART<sup>®</sup> METABONE, CLART<sup>®</sup> CMA NRAS-iKRAS, CLART<sup>®</sup> CMA KRAS-BRAF-PI3K and CLART<sup>®</sup> CMA EGFR.

### Other commercialized molecular amplification assays/platforms

In addition to the integrated NAAT and iNAAT systems described above, and in addition to purely LDTs, there are manufacturers of assay kits for detecting bacterial (and other) pathogens that are validated for use on certain commercially available open systems for sample preparation, nucleic acid extraction and amplification/detection. These include the following:

- **Fast Track Diagnostics (Siemens, USA):** Siemens recently acquired Fast Track Diagnostics, Ltd. (Malta), which offers a wide array of real-time PCR multiplex IVD kits for syndromic testing. These include test kit panels for respiratory infections (e.g., *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmo-*

*nella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae*), gastroenteritis (*Campylobacter coli/jejuni/lari*, *Clostridium difficile*, *Salmonella* spp. and *Shigella* spp.), STIs (NG), eye infections, fever/rash/childhood infections, tropical fever, hepatitis, infections of the immunosuppressed, meningitis infections and human adenovirus. The assays can be used on a range of samples, as appropriate, including tissue from biopsies, blood, faeces, sputum, CSF and swabs of mucosal surfaces. With the exception of kits for HBV/HCV and Rift Valley fever virus (RVFV), all kits are CE labelled for IVD use.

The assay kits are for use with UgenTec's FastFinder<sup>™</sup>, a PCR interpretation platform. Depending on the assay, tests can be run on the following real-time PCR platforms: ABI 7500 (Thermo Fisher Scientific, USA), LightCycler<sup>®</sup> 480 II (Roche, USA) and CFX96<sup>™</sup> (Bio-Rad, France).

- **SepsiTest<sup>™</sup>-UMD (Molzylm Molecular Diagnostics, Germany):** Molzylm offers SepsiTest<sup>™</sup>-UMD, which is a CE-IVD-marked PCR test for detecting bacterial and fungal DNA in 1 mL of K-EDTA (potassium-EDTA) or citrate-treated whole blood; it can also use blood culture, CSF, sputum and nasal swabs among other specimen types. The test is able to identify species from more than 200 genera of bacteria and 65 genera of fungi.

SepsiTest<sup>™</sup>-UMD utilizes three processes: extracting and purifying microbial DNA using centrifugation, universal PCR and Sanger sequencing (discussed below). The PCR result can be available in 4 hours. Amplicons from positive samples are then sequenced to confirm the PCR result and to determine which bacterial or fungal species are present. Where readable sequences are available from sequence analysis, bacteria and fungi can be identified using the SepsiTest<sup>™</sup>-BLAST online tool. Sequencing results are typically available in 3–4 hours, depending on the analyser used, making a total TAT of about 8 hours or slightly more depending on the workflow in the laboratory.

SepsiTest<sup>™</sup>-UMD can be performed on a number of real-time PCR instruments, including LightCycler<sup>®</sup> platforms, CFX96<sup>™</sup>, AriaMx and Mx3005P (Agilent, USA), ABI 7500 Fast (Thermo Fisher Scientific, USA), and RotorGene<sup>®</sup> Q (QIAGEN, Germany). For sequencing, SepsiTest<sup>™</sup>-UMD has been validated for use with the ABI 3730xl and ABI Prism<sup>®</sup> 310 DNA analysers together with the BigDye<sup>®</sup> Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific, USA).

- **Cognitor® Minus (Momentum Bioscience Ltd., UK):** Momentum has developed an assay, the Cognitor® Minus test, that is designed to provide rapid, universal detection of viable microorganisms without the need for preselection. The test is performed on any standard thermocycler on negative blood culture samples after 12 hours of incubation and is used to confirm a negative BSI result. The technology uses Enzymatic Template Generation and Amplification (ETGA®) to detect microbial DNA polymerase activity common to a wide range of bacteria and fungi from blood culture after amplification by PCR. The assay is CE-IVD marked.

The company is also planning to develop technology for the detection, ID and AST of positive blood cultures. No timeframe for this is available.

- **xTAG® technology (Luminex, USA):** Luminex offers a group of in vitro diagnostic assays that combines multiplex reverse transcription and RT-PCR with its proprietary universal tag system that allows easy development and optimization of nucleic acid assays. The assays consist of kit reagents and software. The assay of relevance to this report is the FDA-cleared xTAG gastrointestinal pathogen panel (GPP), which is a multiplexed nucleic acid test intended for simultaneously detecting and identifying 14 viral, bacterial and parasitic nucleic acids in human stool specimens or human stool in Cary-Blair media from individuals with signs and symptoms of infectious colitis or gastroenteritis. In addition to certain viruses and parasites, bacteria in the panel include *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only), *tcdA* and *tcdB*, *Escherichia coli*, *Salmonella* and *Shigella*. The assay can aid in diagnosing gastrointestinal infection in conjunction with clinical evaluation.

The xTAG® technology is not an integrated system. Each sample is pretreated prior to extraction; extraction purification is then done using the bioMérieux NucliSENS® easyMAG® kit. This is followed by multiplex amplification and bead hybridization and detection. Finally, xTag data analysis software for the GPP analyses the data and provides a report summarizing which pathogens are present. Data acquisition and analysis can be done by Luminex MAGPIX® or Luminex 200™ instruments. TAT is approximately 5 hours for 24 samples.

The use of the systems described immediately above is most appropriate for sophisticated laboratories with strong laboratorians and quality assurance in place.

## Pipeline molecular systems for identifying pathogens

In addition to the commercialized molecular systems for identifying pathogens detailed above, there are a good number of systems in the diagnostic pipeline. Many of these platforms are currently designed to perform individual tests; others can multiplex. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established.

- **Lucira Health, Inc. (USA)** is developing a disposable test kit to detect DNA and RNA of infectious disease pathogens. As pictured in Fig. 51, the platform is a NAAT-based platform designed to look like a lateral flow device. The company has used both RPA and LAMP technologies.

Fig. 51. Lucira test



The company is developing a test for influenza A and B direct from swabs; additional tests are planned for RSV and strep throat. No additional information is available.

- **FAST-ID™ (Qvella, Canada)** is developing a rapid, easy-to-use multiplex PCR assay system called FAST-ID™, pictured in Fig. 52. The first panel planned for the system is the FAST-ID™ BSI panel, which, per Qvella, will detect more than 95% of sepsis-causing pathogens direct from whole blood. The technology uses direct aspiration from a closed sample tube, which simplifies test setup and minimizes potential contamination. The system features fully automated intact pathogen recovery integrated with e-lysis™, which results in PCR-ready lysate™, eliminating the need for multistep extractions. The test cartridge is disposable. TAT is approximately 1 hour.

Fig. 52. FAST-ID™ PCR system



- NanoDetection Technology (USA) is developing a novel diagnostic system that initially will be applied to detecting human infectious diseases. At the core of the system is a novel biochip technology by which 25 discrete light-sensing diodes enable multiplex testing with high sensitivity. The system, pictured in Fig. 53, allows labs to detect multiple diseases using analyte-specific cassettes.

Fig. 53. NanoDetection instrument



Workflow for the system is simple and requires the user to collect a patient specimen, prepare the sample of the system's cassette and insert the cassette into the instrument, as shown above. The proposed menu of assays for the instrument includes MRSA screening, MRSA/methicillin-susceptible *Staphylococcus aureus* (MSSA) dual screening, sepsis, influenza A and B, HCV, STIs and dengue.

- Biospectrix (3i Diagnostics, Inc. [3iDx], USA)  
3iDx is an early-stage company that is dedicated to achieving faster diagnosis of BSIs direct from whole blood. The company's technology, Biospectrix, employs a microfluidics chip with microchannels and reservoirs etched into plastic tags that capture the sample of whole blood. Biospectrix then uses inertial forces to separate blood cells by size. After this separation, blood cells are forced through a nanoscale filter that lyses blood cells into fragments smaller than bacteria, or other microbes, allowing the microbes to collect intact. Blood cell debris is then removed with another filtering process, and excess water is removed to leave concentrated microbes on a surface made transparent for infrared examination. Infrared is used to perform a spectrometry analysis where the absorption of rays by the captured microbes form unique molecular signatures. The company indicates that these signatures allow it to identify a broad range of bacteria.

The proposed workflow for Biospectrix is simple and can produce results within an hour. 3iDx also indicates that the platform is portable, easy to use and will be cost-effective.

The diagnostic platforms described above are but a few of the potential systems for identifying human infectious diseases in the pipeline. Others are earlier stage, have no plans to develop assays relevant to this report or otherwise are less relevant. These include, but are by no means limited to, the following: BLINK ONE (BLINK DX, Germany), Genedrive® (Genedrive plc, UK), Spartan Cube (Spartan Bioscience, Inc., USA), Accula™ Dock (Mesa Biotech, USA), Puckdx™ (TTP PLC, UK), Polyvalent Analyzer (PAX) platform (ChipCare, CA) and BluBox (BluSense Diagnostics, Denmark). Nonetheless, these and other platforms should be watched as at least some of them could be further developed over the next few years, specifically for use at POC in LMICs.

## Sequencing methods

In addition to the molecular hybridization and amplification testing methods discussed above, sequence analyses of genes or the whole genome of pathogens are also being used in microbiology laboratories, primarily for research. Sequencing methods are able to identify bacterial pathogens that cannot be successfully cultured and to detect changing genetic features in evolving pathogens that cannot be detected by molecular testing (40).

Nucleic acid sequencing involves methods that determine the exact nucleotide sequence of a microorganism's genome, which is the blueprint for the

organism. As such, sequencing can, among other things (i) identify bacteria by sequence analysis of the 16S rDNA; (ii) detect mutations in viral or bacterial genomes that could lead to resistance against antivirals or antibiotics; (iii) detect and classify previously unknown human pathogens; and (iv) establish the genetic relationship of either bacteria or viruses.

In the early years of sequencing, the most commonly used method was Sanger sequencing. In this method, target DNA is copied many times, making fragments of different lengths; fluorescent “chain terminator” nucleotides mark the end of the fragments and allow the sequence to be determined. However, Sanger sequencing is laborious and slow, and it has largely been replaced by new, automated methods, referred to as next-generation sequencing (NGS). Using NGS, an entire human genome can be sequenced in a single run. While this means that NGS does not require target-specific primers, it does require the preparation of libraries in which fragments of DNA or RNA are fused to adapters and barcodes to distinguish the DNA of the sequenced isolate after sequencing (40). Data analysis following sequencing remains a challenge with NGS, as it requires bioinformatics skills and computational resources to analyse large data sets. This analysis is also very time-consuming, taking up to 4–5 days (40).

There are currently two sequencer platforms in relatively common use in sophisticated laboratories: Illumina MiSeq™Dx (Illumina, Inc., USA), which has commercially available IVD applications; and Ion PGM™ from Thermo Fisher Scientific (USA), the applications for which are for research use only (RUO). They are discussed briefly below.

### MiSeq™ Dx instrument

The MiSeq™Dx instrument, pictured in Fig. 54, uses a fluorescence-based approach to reading the bases in a nucleotide sequence. It is the first FDA-cleared and CE-IVD-marked platform for NGS. The MiSeq™Dx is a compact benchtop sequencer with a relatively simple, three-step workflow and an integrated software design, which enables sample tracking, user traceability and results interpretation. The workflow starts with gDNA extracted from human peripheral whole blood specimens or formalin-fixed, paraffin-embedded (FFPE) tissues. The MiSeq™Dx instrument is built on Illumina sequencing by synthesis, which compared to Sanger sequencing, can provide a broader range of DNA variants in less time and with fewer hands-on steps.

Fig. 54. MiSeq™Dx instrument



Illumina currently offers a small menu of IVD assays and kits. These include the MiSeq™Dx Cystic Fibrosis 139-Variant Assay and the MiSeq™Dx Cystic Fibrosis Clinical Sequencing Assay. The company also offers the TruSeq™ Custom Amplicon Kit Dx, which facilitates LDTs that target the genetic variants most important to any given laboratory.

### Ion PGM™ Dx system (Thermo Fisher Scientific, USA)

The Ion PGM™ Dx system is composed of a sequencing instrument that measures the hydrogen ions generated during the incorporation of nucleotides in the DNA sequencing reaction and the ancillary instrumentation necessary for sample processing. More specifically, the system employs Ion Torrent™ technology, which uses pH measurements to read nucleotide sequences using semiconductors, rather than the optics or modified nucleotides used in many other NGS technologies. Ion Torrent™ technology directly translates chemically encoded information (A, C, G, T) into digital information (0, 1) on a semiconductor chip, which per the company is simpler, faster, and more cost-effective and scalable than other NGS methods.

The Ion PGM™ Dx system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, Ion 318™ Dx Chip Kit and data analysis software. The Ion PGM™ Dx system is intended for targeted sequencing of human gDNA derived from peripheral whole blood, and DNA and RNA extracted from FFPE samples. The Ion PGM™ Dx system is not intended for whole genome or de novo sequencing.

Of note with regard to this report is that the Ion S5™ instrument, pictured in Fig. 55, performs targeted sequencing of bacteria, viruses or fungi from biological specimens without the need for culture. It could be of interest if commercialized IVDs become available for the platform.

Fig. 55. Ion S5™ system



using the platform and other platforms for NGS to create LDTs. There are no commercially available IVD applications for the Ion S5™.

In their present configurations, these NGS systems are used in highly sophisticated laboratories primarily for research. There are additional systems available, including MinION (Oxford Nanopore Technologies, UK) and Sequel (Pacific Biosciences, USA), but these systems are not yet used in clinical microbiology labs for reasons of affordability, lower quality of sequences and low throughput (40).<sup>7</sup> Some studies have shown applicability of NGS in drug-resistance testing (48).

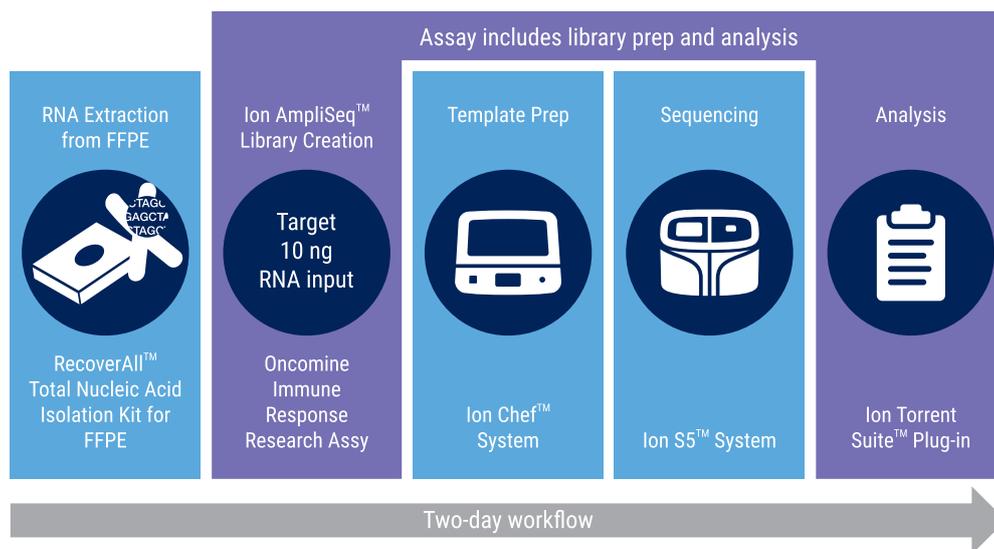
### Other methods of identifying bacterial pathogens

The NGS workflow is illustrated in Fig. 56 for the Oncomine™ Immune Response Research Assay (Thermo Fisher Scientific, USA), a precancer gene expression assay that enables quantitative evaluation of the expression of markers associated with tumour progression. The assay contains the reagents for manual library construction using Ion AmpliSeq™ and a single pool of primers used to perform multiplex PCR for preparation of amplicon libraries using the Ion S5™ instrument. The assay is supported by informatics analysis on the Ion Torrent Suite™ plug-ins. Time to results is about 48 hours.

In addition to the phenotypic, immunoassay and molecular methods of identifying bacterial pathogens discussed above, other techniques are also available. One of these is MS, an analytical technique in which chemical compounds are ionized into charged molecules and the ratio of their mass to charge (m/z) is measured (49).<sup>8</sup> In the 1980s, two methods of MS were developed that have brought these methods into the clinical microbiology laboratory: electron spray ionization (ESI) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS. Of the two methods, MALDI-TOF MS has the advantage of being completely automated, allowing for high throughput and speed, which means TATs are typically

To date, the Oncomine™ assay and other assays for the S5™ Dx instrument are RUO. Laboratories are

Fig. 56. Oncomine™ assay workflow



<sup>7</sup> For detailed information on the future impact of NGS, see Deurenberg, Bathoorn, Chlebowicz et al. (47)

<sup>8</sup> For a detailed description of the principles and methodology of MALDI-TOF MS, see Singhal, Kumar, Kanaujia et al. (49).

reduced by at least one working day compared to conventional ID methods (50). MALDI-TOF is the MS method most often used to identify bacterial species in large clinical laboratories.

## MALDI-TOF MS

MALDI-TOF ionizes target pathogen proteins, and a mass profile of the resulting fragments is then created and compared to a large database of organism profiles for ID. It consists of three basic principles: ionization, separation and detection. MALDI-TOF MS is able to identify bacterial and other pathogens most commonly from isolates cultured on solid media, and it has been widely adopted by clinical microbiology laboratories for this purpose (50, 51). Research has shown that MALDI-TOF can also be used for identifying bacteria from other specimen types (e.g., blood, urine, CSF) to detect UTIs, respiratory tract infections and enteric infections (49); however, when subculture isolates are used, it means a delay of 24–36 hours from blood culture positivity before ID results can be obtained. MALDI-TOF generally cannot be used alone for AST, but there are some software systems available to allow interface of MALDI-TOF testing modules with certain automated AST systems after pathogen ID (52).<sup>9</sup>

There are two well established, commercially available MALDI-TOF systems: (i) Bruker MALDI Biotyper<sup>®</sup> (Beckman Coulter, a Danaher Corporation, USA) and (ii) VITEK<sup>®</sup> MS (bioMérieux, France). Both platforms are FDA cleared and approved for use as IVDs. Each of these systems uses a different database, ID algorithm and instrument.

### Bruker MALDI Biotyper<sup>®</sup> system

The Bruker MALDI Biotyper<sup>®</sup> system identifies microorganisms (bacteria and yeast) using MALDI-TOF MS to determine the unique protein fingerprint of an organism. In particular, the Bruker MALDI Biotyper<sup>®</sup> system measures highly abundant proteins found in all microorganisms. The characteristic patterns of these proteins are then used to identify a particular microorganism by matching it against a microorganism reference library/database. The Bruker MALDI Biotyper<sup>®</sup> system has received FDA clearance and CE-IVD marking for the identification of gram-negative and gram-positive bacteria, anaerobic bacteria and yeast cultured from human specimens. The device is to be used in conjunction with other clinical and laboratory findings to aid in diagnosing bacterial and yeast infections. The Bruker MALDI Biotyper<sup>®</sup> system can identify 333 species or species groups, covering 424 clinically relevant bacteria and yeast species. Per

the company, these represent more than 98% of the typical bacterial ID workflow of clinical microbiology laboratories.

The Bruker MALDI Biotyper<sup>®</sup> system comprises the Bruker MALDI-TOF benchtop microflex<sup>™</sup> LT/SH or the microflex<sup>™</sup> smart LS (pictured in Fig. 57), software, IVD-labelled reagents, a disposable MBT Biotarget 96 or a reusable 48-spot MALDI target plate, and the FDA-cleared microorganism reference library.

Fig. 57. microflex<sup>™</sup> smart LS



The microflex<sup>™</sup> LT/SH is a high-performance system designed for basic applications, while the microflex<sup>™</sup> LT/SH has been designed for applications where a short TAT is required. It utilizes a fast, solid-state Bruker smartbeam<sup>®</sup> laser and high-performance vacuum system.

The Bruker MALDI Biotyper<sup>®</sup> system does not perform AST, but it can be used in conjunction with the MBT STAR-Carba IVD kit and the MBT STAR-BL IVD kit software. Collectively, these allow for rapid microorganism ID and detection of carbapenemase activity in a single workflow.

The MBT-STAR Carba kit utilizes imipenem as the benchmark carbapenem antibiotic. Bacteria from overnight cultures or positive blood cultures are incubated in an imipenem solution. Bacteria with active carbapenemase will inactivate imipenem by hydrolysis of the beta-lactam ring, which is associated with a mass shift. In assays with bacteria without active carbapenemase, only peaks corresponding to the intact antibiotic will be present in the mass spectrum; whereas in assays with bacteria with active carbapenemase, peaks of the intact antibiotic will decrease.

<sup>9</sup> For more information on the use of resistance and/or AST-like methods in combination with MALDI-TOF, see Vrioni, Tsiamis, Oikonomidis et al. (52).

The intensity ratio of hydrolysed to intact antibiotic signals indicates the level of carbapenemase activity. The MBT STAR-BL IVD software module monitors the activity on acquired mass spectra of aliquots of the co-incubation solution by automatically calculating the intact imipenem intensity and corresponding ratio of hydrolysed to nonhydrolysed signals.

The MBT-STAR Carba kit can be used to rapidly detect prevalent Class A, B or D carbapenemase activity in gram-negative *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp.

### VITEK® MS

VITEK® MS is a mass spectrometry system using MALDI-TOF MS to identify microorganisms cultured from human specimens. The system is a qualitative in vitro diagnostic device indicated for use in conjunction with other clinical and laboratory findings to aid in diagnosing bacterial, yeast and mould infections.

The VITEK® MS process is as follows. Depending on the isolate culture, the analyte sample may be directly spotted to a target slide; some analyte specimens (e.g., *Mycobacterium*) must first be processed before spotting. The slide is then loaded onto the MS VITEK® MS instrument, pictured in Fig. 58, where a laser targets the sample spot and pulses the isolate spot, resulting in vibrational excitation of matrix and analyte molecules. The matrix transfers protons to the analyte, resulting in a positive charge. The ionized molecules are then accelerated in an electromagnetic field and a grid electrode in the ionization chamber. The time of flight is then measured by the arrival of ions in a particle detector. Based on the time of flight, the m/z ratio of each particle can be determined and a mass spectrum of the analyte sample mixture is generated. The mass spectra are sufficiently unique to allow taxonomic characterization at the genus and species level. The instrument runs up to 192 isolates per run and delivers results in minutes.

Fig. 58. VITEK® MS instrument



The VITEK® MS system has received FDA clearance and CE-IVD marking for identifying an extensive number of bacterial pathogens. These include, but are not limited to, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella* (which are both characterized as *E. coli*), *Klebsiella pneumoniae*, *Clostridium difficile*, NG (for which a confirmatory test is recommended), *Enterococcus faecium*, *Staphylococcus aureus*, *Campylobacter* spp., *Salmonellae* (for which a confirmatory test is recommended), *Streptococcus pneumoniae*, *Haemophilus influenzae* and MTB complex.

In addition to the VITEK® MS instrument, the VITEK® MS system consists of the VITEK® MS preparation station, which is used to prepare the target slides, and the VITEK® MS acquisition station, which is connected to the instrument and displays its status and the spectra from the instrument. The spectra are then sent to MYLA from the acquisition station for analysis. The MYLA® server contains the MYLA® software and is a middleware solution. MYLA® is connected to both the VITEK® MS preparation station and VITEK® MS acquisition station, providing complete integration and traceability of all individual patient samples and their results.

### Other MS methods

In addition to MALDI-TOF MS, other methods of MS have been developed. These include electrospray ionization MS (ESI-MS) and surface-enhanced laser desorption/ionization (SELDI). Both of these are variations on MALDI-TOF MS. ESI-MS uses a multiplex pool of PCR primers that target conserved sequences in bacterial genomes together with high-precision EIS-MS to identify and group organisms. SELDI is a technique for enriching proteins with specific chemical characteristics and combines chromatography with MS (53). While these methods are interesting and could be used in clinical microbiology, no currently available commercialized platforms were found.

## Conclusion

Immunoassays for bacterial detection are easy to use and fast, with very short TAT. In addition, because the assays generally do not require equipment beyond a test cartridge, the tests can be used at primary care facilities in LMICs. Their utility is somewhat limited, however, because the tests are monoplexes, which can only identify a single bacterial pathogen. In addition, because the performance of some assays has proven to be inadequate, this must be considered with respect to the implementation of the tests.

Molecular-based testing, sequencing and MS, in particular MALDI-TOF MS, are all more rapid IVD pathogen ID methods than traditional phenotypic

methods, and in many cases they can identify certain pathogens, including fastidious or slow-growing bacteria, that cannot be readily identified by culture methods. However, like traditional culture techniques, these methods are most appropriate for use in medium- to high-throughput, sophisticated laboratories with significant infrastructure, consistent electricity, climate control and refrigeration, and well trained laboratory technicians. In resource-limited settings, this would mean that the tests and test platforms would be best positioned in Level III and, more likely, Level IV settings.

Of the platforms described above, there are several diagnostic platforms/systems that have the potential to be used at or near POC. These include the cobas® Liat® system, Solana® platform and revogene®, for which there are no multiplex panels currently available, as well as the Novodiag® system, STAT-Dx and Vivalytic analyser/Bosch Healthcare Solutions, for which multiplex, syndromic testing panels are already available, although not for BSIs. With the exception of the MR platform from T2 Biosystems, none of the platforms can detect BSIs, including *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, from whole blood.

Therefore, for now, there are gaps in the ability of these platforms to identify many of the bacterial pathogens prioritized by WHO. In any event, these platforms do not perform resistance testing and would have to be supplemented with reflex testing to identify genes that directly confer bacterial resistance.

# IVDs for AST and antibiotic resistance testing of bacterial pathogens

Given the ability of bacterial and other pathogens to cease to be susceptible to, and/or acquire and express resistance to, the antimicrobial agents used to treat infections, once pathogen ID has been completed, it is important to do an AST profile of the organism in order to confirm susceptibility to empirical antimicrobial agents or to detect resistance in individual bacterial isolates. Phenotypic AST methods are most commonly used for these purposes. Like bacterial ID, AST can be performed manually or using more rapid, growth-dependent automated instruments in the microbiology laboratory. While generally effective, these phenotypic methods are slow and can be costly; they also require a relatively large number of viable microorganisms and have a limited organism spectrum (54).

Since all phenotypic traits of microorganisms, including those that render them resistant to antimicrobial agents, are encoded on specific genes, genotypic methods can be used to detect the genes coding antimicrobial resistance (28). These methods include molecular methods like PCR and DNA microarrays, as well as, to a limited extent, MALDI-TOF MS and NGS (48, 53). Although faster than phenotypic methods, these methods still have certain downsides. For example, the presence or absence of a gene or a mutation that affects gene function or regulation cannot always accurately predict antibiotic resistance. These methods are also costly.

## Phenotypic methods of AST

At the most fundamental level, phenotypic AST methods bring together the antimicrobial agent of interest and the bacterial microorganism in the same in vitro environment to determine the effect of the presence of the antimicrobial on bacterial growth or viability (28). The bacterial growth is then measured along with the organism's resistance or susceptibility to the antimicrobial agent. In particular, the minimum inhibitory concentration (MIC) is measured. The MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (55). This is usually translated into a sus-

ceptibility testing category based on well-established studies and criteria for an array of antimicrobial agents.<sup>10</sup> The primary interpretive categories, also referred to as break points, are susceptible, intermediate or resistant.

Regardless of the AST method used, certain antimicrobial agents will be selected for use against a particular bacterial isolate. These agents are referred to as the antimicrobial battery or panel. Antimicrobials to which the bacterial isolate is intrinsically resistant are excluded from the test panel (e.g., vancomycin with respect to gram-negative bacilli). By the same token, some antimicrobials have been developed specifically for use against particular bacterial isolates, but not against others (e.g., ceftazidime for use against *Pseudomonas aeruginosa*, but not against *Staphylococcus aureus*) (28). These and other considerations guide the selection of the ultimate antimicrobial test battery used.

As indicated above, several methods are available for AST: classical susceptibility testing methods using solid media (e.g., disk diffusion and antimicrobial gradient), liquid media (broth dilution) and chromogenic media, as well as manual and automated commercial susceptibility testing systems, which use various media.

## Classical methods of AST

The following manual methods bring together antimicrobial agents and bacterial isolates in solid media. In some cases, commercial methods are available to speed and improve the test procedures. These are noted below.

- **Agar dilution:** In agar dilution testing, bacteria are inoculated into an agar medium containing various antimicrobial concentrations. The method is laborious as with each doubling dilution of an antimicrobial agent, it is incorporated into a single agar plate, which means that testing a series of six dilutions of one antimicrobial agent requires the use of six plates, plus a positive growth control plate. Following incubation,

<sup>10</sup> Note that in the United States, the Clinical and Laboratory Standards Institute (CLSI) publishes a series of documents on AST that, among other things, provide lists of potential antimicrobial reagents for routine testing and interpretive criteria for an array of antimicrobial agents, which are updated annually.

the plates are examined for growth and the MIC break points are determined and interpreted using CLSI, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or other established criteria.

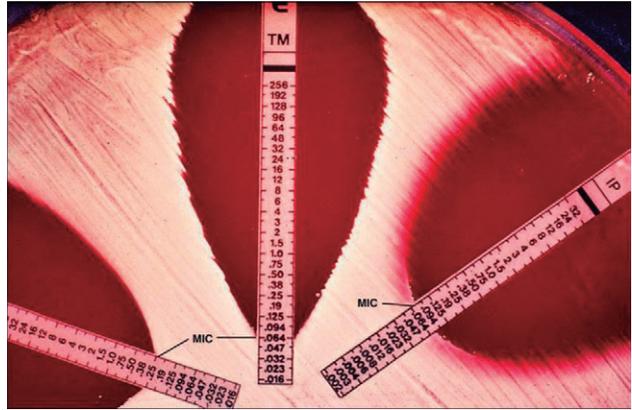
- **Disk diffusion test:** In disk diffusion AST, which is used to test common, rapidly growing bacterial pathogens (4), antibiotic-impregnated filter paper disks (antibiotic discs) are placed on the surface of an agar plate seeded with the bacterial isolate being investigated. With incubation, the bacteria grow on the surface of the plate except where the antibiotic concentration in the gradient around each disk is high enough to inhibit growth. After 16–24 hours of incubation, the diameter of the zone of inhibition around each disk is measured and interpreted using CLSI, EUCAST or other established criteria. While this method is easier, more flexible and less expensive than agar dilution, it provides only qualitative results (susceptible, intermediate or resistant).

The BIOMIC® V<sup>3</sup> microbiology system (Giles Scientific, USA) automates reading, interpreting and expert review of CLSI or EUCAST antibiotic disk diffusion (Kirby-Bauer) tests on 90–150 cm plates. An enlarged plate image with antibiotic disks and zone diameter reading can be displayed on the instrument's screen to provide high-resolution images. It is also possible to combine the results with computer software to produce MIC values. BIOMIC® V<sup>3</sup> can also provide antibiotic resistance monitoring following CLSI or EUCAST guidelines, including a variety of antibiogram reports.

- **Antimicrobial gradient method:** The antimicrobial gradient method is a diffusion method that establishes an antimicrobial concentration gradient in an agar medium in order to determine susceptibility. Unlike the manual disk diffusion test, the antimicrobial gradient method can be used to determine quantitative MICs, which may be necessary in some situations.

Etest® (bioMérieux, France) is a commercial version of the antimicrobial gradient method. It uses thin plastic strips, one side of which is impregnated with a dried antibiotic concentration gradient and the other side of which contains a numerical concentration scale. Five or six strips may be placed radially on an appropriately inoculated agar plate; this permits multiple antimicrobials to be tested on a single bacterial isolate. Following overnight incubation, the plate is examined and read by viewing the strips from the top of the agar plate. The MIC is determined by reading the number on the concentration scale where the border of growth/inhibition edge intersects with it, which is illustrated in Fig. 59.

Fig. 59. Etest® strips and interpretation scale



In addition to AST testing using solid media, broth dilution is also an available manual technique.

- **Broth dilution:** In broth dilution methods, the bacterial organism of interest is challenged with antimicrobial agents in a liquid growth medium. In macrobroth or tube dilution, dilutions of antibiotics are dispensed into test tubes. Each antimicrobial agent is tested using a range of concentrations. Following an incubation time of 20–24 hours, tubes are examined for visible bacterial growth, with the lowest concentration of antibiotic that prevented growth recorded as the MIC. Macrobroth dilution testing produces a quantitative result, but it is laborious and requires considerable space in the laboratory.

In microbroth dilution testing, the methods are the same as macrobroth dilution, but the total broth volume required is much smaller, effectively miniaturizing the procedure. The testing has been standardized and can be done in small, disposable “microdilution” trays, like the one pictured in Fig. 60, which contain 96 wells and predetermined antibiotic concentrations. Moreover, there are commercially supplied frozen or dried microdilution panels available. These include the following: Sensititre™ (Thermo Fisher Scientific, USA) and MicroScan TouchScan (Siemens, USA).

Fig. 60. Broth microdilution tray



### Chromogenic culture media

The use of chromogenic culture media for bacterial pathogen ID and AST is a manual method that utilizes synthetic chromogenic enzyme substrates to specifically identify pathogenic microorganisms based on their enzymatic activity. The majority of chromogenic media, which may be solid or liquid, are both selective and differential, accommodating the inhibition of nontarget microorganisms while enabling target pathogens to grow as coloured colonies due to their metabolism. The method exhibits high specificity.

Although chromogenic media are generally more expensive than traditional media, use of a single chromogenic medium rather than two or three selective ones reduces the cost of sample processing. Further, because only target pathogens should generate colonies of a particular colour, the number of colonies in a polymicrobial culture that require further examination should be reduced. This often results in cost savings from reduced labour time. Because the use of chromogenic media may eliminate certain steps in sample processing (e.g., subculturing and biochemical testing), it can also contribute to quicker confirmation of pathogens and reduce the overall time to result compared with conventional culture methods (56).

There are a wide range of chromogenic media commercially available to clinical laboratories from manufacturers, including bioMérieux, Thermo Fisher Scientific and Bio-Rad. These can be used to detect numerous bacterial pathogens. These include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium difficile*, *Campylobacter* spp., *Salmonella* spp., *Shigella* spp. and Shiga toxin-producing *Escherichia coli*.

Chromogenic media can also be used to screen pathogens with acquired antimicrobial resistance, including VRE, ESBL-producing and carbapenemase-producing *Enterobacteriaceae*, carbapenem-resistant *Acinetobacter* spp. and MRSA.<sup>11</sup>

Culture using chromogenic media, like other culture methods, is used in conjunction with methods of bacterial pathogen ID, including molecular methods and MALDI-TOF MS. The convenience of molecular methods for the ability to screen for a wide variety of pathogens – e.g., enteric and respiratory – simultaneously and effectively is of great value, but the use of chromogenic media to complete pathogen analysis – e.g., for AST following ID by PCR or other molecular methods – is also required. As Perry concludes: “Chromogenic media should no longer be assessed as individual tools but as potentially useful components within diagnostic algorithms” (56).

### MICRONAUT ID and AST (MERLIN Diagnostika GmbH, a Bruker company, Germany)

MERLIN offers a number of MICRONAUT assays for ID and susceptibility testing of bacteria and yeasts. These assays are primarily manual, but can be combined with some automated instrumentation.

The test principle of MICRONAUT ID systems is based on phenotypic detection of the biochemical properties of microorganisms. Various substrates are placed in dehydrated form into the wells of microtitration plates and are dissolved by adding bacterial suspensions. Following an incubation period of 5 to 24 hours, depending on the test system, the ID plate is measured in a commercially available photometer (not provided by MERLIN) and the test is evaluated by MICRONAUT software (for which an external computer is required).

All MICRONAUT ID systems offer a standardized procedure and optimized software-controlled reading and interpretation of results. Available assays include:

- **MICRONAUT-GNE:** identifies *Enterobacteriaceae* and other gram-negative bacteria. Identification takes place via 24 biochemical reactions (chromogene substrate, decarboxylases, classical reactions and fermentations after 18–24 hours; 65 different taxa are included in the database.
- **MICRONAUT-NF:** identifies nonfermenting gram-negative and some glucose-fermenting bacteria via 27 biochemical reactions (decarboxylases, fermentation, assimilations, glucosidases/esterases, classical reactions). TAT is 24 hours; 62 different taxa are included in the database.

<sup>11</sup> For a review a various media available for bacterial pathogens and their performance, see Perry (56).

- **MICRONAUT-STAPH:** identifies relevant staphylococci within 6 or 18–24 hours via 21 biochemical reactions; 21 different taxa are included in the database.
- **MICRONAUT-IDS:** identifies clinically important *Enterobacteriaceae*, nonfermenters, staphylococci, enterococci and streptococci via 23 biochemical reactions (peptidases, decarboxylases, glucosidases/esterases, fermentations and classical reactions). TAT is 5–6 hours.

In addition to the MICRONAUT ID systems, MICRONAUT also offers AST systems. The test principle of the MICRONAUT system for AST is based on classical phenotypic detection of resistance as expressed by microbial growth in the presence of antibiotic compounds. The microdilution procedure used is a standardized method with accepted reference systems (EUCAST or CLSI) for determining MICs.

For all MICRONAUT AST systems, the various antibiotics are placed with or without broth in a dehydrated form into the wells of microtitration plates supplied by the company and are dissolved by adding bacterial suspensions. After 6 hours (with rapid AST) or 18–24 hours of incubation at 35–37 °C, the AST plate is measured in a photometer (commercially available), and the test is evaluated with the MICRONAUT software or read visually. Users can choose between EUCAST or CLSI standards, and customize antibiograms from more than 200 antibiotics or select from a wide variety of standard layouts.

MERLIN offers MICRONAUT AST microplates for automated or manual susceptibility testing of bacteria, MICRONAUT-S plates and MICRONAUT-SB plates; the latter offers a shorter incubation period of 6 hours for rapid AST. In addition to these two AST systems, there is also a manual MICRONAUT MIC-Strip Colistin test that enables AST against the backup antibiotic colistin by using the broth microdilution method for *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* group. The assay, which is CE-IVD marked, allows phenotypic detection of the *mcr-1* colistin resistance gene using standardized MIC determination of colistin according to EUCAST recommendations in a test strip format.

In addition, the MICRONAUT-UR test system is designed to both identify and detect susceptibility in UTIs. Fifty taxa, including *Acinetobacter* spp., *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*, are identified using 24 reactions. Further, AST of all relevant gram-negative bacilli and gram-positive cocci is done using 22 antibiotics, including MRSA detection and ESBL screening. With the exception of automated reading, evaluation and interpretation of results, the test is manual.

MERLIN also offers the MICRONAUT ASTroID, which is an automated workflow combining MALDI-TOF MS for microbial ID and MICRONAUT for AST. Procedures are standardized, and identical samples are used for ID and AST.

Given the manual nature of the MICRONAUT systems, their use is generally most suited to sophisticated laboratories with well trained laboratory technicians. In addition, because the system uses traditional phenotypic methods of microbial ID and AST, results generally take a day or more.

## Automated combined ID and AST systems

In addition to conventional manual methods of AST, there are four automated combined ID and AST instruments: VITEK® 2 system (bioMérieux, France), BD Phoenix™ automated microbiology system (BD, USA), MicroScan systems (Beckman Coulter, a Danaher Corporation, USA) and Sensititre™ ARIST™ 2X (Thermo Fisher Scientific, USA). These systems, which are relatively easy to operate and provide a streamlined workflow and quantitative results, are in widespread use in clinical microbiology laboratories in the United States, but are not generally available in resource-limited settings (4, 5). Nonetheless, the automated systems, like current manual AST systems, require the use of cultured bacterial isolates, and AST is based on bacterial growth and turbidity changes. Therefore, even the commercially available, automated systems are plagued by slow TAT and low sensitivity (4).

The primary commercialized automated AST systems are described below. They use varying degrees of automation of inoculum preparation and inoculation, varying methods to detect growth, and varying algorithms to interpret and determine MIC values as well as categorical findings (i.e., susceptible, intermediate or resistant).

### VITEK® 2 system

The VITEK® 2 system is a family of automated instruments (VITEK® 2 Compact, VITEK® 2 and VITEK® 2 XL), which differ with respect to levels of automation and capacity. All three systems use the same compact, colorimetric reagent cards (about the size of a credit card), which are incubated and interpreted automatically. While the VITEK® 2 Compact is primarily for industrial use, it could be used in low- to medium-volume clinical laboratories; the VITEK® 2, pictured in Fig. 61, and VITEK® 2 XL are primarily for use in large clinical microbiology laboratories.

Fig. 61. VITEK® 2 system



The VITEK® 2 system reagent cards, pictured in Fig. 62, have 64 wells that can each contain an individual test substrate. Each card has a pre-inserted transfer tube used for inoculation. Cards also have barcodes that contain information on product type, lot number, expiration date and a unique identifier. There are currently five reagent cards available for identifying different organism classes: (i) gram-negative fermenting and nonfermenting bacilli – 76 antimicrobials and ESBLs; (ii) staphylococci and/or enterococci – 55 antimicrobials, four high-level aminoglycoside screens and an inducible clindamycin resistance (ICR) test; (iii) streptococci – 14 antimicrobials and ICR test and gentamicin synergy; (iv) *Streptococcus pneumoniae* – 23 antimicrobials; and (v) yeasts – six antifungals.

Fig. 62. VITEK® 2 colorimetric ID cards



ID cards are inoculated with microorganism suspensions using an integrated vacuum device, and a test tube containing the microorganism suspension is placed into a special rack (cassette). The ID card is placed in a neighbouring slot. The VITEK® 2 and VITEK® XL cassettes can accommodate up to 15 tests; VITEK® 2 Compact cassettes can accommodate up to 10 tests. Inoculated cards are then incubated using repetitive turbidimetric monitoring of bacteria grown during an incubation period of between 4 and 10 hours; the carousel incubator can accommodate up to 30 or up to 60 cards.

During incubation, the VITEK® 2 systems perform optical readings every 15 minutes to measure the light transmitted through each well. Algorithmic analysis of the growth kinetics in each well is performed by the system's software to derive the MIC results, which are validated with the VITEK® Advanced Expert System™ (AES) software. An interpretation category is assigned, and the organism's antimicrobial resistance patterns are reported.

The VITEK® 2 instruments can be linked with the VITEK® MS ID system using MYLA connectivity. The VITEK® MS ID system and MYLA® are detailed in the previous section of this report.

### BD Phoenix™ automated microbiology system

The BD Phoenix™ automated microbiology system is an automated ID and susceptibility system for testing clinically relevant bacterial isolates. It is intended for in vitro rapid ID and quantitative determination of antimicrobial susceptibility by the MIC of certain bacterial pathogens. The system comprises the BD Phoenix™ instrument, pictured in Fig. 63, and software, disposable panels containing biochemicals for organism ID testing and antimicrobial agents for AST determinations, broths for ID and AST, and a susceptibility testing indicator.

Fig. 63. BD Phoenix™ automated microbiology system instrumentation



The BD Phoenix™ system identifies a broad range of gram-positive (including genera *Staphylococcus*, *Streptococcus* and *Enterococcus*) and gram-negative (15 different genera, including *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Salmonella* and *Shigella*) bacteria using modified conventional, fluorogenic and chromogenic substrates. The instrument can analyse up to 100 ID and AST combination panels at the same time. The BD Phoenix™ disposable test panel, pictured in Fig. 64, is a sealed, self-inoculating moulded polystyrene tray with 136 microwells containing dry reagents and is available in ID-only, ID/AST and AST-only formats.

Fig. 64. BD Phoenix™ AST panels



Bacteria for susceptibility testing must be a pure culture and already preliminarily identified as either a gram-negative or gram-positive isolate. The BD Phoenix™ AST method is a broth-based microdilution test that uses a redox indicator to detect organism growth in the presence of an antimicrobial agent. It monitors and reads each panel every 20 minutes using measurements of changes to the indicator as well as bacterial turbidity to determine bacterial growth. The readings are interpreted to provide (i) an ID of the bacteria isolate; (ii) MIC values; and (iii) categorical interpretations (susceptible, intermediate, resistant or not susceptible) of bacterial growth. MIC results are generated in 6–16 hours.

### MicroScan systems

Beckman Coulter currently offers three MicroScan systems for ID/AST of clinically relevant bacterial isolates, including gram-positive (*Staphylococcus* and related genera and *Streptococcaceae*) and gram-negative glucose fermenting as well as glucose nonfermenting bacteria. The systems are the DxM MicroScan WalkAway system, pictured in Fig. 65, the MicroScan WalkAway *plus* system, and the autoSCAN-4 system. The DxM MicroScan WalkAway and the MicroScan WalkAway *plus* systems are automated systems, which consist of MicroScan panels (microwells), an inoculator, a MicroScan WalkAway instrument and a Lab-Pro information system. The autoSCAN-4 system is a smaller, manual reading system only, with a separate, stand-alone incubator device. The two automated systems are self-contained incubators/readers that can incubate and analyse 40–96 MicroScan panels at a time.

Fig. 65. DxM MicroScan WalkAway system



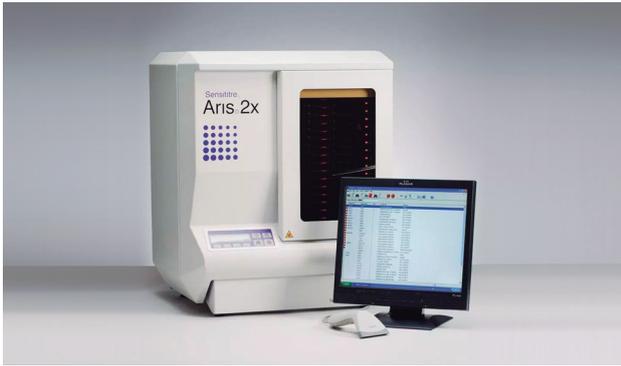
With an incubation time of 16–18 hours, the two automated MicroScan systems are overnight testing systems that offer either a full MIC panel or combination ID and AST panels, which are manually inoculated with bacteria isolated from clinical specimens and then placed in one of the incubator slots in the instrument. The panels contain ID media consisting of preloaded substrates and/or growth inhibitors, which will exhibit colour changes (fluorogenic substrates) or increases in turbidity (if using turbidimetric endpoints). The panels may also contain series of antimicrobial agents in specified concentrations for AST. The instrument incubates the trays for an appropriate time depending on the panel, examining them periodically with either a photometer or fluorometer to determine growth development, and determines the MIC. The company cites as an advantage of their systems that the MIC technology on them detects emerging resistance as it occurs, without reliance on historical data or virtual MIC.

The DxM MicroScan WalkAway and the MicroScan WalkAway *plus* systems are intended for use in medium- to high-capacity laboratories, while the autoSCAN-4 device is intended for small-capacity laboratories. For optimized, high-volume testing, the DxM MicroScan WalkAway system can be paired with the Copan WASP® DT and the Bruker MALDI Biotyper®, both of which were described earlier in this report.

### Sensititre™ ARIS™ 2X

The Sensititre™ ARIS™ 2X, pictured in Fig. 66, is an automated benchtop in vitro diagnostic instrument for ID and clinical susceptibility testing of nonfastidious gram-negative isolates (*Enterobacteriaceae*, *Pseudomonas aeruginosa* and other non-*Enterobacteriaceae*) and of nonfastidious gram-positive isolates (*Staphylococcus* spp., *Enterococcus* spp. and beta-haemolytic streptococci other than *Streptococcus pneumoniae*). Additional testing capabilities are for yeasts (*Candida* spp.) and MTB. Not all tests are FDA cleared; some are CE-IVD marked or have RUO designations.

Fig. 66. Sensititre™ ARIS™ 2X instrument



The Sensititre™ ARIS™ 2X is an overnight incubating and reading device that can accommodate 64 MIC, break point or ID plates, for a combination of 192 possible tests on a single instrument. The instrument is part of the Sensititre™ system, which includes the Sensititre™ AIM™ system for automated inoculation, the OptiRead™ automated fluorometric plate reading system, the Vizion™ MIC viewing system, which facilitates reading of all organisms and the Sensititre™ Windows® (SWIN) software system, which combines manual, semi-automated and fully automated read options onto a single software platform.

Inoculation of the Sensititre™ ARIS™ 2X instrument panels (96-well plates) may be done using the automated Sensititre™ AIM™ instrument or manually. The ID system is based on 32 biochemical tests pre-dosed and dried in the Sensititre™ plates; the plates may be read manually or using the automated OptiRead™ instrument. The Sensititre™ ARIS™ 2X is based on fluorescence measurement and detects bacterial growth by monitoring the activity of specific surface enzymes produced by the test organisms. Growth can be measured after 18–24 hours of incubation time.

## Novel AST methods

In addition to classical AST methods or automated phenotypic methods of ID/AST, there are interesting new methods, including imaging-based and non-imaging-based techniques, which are designed to deliver faster results. Some of these technologies are already in the market, while some are in the near-term development pipeline. These are discussed below.

### Imaging-based ID/AST or AST only

#### Accelerate Pheno™ system (Accelerate Diagnostics, USA)

The Accelerate Pheno™ system is a relatively new diagnostic device that can perform rapid bacterial ID of organisms that cause BSIs and can provide AST; it

is CE marked and FDA cleared. The system provides qualitative ID of organisms utilizing FISH probes targeting organism-specific rRNA sequences and quantitative morphokinetic cellular analysis using time-lapse imaging for AST. The Accelerate Pheno™ system uses electrokinetic concentration technology to direct microbial cells to a surface and hold them in position for image capture and analysis as they grow.

Accelerate Diagnostics offers the Accelerate PhenoTest™ BC, which is a multiplexed in vitro diagnostic test capable of simultaneously detecting and identifying multiple microbial targets followed by susceptibility testing of the appropriate detected bacterial organisms. For each drug-specific AST, a single concentration of antibiotic is used to provide MICs and categorical interpretations (susceptible, intermediate or resistant) per FDA and/or CLSI break points. The PhenoTest™ BC is performed directly on blood culture samples and is intended for use with the Accelerate Pheno system.

The PhenoTest™ BC can identify 16 organisms – six gram-positive and eight gram-negative bacteria, as well as two *Candida* species. The gram-positive bacteria are CNS, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus lugdunensis* and *Streptococcus* spp. The gram-negative bacteria are *Acinetobacter baumannii*, *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Serratia marcescens*. The *Candida* species are *Candida albicans* and *Candida glabrata*.

The PhenoTest™ BC provides AST data for six gram-positive antimicrobial agents (including ampicillin, ceftaroline, erythromycin, vancomycin and methicillin), two gram-positive resistance phenotype markers (MRSA and MLSb [macrolide-lincosamide-streptogramin B resistance]) and 12 gram-negative antimicrobial agents (including amikacin, ceftazidime, ceftriaxone, ciprofloxacin and gentamicin).

The PhenoTest™ system comprises the Accelerate Pheno™ instrument, pictured in Fig. 67, software, host computer, analysis computer and the Accelerate PhenoTest™ BC kit.

Fig. 67. Sensititre™ ARIS™ 2X instrument



The Pheno™ instrument is a compact benchtop device. The PhenoTest™ BC kit contains a sample vial, a 48-channel disposable test cassette and a reagent cartridge needed to test samples from a blood culture bottle that has been determined to be positive by a continuous monitoring blood culture system. All ID and AST is performed in individual flow cells of the test cassette. The reagent cartridge contains gel electrofiltration stations, FISH probes, antibiotics and reagents for automated sample preparation, ID of bacterial and fungal target organisms, and AST and phenotypic resistance detection testing for bacterial target organisms. The user loads an aliquot of the positive blood culture in the sample vial, places the test cassette, reagent cartridge and sample vial into the Accelerate Pheno™ system device, presses the button on the device to close the door and starts the run. The remainder of the operations are automated. Organism ID is available within 90 minutes, and AST is available in approximately 7 hours from a positive blood culture specimen (57).

#### oCelloScope (BioSense Solutions ApS, Denmark)

The oCelloScope, pictured in Fig. 68, is a small, portable optical imaging instrument that is based on imaging the growth over time of a population of bacterial cells in a fluid sample containing antibiotics in a 96-well microtitre plate. It is essentially an automated microscope using digital time-lapse technology that scans through a fluid sample, generating a series of images.

Fig. 68. oCelloScope instrument and microwell plate



The optical axis is tilted 6.25° relative to the horizontal plane, which facilitates scanning of volumes by recording a series of images to form an image stack. The image acquisition process is repeated every 15 minutes over time (generally a period of 12 hours), and the time-lapse sequence of best-focus images is used to generate a video. All of the images are saved, and the data is processed using imaging algorithms to

quantify changes in the area occupied by a growing population of cells. The oCelloScope's UniExplorer software is used for this purpose.

The oCelloScope can be used for real-time monitoring of microbial growth and growth inhibition as well as quantification of morphological features of up to 96 specimens at a time using standard microtitre plates. In a study to evaluate oCelloScope for use in BSIs, AST results were available from blood culture specimens in 1–4.2 hours following incubation, depending on the bacteria-antibiotic combination and whether the bacteria had reached the stationary growth phase prior to testing (58).

The oCelloScope is not commercially available. Additional studies of the technology are ongoing.

#### Sidecar, Alfred 60<sup>AST</sup> and HB&L systems (Alifax SPA, Italy)

Unlike other AST platforms that rely on specimens cultured for more than 10–12 hours until bacterial colonies form, Alifax manufactures three AST instruments that rely on “rapid bacterial culture”, which is based on the concept of monitoring bacterial culture in real time. Alifax claims that this method results in faster TAT, since only the minimum required concentration of bacteria are allowed to grow (rather than a full colony). Alifax makes three instruments, pictured in Fig. 69, that utilize this rapid bacteria culture method:

- Sidecar: a fully integrated system with an automated streaker of solid culture plates, incubator, tube inoculator and turbidity meter;
- Alfred 60<sup>AST</sup>: a fully automated system that consists of an incubator, inoculator and turbidity meter; and
- HB&L: a semi-automated system consisting of an incubator and turbidity meter.

Each instrument uses turbidity measurements (light scattering) to determine the concentration of bacteria in tubes that have been inoculated with specimens. The concentration of microorganisms is proportional to the amount of light scattered, which is detected by the instruments. Quantitative bacterial count results are reported in culture-forming units (CFU) per millilitre. Turbidity levels in samples are converted to McFarland (McF) standards, and when samples reach a bacterial concentration of 0.5 McF, the sample is then used to inoculate another tube (manufactured by Alifax) containing predefined antibiotics and growth media. The growth within these tubes is then monitored again, using turbidity to assess growth in the presence of the antibiotic. Currently, Alifax manufactures tubes for 33 different EUCAST drugs and 31 CLSI drugs.

Fig. 69. Alifax instruments: Sidecar (left), HB&amp;L (centre) and Alfred 60AST (right)



Alifax instruments do not identify bacterial pathogens, which must be done using other methods – e.g., MALDI-TOF MS.

Tests and applications available for the Alifax systems include:

- urine screening (culture)
- susceptibility test in urine (Uro-Quick)
- susceptibility test in blood culture
- residual antimicrobial activity (RAA)
- human biological liquids bacterial culture
- multidrug-resistant organisms (MRSA, ESBL/AmpC, carbapenem and VRE [vancomycin-resistant enterococci] screening kit [pipeline]).

TAT for results (e.g., urine screening, human biological liquids, bacterial culture and multidrug-resistant organisms) ranges from 3 hours to about 6.5 hours. All assays and systems are CE-IVD marked.

## Nonimaging AST

### LifeScale® (Affinity Biosensors, USA)

Affinity Biosensors offers the LifeScale®, pictured in Fig. 70, which is an automated system for rapid AST. It uses microelectromechanical systems (MEMS) technology. Unlike optical techniques, which depend on the optical properties of the inoculum to determine microbe concentration, LifeScale® employs the principle of resonant mass measurement to determine microbe mass, and at the same time counts each microbe to yield both the microbe concentration and the mass distribution of the population. The LifeScale®-AST is able to provide a MIC result for most gram-negative organisms in 3–3.5 hours, although some organisms, e.g., *Pseudomonas* spp. may take longer.

Fig. 70. LifeScale® instrument



The LifeScale® instrument is a benchtop direct-from-blood analyser that uses custom-designed Thermo Fisher Sensititre 96-well broth microdilution plates. After blood specimens are prepared for the plates, a simple centrifuge step is added in order to remove red blood cells. LifeScale® generates and automatically measures the MIC for each of the antimicrobials on the panel. Once a species ID has been made, the LifeScale® instrument produces a final report with both MIC and interpretive results, based on either FDA, CLSI or local break points.

LifeScale®-AST is currently RUO; no IVD is available commercially. Initially, LifeScale®-AST will target gram-negative rods from positive blood culture; however, the company indicates that the instrument is capable of determining bacterial growth and, therefore, phenotypic AST from gram-negative and gram-positive species.

In addition, there are novel technologies available for detecting resistance to particularly critical compounds, including phenotypic carbapenemase detection in *Enterobacteriaceae* and nonfermenters. These include:

### RAPIDEC® CARBA NP (bioMérieux, France)

RAPIDEC® CARBA NP is a colorimetric phenotypic in vitro diagnostic test for qualitatively detecting carbapenemase enzymes in *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies that have MIC values to any carbapenem. It is intended as an aid in preventing and controlling infection, in particular, HAIs caused by carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*, and not to guide or monitor the treatment of these bacterial infections. The test is intended to be used in conjunction with other laboratory tests, including AST.

The RAPIDEC® CARBA NP test, pictured in Fig. 71, is a strip test that can be performed directly from bacterial colonies grown on selective or nonselective agar plates. It comprises five wells prepared with premeasured portions of the necessary substrates for the reactions. Test results are available in 30 minutes to 2 hours. The test is FDA cleared.

Fig. 71 RAPIDEC® CARBA NP test



### Rapid CARB Blue kit (Rosco Diagnostica, Denmark)

Similar to RAPIDEC® CARBA NP, the Rapid CARB Blue test is a colorimetric phenotypic in vitro diagnostic test, pictured in Fig. 72, for qualitatively detecting carbapenemase enzymes in *Acinetobacter* spp., *Enterobacteriaceae* and *Pseudomonas aeruginosa* colonies. The test is based on hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lysis), which is detected by changes in a pH indicator (bromothymol blue). The test is intended for use as an aid in preventing and controlling HAIs. It is CE-IVD marked.

The Rapid CARB Blue kit is in a tablet format; the antimicrobial agent is encapsulated in the tablet. Bacterial suspensions are prepared from isolates grown

on agar plates. In two separate tubes, the suspension is vortexed briefly with an imipenem bromothymol Rapid CARB Blue tablet and a Carb Negative Control Blue Diatab, respectively, and further centrifuged. Results are then interpreted after 15 minutes, 30 minutes or 1 hour of incubation at 37 °C, and change in colour (e.g., from blue to yellow) is considered a positive result.

Fig. 72. Rapid CARB Blue suspensions



Two additional kits for detecting bacterial resistance are available from the company: the Rapid ESBL Screen kit and Neo-Rapid CARB kit.

## Pipeline technologies for AST

In addition to the emerging AST technologies discussed above, other technologies are being developed and refined that are early-stage systems. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established. A few of these pipeline technologies focused on rapid AST are described briefly below.

- **QMAP (QuantaMatrix, Inc., Korea):** QuantaMatrix uses microfluidic agarose channel (MAC) technology to detect MIC and systemic inflammatory response syndrome (SIRS) while observing bacterial growth in real time using a proprietary microfluidic chip. The MAC chip, which is composed of microfluidic channels containing bacteria in agarose and a well to supply antimicrobials and nutrients, is integrated with a 96-well platform for high-throughput analysis. The imaging region is the interface between the liquid medium and the microfluidic channel. The immobilized bacterial cells at the bottoms of the channels are monitored for single-cell morphological analysis (SCMA) by time-lapse bright-field microscopy. SCMA can determine AST by analysing and categorizing the morphological changes in single bacterial cells under various antimicrobial conditions. The company's dRAST system captures an image every

hour and can determine MIC and SIRS after approximately 6 hours from blood culture specimens. The system also has applications for TB.

- **QuickMIC™ AST system (Gradientech AB, Sweden):** Gradientech is developing a novel and proprietary microfluidic technology solution to create stable substances for rapid AST from blood culture samples. The system, called QuickMIC™, will monitor and quantify bacterial growth of microcolonies within precise antibiotic gradients providing AST within 2 hours. The QuickMIC™ solution can be applied in parallel with, or following bacterial ID, with a system like MALDI-TOF MS. Panels for gram-negative and gram-positive bacteria will also be available. Per the company, QuickMIC™ is a universal AST solution that detects any functional bacterial resistance; it is not based on specific probes or image database comparison.
- **Captiver™ system (Astrego Diagnostics AB, Sweden):** Astrego is a young diagnostics company that is entirely dedicated to AST solutions. The Astrego technology is based on microfluidics and imaging analysis techniques. In simple terms, a sample is loaded onto a microfluidic chip; bacteria present in the sample are caught in bacteria-sized “traps”. Trapping of bacteria is monitored, and the loading time gives an estimate of the bacterial density in the sample. A fraction of the trapped bacteria are exposed to a candidate antibiotic. Bacterial growth is monitored in each trap, both with and without the candidate antibiotic. The average bacterial growth rates, with and without candidate antibiotics, are calculated in real time. Bacteria are considered susceptible if their growth is duly inhibited. To date, Astrego has focused primarily on UTIs using its Captiver™ system, but it is also focusing on AST for sepsis from positive blood cultures.

Also under development are AST products using microcantilevers, plasmonic imaging and tracking, flow cytometry and isothermal microcolorimetry.

These and other future technologies for AST are discussed in some detail in Syal and colleagues and van Belkum and Dunne (4, 54).

## Conclusion

Phenotypic ID of pathogens and AST are mainstays of the clinical microbiology laboratory. Their advantages over genotypic methods include the ability to predict both drug resistance and drug susceptibility as well as the ability to quantify the level of susceptibility of a bacterial isolate to individual antimicrobial agents (50). However, phenotypic pathogen ID typically takes 24 hours, with another 24–48 hours or more for susceptibility testing (Fig. 73). These delays in test results can lead to longer hospital stays, increased cost and patient mortality.

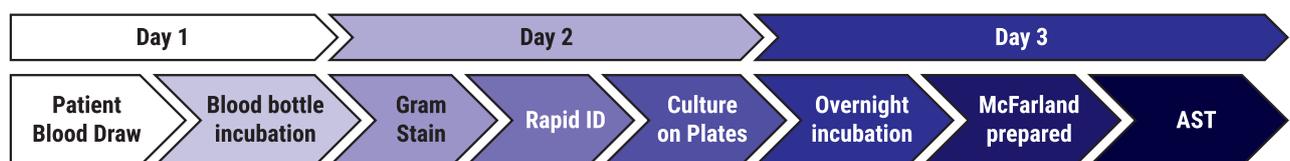
As described above, the introduction of automated phenotypic ID and AST systems have helped to improve TAT, and newer technologies, like the Accelerate Pheno™ system, which combines FISH for ID and multiplexed automated digital microscopy for susceptibility determination, provide both ease of use and faster results. Additional novel phenotypic technologies are under development.

Molecular-based assays are now being used routinely in clinical microbiology laboratories both for ID and resistance testing of bacterial, viral and other pathogens. These methods are discussed below.

## Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance

NAAT-based methods, especially PCR, are the genotypic methods that have gained the most widespread acceptance for both pathogen ID and characterization of bacterial resistance. For example, as described earlier in this report, multiplex PCR has made simultaneously detecting multiple AMR genes in bacteria possible. NAAT-based methods that can identify bacterial pathogens and the genes that directly confer bacterial resistance from positive blood cultures are

Fig. 73. Rapid CARB Blue suspensions



an important target of commercial systems. It should be kept in mind that while these methods can provide information on drug resistance, they cannot provide information on possible acquired resistance markers. NAAT-based testing does not provide information on susceptibility nor does it permit quantification of susceptibility to specific antibiotics, i.e., it is not possible to determine MICs (50, 51). As a result, most commercial assays target positive blood cultures yielding growth of cluster-forming gram-positive cocci, as opposed to gram-negative organisms (50).

## Molecular platforms for identifying pathogens and characterizing bacterial resistance from blood culture

### Verigene® (Nanosphere/Luminex, USA)

Luminex offers a number of syndromic test panels for use on its Verigene® system. These include both a Gram-Positive Blood Culture Nucleic Acid (BC-GP) test and a Gram-Negative Blood Culture Nucleic Acid (BC-GN) Test. Both assays are qualitative multiplexed in vitro diagnostic tests for simultaneously detecting and identifying selected gram-positive or gram-negative bacteria, as the case may be, along with resistance markers from blood cultures. The assays are intended to aid in diagnosing BSIs when used in conjunction with other clinical and laboratory findings, but are not used to monitor these infections. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing, identify organisms not detected by the assays and differentiate mixed growth.

In addition to certain other gram-positive bacterial pathogens, the BC-GP test detects and identifies the following bacterial genera and species from the WHO priority list: *Staphylococcus aureus* and *Enterococcus faecium*. It also detects the *mecA* resistance marker, inferring *mecA*-mediated methicillin resistance, and the *vanA* and *vanB* resistance markers, inferring *vanA/vanB*-mediated vancomycin resistance. Time to result is 2.5 hours.

The BC-GN test detects and identifies the following bacterial genera and species from the WHO priority list: *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (but does not distinguish *Escherichia coli* from *Shigella* spp.). The assay also detects and identifies the following resistance markers: CTX-M (*bla*<sub>CTX-M</sub>), KPC (*bla*<sub>KPC</sub>), NDM (*bla*<sub>NDM</sub>), VIM (*bla*<sub>VIM</sub>), IMP (*bla*<sub>IMP</sub>) and OXA (*bla*<sub>OXA</sub>). Time to result is 2 hours.

Additional tests that can be performed on the Verigene® system include a respiratory panel, the Verigene® Respiratory Pathogens Flex (RP-Flex) nucleic acid test, and an enterics panel, the Verigene®

Enteric Pathogens (EP) nucleic acid test, as well as a test for *Clostridium difficile*, the Verigene® *Clostridium difficile* (CDF) nucleic acid test.

- **Verigene® RP-Flex:** a qualitative in vitro diagnostic test for detecting and identifying multiple respiratory pathogen nucleic acids in nasopharyngeal swabs collected in viral transport media and obtained from individuals suspected of respiratory tract infections. It detects 13 viruses, including influenza A and B, and RSV A and B; it also detects four bacteria: *Bordetella parapertussis/bronchiseptica*, *Bordetella holmesii* and *Bordetella pertussis*. Time to result is 2 hours.
- **Verigene® EP:** a qualitative test for simultaneously detecting and identifying common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection. The assay identifies the following pathogenic bacteria: *Campylobacter* group (comprising *C. coli*, *C. jejuni* and *C. lari*), *Salmonella* spp., *Shigella* spp. (including *S. dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri*), *Vibrio* group (comprising *V. cholerae* and *V. parahaemolyticus*) and *Yersinia enterocolitica*. Time to result is 2 hours.
- **Verigene® CDF:** a qualitative multiplexed in vitro diagnostic test for rapidly detecting *tcdA*, *tcdB* and *tcdC* sequences of toxigenic strains of *Clostridium difficile* and for presumptive ID of PCR ribotype 027 strains from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. The CDF test is indicated for use as an aid in diagnosing CDI; detection of PCR ribotype 027 strains of *C. difficile* by the CDF test is solely for epidemiological purposes and is not intended to guide or monitor treatment of *C. difficile* infections. Time to result is less than 2 hours.

All of the above assays are intended to be performed on the Verigene® system, pictured in Fig. 74, which is a benchtop sample-to-result molecular diagnostics workstation that consists of two modules: one or more multiple Verigene® Processor SP modules or units and one Verigene® Reader. The Verigene® Processor SP automates the steps of (i) sample preparation and, if required, target amplification (i.e., cell lysis and magnetic-bead-based bacterial DNA isolation and nucleic acid amplification); and (ii) hybridization (i.e., detecting and identifying bacterial-specific DNA in a microarray format using gold nanoparticle probe-based technology, called NanoGrid Technology).

Fig. 74. Verigene® system



Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of trays and finally loads the specimen into a test cartridge for hybridization. The Verigene® test cartridges, pictured in Fig. 75, are single-use, self-contained test units made up of (i) a microfluidic cassette that contains all of the hybridization reagents needed for a single test and captures the waste materials generated during test processing, and (ii) a substrate holder that contains a glass slide that serves as a solid support for the microarray used to capture targeted nucleic acids.

Fig. 75. Verigene® test cassette



To obtain the test results after test processing is complete, the user removes the test cartridge from the Processor SP and inserts the substrate holder into the Verigene® Reader for analysis. Light scatter from the capture spots is imaged by the device, and intensities from the microarray spots are used to make a determination regarding the presence or absence of a bacterial nucleic acid sequence/analyte. The determination is automated and is made by means of a software-based decision algorithm in the Verigene® Reader.

The complexity of the overall system suggests that it is only appropriate for use in a sophisticated clinical laboratory. The Verigene® assays described above are all FDA cleared, and all except the *Clostridium difficile* assay are CE-IVD marked.

### BioFire® FilmArray® (bioMérieux, France)

BioFire® Diagnostics offers its FilmArray® system, which is a multiplex nucleic acid-based sample-to-answer diagnostic platform with an emphasis on syndromic test panels. Of particular significance for this report is the FilmArray® Blood Culture Identification (BCID) panel, which is intended for use with the FilmArray® instrument for the qualitative in vitro detection and ID of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BCID assay is performed on positive blood culture samples that determine the presence of organisms by a continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain.

The BCID panel simultaneously tests a single blood culture sample for 24 different organisms and organism groups that cause BSIs as well as three genetic markers that confer AMR. TAT is approximately 1 hour. Note that both gram-positive and gram-negative bacteria are included in the same panel. These are listed in Table 1.

Additional BioFire® test panels intended for use with the BioFire® FilmArray® instrument are the FilmArray® Gastrointestinal (GI) panel, FilmArray® Respiratory panel (RP), FilmArray® Meningitis/Encephalitis (ME) panel and FilmArray® Pneumonia panels. TAT for each of the panels is approximately 1 hour. All of the panels are both FDA cleared and CE-IVD marked, with the exception of one of the pneumonia panels that to date is only CE-IVD marked.

- **FilmArray® GI panel:** is a qualitative multiplexed in vitro diagnostic test that can simultaneously detect and identify nucleic acids from 22 bacteria, viruses and parasites directly from stool samples in Cary-Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The GI panel identifies the following bacteria from the WHO priority list: *Campylobacter* (*C. jejuni*, *C. coli*, *C. upsaliensis*), *tcdA/tcdB*, *Salmonella* and several diarrhoeagenic *Escherichia coli*/*Shigella* pathotypes. The GI panel is indicated as an aid in diagnosing specific agents of gastrointestinal illness, and results are meant to be used in conjunction with other clinical, laboratory and epidemiological data. The device is not intended to monitor or guide treatment for *Clostridium difficile* infection.
- **FilmArray® RP:** is a multiplexed in vitro diagnostic for the simultaneous qualitative detection and ID of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs obtained from individuals suspected of respiratory tract infection. The respiratory panel is able to detect 17 viruses and three bacterial pathogens

Table 1. Organisms and organism groups and genetic markers identified by FilmArray® BCID<sup>12</sup>

Gram-positive bacteria	Gram-negative bacteria	Yeast
<i>Enterococcus</i>	<i>Acinetobacter baumannii</i>	<i>Candida albicans</i>
<i>Listeria monocytogenes</i>	<b>Enterobacteriaceae</b>	<i>Candida glabrata</i>
<b>Staphylococcus</b>	<i>Enterobacter cloacae</i> complex	<i>Candida krusei</i>
<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida parapsilosis</i>
<b>Streptococcus</b>	<i>Klebsiella oxytoca</i>	<i>Candida tropicalis</i>
<i>Streptococcus agalactiae</i>	<i>Klebsiella pneumoniae</i>	<b>Antimicrobial resistance genes</b>
<i>Streptococcus pneumoniae</i>	<i>Proteus</i>	<i>mecA</i> – methicillin resistance
<i>Streptococcus pyogenes</i>	<i>Serratia marcescens</i>	<i>vanA/B</i> – vancomycin resistance
	<i>Haemophilus influenzae</i>	KPC – carbapenem resistance
	<i>Neisseria meningitidis</i> (encapsulated)	
	<i>Pseudomonas aeruginosa</i>	

– *Bordetella pertussis*, *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae*. The results of the panel are for use as an aid in diagnosing respiratory infection if used in conjunction with other clinical and epidemiological information.

- **FilmArray® ME panel:** is a qualitative multiplexed nucleic acid-based in vitro diagnostic test for simultaneously detecting and identifying multiple bacterial, viral and yeast pathogens directly from CSF. The panel identifies six bacteria: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae* and *Streptococcus pneumoniae*, as well as six viruses and one yeast. Results of the panel are not intended to be used as the sole basis for diagnosis, treatment or other patient management decisions, but are intended as an aid in diagnosis along with other clinical and epidemiological information.
- **FilmArray® Pneumonia panels:** BioFire® has developed two pneumonia panels, the FilmArray® Pneumonia Panel and the FilmArray® Pneumonia Panel *plus*. The former is FDA cleared, and the latter has recently received CE-IVD marking. Both of the panels detect 18 bacteria and eight viruses, as well as seven genetic markers of antimicrobial resistance, from sputum (including endotracheal aspirate) and bronchoalveolar lavage (including mini-BAL). The *plus* version of the panel also detects MERS-CoV (Middle East

respiratory syndrome-coronavirus). The assays yield semi-quantitative levels for 15 of the bacterial targets, including *Acinetobacter calcoaceticus-baumannii* complex, *Klebsiella pneumoniae* group, *Escherichia coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Antimicrobial resistance genes detected include *mecA/C* and *mec* right extremity junction (MREJ), KPC (*bla<sub>KPC</sub>*), NDM (*bla<sub>NDM</sub>*), CTX-M (*bla<sub>CTX-M</sub>*), VIM (*bla<sub>VIM</sub>*) and IMP (*bla<sub>IMP</sub>*).

The FilmArray® panels described above are intended to be used with the FilmArray® Torch or FilmArray® 2.0, collectively, the FilmArray® systems. Each panel includes a pouch which contains the freeze-dried reagents necessary to perform nucleic acid purification and nested, multiplex PCR with DNA melt analysis. Certain manual steps are required. A test is initiated by loading hydration solution into one port of the pouches and a sample mixed with the provided sample buffer ampoules into the other port of the pouch, which rehydrates the reagents, and placing it in the FilmArray® instrument. After the pouch is prepared, the FilmArray® software on the FilmArray® systems guides the user through the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample ID and initiating the run on the FilmArray® systems.

The FilmArray® instruments (the FilmArray® 2.0 is pictured in Fig. 76) contain a coordinated system of inflatable bladders and seal points that act on the

<sup>12</sup> The organisms/genetic markers highlighted in blue are on the WHO priority list of bacterial pathogens.

pouch to control the movement of liquid between the pouch blisters. Nucleic acid extraction occurs within the pouch using mechanical and chemical lysis, followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplex PCR is executed in two stages. During the first stage, the system performs a single, large volume, multiplexed reaction. The products from the first-stage PCR are transferred to an array with approximately 100 wells, prespotted with second-stage PCR primers. The second-stage PCR is performed in each well of the array. The array is then interrogated by melt curve analysis to detect signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the second-stage PCR reactions, and software interprets the data.

Fig. 76. BioFire® FilmArray® 2.0 instrument



The FilmArray® systems consist of three instruments: the FilmArray® Torch, the FilmArray® 2.0 and the FilmArray® EZ Configuration. The FilmArray® Torch is a larger, fully integrated random access instrument, while the FilmArray® 2.0 is a high-throughput, smaller benchtop system that uses single database management for up to eight instruments per computer. It has LIS-interfacing capabilities. Finally, the EZ Configuration is a CLIA-waived system for near-patient molecular testing. It is currently only used with the Respiratory Panel EZ, which tests for a comprehensive set of 14 respiratory, viral and bacterial pathogens.

### iC-System™ (iCubate, Inc., USA)

iCubate offers the iC-GPB Assay™ for use on its iC-System™. The iC-GPC Assay™, which is performed directly on positive blood cultures, is a qualitative, multiplexed in vitro diagnostic test for detecting and identifying potentially pathogenic gram-positive bacteria which may cause BSI. The iC-GPC Assay™ is validated for use on the BACTEC™, BACT/ALERT®

and VersaTREK™ blood culture bottles. Together with other clinical laboratory findings, such as blood culture isolate ID and AST, it is intended to aid in diagnosing bacterial BSIs, but not to monitor such infections.

The iC-GPC Assay™ detects an organism's DNA and identifies the following species: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium*. It also identifies the following resistance markers: *mecA*, inferring *mecA*-mediated methicillin resistance, as well as *vanA* and *vanB*, inferring *vanA/vanB*-mediated vancomycin resistance.

The iC-GPC Assay™ is intended to be used on the iC-System™, which comprises the iC-Processor™, the iC-Reader™ and assay cartridges, pictured in Fig. 77. A separate iMac computer is also required. The iC-System™ utilizes PCR for multiplex amplification of pathogens and detects amplified targets with microarray hybridization. More specifically, the system utilizes proprietary ARM-PCR (amplicon rescue multiplex PCR) technology; this technology allows multiple targets to be amplified in one reaction, but requires two rounds of amplification. Testing is performed using a self-contained disposable cassette that is processed by the iC-Processor™. After processing, the cassette is transferred to the iC-Reader™, where it is read. Data is then transferred from the iC-Reader™ to an iMac computer on which data is analysed using iC-Report™ software, and a final result is generated. TAT is approximately 4.5 hours. The iC-GPC Assay™ is FDA cleared.

Fig. 77. The iC-Reader™ (left), iC-Processor™ (right rear) and iC assay cartridges (right front)



In addition to the iC-GPC Assay™, iCubate has several assays under development. The iC-GN Assay™ for detecting and identifying gram-negative rods is anticipated to be FDA cleared in 2019. Specifically, the iC-GN Assay™ will detect *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Serratia marcescens*.

Additional assays in the pipeline include *Mycobacterium*, gastrointestinal and respiratory tests.

### Sepsis Flow Chip (Master Diagnóstica, Spain)

Master Diagnóstica offers the Sepsis Flow Chip (SFC) assay, which is an IVD for the simultaneous rapid detection from positive blood culture of 40 bloodstream pathogens in the same assay, including gram-positive and gram-negative bacteria as well as fungi, and for the detection of 20 antibiotic resistance genes, including MRSA, *mecA*, *vanA*, *vanB*, *ESBL* and carbapenems. The SFC assay, which is CE-IVD marked, is based on multiplex PCR using biotinylated primers followed by automatic reverse dot-blot hybridization to a low-density DNA array. Table 2 lists the pathogens and resistance markers detected by the SFC assay.

Table 2. Clinical pathogens and resistance markers detected by SFC assay	
Pathogen ID	Genetic resistance markers
<b>Gram-positive bacteria</b>	
<i>Streptococcus pneumoniae</i>	
<i>Streptococcus agalactiae</i>	
<i>Streptococcus</i> spp.	
<i>Staphylococcus aureus</i>	<i>mecA</i>
<i>Staphylococcus</i> spp.	
<i>Enterococcus</i> spp.	<i>vanA/vanB</i>
<i>Listeria monocytogenes</i>	
<b>Gram-negative bacteria</b>	
<i>Stenotrophomonas maltophilia</i>	<i>bla</i> <sub>CTX</sub> , <i>bla</i> <sub>SHV</sub> , <i>bla</i> <sub>SME</sub> , <i>bla</i> <sub>KPC</sub> , <i>bla</i> <sub>NMC/IMI</sub> , <i>bla</i> <sub>GES</sub> , <i>bla</i> <sub>IMP</sub> , <i>bla</i> <sub>GIM</sub> , <i>bla</i> <sub>VIM</sub> , <i>bla</i> <sub>SPM</sub> , <i>bla</i> <sub>SIM</sub> , <i>bla</i> <sub>NDM</sub> , <i>bla</i> <sub>OXA23</sub> , <i>bla</i> <sub>OXA24</sub> , <i>bla</i> <sub>OXA48</sub> , <i>bla</i> <sub>OXA51</sub> and <i>bla</i> <sub>OXA58</sub>
<i>Serratia marcescens</i>	
<i>Escherichia coli</i>	
<i>Klebsiella pneumoniae</i>	
<i>Morganella morganii</i>	
<i>Proteus</i> spp.	
<i>Enterobacteriaceae</i>	
<i>Acinetobacter baumannii</i>	
<i>Pseudomonas aeruginosa</i>	
<i>Neisseria meningitidis</i>	
<b>Fungi</b>	
<i>Candida albicans</i>	

In addition to the SFC assay, Master Diagnóstica also offers several other CE-IVD-marked test panels. For purposes of this report, the most relevant panel is the Bacterial CNS Flow Chip, which detects *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Treponema pallidum*, MTB, *Coxiella burnetii*, *Cryptococcus neoformans* (hongo) and *Borrelia burgdorferi*. Additional assays available are the HPV Direct Flow Chip, Viral CNS Flow Chip and Tick-Borne Bacteria Flow Chip.

The flow chip assays/panels must be run on a combination of instruments that are not integrated, which means the system is suitable only for a sophisticated clinical laboratory. Following Gram staining and organism ID, positive blood culture samples are amplified using a commercially available thermal cycler (e.g., ABI Veriti™ Dx [Thermo Fisher Scientific]). Subsequently, reverse dot-spot hybridization and analysis of the results can be conducted with the fully automated hybriSpot HS24™ platform or semi-automated hybriSpot 12™ instrument from Master Diagnóstica. The TAT to obtain a result from a positive blood culture takes from 30 to 120 minutes on the HS24 platform, which can process up to 24 samples simultaneously. If using the HS12 instrument, 1–24 samples can be done per run in 20–120 minutes.

### Unyvero™ system (Curetis GmbH, Germany)

The Unyvero™ system is a cartridge-based molecular diagnostic platform for simultaneously detecting and identifying gram-positive and gram-negative bacteria, mycobacteria and fungi as well as antibiotic resistance markers. It is a qualitative test. The Unyvero™ BCU blood culture application, which is CE-IVD marked, includes 36 analytes covering more than 50 pathogens, and 16 antibiotic resistance gene markers for detection from positive blood culture bottles; Gram stain is not required. TAT is approximately 5 hours. Of interest for this report, the BCU application can detect and identify the bacteria listed in Table 3, among other microorganisms:

Table 3. Select list of clinical pathogens identified by Unyvero BCU application

Group	Pathogen
	<i>Staphylococcus aureus</i>
	<i>Streptococcus</i> spp.
	<i>Streptococcus agalactiae</i>
Gram-positive bacteria	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes/dysgalactiae</i>
	<i>Enterococcus</i> spp.
	<i>Enterococcus faecalis</i>

(Table 3, continued)

	<i>Enterobacteriaceae</i>
	<i>Escherichia coli</i>
	<i>Klebsiella pneumoniae</i>
Gram-negative bacteria	<i>Acinetobacter baumannii</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Haemophilus influenzae</i>

Resistance markers detected and identified by the Unyvero™ BCU application include *mecC* (LGA251), *vanA*, *vanB*, CTX-M (*bla<sub>CTX-M</sub>*), KPC (*bla<sub>KPC</sub>*), IMP (*bla<sub>IMP</sub>*), NDM (*bla<sub>NDM</sub>*), OXA-23 (*bla<sub>OXA-23</sub>*), OXA-24/40 (*bla<sub>OXA-24/40</sub>*), OXA-48 (*bla<sub>OXA-48</sub>*), OXA-58 (*bla<sub>OXA-58</sub>*) and VIM (*bla<sub>VIM</sub>*).

In addition to the BCU application, Unyvero™ offers several other panels for infectious disease, including an FDA-cleared panel for lower respiratory tract infections, the Unyvero™ LRT application.<sup>13</sup> The LRT application is a qualitative nucleic acid multiplex test intended for simultaneously detecting and identifying nucleic acid sequences from certain microorganisms and antibiotic resistance markers in endotracheal aspirates from adult hospitalized patients with suspected lower respiratory tract infection. The LRT application detects and identifies 19 bacteria and fungi, including, but not limited to, the following: *Acinetobacter* spp., *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. In addition, the panel detects and identifies the following associated resistance markers: *mecA*, CTX-M (*bla<sub>CTX-M</sub>*), KPC (*bla<sub>KPC</sub>*), NDM (*bla<sub>NDM</sub>*),

OXA-23 (*bla<sub>OXA-23</sub>*), OXA-24/40 (*bla<sub>OXA-24/40</sub>*), OXA-48 (*bla<sub>OXA-48</sub>*) and VIM (*bla<sub>VIM</sub>*).

Similarly, Unyvero™ offers a qualitative in vitro diagnostic test for UTIs. The Unyvero™ UTI application, which was recently CE-IVD marked, is targeted at patients with complicated and severe UTIs, including pregnant women and immunocompromised individuals. The Unyvero™ UTI application can detect 88 pathogens, including a broad range of gram-positive and gram-negative bacteria as well as difficult-to-culture anaerobic bacteria, and the fungus *Candida auris*, which is multidrug resistant.

Unyvero™ UTI also detects 15 genetic markers of antibiotic resistance, including the *mcr-1* antibiotic resistance gene, which results in resistance to colistin, one of the few last-resort antibiotics for gram-negative infections. Similar to other Unyvero™ applications, Unyvero™ UTI allows rapid detection of pathogens and genetic resistance markers in a broad range of routinely available patient sample types, such as mid-stream urine, catheter urine, suprapubic aspiration and tissue.

Finally, Unyvero™ also offers two additional test panels: the IAI (intra-abdominal infection) application and the ITI (implant and tissue infection) application. Similar to the other Unyvero™ assays, these applications detect and identify a broad range of microorganisms and resistance markers, which will not be discussed in detail in this report.

Each of the above Unyvero™ IVD applications is performed using the Unyvero™ system; the process includes specimen processing (lysis), genomic bacterial DNA isolation and purification, multiplex PCR, and array hybridization and detection. The system comprises three instruments, pictured in Fig. 78: the

Fig. 78. Unyvero™ system instruments: Unyvero™ L4 Lysator (left), Unyvero™ C8 Cockpit (centre) and Unyvero™ A50 Analyzer (right)



<sup>13</sup> Note that, in Europe, Curetis offers a very similar panel called the Unyvero pneumonia application, which is CE-IVD marked. Both the Unyvero LRT application and the pneumonia application are targeted to hospitalized patients.

Unyvero™ L4 Lysator for specimen processing, the Unyvero™ A50 Analyzer (for amplification and reading) and the Unyvero C8 Cockpit controller, which provides the main user interface for the Unyvero™ system, guides the user through the steps to run the applicable Unyvero™ application, and automatically generates and displays test results.

The workflow for the Unyvero system is as follows. It is composed of both manual and automated elements. A specimen is first pipetted into the Unyvero sample tube and closed with the Unyvero sample tube cap. Closing the sample tube automatically adds the lysis reagent and the internal control gene template to the specimen. The sample tube is then placed on the L4 Lysator. After the specimen is lysed in the Lysator, the sample tube and master mix are loaded into the Unyvero cartridge, pictured in Fig. 79, for automated processing and analysis.

Fig. 79. Unyvero™ cartridge



The remainder of the testing steps are automated by the Unyvero™ A50 Analyzer. The lysed specimen is further processed and then transferred onto a DNA purification column for nucleic acid, and DNA is transferred to separate PCR reaction chambers containing multiple primer pairs. After amplification in the Unyvero™ A50 Analyzer, PCR products are hybridized to the corresponding array probes. Results data are then transferred to the Unyvero™ C8 Cockpit for visualization and results printout.

### GeneXpert® system (Cepheid, a subsidiary of Danaher Corporation, USA)

The Cepheid GeneXpert® system is a fully automated and integrated system for PCR-based NAAT, which currently has 21 FDA-cleared and 27 CE-IVD-marked assays, including the Cepheid Xpert® MRSA/SA blood culture assay. The assays are performed on the Cepheid GeneXpert® instrument systems, which contain between one and 80 modules, depending on the instrument. The instruments include the GeneXpert®

Express, GeneXpert® Dx (the four-module instrument is pictured in Fig. 80), GeneXpert® Infinity-48, GeneXpert® Infinity-48s and GeneXpert® Infinity-80 systems, all of which automate sample preparation, amplification and real-time detection in single-use, disposable cartridges. Select assays available for these instruments relevant to this report are described below.

Fig. 80. Four-module GeneXpert® Dx instrument (left) and cartridge (right)



The Cepheid Xpert® MRSA/SA blood culture assay is a qualitative *in vitro* diagnostic test intended for detecting *Staphylococcus aureus* and MRSA DNA. The assay utilizes automated real-time PCR for amplifying MRSA/*S. aureus*-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive blood culture specimens using BD BACTEC™ Plus Aerobic/F blood culture bottles that are determined as gram-positive cocci in clusters (GPCC) or as gram-positive cocci in singles (GPC) by Gram stain. The Cepheid Xpert® MRSA/SA blood culture assay is not intended to monitor treatment for MRSA/*S. aureus* infections. TAT is approximately 1 hour. The assay is CE-IVD marked and FDA cleared.

In addition to the Xpert® MRSA/SA blood culture assay, Cepheid offers three additional cartridges related to testing for MRSA. The MRSA SA Nasal Complete cartridge and the MRSA/SA SSTI cartridge are each a qualitative *in vitro* diagnostic test for detecting *S. aureus* and MRSA DNA directly from either nasal swabs in patients at risk for nasal colonization (MRSA SA Nasal Complete cartridge) or from skin and soft tissue infection swabs (MRSA SSTI cartridge). Cepheid also offers the Xpert® MRSA NxG (next-generation) assay, which like the MRSA cartridge is a qualitative *in vitro* diagnostic test for detecting *S. aureus* and MRSA DNA directly from nasal swabs in patients at risk for nasal colonization, but which, per the company, is an improved assay because it expands coverage of the test by using an extensive library of

over 195 MRSA strains from around the world. The assay also adds primers and probes to detect sequences within the *mecA* and *mecC* genes in order to reduce false-positive results due to empty cassettes. The MRSA NxG assay is validated for use with both rayon swabs and eSwab™ (Copan Diagnostics, Italy). TAT is approximately 1 hour. All of these assays are FDA cleared and CE-IVD marked.

Cepheid also offers additional assays related to HAIs. These include the Xpert® *vanA* and Xpert® Carba-R assays, both of which are FDA cleared and CE-IVD marked. The Xpert® *vanA* assay is a qualitative in vitro diagnostic test designed for rapidly detecting the *vanA* gene sequence associated with vancomycin resistance in bacteria obtained from rectal swab specimens from patients at risk for intestinal colonization with vancomycin-resistant bacteria. This assay is intended to aid in recognizing, preventing and controlling vancomycin-resistant organisms that colonize patients in healthcare settings, and not for diagnosing infections caused by vancomycin-resistant bacteria nor to guide or monitor treatment for vancomycin-resistant bacterial infections. TAT is 45 minutes.

The Xpert® Carba-R assay is a qualitative in vitro diagnostic test designed for detecting and differentiating the *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>IMP</sub> gene sequences associated with carbapenem nonsusceptibility. The assay can be performed on carbapenem-nonsusceptible pure colonies of *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, when grown on blood agar or MacConkey agar. For testing pure colonies, the assay should be used in conjunction with other laboratory tests, including phenotypic AST. The assay can also be performed on rectal and perirectal swab specimens from patients at risk for intestinal colonization with carbapenem-nonsusceptible bacteria. When performed on rectal and perirectal swab specimens, the Xpert® Carba-R assay is not intended to guide or monitor treatment for carbapenem-nonsusceptible bacterial infections nor to determine infection from carbapenem-nonsusceptible bacteria. TAT is less than 50 minutes.

There are two Cepheid assays for *Clostridium difficile* – the Xpert® *C. difficile* assay and the Xpert® *C. difficile*/Epi assay. The Cepheid Xpert® *C. difficile* assay is a qualitative in vitro diagnostic test for rapidly detecting *tcdB* gene sequences from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. The Xpert® *C. difficile*/Epi assay is a qualitative in vitro diagnostic test for rapidly detecting *tcdB* gene sequences and for presumptive ID of 027/NAP1/BI strains of toxigenic *Clostridium difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. Each assay is intended as an aid in diagnosing CDI. Both tests have a TAT of 45 minutes.

Also of relevance to this report are two additional assays for the GeneXpert® system: the Xpert® MTB/RIF assay and the Xpert® CT/NG assay. The Xpert® MTB/RIF assay is a qualitative nested real-time PCR in vitro diagnostic test for detecting *Mycobacterium tuberculosis* complex (MTB complex) DNA in raw sputum or concentrated sediments prepared from induced or expectorated sputum. In specimens where MTB complex is detected, the Xpert® MTB/RIF assay also detects the rifampin-resistance-associated mutations of the beta subunit of RNA polymerase (*rpoB*) gene. TAT is approximately 2 hours.

The Xpert® CT/NG assay is a qualitative in vitro real-time PCR test for automated detection and differentiation of genomic DNA from CT and/or NG. It is CE-IVD marked and FDA cleared. The assay may be used on the following specimens from both asymptomatic and symptomatic patients: female and male urine, endocervical swab, patient-collected vaginal swab (collected in a clinical setting) and rectal and pharyngeal swab specimens. TAT is approximately 90 minutes.

All of the Cepheid assays described above, as well as additional assays in the Cepheid portfolio, are performed on the GeneXpert® system. The GeneXpert® system consists of a GeneXpert® instrument, personal computer and multichambered fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection. The GeneXpert® system integrates and automates sample preparation, amplification and detection in a single-use, self-contained cartridge. Most liquids and dry reagents along with enzymes are prefilled so that pre-analytical steps are minimized, reducing opportunities for sample mix-ups and operational errors. GeneXpert® cartridges can handle a variety of sample volumes (micro- to millilitre volume range) within macrofluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed.

Further, the GeneXpert® system is modular. Individual modules contain solid-state circuitry that controls temperature, pressure, rotation of the valve that moves the liquid between reservoirs in the cartridge and the detection software. These individual modules are packaged in cabinets that can hold up to 1, 2, 4, 16, 48 or 80 modules, as mentioned above. The latter two systems (Infinity-48 and Infinity-80) are fully automated, walk-away robotic systems, developed for high-throughput laboratory applications. Additionally, the modules can be removed and replaced individually so that the entire system is not incapacitated if one module fails.

Generally, the GeneXpert® systems are best used at district hospitals and above in the tiered laboratory system in-country. The instruments are not as well suited to use at health centres and below for

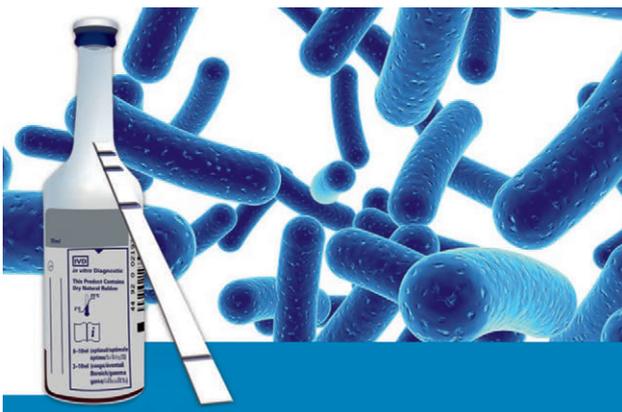
reasons including, the need for stable electricity, temperature conditions and calibration requirements. Training, however, is relatively straightforward and can usually be done in less than a day.

Additionally, GeneXpert® Edge, launched in 2018, is Cepheid's new offering based on the existing GeneXpert® instrument family to move molecular testing beyond the laboratory. By including an easy-to-use touch-screen workflow, external battery pack and dust filter, GeneXpert® Edge enables testing in challenging environments.

### GenoType assays and FluoroType® system (Hain Lifescience GmbH – a Bruker Company, Germany)

Hain Lifescience has developed a large number of CE-IVD-marked in vitro diagnostic tests, its GenoType and FluoroType® assays, of which the latter can be performed on its FluoroCycler® PCR instrument. Among these are GenoType BC gram-negative and gram-positive test kits, pictured in Fig. 81, which can identify 15 specimens of gram-negative rods and 17 specimens of gram-positive cocci, respectively, taken directly from positive BACTEC™ blood culture bottles. Pathogens identified by the GenoType BC positive kit to the species level include *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumoniae*, along with ID of *mecA* and *van* genes. Pathogens identified by the GenoType BC negative kit include *Escherichia coli*, *Enterobacter* spp. (*E. aerogenes*, *E. cloacae* and *E. sakazakii*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Fig. 81. GenoType BC test kit



The GenoType BC assays are based on DNA strip technology that uses DNA multiplex amplification with biotinylated primers followed by hybridization to membrane-bound probes. The testing process is quite manual. A drop of positive blood culture is applied to the Hain Lifescience GenoCard, a special membrane

device. A small piece of the carrier matrix is then punched out and, following a short drying step, is added to a PCR mixture for amplification. Hybridization and detection are then carried out, for which an automated washing and shaking device may be used. GenoCard strips are then air dried and fixed to a data sheet for evaluation by visual examination. TAT is approximately 5 hours.

Hain Lifescience offers a series of GenoType assays that are based on the same technology used in the BC assays and for which the workflow is the same or similar to that described above. Of relevance to this report, these include GenoType:

- MRSA (for directly detecting MRSA from cultured material);
- Staphylococcus (to detect *Staphylococcus aureus* from cultured material);
- HelicoDR (to identify *Helicobacter pylori* from culture and biopsy samples);
- CDiff (to detect *Clostridium difficile* and ribotype 027 from stool and culture samples as well as rectal smears);
- Enterococcus (to identify and differentiate among *E. faecalis*, *E. faecium*, *E. casseliflavus* and *E. gallinarum*, and to identify vancomycin resistance genes from culture, preferably freshly grown); and
- MTB complex: three assays, including, MTBC VER 1.X (to identify and differentiate MTB complex from liquid and/or solid culture); MTBDRsl VER 1.0/2.0 (to identify MTB complex and its resistance to fluoroquinolones, aminoglycosides/cyclic peptides [and ethambutol]); and MTBDRplus VER 1.0/2.0 (to identify MTB complex and its resistance to rifampicin and/or isoniazid).

In addition to the GenoType assays, Hain Lifescience also offers a series of CE-IVD-marked FluoroType® tests to be performed on its FluoroCycler® system. The assays include FluoroType®:

- MTB (to detect MTB complex from decontaminated pulmonary and extrapulmonary patient specimens);
- MTBDR VER 2.0 (to identify MTB complex and its resistance to rifampicin and/or isoniazid);
- MRSA (to detect methicillin-resistant *Staphylococcus aureus* from swab specimens – nose, throat, skin and wounds);
- CDiff (to detect *Clostridium difficile* and *tcdB* from stool samples); and
- NG (to detect *Neisseria gonorrhoeae* from urethral or cervical swabs, as well as urine).

The Hain Lifescience system includes a 96-well format for nucleic acid extraction, the GenoXtract® 96 instrument, and an amplification instrument, the FluoroCycler® 96, which performs subsequent amplification and detection using qPCR. The company also offers a lower-throughput extraction platform and qPCR cyler, the GenoXtract® 12 and the FluoroCycler® 12, respectively, which can process or amplify up to 12 samples at once. The system is pictured in Fig. 82.

The company uses a novel amplification and probe technology – linear-after-the-exponential (LATE)-PCR combined with fluorescence “lights on/lights off” probes that tile side by side on the target region. LATE-PCR is an optimized form of asymmetric PCR, in which a limiting primer and an excess primer are used for exponential amplification of double-stranded DNA, followed by linear amplification of a single strand. Each single-stranded amplified sample can then be detected in real time. With the lights on/lights off probes, fluorescence is either emitted or suppressed, which is reflected through a characteristic fluorescence pattern in melt curve analysis. This allows larger regions of target DNA to be interrogated compared with the conventional method of using a single probe. Finally, test-specific Fluoro-Software® evaluates the test results and displays them automatically. TAT varies with the assay, but it is approximately 2.5 hours for the MTBDR assay.

**ePlex® system (GenMark Diagnostics, USA)**

GenMark offers its ePlex® system, which is a clinical multiplex test system using single-use assay cartridges that incorporate digital microfluidics and GenMark’s proprietary eSensor detection technology. The system is fully automated.<sup>14</sup>

The ePlex® system is based on competitive nucleic acid hybridization and electrochemical detection of

nucleic acids on a microchip in a disposable cassette. Target amplification is done via PCR or RT-PCR. Quantitation is not possible on the system.

The ePlex® system is modular and scalable, and has instrument configurations ranging from 3 to 24 test bays. GenMark recently introduced a small configuration instrument, the ePlex® NP (Near Patient), pictured in Fig. 83, for use at smaller laboratories running as few as 12 patient samples per shift. The ePlex® tower instruments range from a one-tower configuration that can process six cartridges at a time with random access to two-, three- and four-tower configurations containing three to 24 test bays. The system offers bidirectional LIS.

Fig. 83. ePlex® NP platform (left) and test cartridge (right)



GenMark offers a variety of test cartridges. One of these is an FDA-cleared and CE-IVD-marked respiratory pathogen (RP) panel available from GenMark for the ePlex® systems, but the test is targeted primarily at viruses and is not directly relevant to this report. Of relevance to this report are two CE-IVD-marked blood culture assays. These are the ePlex® BCID-GP and ePlex® BCID-GN tests. The ePlex® BCID-GP Panel identifies 26 targets, including *Enterococcus*, *Enterococcus faecium*, *Staphylococcus* and *Staphylococcus aureus*, as well as resistance markers

Fig. 82. Hain Lifescience instruments: GenoXtract® (left), FluoroCycler® (FluoroCycler® 12, centre left; and FluoroCycler® 96, centre right) and separate computer for results display



<sup>14</sup> GenMark also offers a larger system, the XT-8, which performs highly multiplexed post-PCR detection of infectious disease, genetics and pharmacogenetic targets. But the assays for this system are not relevant to this report.

*mecA*, *mecC*, *vanA* and *vanB*. The ePlex® BCID-GN Panel identifies 29 targets, including *Acinetobacter baumannii*, *Enterobacter* (non-*cloacae* complex and *cloacae* complex), *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella*, as well as resistance markers including CPE (*bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>OXA</sub>) and ESBL (*bla*<sub>CTX-M</sub>). Samples are positive blood culture bottles. TAT is approximately 1.5 hours with less than 2 minutes of hands-on time. GenMark has submitted both of its BCID assays to the FDA for clearance.

GenMark also offers a blood culture assay for detecting fungus. In addition, GenMark has a number of assays under development, including (i) a gastrointestinal pathogen panel for bacterial, viral and parasitic targets from stool samples; (ii) a central nervous system panel for bacterial, viral and fungal targets from CSF; and (iii) an HCV genotyping panel from plasma or serum.

### Molecular platforms for identifying pathogens and characterizing bacterial resistance from whole blood and other sample types

In addition to the IVD systems described above that can identify bacterial pathogens and genes that directly confer bacterial resistance from blood culture, there are systems available that can similarly detect such pathogens and resistance genes from whole blood and other specimens. These are described below.

#### Seeplex™, Allplex™, Anyplex™, MagicPlex™ systems (Seegene, Republic of Korea)

Seegene offers numerous highly multiplexed NAAT-based assay kits that use real-time PCR or capillary electrophoresis for amplicon detection. These are the Seeplex™, Allplex™, Anyplex™ and MagicPlex™ test kits, many of which are CE-IVD marked. The company does not, however, supply completely integrated sample-to-result systems. For example, sample prepara-

tion is not provided by Seegene; therefore, a product from another manufacturer must be used for this purpose. While DNA extraction and an initial PCR amplification step can be run on Seegene instruments (SEEPREP12™ and SEEAMP™, respectively, pictured in Fig. 84), additional real-time PCR amplification steps or automated electrophoresis, depending on the assay kit, must be done on other systems validated for use with Seegene kits. For real-time PCR, these include the ABI 7500 Real-time PCR (Thermo Fisher Scientific, USA) and the CFX96™ Real-time PCR (Bio-Rad, USA); for electrophoresis, this includes the MultiNA (Shimadzu Corporation, Japan), an automated microchip electrophoresis system that performs automated high-speed electrophoresis separation and fluorescence detection.

Seegene has developed certain proprietary software, Multiple Detection Temperatures Technology (MuDT™), to discriminate between the 10 channels on the CFX96™ platform. It allows simultaneous ID and quantification of multiple pathogen targets in a single channel without melt curve analysis following amplification. In addition, viewer software analyses the raw data to generate test results from the various Seegene assays. The multiplicity of steps and equipment required to perform the Seegene test kits suggests that they should be used only in the most sophisticated laboratory settings.

Examples of Seegene IVD kits include the CE-IVD-marked Magicplex™ Sepsis real-time test, which is notable because it is able to screen for more than 90 sepsis-causative pathogens at genus level and resistance markers using real-time PCR from EDTA whole-blood samples in approximately 3.5 hours, excluding extraction time. The test is done in two different reactions, one for gram-positive bacteria/drug-resistance genes and one for gram-negative bacteria/fungi. Identification of 27 pathogens at the species level, without additional amplification, takes place via a third step. Pathogens include *Streptococcus pneumoniae*, *Enterococcus faecium*, *Staphylococcus aureus*,

Fig. 84. Equipment used to perform the Magicplex Sepsis real-time test (SEEPREP12™, left; SEEAMP™, centre; and CFX96™, right)



*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*. Resistance markers include *vanA*, *vanB* and *mecA*.

Additional Seegene assays are available and include certain drug-resistance tests, among others:

- **Seeplex™ assays:** Seeplex™ VRE ACE Detection (detects *vanA* and *vanB* genes in enterococci isolated from stool culture) and Seeplex™ *H. pylori*-ClAR ACE Detection (detects two types of permutations causing clarithromycin resistance in *Helicobacter pylori* isolated from a gastric biopsy), as well as CE-IVD-marked multiplex respiratory pathogen assays, STI assays, HSV 1 and 2, HPV (screening and genotyping) and meningitis. Non-CE-marked assays include MTB/nontuberculous mycobacteria (NTM).
- **Anyplex™ assays:** Anyplex™ VanR Real-time Detection (simultaneously detects *vanA*, *vanB* and *vanC* genes from cultured samples of enterococci). CE-IVD-marked assays include multiplex respiratory pathogen assays, STI assays, HPV genotyping, MDR-TB, XDR-TB and MTB/NTM/MDR-TB. These assays require sample extraction and PCR setup on the Microlab NIMBUS (Hamilton, USA), followed by qPCR analysis on an instrument such as the CFX96™ (Bio-Rad).
- **Allplex™ assays:** Allplex™ Entero-DR assay (simultaneously detects eight antibiotic-resistance genes, including CPE [*bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>OXA-48</sub>], VRE [*vanA*, *vanB*] and ESBL [*bla*<sub>CTX-M</sub>]) from rectal swabs. Additional CE-IVD-marked assays include multiplex respiratory pathogen assays, STI assays, a GI assay and a meningitis assay.

### ELITe MGB® kits and panels (ELITechGroup Solutions, France)

ELITechGroup offers a number of IVD kits and panels. Of relevance to this report are several bacterial ID panels as well as several panels solely for identifying bacterial resistance markers. The assays can be performed on the ELITe InGenius® system, pictured in Fig. 85, which is a sample-to-result molecular diagnostics platform.

ELITechGroup offers the following assays for identifying bacterial pathogens primarily for HAIs:

- ***C. difficile* ELITe MGB® kit:** a real-time PCR assay designed for qualitatively detecting and differentiating *Clostridium difficile* *tcdA* and *tcdB*, including the NAP1/B1/027 strain, in stool. The assay is CE-IVD marked.

Fig. 85. ELITe InGenius® platform



- **STI ELITe MGB® panel:** a triplex real-time PCR assay designed to detect and differentiate CT, NG and MG DNAs in urine and cervical swabs (in the pipeline). The assay can be used in combination with the Macrolide-R/MG ELITe MGB® kit for detecting macrolide resistance. The assay is CE-IVD marked.
- **MRSA/SA ELITe MGB® kit:** a PCR assay for the direct, qualitative detection of *Staphylococcus aureus* and MRSA using DNA purified from human nasal swabs. The MRSA/SA ELITe MGB® is not intended to diagnose, guide or monitor MRSA infections nor to provide results of susceptibility to oxacillin/methicillin. Rather, it is intended to aid in preventing and controlling MRSA infections in healthcare settings. The assay is CE-IVD marked and is FDA cleared (when performed with sample processing on the bioMérieux NucliSENS® easyMAG® and performed on the Thermo Fisher Scientific ABI 7500 FAST Dx system, which consists of the 7500 FAST Dx instrument, a personal computer, 96-well plates and seals).

ELITechGroup also offers several assays for detecting resistance genes in bacterial HAIs:

- **CRE ELITe MGB® kit:** a multiplex real-time PCR assay designed to detect and differentiate the conserved regions of carbapenem-resistance genes of *Enterobacteriaceae*: *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>OXA-48</sub>-like genes from rectal swabs and blood culture (in the pipeline). The assay is CE-IVD marked.
- **ESBL ELITe MGB® kit:** a multiplex real-time PCR assay designed to detect ESBL genes of *Enterobacteriaceae*: CTX-M 1, 9, 14 and 15 groups. The assay can be performed on rectal swabs and blood culture (in the pipeline). The assay is CE-IVD marked.

- **Colistin ELITE MGB® kit:** a multiplex real-time PCR assay designed to detect conserved regions of mobilized colistin-resistance genes, *mcr-1* and *mcr-2*, of *Enterobacteriaceae* in rectal swabs. The assay is CE-IVD marked.

Each of the assay kits above is designed to be performed on the ELITE InGenius® platform, which is an integrated benchtop instrument that automatically performs extraction, real-time PCR amplification and results interpretation. The platform has bidirectional connectivity, which enables the laboratory to automatically communicate with an LIS to import testing information and export patient results. Overall TAT from extraction to results analysis is approximately 2.5 hours. Hands-on time is about 2 minutes per sample.

The ELITE InGenius® platform offers random access. One to 12 samples can be processed in parallel in independently controlled real-time PCR units. The platform has a minimum sixplex target capability, which is enhanced with melt curve analysis. The user can potentially mix all kinds of sample matrices and use diverse thermal profiles and even different PCR chemistries at the same time.

### Amplidiag® system (Mobidiag, Finland)

Based on qPCR test panels, Mobidiag offers multiplex test panels for clinically relevant gastrointestinal bacteria (as well as parasites and viruses) and antibiotic resistance. The system is designed for mid- to large-sized laboratories.

Test panels relevant to this report include the following CE-IVD-marked assays:

- **Amplidiag® Bacterial GE:** detects eight bacterial pathogens from DNA extracted from stool (without preculture) in a single test in less than 2 hours. Pathogens identified include *Campylobacter*, *Salmonella* and *Shigella*/EIEC, among others.

- **Amplidiag® *C. difficile*+027:** detects pathogenic *Clostridium difficile* and its hypervirulent 027 ribotype from DNA extracted from stool (without any preculture) in a single test. TAT is less than 2 hours.
- **Amplidiag® *H. pylori*+ClariR:** detects *Helicobacter pylori* and its clarithromycin resistance directly from stool samples or gastric biopsies. TAT is less than 2 hours.
- **Amplidiag® CarbaR+VRE:** detects most relevant carbapenemases and vancomycin resistance from DNA extracted from pure culture in a single test. These include *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>OXA-181</sub>, *Acinetobacter bla*<sub>OXA</sub>, *vanA* and *vanB*. TAT is less than 2 hours.
- **Amplidiag® CarbaR+MCR:** detects clinically relevant carbapenemases and colistin resistance from DNA extracted from stool samples, rectal swabs or pure culture. These include *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>OXA-181</sub>, *Acinetobacter bla*<sub>OXA</sub>, *mcr* and *bla*<sub>GES</sub>. TAT is less than 2 hours.

Test panels for parasites and viruses are also available.

The Amplidiag® test panels can be run on the Amplidiag® system, which is not integrated. As illustrated in Fig. 86, the system comprises (i) sample preparation; (ii) nucleic acid extraction and PCR plate setup on the Amplidiag® Easy instrument, the NucliSENS® easyMAG® (bioMérieux, France) or MagNA pure 96 (Roche, USA); (iii) real-time PCR on compatible/validated instruments, including the Bio-Rad CFX96™, ABI 7500 Fast, Corbett RotorGene (Thermo Fisher Scientific, USA) and QIAGEN Rotor-Gene® Q; and (iv) automated analysis and reporting using Amplidiag® Analyzer software. Up to 48 samples can be processed in about 2 hours.

Fig. 86. Amplidiag® system workflow



### QIASymphony® SP/AS (QIAGEN N.V., Germany)

QIAGEN has a line of assays relevant to this report. They are:

- **artus™ *C. difficile* QS-RGQ kit:** an in vitro diagnostic test for qualitatively detecting toxigenic *Clostridium difficile* *tcdA* and *tcdB* from human liquid or soft stool samples;
- **artus™ CT/NG QS-RGQ kit:** an in vitro diagnostic test for qualitatively detecting CT plasmid and gDNA, and NG gDNA from vaginal swabs and urine; and
- **artus™ VanR QS-RGQ kit:** an in vitro diagnostic test for detecting *vanA* and *vanB* vancomycin-resistance genes from human perianal or rectal swabs.

Each of the assays is CE-IVD marked, and the kits come ready to use with all optimized reagents required to run the test. The kits are designed to be used with the automated extraction and sample preparation system (QIASymphony® SP/AS). The assays must then be run on one of the QIAGEN real-time Rotor-Gene® Q (RGQ) thermocyclers for amplification and detection. An example of a complete QIASymphony® RGQ system is shown in Fig. 87.

Fig. 87. QIASymphony® RGQ system



The QIASymphony® SP/AS instruments provide automated sample preparation and assay setup. The QIASymphony® SP can process 1–96 samples (in batches of 24) with sample volumes up to 1 mL. It is a ready-to-run instrument that requires minimal installation. The SP can be combined with the QIASymphony® AS device in a fully integrated system that can automate the entire workflow. To reduce manual

handling and minimize the risk of sample contamination, samples processed on the SP can be transferred automatically to the AS, or the two instruments can be operated independently. Despite their ease of use, the QIASymphony® system and RGQ instruments are designed for use in sophisticated laboratories.

The QIAGEN real-time PCR cycler, the RGQ, offers a unique centrifugal rotary design. Each tube in the instrument spins in a chamber of moving air, which keeps all samples at precisely the same temperature during rapid thermal cycling. Detection is also uniform. When each tube aligns with the detection optics in the instrument, the sample is illuminated and the fluorescent signal is rapidly collected from a single, short optical pathway. Per the company, the thermal and optical uniformity of the system results in sensitive, precise and fast real-time PCR.

In addition to the assay kits described above, artus™ panels for QIASymphony® RGQ are offered for numerous other assays, including assays for HBV and HCV, as well as assays for detecting and quantifying cytomegalovirus, Epstein-Barr virus, HSV 1 and 2, HIV, varicella-zoster virus and BK virus.

### UtiMax™/BsiMax® (GeneFluidics, USA)

GeneFluidics is an early-stage company that is developing a diagnostics system based on electrochemical measurement of bacterial 16S rRNA for detection, ID and AST. The company currently has one CE-IVD-marked UTI assay on the market for performance on the UtiMax™ lab automation system; TAT is approximately 30 minutes for pathogen ID and 120 minutes for AST.

Fig. 88. UtiMax™ lab automation system



The UtiMax™ lab automation system, pictured in Fig. 88, is a fully automated rapid diagnostic system for identifying uropathogens directly from urine samples. Pathogen ID and AST are performed by the UtiMax™ lab automation system with a reagent kit and disposable sensor array chip. UtiMax™ ID/AST

is an electrochemical-based sandwich hybridization test to quantify species-specific ribosomal 16S rRNA. Each sample is lysed chemically prior to hybridization at high stringency. A built-in multichannel potentiostat reads the electrical current from the steady-state enzymatic cycling amplification: the signal is proportional to the bound 16S rRNA content from lysate and reported in ranges of CFU per millilitre through an established calibration curve.

A follow-up product line, BsiMax<sup>®</sup> (with the additional feature of lysis centrifugation), is in development. The BsiMax panel will include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA, MSSA and *Enterococcus*, among others. The AST panel will include the following antibiotics: gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam. The BsiMax<sup>®</sup> assay can process whole-blood samples for BSIs with a limit of detection (LOD) < 4 CFU/mL with ID in 5 hours and AST in 2 hours. Both UtiMax<sup>™</sup> and BsiMax<sup>®</sup> can be performed using the company's robotic liquid-handling systems, with associated reagent kits and sensor chips.

Per the company, both the BsiMax<sup>®</sup> and UtiMax<sup>™</sup> ID/AST tests can quantify unique species-specific nucleic acid sequences associated with each target pathogen without using PCR, and can conduct AST without the need to obtain a clinical isolate or positive blood or urine culture sample. No peer-reviewed published studies are available on the UtiMax<sup>™</sup> ID/AST assay, and the BsiMax<sup>®</sup> ID/AST assay is still in development.

## Nonphenotypic methods of detecting antibiotic resistance

In addition to some of the systems described above, including the GeneXpert<sup>®</sup> system, Unyvero<sup>™</sup> system, FluoroType<sup>®</sup> system and several systems from Seegene, which can both identify bacterial pathogens and detect resistance genes, other platforms identify multiple genes that directly confer antibiotic resistance but do not identify pathogens.

## Molecular methods of detecting antibiotic resistance

### Check-Direct and Check-MDR assays (Check-Points, Netherlands)

Check-Points manufactures screening assay kits for rapid AMR detection. There is a family of CE-IVD-marked assays for use on the BD MAX<sup>™</sup> system, described earlier in this report. The reagents for these assays come in pre-aliquoted, dried-down format, pictured in Fig. 89, for easy automation on the BD MAX<sup>™</sup> system from BD. The company also offers assays available for use on other systems.

Fig. 89. Check-Direct CPE for BD MAX<sup>™</sup> reagent pouch



The assays for the BD MAX<sup>™</sup> system are:

- **Check-Direct CPE for BD MAX<sup>™</sup>:** detects the clinically most prevalent carbapenemases – *bka*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub> – including the emerging *bla*<sub>OXA-181</sub> variant from culture;
- **Check-Direct CPO for BD MAX<sup>™</sup>:** detects the five most prevalent carbapenemase genes (*bka*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub> and *bla*<sub>IMP</sub>) directly from rectal swabs in about 2.5 hours; and
- **Check-Direct ESBL screen for BD MAX<sup>™</sup>:** detects ESBL genes (*bla*<sub>CTX-M-1</sub> group, *bla*<sub>CTX-M-2</sub> group, *bla*<sub>CTX-M-9</sub> group, *bla*<sub>SHV ESBL</sub>) from rectal swabs or culture.

Check-Points also offers an assay, the Check-Direct CPE, which can be performed using the NucliSENS<sup>®</sup> easyMAG<sup>®</sup> for sample preparation and the ABI 7500, CFX96<sup>™</sup>, LightCycler<sup>®</sup> 480 system I and II (Roche, USA) and Rotor-Gene<sup>®</sup> Q. The assay detects the most prevalent carbapenemases – *bka*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub> and *bla*<sub>NDM</sub> – from rectal swabs or culture. TAT is approximately 2 hours.

In addition, Check-Points offers a family of microarrays for epidemiology and confirmation. These assays are performed from culture using the following equipment: (i) magnetic bead- or column-based methods for sample preparation; (ii) a validated thermocycler, vortex mixer and mini-centrifuge, pre-PCR; and (iii) a validated thermocycler, vortex mixer, mini-centrifuge, thermomixer with active cooling, Check-Points Tube Reader, including E-Ads software, computer with USB drive and Internet connection and barcode reader (optional), post-PCR. The assays offered are:

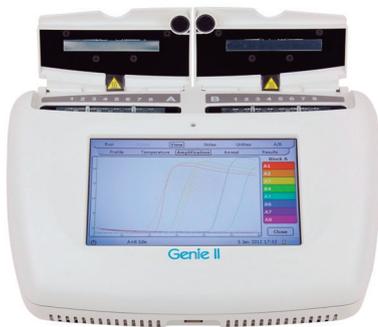
- **CHECK-MDR CT101:** permits investigation of the epidemiology of suspected CTX-M ESBLs, discriminates directly between ESBL and non-ESBL variants of TEM and SHV, and identifies presumptive mobile AmpCs.

- CHECK-MDR CT102: detects the clinically most prevalent carbapenemases and ESBLs in *Enterobacteriaceae*, and discriminates directly between ESBL and non-ESBL variants of TEM and SHV.
- CHECK-MDR CT103 XL: a CE-IVD-marked assay that identifies carbapenemase and ESBL targets, including emerging types. It can identify carbapenemases typically identified in *Acinetobacter baumannii* and carbapenemases and ESBLs found in *Pseudomonas aeruginosa*. The assay also discriminates directly between carbapenemase and ESBL variants of GES-type beta-lactamase (GES).

**eazyplex® (AmplexDiagnostics GmbH, Germany)**

AmplexDiagnostics offers the eazyplex® lyophilized ready-to-use amplification system for which there are numerous test kits available, most of which identify multiple genes that directly confer antibiotic resistance from various specimen types. Tests are validated to be run on the Genie® II instrument (OptiGene, UK), pictured in Fig. 90, for target isothermal amplification and detection. It is a fully portable, compact and light-weight platform designed for use at or near POC.

Fig. 90. Genie® II platform



The eazyplex® tests are qualitative in vitro molecular diagnostic tests to detect bacterial DNA in no more than 30 minutes. No DNA/RNA extraction is required, and eazyplex® test kits can be stored at ambient temperature. Test kits generally consist of eight-microtube test strips containing freeze-dried, ready-to-use reagents for amplifying seven resistance genes and one internal control. The test strips are used with the Genie® II platform, which uses a single-channel fluorescence excitation and detection system, to carry out LAMP of targeted resistance genes. The platform is mains powered, but can be used with a battery as well.

The test process is as follows. Samples are suspended in resuspension and lysis fluid (RALF) buffer

solution and incubated for 2 minutes with thermal lysis. Twenty-five millilitres of the RALF suspension is then added to each tube of the strip containing ready-to-use mastermix. The test strip is then immediately placed into the Genie® II instrument, where it is incubated at 66 °C for 30 minutes with fluorescent monitoring. Isothermal amplification is indicated by a strong increase in fluorescence signal in the form of a typical amplification curve. Different colours are given to each of the tested gene variants. Genie® II has two heating blocks, each of which can process a single eight-microtube test strip. The blocks can be controlled independently or run together to process up to 16 samples.

In addition to the Genie® II platform, OptiGene has introduced the Genie® III, pictured in Fig. 91, which has been developed for use with the eazyplex® test kits. It is smaller and lighter than the Genie® II, and per the company, is suitable for use in demanding environments. The platform includes dual-channel fluorescence measurement to allow use of internal controls and multiplexed assays. It also has positional information through GPS and offers wireless connectivity in the form of Bluetooth and Wi-Fi. Genie® III incorporates a rechargeable lithium-polymer battery that can support operation of the instrument for an 8-hour day. The instrument has a single heating block, which can process a single eight-microtube strip.

Fig. 91. Genie® III platform



Eazyplex test kits of relevance to this report include the following, all of which are validated for use on the Genie platforms. The assays are CE-IVD marked.

SuperBug® tests include three assays that determine the presence of carbapenemase-producing organisms (CPOs) and ESBL genes in people for whom colonization with these organisms is suspected. These are:

- SuperBug® complete A: a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from rectal swabs taken with eSwab™ (Copan) or

bacterial isolates from agar plates. The following carbapenemases are detected: KPC (*bla*<sub>KPC</sub>), NDM (*bla*<sub>NDM</sub>), VIM (*bla*<sub>VIM</sub>) and OXA (*bla*<sub>OXA-23,40,48,58</sub>).

- **SuperBug® complete B:** a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from rectal swabs taken with eSwab™ (Copan) or bacterial isolates from agar plates. The following carbapenemases are detected: KPC (*bla*<sub>KPC</sub>), NDM (*bla*<sub>NDM</sub>), VIM (*bla*<sub>VIM</sub>) and OXA (*bla*<sub>OXA-23,40,48,181</sub>).
- **SuperBug® CRE:** a qualitative IVD for directly detecting carbapenemase-producing bacteria and culture confirmation directly from bacterial isolates from agar plates, blood culture media from positive flagged blood culture bottles, rectal swabs taken with eSwab™ or urine. The following carbapenemases are detected: KPC (*bla*<sub>KPC</sub>), NDM (*bla*<sub>NDM</sub>), VIM (*bla*<sub>VIM</sub>) and OXA (*bla*<sub>OXA-48,181</sub>). In addition, the following ESBL genes are detected: *bla*<sub>CTX-M-1</sub> group and *bla*<sub>CTX-M-9</sub> group.

There are two additional eazyplex® SuperBug® test kits:

- **SuperBug® mcr-1:** a qualitative IVD for confirming *mcr-1*, which confers resistance to colistin (polymyxin B), in gram-negative bacteria from culture; and
- **SuperBug® AmpC:** a qualitative IVD for confirming AmpC beta-lactamases from AmpC-positive *Enterobacteriaceae* in culture.

Eazyplex® also offers two VRE assays. These are:

- **eazyplex® VRE:** a qualitative IVD for detecting VRE from rectal swabs or blood culture. The assay detects *vanA* and *vanB* and has three integrated controls.
- **eazyplex® VRE basic:** a qualitative IVD for confirming VRE directly from agar plates or from positive blood culture. The assay confirms *vanA* or *vanB* in 20 minutes.

Finally, eazyplex® offers two IVDs for MRSA screening, one for screening for MRSA based on detection directly from nasal swabs, and one for confirmation based on culture media. It also offers a family of *Clostridium difficile* test kits and a selection of CSF tests for detecting bacteria and viruses from CSF.

### Carbaplex® IVD PCR (Bruker, Germany)

Bruker offers the Carbaplex® IVD PCR assay for qualitatively detecting CPEs. The test, which is a multiplex real-time PCR assay, detects and differentiates

the five most prevalent carbapenemase genes from a single rectal swab sample. These are KPC (*bla*<sub>KPC</sub>), NDM (*bla*<sub>NDM</sub>), VIM (*bla*<sub>VIM</sub>), OXA (*bla*<sub>OXA-48,181</sub>) and IMP (*bla*<sub>IMP</sub>). The test can also be used for confirmation testing from suspected culture isolates. The assay is CE-IVD marked. TAT is less than 3 hours.

Carbaplex is provided in an easy-to-use master mix format and is designed for use with existing laboratory equipment in large laboratories, including the ABI 7500 and ABI QuantStudio 5, both from Thermo Fisher Scientific, the CFX detection systems (Bio-Rad), and the Rotor-Gene® Q.

### Antibiotic resistance line probe assays (AUTOIMMUN DIAGNOSTIKA GmbH, Germany)

AUTOIMMUN DIAGNOSTIKA offers several antibiotic resistance line probe assays (LPAs) for infectious disease. These assays are designed to be run on end-point PCR equipment, which requires a sophisticated and well equipped laboratory. Especially well trained technicians are important as the system is not integrated.

In addition to several assays for TB, which are not covered in this report, the assays include the following CE-IVD-marked tests for use on automated systems, which are relevant to this report:

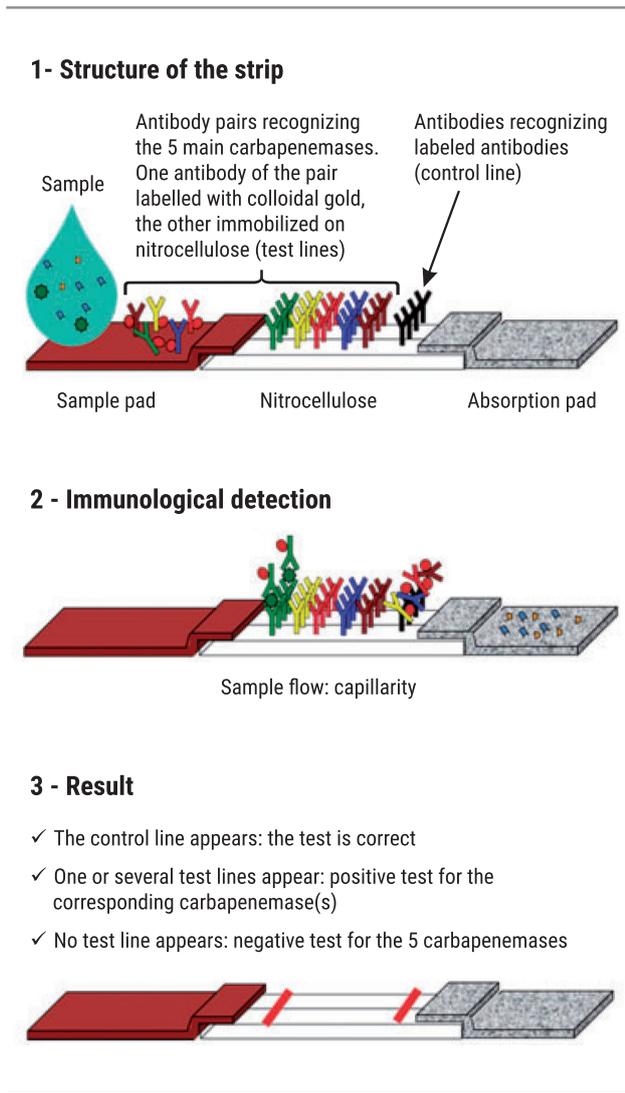
- **AID ESBL:** an assay for rapidly detecting ESBL genes, including *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>KPC</sub> within 5 hours from culture and clinical specimens.
- **AID carbapenemase:** an assay for initial screening of the most frequent carbapenemases from bronchoalveolar lavage, sputum, wound swabs or bacterial culture. The test detects 13 different carbapenem resistances, including *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub> and *bla*<sub>OXA-48</sub>. TAT is less than 4 hours.
- **AID MRSA combi:** an assay to detect the most frequent resistance genes of staphylococci, including *mecA* and *mecC*, and to differentiate *Staphylococcus aureus* and CNS. TAT is approximately 4 hours.

### Immunoassays and other methods for detecting antibacterial resistance

#### Antimicrobial lateral flow immunoassays (LFIAs) (NG Biotech, Netherlands)

NG Biotech offers several in vitro LFIAs for detecting/confirming resistance genes from culture. These are NG-Test CARBA 5, NG-Test CTX-M and NG-Test MCR-1. The assays are qualitative lateral flow strip tests, all of which are CE-IVD marked. TAT is 10 to 15 minutes.

Fig. 92. Test protocol for NG Biotech LFIA<sup>15</sup>



- NG-Test CARBA 5 detects the five main carbapenemases – NDM ( $bla_{NDM}$ ), KPC ( $bla_{KPC}$ ), NDM ( $bla_{NDM}$ ), VIM ( $bla_{VIM}$ ) and OXA-48 ( $bla_{OXA-48}$ ) – from cultured bacterial isolates.
- NG-Test CTX-M detects the ESBL CTX-M ( $bla_{CTX-M}$ ) from cultured bacterial isolates.
- NG-Test MCR-1 detects and confirms *mcr-1*, which confers colistin resistance, in gram-negative bacteria from culture.

The test protocol for these assays is illustrated in Fig. 92.

**RESIST assays (Coris BioConcept, Belgium)**

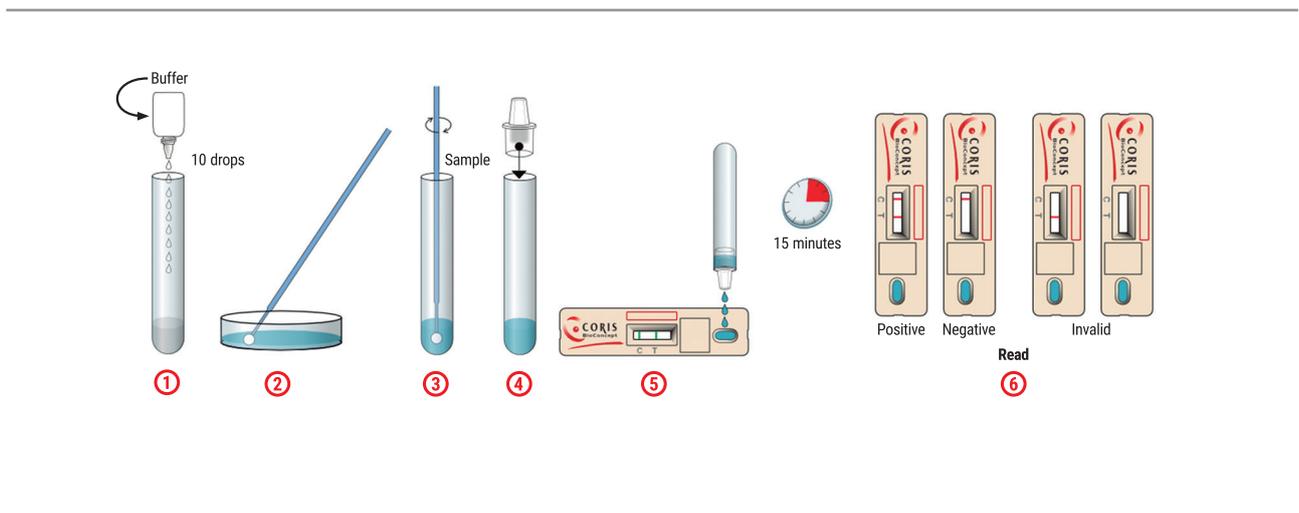
Coris BioConcept offers a range of CE-IVD-marked in vitro cartridge-based LFIA for detecting/confirming select carbapenemase resistance genes from cultured bacterial isolates. The tests are branded “RESIST” and consist of the following:

- OXA 48 K-SeT for detecting  $bla_{OXA-48}$
- KPC K-SeT for detecting  $bla_{KPC}$  only
- RESIST-3 O.K.N. K-SeT for detecting  $bla_{OXA-48-like}$ ,  $bla_{KPC}$  and  $bla_{NDM}$
- RESIST-3 O.O.K. K-SeT for detecting  $bla_{OXA-48-like}$ ,  $bla_{KPC}$  and  $bla_{OXA-163}$
- RESIST-4 O.K.N.V. for detecting  $bla_{OXA-48-like}$ ,  $bla_{KPC}$ ,  $bla_{NDM}$  and  $bla_{VIM}$ .

TAT for each of the tests is approximately 15 minutes. The procedure is illustrated in Fig. 93.

In addition to the RESIST line of assays, Coris BioConcept also offers a family of CE-IVD-marked in vitro diagnostic tests for rapidly detecting various

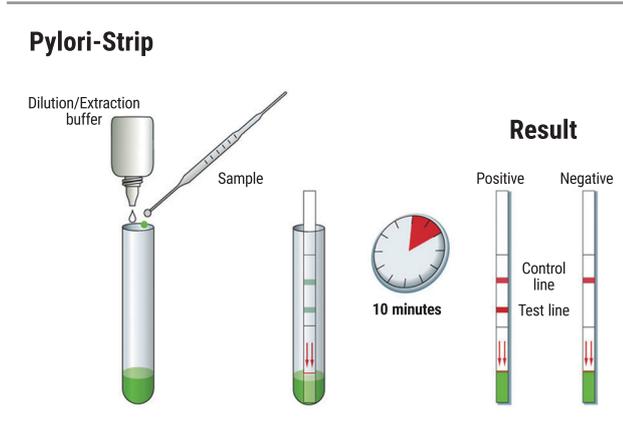
Fig. 93. Test procedure for RESIST range of LFIA from Coris BioConcept



<sup>15</sup> Reproduced from Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OA-48-like carbapenemase-producing *Enterobacteriaceae*. J Antimicrob Chemo. 2018;73(4):909–15.

pathogens (bacteria, virus, parasites). These immunochromatographic assays come in both strip/dipstick and cassette format. Of relevance to this report are the assays for *Helicobacter pylori*, *Escherichia coli* and *Clostridium difficile*. All of the assays require stool specimens, which in the case of the *E. coli* assay must have been broth enriched. TAT ranges from 10 minutes (*H. pylori*) to 15 minutes (for *E. coli* and *C. difficile*). The test procedure is similar for all three of the assays and is illustrated in Fig. 94 for the *H. pylori* assay.

Fig. 94. Coris BioConcept *Helicobacter pylori* strip test procedure



Finally, Coris BioConcept is developing a fully automated system, the TRAPIST V6 instrument and test cassettes, pictured in Fig. 95, for multiplex diagnostic testing. The platform, which is being designed for use in clinical laboratories, uses disposable microfluidic chip technology that combines both molecular assays and immunoassays. To date, the platform has not been commercialized; no assays have FDA clearance or CE-IVD marking. Initial assays for the TRAPIST system are focused on two sepsis panels – multiplex gram-positive cassette (e.g., *Staphylococcus aureus*); multiplex gram-negative cassette (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*) – and resistance markers (e.g., *vanA*, *vanB*, *mecA* and *mecC*) for gram-positive bacteria. Coris BioConcept aims for the TRAPIST system to return results in less than 1 hour. Additional information on these assays is not available.

Fig. 95. Coris BioConcept TRAPIST V6 instrument (left) and test cartridge (right)



## Pipeline technologies for identifying pathogens and/or detecting antibiotic resistance

There are PCR, DNA hybridization, electrochemical detection and other technologies for pathogen ID and detection of genes that directly confer bacterial resistance in the development pipeline. These are discussed below. Unless otherwise noted, all of the test systems described below are under development and not approved for sale; their performance characteristics have not yet been established.

### LabDisk (SpinDiag, Germany)

SpinDiag is a start-up company that was a spin-off from the Hahn-Schickard research institute. SpinDiag is developing a benchtop instrument, the LabDisk (pictured in Fig. 96), currently in prototype form, to test for 25 drug-resistant bacterial strains in 30 minutes at low cost. The technology uses centrifugal microfluidics with a disc-based test cartridge. There are no active components inside the instrument, but rather a simple optical reader.

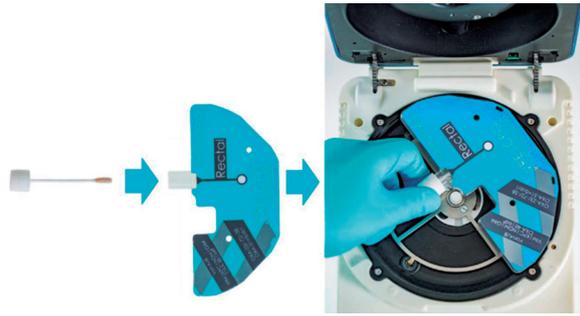
Fig. 96. LabDisk prototype instrument



The LabDisk instrument has a small footprint. As illustrated in Fig. 97, the system also has a simple workflow. Specimen swabs can be directly inserted into the LabDisk cartridge with no manual sample preparation; all dried reagents for sample preparation, amplification and detection are contained in the cartridge. The instrument uses fast, solid-state heating/cooling elements, mechanical lysis for extraction and nested PCR for high sensitivity. It can run 48 PCRs in parallel (composed of 36 parameters from one sample plus 12 internal controls).

To date, having tested 70 samples from 30 patients, the company has only a limited data set for the system. Results have been good, indicating that the instrument can detect down to single pathogens.

Fig. 97. LabDisk workflow



SpinDiag is currently testing for MRSA using nasal swabs and for VRE using rectal swabs. The company expects to be able to launch its first assay, a pan-bacterial test, by 2020. The next assays to be developed will be for respiratory tract infections and STIs. SpinDiag is also testing whether the platform could be used to test whole blood and/or urine specimens. The company believes that quantitation is possible on the LabDisk, but it has not yet been demonstrated.

### FireflyDx™ (ExcitePCR, a subsidiary of Positive ID, USA)

ExcitePCR is developing two diagnostic platforms for use at POC in low-resource settings: the FireflyDX-Portable™ and the FireflyDX-Handheld™. Both are in prototype stage. The FireflyDX-Portable™, pictured in Fig. 98, is a lightweight, “bookbag-sized” system utilizing real-time PCR that is designed to provide integrated sample purification, biological analysis and wireless communication of pathogen detection results in 30 minutes or less. The system incorporates single-use, disposable cartridges containing radio-frequency identification (RFID) chips that encode data. Cartridges will be able to process whole blood, nasal swabs and urine, among other specimens. Per the company, the FireflyDX-Portable™ instrument is an open system and will operate with any commercial assay, including those used by CDC and WHO.

Fig. 98. Rendering of the FireflyDX-Portable™ instrument



To date, the FireflyDX-Portable™ instrument has detected the Ebola and Zika viruses, *Escherichia coli*, influenza, MRSA, MSSA and *Clostridium difficile* on its prototype system. No commercialized assays for the system are available.

The other system being developed by ExcitePCR is the FireflyDX-Handheld™, pictured in Fig. 99. Like the FireflyDX-Portable™, it is a sample-in, result-out platform utilizing real-time PCR and single-use disposable cartridges. Because of its small size, the FireflyDX-Handheld™ can be used at bedside.

Fig. 99. Rendering of FireflyDX-Handheld™ instrument



The company plans multiple applications for the two FireflyDX™ systems, including AMR assays.

### GeneSTAT® analyser system (DxNA, LLC, USA)

DxNA is developing a sample-in, result-out diagnostic instrument platform, the GeneSTAT® analyser, for use at or near POC. The instrument, pictured in Fig. 100, has a small footprint with only four moving parts. Per the company, it requires minimal maintenance and no calibration. The GeneSTAT® analyser uses real-time PCR technology; test results can be read either on the instrument itself or on an attached laptop computer.

Fig. 100. GeneSTAT® analyser



The GeneSTAT® generates, analyses and transmits results to a connected preconfigured computer for presentation to the user. One single-use test cartridge

can be tested at a time in each GeneSTAT® analyser. Up to four GeneSTAT® analysers can be connected to a single computer.

Each GeneSTAT® single-use cartridge contains all required reagents as well as all the information needed to perform a test. In its present design, the cartridge has the capacity to perform up to four results with three analytical targets and one control. Reagents are lyophilized in each of the reaction wells, and once the specimen is placed into the cartridge, the cartridge becomes a closed, pressurized system. Per the company, this eliminates issues that arise from contamination from PCR products produced in the test process. Needed test information is provided on an RFID tag on each cartridge.

In 2017, DxNA received FDA clearance for its in vitro diagnostic assay for detecting *Coccidioides* spp. (valley fever). Currently, DxNA is developing a diagnostic test for *Staphylococcus aureus* that will both identify and differentiate resistant and nonresistant strains of *S. aureus* and CNS from multiple specimen types. The test uses three separate proprietary targets and a proprietary methodology to determine which type(s) of *Staphylococcus* are present and which carry a bacteria-resistant gene. TAT is about 60 minutes.

**ASTar™, ASTrID® (Q-linea AB, Sweden)**

Q-linea is in the process of developing two platforms for detecting BSIs: ASTar™ for AST and ASTrID® for pathogen ID.

The ASTar™ instrument performs phenotypic AST in about 3–6 hours following pathogen ID by current methods, e.g., MALDI-TOF MS, with which ASTar™

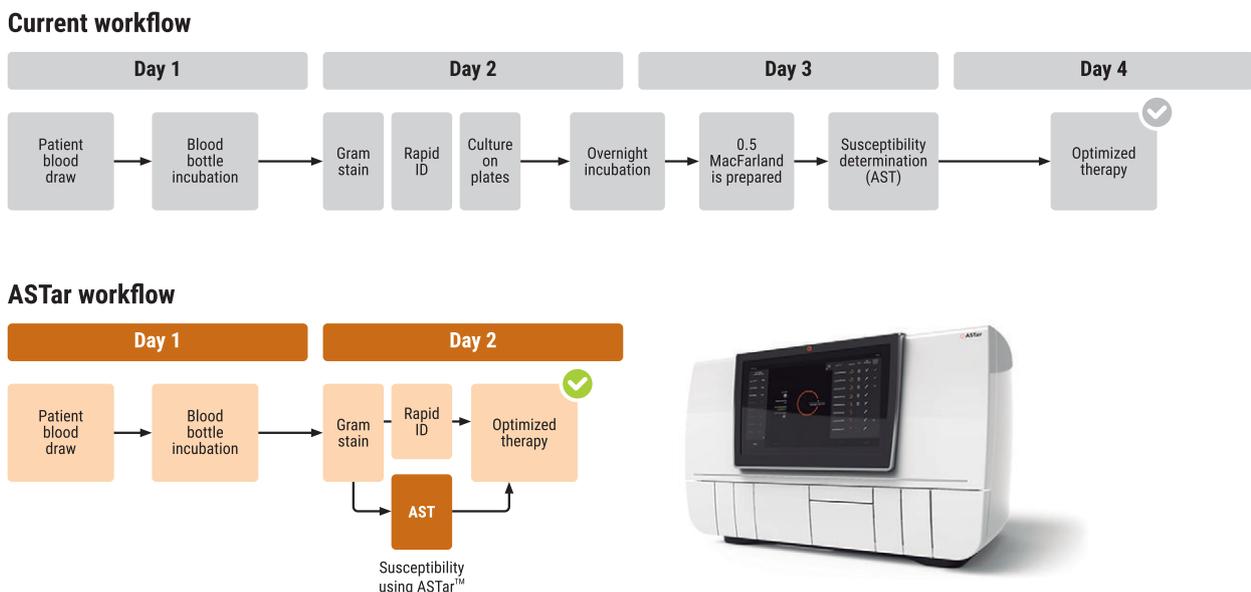
can be combined. The ASTar™ workflow compared to classical phenotypic AST is illustrated in Fig. 101.

The ASTar™ assay is based on broth microdilution and produces reproducible MICs. Currently, the assay takes blood culture samples only, although the company plans to develop an assay for use with whole blood. In internal studies, the ASTar™ assay has shown phenotypic AST results, obtained within 6 hours, with 96% essential agreement and 95% categorical agreement compared to reference broth microdilution with respect to *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* (59).

Q-linea is also developing the ASTrID® platform, pictured in Fig. 102, which will be a fully automated, high-throughput, multiplex benchtop system. The platform is based on Q-linea’s core padlock probe technology and circle-to-circle isothermal amplification (C<sub>2</sub>CA). More specifically, highly specific and selective padlock probes forming circularized DNA strands are amplified via RCA and subsequent C<sub>2</sub>CA. The resulting RCA products are labelled with fluorescence and are detected on a microarray (60–62).

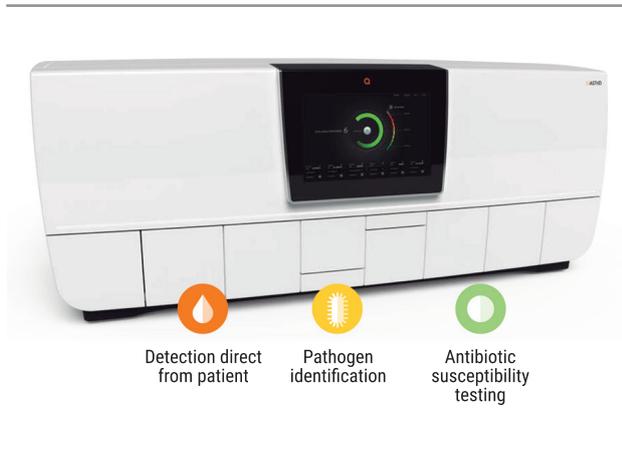
ASTrID® will enable ID of more than 50 sepsis pathogens and selected resistance genes, in addition to phenotypic AST, in 10 hours directly from whole blood. Per the company, the ID panel will cover 95% of relevant pathogens as well as 11 resistance markers; the panel of antibiotic substances will contain more than 30 antibiotics. Susceptibility will be reported as MIC values. Q-linea® has conducted a preclinical study using the prototype ASTrID® instrument that demonstrated that it can deliver pathogen ID directly

Fig. 101. ASTar™ workflow compared to classical phenotypic AST



from clinical blood samples without a positive blood culture, and a susceptibility profile after 6 hours using positive blood cultures.

Fig. 102. ASTriD® platform



### Reveal AST™/ID™ (Specific Diagnostics, USA)

Specific Diagnostics is developing a diagnostic system for ID and AST of pathogens. The system will use a proprietary, novel small molecule sensor (SMS) array that responds to metabolic byproducts produced by microorganisms to identify pathogens, detect their growth and assess their antibiotic efficacy. The SMS array detects low (parts per billion) concentrations of volatile organic compounds (VOCs) in very complex mixtures. A high-dimensional printed array of more than 70 colorimetric chemical indicators embedded in a nanoporous matrix has distinct chemical reactivity with volatile microorganisms and changes colour based on exposure to various VOCs and VOC mixtures. When placed in a culture, the resulting pattern of colour changes comprises a high-dimensional fingerprint of the cell type for bacterial species and strain from which bacteria can easily be differentiated.

The technology will permit Reveal ID™ to identify bacteria from blood culture in 4 hours on average directly in the vial, while Reveal AST™ will provide phenotypic MICs in 4 hours, also directly from positive blood culture or from isolate dilutions. Per the company, the technology has been clinically validated. Specific Diagnostics indicates that the system is easy to use and will be low cost.

### GeneXpert® Omni (Cepheid, a subsidiary of Danaher Corporation, USA)

Cepheid is developing the GeneXpert® Omni system (pictured in Fig. 103). The system leverages existing Xpert® cartridge technology (described earlier in this report). It should be noted, however, that the Omni platform does not use the same cartridge as

that in the original GeneXpert® platforms; therefore, the cartridges are not interchangeable between the two systems.

The GeneXpert® Omni is highly portable, measuring just 9 inches tall (about 23 cm) and weighing 2.2 pounds (about 1 kg). The system is battery operated (with up to 4 hours of operation and a supplemental rechargeable battery with an additional 8 hours of battery life), and is wireless and connectivity enabled. Advanced microfluidics regulate all aspects of the testing process within the test cartridge – from sample preparation and nucleic acid extraction to amplification and detection. Additionally, the platform has solid-state digital electronic architecture, which means it is durable.

Fig. 103. GeneXpert® Omni platform



The GeneXpert® Omni platform will use a dedicated mobile device to control each test module. The provided mobile device can control up to 3 Omni instruments, thus providing scalability and flexibility. The platform will also use a secure, hosted platform that collects and aggregates real-time test and system telemetry information. A single system can store more than 20 000 test results.

The initial assays planned for availability on the system will be the Xpert® MTB/RIF (resistance to rifampicin), Xpert® MTB/RIF Ultra, Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load and Xpert® HCV Viral Load and Xpert® HPV. Over time, it is Cepheid's intent to have the majority of the Xpert® menu available on the GeneXpert® Omni, which could include ID and molecular resistance cartridges.

### binx io® diagnostic system (Binx Health, Inc., formerly Atlas Genetics, UK)

The binx io® platform is a rapid, multiplex, molecular diagnostic system that can deliver laboratory quality results in about 30 minutes. The system consists of a small instrument and disposable cartridge (pictured in

Fig. 104) that contains all reagents necessary to run a test and is designed to be simple and intuitive; the user interacts with the instrument through a touch-screen interface, which then guides the user through the io system process. Once the raw sample has been added to the cartridge and loaded into the instrument, no further interaction is required. The instrument fits easily on a bench-top and is fully integrated, which enables the movement of a sample and reagents within the cartridge.

The cartridge has three main assay steps: sample preparation to isolate and purify target DNA, ultra-rapid PCR, which amplifies specific regions of DNA from the target organisms, and proprietary electrochemical detection to identify the presence of amplified DNA. Once the test is completed, a qualitative 'Detected/Not Detected' result is available with no clinical or laboratorian interpretation needed.

Fig. 104. binx io® instrument and cartridge from Binx Health



Binx Health's core focus is on STIs and their first application is for CT and NG. The binx test is designed to provide a result directly from an unpurified patient sample in about 30 minutes with equivalent accuracy and performance as current standard of care platforms run in central laboratories (which can take seven or more days).

The dual target CT/NG assay received CE marking for use within Europe in April 2019. Binx Health also recently successfully completed a US-based multi-center study of the platform and has submitted an application to the FDA for 510(k) clearance.

Binx continues to develop additional targets to add to its test menu, including an expansion of its current CT/NG multiplex test to include two other STIs with rapidly increasing prevalence: TV and MG. In addition, the company is also developing a NG resistance assay to detect Ciprofloxacin-sensitive strains. This work funded by the National Institute for Health Research (UK), and in collaboration with St. Georges Hospital in London, will allow greater antibiotic stewardship and open the breadth of treatments available to address this major public health crisis.

## Q-POC™ (QuantuMDx Group, UK)

QuantuMDx Group is developing a small benchtop diagnostic device, Q-POC™ and test cassette (pictured in Fig. 105), which can deliver patient results in less than 20 minutes.

Fig. 105. Q-POC™ cassette and device



The Q-POC™ device is a portable sample-to-answer molecular platform that is simple to use and runs end-point PCR chemistries, qPCR chemistries and includes a microarray after the amplification step. It is the first MDx POC platform that combines the ability to quantify pathogens through its six-channel qPCR and additionally perform multiplex detection of approximately 50 markers with its integrated microarray. Because it uses a rapid microfluidic thermal cycler that performs a 35-cycle end-point PCR in a few minutes, the device can run raw sample-to-answer assays in as little as 7–20 minutes, depending on the complexity of the assay.

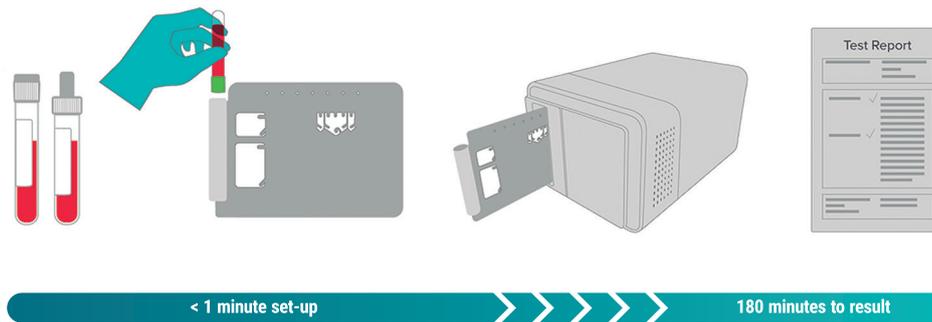
The first assay being developed on the Q-POC™ platform is an HPV genotyping assay that provides individual genotypes for 13 high-risk HPV subtypes in under 20 minutes, direct from a swab sample. The assay is presently in clinical field studies to demonstrate the clinical utility in screen-and-treat programmes in LMICs. The next assay planned for the Q-POC™ platform is a CT/NG/TV triplex assay, also run direct from swab samples in under 15 minutes. The company is developing an AMR NG assay to complement its triplex STI detection assay.

## LiDia® (DNA Electronics, Ltd. [DNAe], UK and USA)

DNAe is developing its LiDia® system for diagnosing infectious diseases using semiconductor genomic analysis. The target assay for its first IVD is the detection of sepsis from whole blood by direct sequencing. Tests for antimicrobial resistance testing (e.g., *mecA*), as well as tests for influenza and liquid biopsies, will follow.

The LiDia® technology uses immunomagnetic beads to capture only intact bacterial (and fungal) pathogens from specimens. Bead cell complexes are

Fig. 106. Workflow for sepsis assay on LiDia® platform



separated from background sample matrices, and human DNA is removed. Pathogens are then lysed, and purified DNA is eluted. Primers are bound onto transistors on a pH-sensing semiconductor chip. The purified pathogen nucleic acid is then amplified in a multiplexed PCR reaction containing primers specific for multiple pathogen targets; the multiplex mixture is split into separate reaction vessels, each with a specific nested PCR target assay. Detection of changes in pH associated with the release of hydrogen ions during PCR provides a real-time readout of detection events. The technology requires no optics or fluorescent labels.

The planned workflow with respect to the sepsis assay, illustrated in Fig. 106, consists of inserting a 10 mL blood Vacutainer® or Monovette® into a disposable cartridge, loading the cartridge into the LiDia® instrument and reading the result in about 3 hours. The company indicates that only about 1 minute of setup time is required.

### Smarticles™ technology (Roche, USA)

In 2015, Roche acquired GeneWeave Biosciences, which was developing Smarticles technology, a class of molecular diagnostics that can quickly identify multidrug-resistant pathogens and can assess AST directly from clinical samples without the need for traditional enrichment, culture or sample preparation processes. GeneWeave was also developing a platform called vivoDx, a fully automated random-access system.

Smarticles™ are nonreplicative transduction particles (phages) that bind specifically to bacteria and deliver DNA that contains a reporter luciferase gene that is expressed in the bacteria. It is complex technology in which each organism requires a unique phage.

Roche has indicated that its first assay for its cobas® vivoDx platform will be for MRSA; assays for CRE and VRE are in development. No additional information is available.

## Conclusion

There are numerous nonphenotypic assays and platforms for identifying bacterial pathogens as well as for identifying the genes that directly confer antibiotic resistance. Most of the platforms, especially those for BSIs, are systems best used in sophisticated laboratory settings with strong infrastructure and well trained laboratory staff. A few platforms, including the GeneXpert® system and FilmArray® EZ Configuration, can be used in near-patient settings, including Level II facilities in LMICs, but not for assays requiring culture. All of the platforms offer faster results than phenotypic methods.

Molecular and immunoassay platforms for detecting antibiotic resistance only, including Check-Direct and eazyplex®, as well as lateral flow assays from NG Biotech, are suitable for near-patient testing in LMICs. They do not, however, provide pathogen ID, which must be done on separate instrumentation.

Finally, a number of molecular systems in the development pipeline are designed for use in LMICs. The systems are smaller and simpler to use than conventional systems designed for use in large laboratories. Some of these pipeline diagnostics provide pathogen ID as well as resistance testing capabilities; some do not. Some perform multiplex testing only and some will only process sample matrices such as swabs and urine, which limits the pathogens they are able to detect. Some systems will process complex matrices, including whole blood, which would offer the possibility of avoiding culture. However, detecting and identifying bacteria direct from whole blood with performance at least equivalent to blood culture has proven to be very difficult. It is a challenge that has not yet been met.

# Host response assays

The diagnostic systems mapped in this report so far have focused on specific pathogen ID approaches. There are other rapid, easy-to-use diagnostics that might have value in combating ABR that are suitable for use at Level I and Level II settings in LMICs that are not direct pathogen detection methods. These include host immune response assays, including tests to detect blood-based host-derived biomarkers. Some of these tests are already commercialized; some are in the development pipeline.

Host-derived biomarkers of infection include white blood cell levels, erythrocyte sedimentation rate, CRP, PCT, presepsin, CD64 and proADM, among others (63). Of particular interest for this report are CRP and PCT, the levels of which increase with bacterial infection. CRP is a nonspecific, inflammation-related protein that is produced in the liver and regulated by plasma interleukin-6 (IL-6). It increases with bacterial infections, postoperative conditions or tissue injury (64). PCT is a glycoprotein with no hormonal activity. It demonstrates high sensitivity to viral and bacterial infections. These host-derived biomarkers may reflect the severity of the infection/condition (e.g., immune activation), but cannot determine etiology. Nonetheless, in the appropriate clinical context, such host response assays can help guide appropriate antibiotic use by ruling in or ruling out a serious bacterial infection (65).

## CRP tests

The use of CRP assays to guide antibiotic treatment has been examined extensively (66). Studies have generally focused on the use of CRP testing in the context of sepsis, particularly in hospital settings, or for both lower and upper acute respiratory tract infection (ARI) in the context of primary care settings; most studies have been done in HICs (64, 66). The majority of studies on CRP have shown statistically significant differences in CRP levels in patients with bacterial infections as opposed to those with nonbacterial infections (66). A recent Cochrane review found that the use of CRP at POC can significantly reduce prescription of antibiotics in ARIs (64). However, as Cooke and colleagues caution, CRP is “not a substitute for a proper clinical examination” (67).

There are commercialized rapid diagnostic CRP tests that can be used at or near the point of patient care. These include qualitative and semi-quantitative

lateral flow tests as well as fully quantitative test systems. Some of these tests are described below.

## Qualitative or semi-quantitative tests

- DTS233 (Creative Diagnostics, USA): a qualitative (single threshold of 10 mg/L), disposable rapid test for detecting CRP abnormality in whole blood, serum or plasma, as an aid in the clinical diagnosis of CRP. It is based on the principle of colloidal gold immunochromatography. It is a one-step test with a TAT of 10–15 minutes. Test results are read visually without any instrument. The test is currently RUO.
- WD-23 (Assure Tech, China): a CE-marked, semi-quantitative (four CRP concentration ranges: <10 mg/L; 10–30 mg/L; 30–80 mg/L; >80mg/L) rapid diagnostic immunoassay for detecting CRP in whole blood, serum or plasma specimens. The assay utilizes a combination of colloidal gold conjugate and anti-CRP antibodies. TAT is between 5 and 7 minutes. Assure Tech also offers a similar CE-marked rapid diagnostic assay for PCT.
- bioNexia® CRPplus (bioMérieux, France): a semi-quantitative (four CRP concentration ranges: <10 mg/L; 10–40 mg/L; 40–80 mg/L; ≥80 mg/L) rapid lateral flow diagnostic test for detecting inflammatory reaction from whole blood. TAT is 5 minutes.
- Actim® CRP (Medix Biochemica, Finland): a semi-quantitative (three concentration ranges: 10–40 mg/L; 40–80 mg/L; >80 mg/L) rapid assay for detecting inflammatory reaction from fingerstick blood (EDTA, citrate or heparin blood can also be used). TAT is 5 minutes. The test requires no laboratory equipment.

Assuming the tests above are available for in vitro use and are easy to use, they would be suitable for use in primary care settings. Performance would also be a factor in their selection and implementation.

## Quantitative tests

- Alere Afinion™ CRP (Abbott, USA): a CE-marked in vitro diagnostic test to determine the amount of CRP in human whole blood, serum

or plasma. It is a solid-phase immunochemical assay that uses a membrane coated with anti-human CRP antibodies, which react with CRP in the sample. The test is intended to be performed on the Afinion™ AS100 analyser, pictured in Fig. 107, which measures the colour intensity of the membrane and is proportional to the amount of CRP in the sample. The test cartridge contains all the reagents needed to measure CRP in a blood sample. The CRP concentration is displayed on the Afinion™ AS100 analyser within 4 minutes. The measurement range is 5–200 mg/L for whole blood and 5–160 mg/L for serum and plasma samples.

Fig. 107. Alere Afinion™ AS100 analyser and cartridges



The Afinion™ system is appropriate for use at or near POC. In addition to the Alere Afinion™ CRP assay, two other assays are available: (i) Alere Afinion™ ACR for detecting albumin, creatinine and albumin/creatinine ratio (ACR) in human urine; and (ii) Alere Afinion™ HbA1c for quantitatively determining glycated haemoglobin (HbA1c) in human whole blood.

- QuikRead go CRP (Orion Diagnostica Oy, Finland): an FDA-cleared and CE-IVD-marked in vitro diagnostic test for determining the level of CRP from fingerstick blood, venous blood, plasma or serum of people who present with symptoms of infection. The CRP measurement range is 5–200 mg/L. The assay is a particle-enhanced immunoturbidimetric assay that uses nanoparticles coated with anti-human CRP fragments, which react with CRP in the sample. The QuikRead go CRP assay is intended to be run on the QuikRead go instrument, pictured in Fig. 108, which is a photometer that is calibrated for both photometric and turbidimetric measurement. The instrument measures the change in turbidity of the sample solution and converts the value into a concentration value on the basis of preset test calibration data. TAT is

2 minutes. Results are automatically stored in the instrument's memory, along with user and patient ID. The QuikRead go instrument also features both unidirectional and bidirectional connectivity.

Fig. 108. QuikRead go instrument



Orion also offers a combined CRP + Hb assay for use on the QuikRead go instrument and offers a CRP assay for use on its QuikRead 101 instrument, pictured in Fig. 109, a portable device that works on the same principle as the QuikRead go instrument. The QuikRead 101 instrument and CRP test are CE-IVD marked, but not FDA cleared.

Fig. 109. QuikRead 101 instrument



- AQT90 FLEX CRP (Radiometer Medical ApS, Denmark): an in vitro CE-marked diagnostic test for determining the concentration of CRP from venous whole blood or plasma in people presenting with symptoms of infection. The CRP measurement range is 5–200 mg/L. The test is designed to be run on the AQT90 FLEX immunoassay analyser, pictured in Fig. 110, which is based on time-resolved fluorescence using a

europium chelate as the fluorescent label. The instrument can process up to 30 samples per hour, has full connectivity capabilities and, per the company, can be used in near-patient settings. TAT is less than 13 minutes per test. Radiometer also offers additional assays for the system, including PCT, troponin and D-dimer.

Fig. 110. AQT90 FLEX analyser



- iChroma™ CRP (Boditech Med, Korea): an in vitro diagnostic test to determine the concentration of CRP from whole blood (EDTA whole blood or capillary blood), serum or plasma in people presenting with symptoms of infection. The test is based on laser-induced immunofluorescence and uses a method of immunodetection which, by mixing whole blood with a detection buffer in a test tube, binds fluoresced anti-CRP antibodies in the buffer to the CRP antigen in the blood sample. The CRP measurement range is 2.5–300 mg/L. The test can be read on the iChroma™ II, pictured in Fig. 111. The instrument is a semi-automated or automated portable desktop fluorescence scanning device/reader that measures the fluorescence intensity of the test sample in a test cartridge. The device calculates the concentration of the analyte according to a preprogrammed calibration equation and displays the result. TAT for each CRP test is approximately 3 minutes.

Fig. 111. iChroma™ II immunoassay reader



The iChroma™ II is suitable for use in near-patient settings. It is easy to use with simple user interface and backup battery power. It has a built-in printer and wired or wireless connectivity.

Additional tests that can be performed on the iChroma™ II include PCT, antistreptolysin O (ASO), as well as a variety of viruses and cardiac markers. All iChroma assays are CE-IVD marked, and the iChroma™ II reader and CRP assay are FDA cleared.

- NycoCard™ CRP (Abbott, USA): a CE-marked in vitro immunochemical assay for quantitatively determining CRP in whole blood, serum and plasma. The assay uses a dilution liquid to make cells soluble, a membrane-bound antibody that binds CRP and a gold-conjugated antibody for making the bound CRP visible. The CRP measurement range from whole blood is 8–200 mg/L; from serum/plasma it is 5–129 mg/L. TAT is less than 3 minutes. The test is read on the NycoCard™ Reader II, pictured in Fig. 112, which is a small battery-powered instrument. It comprises two units: the instrument box, which is the operational and calculating unit, and the reader pen, which detects the signal.

Fig. 112. NycoCard™ Reader II



The NycoCard™ system is suitable for use in primary care settings. Additional tests for the system include HbA1c, U-albumin (urine albumin) and D-dimer.

- CRP test kit (Eurolyser Diagnostica GmbH, Germany): Eurolyser offers a number of test kits for use on its CUBE and Smart analysers. One of these is a CE-marked in vitro assay for the kinetic determination of CRP from whole blood and serum. The test is an immunoturbidimetric assay that uses photometric measurement at 546 nanometres (nm) or 700 nm of

antigen-antibody reaction between antibodies to human CRP bound to polystyrene particles and CRP present in the sample. The test has two ranges: (i) serum: 0.5–120 mg/L for 546 nm and 1.0–120 mg/L for 700 nm; and (ii) whole blood: 2.0–240 mg/L for 546 and 700 nm.

The Eurolyser CRP test can be run on any of several instruments available from the company. For purposes of providing rapid testing at primary care, the CUBE-S instrument, pictured in Fig. 113, is of most interest. It is a lightweight, easy-to-use sample-in, result-out device that the company refers to as a “pocket-sized laboratory”. It employs RFID technology and Android app-based operation. It is Bluetooth and USB enabled with data transfer to a printer or host. With respect to the CRP assay, the CUBE-S automatically includes a patient’s individual haematocrit values when calculating CRP. In addition to CRP, multiple CE-marked tests can be run on the CUBE-S. These include ASO, haemoglobin, HbA1c and D-dimer, among others.

Fig. 113. Eurolyser CUBE-S instrument



- CRP IS - InnovaStar® (DiaSys Diagnostic Systems GmbH, Germany): a CE-marked in vitro immunoturbidimetric assay for quantitatively determining CRP in whole blood or plasma. The CRP measurement range from whole blood is 5–400 mg/L; from plasma it is 2–160 mg/L. TAT is approximately 7 minutes. The test can be run on the company’s InnovaStar® clinical chemistry analyser, pictured in Fig. 114. The instrument is a compact sample-in, result-out benchtop analyser with fully automated measurement. For ease of use, the system uses precalibrated methods and prefilled unit dose reagents. Additional assays available for the InnovaStar measure HbA1c and glucose/haemoglobin, both of which are CE marked.

Fig. 114. InnovaStar instrument



- spinit® (biosurfit, Portugal): biosurfit has developed a multiplex, multianalyte diagnostic platform. The system is a centrifugal microfluidic platform that employs three different technologies: (i) immunoassays performed with surface plasmon resonance using a polarized laser beam; (ii) clinical chemistry performed by measuring absorbance at multiple wavelengths using LEDs; and (iii) haematology performed using an integrated microscopy module and standard dyes. The spinit® instrument is a compact sample-in, result-out platform that is suitable for use in near-patient testing.

Several CE-IVD-marked assays are available for use on the spinit® platform, pictured in Fig 115. One of these is a quantitative in vitro diagnostic for measuring CRP in whole blood (venous and capillary) or, alternatively, in serum and plasma. The CRP measurement range from whole blood is 2–180 mg/L. The spinit® test cartridge, also pictured in Fig. 115, is a microfluidic disc (similar to a DVD), and allows automated sample processing and assay performance based on antibody-antigen reaction on the spinit® instrument. The instrument uses an optical-based (photometry) detection system. CRP concentration is determined from reaction data. TAT is less than 4 minutes.

Fig. 115. spinit® instrument and cartridges



### PCT tests

Like CRP, the use of PCT as a host-derived biomarker to determine bacterial infection has been extensively examined (66, 68, 69). Higher levels of PCT are generally found in severe bacterial infections, but remain relatively low in nonspecific inflammatory diseases. Studies have demonstrated that PCT may be used to support clinical decision-making with respect to starting and/or stopping antibiotic therapy in various types of infections in a variety of settings, including primary care, emergency rooms and hospital wards (70, 71). PCT is generally more specific for bacterial infections than other inflammatory markers, including CRP (69). A recent Cochrane review found that the use of PCT to guide initiation onto, and duration of, antibiotic treatment of ARIs “results in lower risks of mortality, lower antibiotic consumption and lower risk for antibiotic-related side effects” (68). Rhee concludes that the use of PCT to guide antibiotic therapy is most useful in two contexts: (i) noncritically ill patients with suspected or proven ARI, and (ii) critically ill patients with suspected infection/sepsis (69).

Commercial diagnostic PCT tests are available. Of these, at least two assays are disposable rapid diagnostic tests, one from Assure Tech and one from Cortez Diagnostics, Inc. (USA). There is no available performance data on these assays. In addition to the rapid diagnostic assays, a number of quantitative and semi-quantitative assays are available for use on various instrument systems, most of which are designed for use in sophisticated clinical laboratories. However, a few systems could be used for near-patient testing. Some of these assays and systems are described below.

- B·R·A·H·M·S PCT™ direct assay (Thermo Fisher Scientific, USA): an automated in vitro immunochromatographic sandwich assay for determining PCT in human whole blood (capillary or venous). The assay is intended to be performed on the B·R·A·H·M·S™ direct reader, pictured in Fig. 116, which is designed for use at POC. The reader offers data input via a scanner and reader connectivity to an LIS. The assay takes only 20 µL of whole blood. TAT is about 20 minutes. The assay is CE marked, but not FDA cleared.

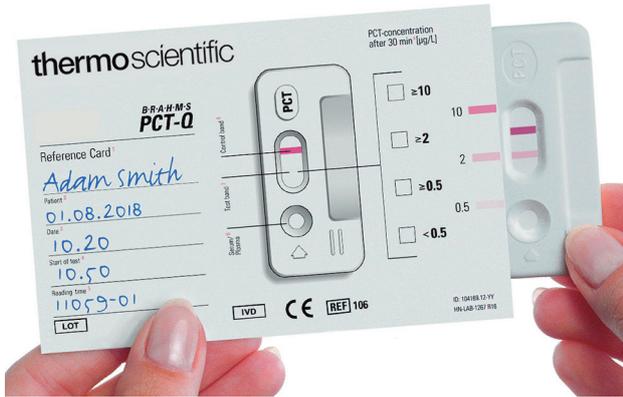
Fig. 116. B·R·A·H·M·S™ direct reader



- Thermo Fisher Scientific offers a number of PCT assays, including the B·R·A·H·M·S PCT™ sensitive KRYPTOR™ assay, for use on its large, high-throughput instruments, including the B·R·A·H·M·S KRYPTOR™ Gold. It should be noted that Thermo Fisher Scientific has a patent for using PCT as a biomarker for sepsis. Other companies, including Abbott, Siemens, bioMérieux, Roche and DiaSorin, license the use of PCT and its antibodies from Thermo Fisher Scientific. All of the commercial quantitative B·R·A·H·M·S PCT™ assays, including the B·R·A·H·M·S PCT™ sensitive KRYPTOR™ (Thermo Fisher Scientific), ADVIA Centaur® and ATELLICA® IM B·R·A·H·M·S PCT™ assays (Siemens), ELECYs® B·R·A·H·M·S PCT™ assay (Roche), LIAISON® B·R·A·H·M·S PCT™ assay (DiaSorin, Italy) and VIDAS® B·R·A·H·M·S PCT™ assay (bioMérieux), use the same sandwich ELISA principle to quantify PCT by forming antibody-PCT-antibody complexes. The primary difference among these assays is the mechanism of detection of the complexes. All of the instrument systems for which the assays above are described are designed for use in relatively large clinical laboratories and would be best used in Level III and Level IV laboratories in LMICs. They are not described in detail in this report. The assays/systems from Thermo Fisher Scientific and its licensing partners are FDA cleared.
- B·R·A·H·M·S PCT™ LIA (Thermo Fisher Scientific): an immunoluminescence assay used to determine the concentration of PCT in human serum and plasma. The B·R·A·H·M·S PCT™ LIA is intended for use in conjunction with other laboratory findings and clinical assessments to aid in assessing risk for progression to severe sepsis and septic shock of critically ill patients on their first day of admission into an intensive care unit (ICU). The assay uses a coated tube system with two monoclonal antibodies (sandwich principle). The PCT concentration is quantified by measuring the luminescence signal using a luminometer and B·R·A·H·M·S™ Basiskit LIA reagents and calculating the resolves from the standard curve. TAT is 60 minutes at room temperature. The assay is CE marked and FDA cleared.
- B·R·A·H·M·S PCT-Q (Thermo Fisher Scientific): a semi-quantitative immunochromatographic assay for determining the concentration of PCT in human serum and plasma. The assay is a cartridge-based, one-step test that uses the sandwich principle with immunogold labelling. TAT is 30 minutes at room temperature. The user

determines the PCT concentration range of the sample by comparing the colour intensity of the best band on the cartridge with colour blocks on the reference card, illustrated in Fig. 117. The assay is CE marked, but not FDA cleared.

Fig. 117. B-R-A-H-M-S PCT-Q test cassette and reference card



- **Diazyme PCT assay (Diazyme Laboratories, Inc., USA):** an in vitro latex-particle-enhanced immunoturbidimetric assay intended for quantitatively determining PCT in human serum, EDTA or lithium heparin plasma. The test is intended for use on the first day of ICU admission for progression to severe sepsis and septic shock. TAT is 10 minutes.

The Diazyme PCT assay is intended to be used on validated chemistry analysers, including the Olympus AU 400 instrument (Beckman Coulter). The test can be run on the Diazyme CUBE-A and SMART instruments. With respect to the CUBE-A and SMART systems, the instrument calculates the PCT concentration of a patient sample by utilizing a lot-specific calibration curve that is stored on an RFID card provided with each instrument kit. The assay is FDA cleared for use on FDA-approved instruments. The assay is CE marked for use on the CUBE-A and SMART systems.

As indicated above in the description of commercially available quantitative CRP assays, quantitative PCT assays are also available for the AQT90 FLEX immunoassay analyser (Radiometer) and for the iChroma™ II analyser.

### Novel host response tests

In addition to tests for CRP and PCT, some diagnostic tests are available that measure novel host-derived biomarkers, a combination of host biomarkers, or combinations of protein biomarkers and gene classifiers. These are described below.

- **ImmunoXpert™ (MeMed BV, Israel):** an in vitro diagnostic test to distinguish between bacterial and viral infections. The assay measures three human immune system biomarkers in serum: (i) tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), (ii) interferon gamma-induced protein-10 (IP-10) and (iii) CRP. A computer algorithm is then used to compute a score indicating the likelihood of a bacterial versus viral (or other nonbacterial) immune response. The company emphasizes that the test is not intended as a stand-alone diagnostic tool and should be used in conjunction with other clinical data; in addition, the test is not intended to distinguish between infectious and noninfectious etiologies.

The ELISA format version of ImmunoXpert™ is CE marked, but not FDA cleared. TAT is 99 minutes. ImmunoXpert™ is suitable for use in centralized laboratories, although the company is developing the ImmunoPoc™ device, pictured in Fig. 118, which would be suitable for use in near-patient settings.

Fig. 118. Prototype ImmunoPoc™ device and cartridge



Also in the pipeline from MeMed are two additional assays: (i) MeMed Sepsis™ and (ii) MeMed Neo™ for bacterial infections in neonates.

- **FebriDx® (RPS Diagnostics, USA):** an in vitro single-use, qualitative disposable rapid test to identify patients who have a clinically significant underlying infection and to help differentiate a clinically significant immune response to viral and/or bacterial ARI from fingerstick blood. The test detects elevated levels of myxovirus resistance A (MxA), a nonspecific inflammatory protein, which is a derivative of interferon. MxA becomes elevated in the presence of acute viral infection, and CRP. TAT is approximately 10 minutes. The assay is CE marked, but is not FDA cleared.

The assay is contained in an all-in-one plastic cassette housing, pictured in Fig. 119, that includes a built-in safety lancet, blood collection and delivery system, and integrated push-button buffer delivery features, which make the test easy to use. The FebriDx<sup>®</sup> assay requires no additional equipment to perform the test or to interpret results, making it appropriate for use at POC.

Fig. 119. FebriDx<sup>®</sup> test cassette



- **SeptiCyte<sup>™</sup> LAB (Immunexpress, USA):** an in vitro gene expression assay using real-time, reverse-transcription PCR to quantify the relative expression levels of four host response genes (*CEACAM4*, *LAMP1*, *PLAC8* and *PLA2G7*) isolated from whole blood collected in PAXgene blood RNA tubes. The SeptiCyte<sup>™</sup> LAB assay is used in conjunction with clinical assessments and other laboratory findings as an aid in differentiating infection-positive (sepsis) from infection-negative systemic inflammation in patients suspected of sepsis on their first day of admission into ICU. The SeptiCyte<sup>™</sup> LAB assay generates a score (SeptiSCORE<sup>™</sup>) that falls within one of four discrete Interpretation bands based on the increasing likelihood of infection-positive systemic inflammation; it uses a binary cut-off of 3.1 to classify patients as high or low risk for sepsis. TAT is approximately 6 hours.

The SeptiCyte<sup>™</sup> LAB sepsis assay is FDA cleared and validated for use on the ABI 7500 Fast. As such, it is appropriate for use in centralized clinical laboratories. However, in January 2018 Immunexpress entered into an agreement with Biocartis Group NV (Belgium) to develop and commercialize the Immunexpress SeptiCyte<sup>™</sup> assay for use on Biocartis's sample-to-result Idylla<sup>™</sup> platform, pictured in Fig. 120. The platform is a fully automated molecular system using real-time PCR to identify up to 30 targets. It features minimal hands-on time of approximately 2 minutes. TAT is between 90 and 150 minutes depending on the assay.

Fig. 120. Idylla<sup>™</sup> platform



- **abioSCOPE<sup>®</sup> (Abionic, Switzerland)** is a multiplex immunoassay platform. One of the assays for the abioSCOPE<sup>®</sup> platform measures a sepsis risk biomarker, pancreatic stone protein, in whole blood (capillary or venous) or serum/plasma samples. TAT is 5 minutes. The assay is CE-IVD marked, but has not yet been commercialized.

The abioSCOPE<sup>®</sup> platform uses fluorescent nanofluidic immunoassay technology. Fluorescent molecular complexes are formed on a nanosensor. These complexes are then detected and quantified optically using an integrated microscope laser. Because of the nanofluidic configuration of the platform, biomolecular interactions are accelerated, leading to quick TATs. The system uses disposable “capsules” (cartridges) that are placed into a disc-shaped mounting plate and then inserted into the instrument.

The abioSCOPE<sup>®</sup> instruments, pictured in Fig. 121, come in three configurations for use in different settings – pharmacy, hospital/ICU and physician's office. The instruments all use the same technology, but have different displays and assay availability.

Fig. 121. abioSCOPE<sup>®</sup> instruments



In addition to the sepsis assay, Abionic has two additional CE-IVD-marked assays, one for allergy and one for iron deficiency (ferritin). Additional assays, including CRP and D-dimer, are in the development pipeline.

Finally, there are two interesting host response assays in development:

- HostDx sepsis and fever assays (Inflammatix, USA) is developing two assays that will use a host response marker, specifically an mRNA signature, to identify acute infection. They are the HostDx Sepsis and HostDx Fever assays. The tests will use quantitative multiplex gene expression to analyse a patient's immune system – the host response – rather than directly identifying pathogens. The assays will be run on a molecular, multiplex platform from white blood cells. The HostDx Sepsis assay will be targeted at hospitalized patients with acute infection, while the HostDx Fever assay will help clinicians decide whether to administer antibiotics for patients presenting with fever in primary care settings. Inflammatix indicates that it is working with partners that are already building platforms suitable for integrating its assays. The company is in the in vivo assay development phase in which it is developing its assays (wet lab) based on a locked set of genes and algorithms and working with instrument partners to finalize the assays. No timeline for launch of either the HostDx Sepsis or HostDx Fever assay is known.
- UTRiPLEX (Mologic, UK) is developing a qualitative lateral flow test, UTRiPLEX, in dipstick format that uses the presence of three biomarkers to rule out UTIs. TAT is 6 minutes. The test is not commercially available; it is currently undergoing clinical evaluation.

Numerous additional host-derived biomarkers are being studied for use in diagnostic assays. These include CH13KI plus CRP, haptoglobin-related protein (Hpr), Lpc-2 plus Hpr, heparin-binding protein and more. Most of this work is being done in academic institutions and has not yet been translated into diagnostic products.

## Conclusion

Some assays, in particular disposable RDTs to detect CRP, could be used in primary care settings (Level I) to help target the need for antibiotics in patients presenting with febrile and respiratory illness. There are also a few instrument-based platforms that could be implemented in Level II settings. These include the iChroma™ CRP test and immunoassay reader and the NycoCard™ CRP test and reader. Unlike the

RDTs available for CRP testing, there are very few such tests available for PCT testing, although a few platforms, like the B·R·A·H·M·S PCT™ direct assay and reader and the B·R·A·H·M·S PCT-Q, which requires no instrumentation but does require a serum or plasma sample, could be used in Level II settings. In all cases, tests would need to be used in an appropriate context and algorithm.

In addition, some interesting novel host response tests/platforms that are commercially available or in the pipeline could also be used to help make an initial determination as to whether an infection is bacterial or nonbacterial for patients presenting with symptoms of infection. For example, the FebriDx single-use qualitative test could be used in Level I settings to help distinguish a clinically significant host immune response to viral and/or bacterial ARIs, which should help determine whether antibiotics are needed. In general, these tests utilizing new host-derived biomarkers or combinations of such biomarkers have not been widely studied, and performance data is still lacking.

Finally, none of these tests is a complete solution, because none of the tests can identify bacterial pathogens nor can they determine AST. Further testing would be required.

# Discussion

This landscape report maps current diagnostic methods for identifying bacterial pathogens, including manual and automated phenotypic methods primarily done at higher levels of the healthcare system in LMICs, with a primary focus on commercialized platforms. The landscape also maps current immunoassay, molecular and other methods of identifying bacterial pathogens, with a focus on commercial systems. Emerging and pipeline diagnostics are also considered.

The landscape maps current and emerging phenotypic methods of AST, including both manual and automated methods, as well as commercial platforms combining bacterial ID and AST. It considers pipeline AST technologies that may be used in primary and secondary care settings, particularly in LMICs. The landscape then maps commercially available and pipeline nonphenotypic methods for simultaneous pathogen ID and detection of ABR or methods for identifying genes that directly confer antibiotic resistance only. In both cases, pipeline technologies are reviewed.

The landscape therefore provides a reasonably comprehensive picture of the test systems for combating ABR available at all levels of the healthcare system and what is in the pipeline; these are summarized in Annex II. This provides a backdrop from which to identify gaps in diagnostics in the context of, and in consideration of, certain key parameters of interest to WHO:

- diagnostics that target high-priority drug-resistant bacterial pathogens identified by WHO, CDC and ECDC and set out in Annex I;
- the need for diagnostics to improve clinical/syndromic management of patients to reduce over-prescribing of antibiotics, i.e., in the context of antimicrobial stewardship;
- the need for IVDs that can be performed at primary and secondary healthcare facilities (Level I and Level II laboratories) in LMICs (as described in this report); and
- given the needs in primary care facilities, focuses on priority bacterial pathogens that are primarily community acquired, including *Escherichia coli*, NG, *Helicobacter pylori*, *Campylobacter* spp., *Salmonella* spp., *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Shigella* spp. and *Staphylococcus aureus*.

As noted earlier in this report, TB is a high-priority multidrug-resistant pathogen. However, extensive landscaping of diagnostics and drug-resistance testing for MTB has already been performed, and a number of TPPs have been developed for priority diagnostic needs. Given the work that has already been done with respect to diagnostics for MTB, they are not a focus of this report. Nonetheless, because of the importance of TB, the report highlights priorities for TB diagnostics R&D as well as priorities for other targeted bacterial pathogens.

In brief, the question that frames this report is, What are the gaps in diagnostics to combat ABR for prioritized drug-resistant bacterial pathogens, with an emphasis on CAIs, at Levels I and II of the healthcare system in LMICs? Based on this landscape report, the short answer is that there are many commercially available test systems, both phenotypic and non-phenotypic, for identifying and/or performing AST/resistance testing with respect to virtually all priority bacterial pathogens. But most systems are predicated on sophisticated, well equipped laboratories with well trained laboratory staff. Therefore, most test systems are only of practical use at Level III and Level IV of the laboratory systems in LMICs. This is shown in Annex III, which summarizes diagnostic platforms for combating ABR that are potentially suitable for Level I and/or Level II in LMICs.

Despite the availability of test systems for sophisticated clinical laboratories, classical phenotypic test methods utilizing culture and AST are considered to be too slow and cumbersome, especially when done manually. This is particularly problematic for patients suspected of sepsis, where time is of the essence in diagnosis.

The introduction of automated phenotypic ID and AST systems has helped to reduce time to result; and newer technologies, like the Accelerate Pheno system, which combines FISH for microorganism ID and multiplexed automated digital microscopy for susceptibility determination, provide both ease of use and faster results. Some of these platforms are compact, easy to use and robust, but they still require samples from culture before AST can be performed. In particular, blood culture is still the gold standard for detecting BSIs and AST (71). However, since culture is generally only performed at Level III and Level IV in LMICs, this means that the systems are not appro-

priate for use in primary and most secondary public healthcare facilities.

It has been generally agreed that simpler and faster methods of bacterial pathogen ID and AST are needed at all levels of the healthcare system, particularly at Level II, and nonphenotypic methods of bacterial ID and/or AST/resistance testing have helped to make this possible. As shown in this report, there are a myriad of such systems, including molecular-based testing, NGS and mass spectrometry, in particular MALDI-TOF MS, all of which are more rapid bacterial pathogen ID methods than traditional phenotypic methods.

Some of these diagnostic systems are only for bacterial ID and perform no resistance testing. In this category are several diagnostic platforms/systems that have the potential to be used in secondary, or possibly primary, care settings. These include the cobas<sup>®</sup> Liat<sup>®</sup> system, Solana<sup>®</sup> platform and revogene<sup>®</sup>, which are monoplexes, identifying one pathogen at a time, as well as the Novodiag<sup>®</sup> system, QIAstat-Dx<sup>™</sup> and Randox assays for the Vivalytic platform for which multiplex, syndromic testing panels are already available. At Level II, for example, these platforms can be useful in identifying multiple gastrointestinal and respiratory pathogens from stool and swabs. However, with the exception of the platform from T2 Biosystems, which is suited only to centralized laboratories, none of the platforms can detect priority BSIs, including *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, from whole blood, i.e., independent of culture.

There are also nonphenotypic systems that combine bacterial ID and AST/resistance testing capabilities. For example, the GeneXpert<sup>®</sup> system and FilmArray<sup>®</sup> EZ Configuration can be used in near-patient settings, including Level II facilities in LMICs, but only for assays not requiring culture samples. Alternatively, diagnostic systems can be combined in the laboratory. A common example in HICs is to combine a high-speed, completely automated bacterial ID platform, like a MALDI-TOF MS platform, with a rapid, automated AST system. However, for reasons of required infrastructure and complexity, among others, most of these combined, nonintegrated systems are appropriate for use only in Level III and, in some cases, only in Level IV settings.

Finally, there are diagnostic platforms that perform resistance testing or AST only. Some of these, including RESIST assays from Coris BioConcept, are LFIA that require no equipment. However, in many cases, a culture sample is required, which for the most part limits the use of these tests in LMICs to Level III settings and above.

There are interesting and novel diagnostic products in the pipeline targeted for use at Levels I and II.

Some of these will perform both pathogen ID and AST or resistance testing (ASTriD<sup>®</sup>, ASTar<sup>™</sup> and GeneXpert<sup>®</sup> Omni); some will only perform ID (FireflyDx<sup>™</sup>, Qvella); some will only perform AST (LabDisk, LiDia<sup>®</sup> and Astrego). Generally speaking, however, most of these platforms will process only swabs and urine, which limits the breadth of bacterial pathogens covered; some are targeting ID from whole blood (ASTriD<sup>®</sup> and LiDia<sup>®</sup>). At this point, none of these platforms has proven capabilities, especially for ID and resistance testing, from whole blood.

At the other end of the spectrum, considering diagnostic tests currently available for use at Level I and Level II, some immunochromatographic and other RDTs are available for a number of individual bacterial pathogens that do not require culture specimens. For HAIs, these include tests for *Clostridium difficile*; for CAIs, these include tests for *Helicobacter pylori*, NG, *Salmonella typhi*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Escherichia coli*. Some of these tests have not exhibited adequate performance (sensitivity/specificity), which can limit their efficacy and needs to be taken into consideration before such tests are adopted.

Of particular concern in this regard are RDTs for NG. In 2016, WHO estimated that approximately 87 million new cases of NG were diagnosed worldwide (72). Yet, many researchers have concluded that although current RDTs for NG often have specificities >90%, sensitivities are often 50% or lower, and as such, they do not perform adequately to be used as screening tests (73, 74). In addition, AMR in NG is particularly problematic. With resistance to both cephalosporins, including third-generation extended spectrum cephalosporins, as well as fluoroquinolones, NG is a multidrug-resistant pathogen. Resistance appears to be outpacing new antibiotics for NG. Although the Xpert<sup>®</sup> CT/NG assay, described earlier in this report, is FDA cleared, its cost is a limiting factor to uptake. In addition, it is targeted at Level II settings, while patients presenting with symptoms of NG generally come to primary healthcare settings for diagnosis and treatment. Improved RDTs for NG and AST are needed at Level I.

There are also a good number of RDTs available to measure the presence of certain biomarkers reflective of host response, including CRP. Assuming adequate performance, these assays can be useful at Level I, in particular, for initial screening to determine whether a patient is likely to have a bacterial or nonbacterial infection. The ability to classify infection has the potential to enhance antibiotic stewardship. Although there are currently few, if any, RDTs available for PCT, there is at least one platform, B.R.A.H.M.S PCT<sup>™</sup> direct assay, that could be used at Level II and possibly Level I.

Lastly, some interesting novel host response assays/platforms are commercially available or in the pipeline that could also be used to help make an initial determination as to whether an infection is bacterial or not for patients presenting with symptoms of infection. For example, the FebriDx<sup>®</sup> is interesting in this respect. However, none of these tests can identify bacterial pathogens nor can they determine AST. Further patient testing would be required.

What emerges from this assessment of available diagnostics to combat ABR, as well as from additional work by WHO with respect to TB, is that there are significant gaps in tests and testing platforms for use at Level I and Level II in LMICs. These include:

- inadequate near-patient testing for (i) biomarker-based, non-sputum-based detection of TB; (ii) patient triage evaluation for TB; (iii) sputum-based replacement for AFB smear microscopy; and (iv) TB drug susceptibility (see [https://www.who.int/tb/publications/tpp\\_report/en/](https://www.who.int/tb/publications/tpp_report/en/));
- little or no ability to perform simplified phenotypic bacterial ID or AST to enable definitive therapeutic decision-making at Level III, and potentially Level II, in LMICs, especially in the context of BSIs, in particular sepsis;
- inadequate near-patient testing options for ID and susceptibility testing for multidrug-resistant NG;
- Few RDTs or easy-to-use, robust diagnostic platforms for use in primary (or secondary) healthcare settings that can reliably distinguish bacterial and nonbacterial infections from accessible, minimally invasive clinical specimens (e.g., whole blood, urine, stool and nasal swabs);
- no multiplex platform suitable for Level II and/or Level I settings to detect bacterial pathogens, including BSIs, from whole blood (no culture required) with AST/resistance testing done on a separate platform or combined with AST/resistance testing on the same platform; and
- no simple, easy-to-use test/platform suitable for use in Level II and/or Level I settings for AST from whole blood or other sample matrices (urine, stool and respiratory specimens) for which culture is not required.

# Priority diagnostics for R&D

The AMR diagnostic gaps listed above suggest the following R&D priority diagnostics against AMR for primary and secondary healthcare facilities over the next 3–5 years for which consensus TPPs to stimulate product development are proposed:

- **Improved near-patient testing for TB.** Globally, a third of all TB cases are not notified, and the samples of many patients are not tested for drug susceptibility. In order to achieve the global strategy for TB prevention, care and control, new diagnostics are needed. Based on diagnostic needs expressed by the TB community, WHO has developed consensus-driven TPPs to enable POC assays capable of (i) detecting all forms of TB by identifying characteristic biomarkers or biosignatures in specimen(s) other than sputum; (ii) low-cost patient triage by first-contact healthcare providers to identify those patients who need further testing; (iii) replacing AFB smear microscopy for detecting pulmonary TB; and (iv) determining first-line regimen-based therapy via DST that can be used at the microscopy-centre level of the healthcare system.

**Suggested action:** Consensus TPPs have been developed. No further action is required.

For detail, see [https://www.who.int/tb/publications/tpp\\_report/en/](https://www.who.int/tb/publications/tpp_report/en/).

- **Simplified phenotypic ID and AST.** As indicated above, phenotypic methods of bacterial pathogen ID and AST are the gold standard. In particular, for identifying bacterial pathogens associated with BSIs, which are a common cause of morbidity and mortality worldwide, with an estimated mortality rate of 15–30% (75), blood culture is essential. Further, ABR is a particular problem for ESBL *Escherichia coli* and *Klebsiella* spp. and CRE. Yet, in LMICs, culture and AST are generally only performed at Level III and Level IV laboratory facilities. In a recently published study by Dailey and colleagues, specialists in Africa and Asia (e.g., infectious disease doctors, public health/clinical microbiologists, clinical researchers and technology experts) suggested that blood culture should be available at district hospitals (i.e., at Level II laboratories in LMICs) to support both patient management and surveillance (36). Expanding phenotypic

methods to Level II laboratories would require a simpler culture system. Dailey and colleagues have put forward a proposed TPP for a diagnostic test for such a simplified blood culture system (36). The minimal standard is a culture system for detecting the culture positivity and gram status of bacterial pathogens suitable for Level III settings. The optimal standard is a culture system for pathogen ID and AST for key resistance categories (e.g., ESBL and CRE) targeted at Level II settings. The target population is any individual presenting with fever.

**Suggested action:** Review existing TPP for a simplified blood culture system to address BSIs, in particular sepsis, for definitive therapeutic decision-making at Level II and higher facilities, and consider whether revisions and a broader consensus process are required.

- **Improved diagnostics and AST for NG.** As discussed earlier in this report, NG is an STI that results in significant morbidity and mortality. Yet, the ability to diagnose the infection, and to diagnose and distinguish it from CT effectively, is limited to molecular testing, at best available at Level II settings and above. Similarly, there is essentially no ability to perform AST, despite the fact that NG is a multidrug-resistant bacterial pathogen. In addition to being on the WHO priority pathogen list (Annex I), it is also a priority organism for AMR monitoring in GLASS as well as GARDP. WHO, FIND and GARDP have identified key TPPs to be developed with respect to NG. These comprise two TPPs: (i) a simple diagnostic test to detect CT/NG infection in symptomatic patients (minimally) and asymptomatic patients (optimally) for use at Level I, and (ii) a comprehensive test that would confirm NG infection for patients presenting with treatment failure (minimally) for use at Level II and detect both NG and CT infection and identify AST for NG (optimally) for use at Level I. The importance of such tests is critical for the effort to protect a next-generation antibiotic for NG due to be launched in 2020.

**Suggested action:** Since a TPP for a CT/NG rapid test for use in primary care settings and a second TPP for a comprehensive test that would both confirm NG infection and enable genotypic

resistance testing of NG infection are already being developed from the combined efforts of WHO, FIND and GARDP, assuming alignment with these efforts, support this work as needed, but do not undertake a separate initiative.

- Host response tests. To date, there is still not an RDT to reliably distinguish between bacterial and nonbacterial infections for use at primary healthcare settings, a test that is often referred to as the Holy Grail. There are, however, RDTs in the form of host response tests that incorporate specific host-derived biomarkers, including CRP, PCT and a few tests combining such biomarkers, that show promise in distinguishing between bacterial and nonbacterial infections. Assuming adequate performance, these tests could be an important way to triage patients presenting with fever at Level I healthcare settings. A consensus TPP for a rapid biomarker-based test, which was the result of a Delphi process, was published in 2016 (76).

Suggested action: Consider whether the existing TPP for a rapid biomarker-based test should be refined/revised. If not, bring renewed attention to the existing TPP.

- Multiplex diagnostic platform to identify bacterial pathogens and perform AST/resistance testing independent of culture. There are effectively no multiplex diagnostic platforms that can be used at Level II of the laboratory system to simultaneously identify bacterial pathogens causing BSIs from whole blood (without culture). Such a system could detect *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., MRSA and *Enterococcus*, among others, on a single assay panel. As illustrated in Annex III, there are a few multiplex systems that can identify respiratory pathogens or gastrointestinal panels from swabs (nasal or stool), but culture is required in the context of BSIs. Similarly, there are multiplex platforms that can identify a number of drug-resistant bacterial strains from nasal and stool swabs, but not from whole blood. There are a few platforms in the pipeline that are striving to perform both ID and AST/resistance testing, but they are not yet a reality.

Even if a simplified phenotypic and AST system were developed for use at district hospitals, it would still be important to have a platform suitable for near-patient testing that could identify a broad range of bacterial pathogens from whole blood, as well as from other accessible, minimally invasive clinical specimens (e.g., urine, stool and nasal swabs), independent of culture – and that ideally could perform AST/

resistance testing on the same platform. Such a system would have value at Level III as well.

Suggested action: Develop a consensus-driven TPP for a multiplex diagnostic platform suitable for use at Level II that can simultaneously identify multiple bacterial pathogens, including bacterial pathogens associated with BSIs, from whole blood and other sample matrices without culture (minimum standard), and that can both identify multiple bacterial pathogens, including bacterial pathogens causative of BSIs, and can perform AST/resistance testing from whole blood and other sample matrices (optimal standard) without culture.

- Simple AST assay from whole blood. While there are existing, and a few pipeline, phenotypic and nonphenotypic AST/resistance-only platforms commercially available, they are generally not designed for Level II settings and below, and to date all require culture samples for AST/resistance testing of BSIs. It would be useful to encourage development of such a platform that minimally could perform AST from accessible, minimally invasive clinical specimens (e.g., urine, stool and nasal swabs), but that optimally could perform AST from whole blood.

Suggested action: Develop a consensus TPP for a simple stand-alone AST device not requiring culture isolates for use following bacterial pathogen detection on a separate instrument or RDT.

## Annex I – ABR prioritization

Bacteria	Gram stain	HAI or CAI <sup>16</sup>	WHO (11)	US CDC (12)	EU (ECDC) (13)
<i>Mycobacterium tuberculosis</i> , drug resistant <sup>17</sup>	N/A	CAI/HAI	Global priority <a href="http://apps.who.int/medicinedocs/en/m/abstract/Js23298en/">http://apps.who.int/medicinedocs/en/m/abstract/Js23298en/</a>	Serious	
<i>Acinetobacter baumannii</i> , carbapenem resistant	Neg	HAI	Critical	Serious	✓
<i>Pseudomonas aeruginosa</i> , carbapenem resistant	Neg	HAI	Critical	Serious	
<i>Enterobacteriaceae</i> , carbapenem resistant/ESBL producing	Neg	HAI/CAI <i>Klebsiella pneumoniae</i> (HAI); <i>Escherichia coli</i> (HAI/CAI)	Critical	Critical/Serious	✓ ( <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> )
<i>Clostridium difficile</i>	Pos	HAI		Critical	
<i>Neisseria gonorrhoeae</i> , cephalosporin-resistant, fluoroquinolone-resistant	Neg	CAI	High	Serious	
<i>Enterococcus faecium</i> , vancomycin-resistant	Pos	HAI	High	Serious	✓
<i>Staphylococcus aureus</i> , methicillin-resistant/vancomycin-intermediate and resistant	Pos	HAI/CAI	High	Serious/Concerning	✓
<i>Helicobacter pylori</i> , clarithromycin-resistant	Neg	CAI	High		
<i>Campylobacter</i> spp., fluoroquinolone-resistant	Neg	CAI	High	Serious	
<i>Salmonella</i> spp., fluoroquinolone-resistant	Neg	CAI	High	High	
<i>Streptococcus pneumoniae</i> , penicillin-non-susceptible	Pos	CAI	Medium	Serious	
<i>Haemophilus influenzae</i> , ampicillin-resistant	Neg	CAI	Medium		
<i>Shigella</i> spp., fluoroquinolone-resistant	Neg	CAI	Medium	Serious	

<sup>16</sup> Indicates the resistant pathogen is most often nosocomial or community acquired, as the case may be. Some pathogens are found commonly in both settings.

<sup>17</sup> In the 2017 WHO report *Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis*, MTB was listed as a global priority for R&D ([https://www.who.int/medicines/areas/rational\\_use/prioritization-of-pathogens/en/](https://www.who.int/medicines/areas/rational_use/prioritization-of-pathogens/en/), accessed 19 June 2019).

# Appendix II – Diagnostic platforms for all levels of healthcare system

Diagnostic platforms to combat ABR suitable for all levels of the healthcare system				
Phenotypic methods				
Automated Gram staining				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
bioMérieux	PREVI® COLOR GRAM	I/II	Automated Gram staining via spray technology	Pan-bacteria
ALL.DIAG - Biosynex	MULTISTAINER®	I/II	Gram stain and fast staining	Pan-bacteria
ELITechGroup Solutions	Aerospray® Gram series 2	I/II	Gram stain	Pan-bacteria
Hardy Diagnostics	QuickSlide™ GramPRO 1™	I/II	Gram stain	Pan-bacteria
Automated specimen processing and inoculation of media				
BD	BD™ Inova automated microbiology specimen processor	III/IV	Processing of liquid specimens and automated streaking	Pan-bacteria
BD	BD Kiestra™ InoqulA+™	III/IV	Processing of liquid and nonliquid specimens	Pan-bacteria
bioMérieux	PREVI® Isola	III/IV	Processing of liquid and nonliquid (e.g., swabs) specimens	Pan-bacteria
Beckman Coulter	Copan WASP® DT: Walk-Away Specimen Processor	III/IV	Accommodates most specimen types, including swabs, urine, faeces, sputum, body fluids and pre-enrichment broths	Pan-bacteria
Phenotypic bacterial ID				
Automated culture systems				
BD	BD BACTEC™ FX	III/IV	Automated culture using fluorescent sensing of CO <sub>2</sub> production	Pan-bacteria, fungi, yeast
bioMérieux	BACT/ALERT® 3D	III/IV	Automated culture using colorimetric sensing of CO <sub>2</sub> production	Pan-bacteria, fungi, yeast
bioMérieux	BACT/ALERT® VIRTUO®	III/IV	Automated culture using colorimetric sensing of CO <sub>2</sub> production	Pan-bacteria, fungi, yeast
Thermo Fisher Scientific	VersaTREK™	III/IV	Automated culture that senses all microorganism gas production, including CO <sub>2</sub>	Pan-bacteria, fungi, yeast and mycobacteria

(Appendix II, continued)

Manual biochemical bacterial ID systems				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
bioMérieux	API®	Level III/IV	Detects enzymatic activity or fermentation of carbohydrates	<p>API gram-negative ID:                      API 20E – <i>Enterobacteriaceae</i> and other nonfastidious gram-negative bacteria                      API Rapid 20E – <i>Enterobacteriaceae</i>                      API 20NE – gram-negative non-<i>Enterobacteriaceae</i>                      API NH – <i>Neisseria/Haemophilus</i>                      API gram-positive ID:                      API Staph – staphylococci and micrococci                      RAPIDEC® Staph – commonly occurring staphylococci                      API 20 Strep – streptococci and enterococci                      API anaerobe ID:                      API 20A – anaerobes                      Rapid ID 32A – anaerobes</p>
BD	BBL™ Crystal™	Level III/IV	Utilizes miniaturized fluorogen and/or chromogen-linked substrates to detect enzymes that bacteria use to metabolize a variety of substrates	<p>Enteric/nonfermenter (E/NF): clinically significant aerobic gram-negative <i>Enterobacteriaceae</i> isolates and nonfermenting gram-negative rods                      Rapid stool/enteric (RS/E): clinically significant aerobic gram-negative bacteria of the <i>Enterobacteriaceae</i> family as well as most pathogens isolated from stool specimens  <i>Neisseria/Haemophilus</i> (N/H): <i>Neisseria</i>, <i>Haemophilus</i> and other fastidious bacteria                      Gram-positive ID system: both gram-positive cocci and bacilli                      Rapid gram-positive ID kit: gram-positive bacterial isolated from clinical specimens                      Anaerobe ID kit: clinically significant anaerobic organisms</p>
Thermo Fisher Scientific	RapID™ systems	Level III/IV	Detects enzymatic activity	<p>RapID™ ONE system: over 70 medically important, oxidase-negative, gram-negative bacilli                      RapID™ ANA II: over 90 clinically important anaerobes                      RapID™ NH system: 30 taxa, including <i>Neisseria</i>, <i>Moraxella</i>, <i>Haemophilus</i> and related microorganisms                      RapID™ NF PLUS system: over 70 clinically important oxidase-positive, gram-negative bacilli, including <i>Vibrio</i> spp.                      RapID™ STAPH PLUS system: 40 different staphylococci and related genera                      RapID™ STR system: streptococci and related genera                      RapID™ SS/u system: commonly isolated urinary tract pathogens in 2 hours</p>

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Thermo Fisher Scientific	Oxoid™ Microbact™ biochemical systems	Level III/IV	Measures pH changes in various substrates and substrate utilization tests	Microbact™ GNB kit: <i>Enterobacteriaceae</i> and common miscellaneous gram-negative bacilli Oxoid™ Microbact™ Staphylococcal 12S kit: staphylococci, including <i>Staphylococcus aureus</i> and CNS
<b>Automated biochemical bacterial ID systems</b>				
Biolog, Inc.	Biolog microbial ID systems	Level III/IV	Uses oxidation-reduction chemistry	Broad range of gram-positive and gram-negative bacteria
MIDI, Inc.	Sherlock™ microbial ID system	Level III/IV	Uses GC analysis of extracted FAME	Broad range of gram-positive and gram-negative bacteria
BacterioScan	216Dx UTI system	Level III/IV (culture)	FLLS	Qualitative diagnosis of bacteriuria (UTIs)
<b>Nonphenotypic methods of bacterial ID</b>				
<b>Immunoassays</b>				
Thermo Fisher Scientific	Oxoid™ PBP2' latex agglutination test	Level III (culture)	Latex slide agglutination test	MRSA and CNS from culture
Abbott	Clearview Exact PBP2a test	Level III (culture)	Immunochromatographic test	MRSA from culture
Thermo Fisher Scientific	RAPID™ Hp StAR™	Level I	Immunochromatographic test	<i>Helicobacter pylori</i> from stool
Otsuka Pharmaceutical Co., Ltd.	RAPIRUN® <i>H. pylori</i> antibody detection kit	Level I	Immunochromatographic test	<i>Helicobacter pylori</i> from stool
Meridian Bioscience, Inc.	ImmunoCard STAT!® CAMPY	Level I	Immunochromatographic test	<i>Campylobacter</i> antigens ( <i>C. jejuni</i> and <i>C. coli</i> ) from stool
Abbott	<i>C. DIFF QUIK CHEK COMPLETE</i> ®	Level I	Immunochromatographic test	<i>Clostridium difficile</i> (TcdA and TcdB) from stool
Thermo Fisher Scientific	Xpect® <i>C. difficile</i> Toxin A/B test	Level I	Immunochromatographic test	<i>Clostridium difficile</i> (TcdA and TcdB) from stool
Meridian Bioscience, Inc.	ImmunoCard® Toxins A&B	Level I	Horizontal flow enzyme immunoassay	<i>Clostridium difficile</i> (TcdA and TcdB) from stool
Thermo Fisher Scientific/ BioStar	BioStar® OIA GC	Level I	Optical immunoassay	NG from female endocervical swabs and male urine specimens
Abbott	BinaxNOW® <i>Streptococcus pneumoniae</i>	Level I	Immunochromatographic assay	<i>Streptococcus pneumoniae</i> from urine
BIOSYNEX	BIOSYNEX <i>S. pneumoniae</i>	Level I	Immunochromatographic test	<i>Streptococcus pneumoniae</i> from urine and CSF
Malaysian Biodiagnostic Research	Typhidot®	Level II, possibly Level I	ELISA	<i>Salmonella typhi</i> in serum
IDL Biotech	TUBEX®	Level I	IMBI	<i>Salmonella typhi</i> in serum
Thermo Fisher Scientific	Wellcogen™ Haemophilus influenzae b Rapid latex agglutination test	Level I	Latex diagnostic test	<i>Haemophilus influenzae</i> type b from CSF, serum, urine or blood cultures
Coris BioConcept	O157 Coli-Strip	Level III (broth dilution)	Immunochromatographic test	<i>Escherichia coli</i> in stool (after broth enrichment)

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Meridian Bioscience, Inc.	ImmunoCard STAT!® E. coli O157 Plus	Level I	Horizontal-flow enzyme immunoassay	<i>Escherichia coli</i> in stool or culture
<b>Molecular methods</b>				
<b>Hybridization methods</b>				
OpGen	AdvanDx PNA FISH® QuickFISH®	Level III/IV	FISH with PNA probes	AdvanDx PNA FISH®: <i>Staphylococcus aureus</i> /CNS, <i>Enterococcus faecalis</i> /OE (other enterococci), gram-negative organisms and <i>Candida</i> QuickFISH®: <i>Staphylococcus</i> , <i>Enterococcus</i> , gram-negative organisms and <i>Candida</i> <i>mecA</i> XpressFISH: <i>mecA</i> mRNA from MRSA
<b>Amplification methods – integrated systems</b>				
Abbott	Abbott m2000 System	Level III/IV	Multiplex real-time PCR followed by fluorescent probe-based detection	CT/NG
Roche	cobas® 6800/ cobas® 8800 systems	Level III/IV	Real-time PCR	CT and/or NG
	cobas® 4800 system	Level III/IV	Real-time PCR and nucleic acid hybridization	CT/NG, <i>Clostridium difficile</i> , MRSA
	cobas® Liat® system	Level II/ possibly Level I if cold chain	Real-time PCR	<i>Clostridium difficile</i>
Hologic	Hologic Panther® Aptima Combo 2 system	Level III/IV	TMA followed by fluorescent probe-based detection	CT and/or NG
	Hologic Panther Fusion® system	Level III/IV	TMA and PCR	MRSA
BD	BD ProbeTec™ ET system	Level III/IV	ET	CT and GC DNA
	BD Viper™ system	Level III/IV	SDA and ET	NB/CT
	BD MAX™ system	Level III/IV	Real-time PCR	CT/GC/TV, <i>Clostridium difficile</i> , enteric bacterial panel, extended enteric bacterial panel, MRSA, StaphSR (for surveillance)
Vela Diagnostics	Great Basin analyser system	Level III/IV	Hot-start PCR and hybridization probes	Stool panel: <i>Campylobacter</i> ( <i>C. coli</i> and <i>C. jejuni</i> ), <i>Salmonella</i> , <i>stx1</i> , <i>stx2</i> , <i>Escherichia coli</i> serotype O157 and <i>Shigella</i> Blood culture panel: <i>Staphylococcus aureus</i> , <i>S. lugdunensis</i> , various <i>Staphylococcus</i> spp. and <i>mecA</i> gene <i>Clostridium difficile</i> , Shiga toxin, <i>Bordetella pertussis</i> , Group B Strep
Meridian Bioscience, Inc.	Alethia™ platform	Level III/IV	iNAAT using LAMP	<i>Clostridium difficile</i> , Group A Strep, Group B Strep

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Quidel Corporation	Solana® platform	Level II	HDA	<i>Clostridium difficile</i> , Group A Streps, Group B Strep
	AmpliVue® platform	Level II	HDA and lateral flow	<i>Clostridium difficile</i> , Group A Strep, Group B Strep
Siemens	VERSANT® kPCR molecular system	Level III/IV	Real-time PCR	CT, NG
Luminex	ARIES® and ARIES® M1 systems	Level II	Real-time PCR	<i>Clostridium difficile</i> , Group A Strep
Mobidiag	Novodiag® platform	Level II	Real-time qPCR and microarray	<i>Clostridium difficile</i> , Bacterial GE+ assay
Randox Laboratories/ Bosch Healthcare Solutions	Respiratory Multiplex Array II/Vivalytic analyser	Level III/IV	Multiplex PCR and biochip array hybridization	Respiratory multiplex array, STI array (CT, NG and others)
QIAGEN NV	QIAstat-Dx™	Level II	Real-time PCR with optical sensor	Respiratory panel, gastrointestinal panel, sepsis panel
GenePOC	revogene®	Level III	Fluorescence-based real-time PCR	<i>Clostridium difficile</i> , Group B Strep
T2 Biosystems	T2Dx® instrument	Level IV	T2 magnetic resonance	Bacteria panel ( <i>Enterococcus faecium</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> ); <i>Candida</i> panel
Abacus Diagnostica	GenomEra™ CDX system	Level III/IV	Fluorescent lanthanide levels, thermal cycling, RT PCR	<i>Clostridium difficile</i> , MRSA SA SC (with <i>mecA</i> and <i>mecC</i> ), MRSA/SA Multi Swab and <i>Streptococcus pneumoniae</i>
GENOMICA S.A.U.	CLART® technology	Level III/IV	Genetic amplification and visualization	CLART® EnteroBac: <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Escherichia coli</i> enteropathogenic, <i>Campylobacter</i> spp. and <i>Clostridium difficile</i> CLART® SeptiBac: <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp. (including methicillin-resistance marker <i>mecA</i> ), <i>Enterococcus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Salmonella enterica</i> , <i>Enterobacter</i> spp., <i>Haemophilus</i> spp., <i>Acinetobacter baumannii</i> and <i>Pseudomonas</i>
<b>Amplification systems – not integrated</b>				
Siemens	Fast Track Diagnostics	Level III/IV	Real-time PCR	Respiratory infections: e.g., <i>Haemophilus influenzae</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> and <i>Streptococcus pneumoniae</i> Gastroenteritis: <i>Campylobacter coli/jejuni/lari</i> , <i>Clostridium difficile</i> , <i>Salmonella</i> spp. and <i>Shigella</i> spp. STIs: NG
Molzym Molecular Diagnostics	SepsiTest™-UMD	Level III/IV	Universal PCR, Sanger sequencing	200 genera of bacteria

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Momentum Bioscience Ltd.	Cognitor® Minus	Level III/IV (requires culture)	ETGA® to detect microbial DNA polymerase activity	Confirms negative blood BSI result Pipeline: assay for detection, ID and AST of positive blood cultures
Luminex	xTAG® technology	Level III/IV	RT-PCR	Gastrointestinal panel: <i>Campylobacter jejuni</i> , <i>C. coli</i> and <i>C. lari</i> only, <i>tcdA</i> and <i>tcdB</i> , <i>Escherichia coli</i> , <i>Salmonella</i> and <i>Shigella</i>
<b>Molecular methods – pipeline</b>				
Lucira Health	Disposable (no name)	Level I/II	RPA and LAMP	CT and NG, <i>Streptococcus</i> (swabs)
Qvella	FAST-ID™	Level II	PCR	Sepsis pathogens (whole blood)
NanoDetection Technology	No name	Level II (?)/Level III	Biochip	MRSA screening, MRSA/MSSA dual screening, sepsis, influenza A and B, HCV, STIs and dengue
Biospectrix	3iDx	Level II/Level I (?)	Microfluidics and nanotechnology	BSIs (whole blood)
<b>Sequencing methods</b>				
Illumina, Inc.	MiSeq™ Dx instrument	Level IV	NGS	LDTs; no bacterial pathogen kits
Thermo Fisher Scientific	Ion PGM™ DX system	Level IV	NGS	RUO; no commercialized IVDs
<b>MS</b>				
Beckman Coulter	Bruker MALDI Biotyper® system	Level IV	MS	424 clinically relevant bacteria and yeast species
bioMérieux	VITEK® MS	Level IV	MS	<i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> and <i>Shigella</i> (which are both characterized as <i>E. coli</i> ), <i>Klebsiella pneumoniae</i> , <i>Clostridium difficile</i> , NG (for which a confirmatory test is recommended), <i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Campylobacter</i> spp., <i>Salmonellae</i> (for which a confirmatory test is recommended), <i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> and MTB complex – among others
<b>Phenotypic methods of ID and AST</b>				
<b>Semi-automated classical phenotypic methods</b>				
MERLIN Diagnostika GmbH	MICRONAUT ID and AST (semi-automated)	Level IV	Phenotypic biochemical methods and photometry	<i>Enterobacteriaceae</i> and other gram-negative bacteria; nonfermenting gram-negative and some glucose-fermenting bacteria; <i>Staphylococcus</i> , among others

(Appendix II, continued)

Automated classical phenotypic methods				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
bioMérieux	VITEK® 2 system	Level III/IV	Automated phenotypic biochemical methods	Gram-negative fermenting and nonfermenting bacilli: 76 antimicrobials and ESBL  Staphylococci and/or enterococci: 55 antimicrobials, four high-level aminoglycoside screens and inducible ICR test  Streptococci: 14 antimicrobials and ICR test and gentamicin synergy <i>Streptococcus pneumoniae</i> : 23 antimicrobials and yeasts – six antifungals
BD	BD Phoenix™ automated microbiology system	Level III/IV	Broth-based microdilution method	Gram-positive bacteria (including genera <i>Staphylococcus</i> , <i>Streptococcus</i> and <i>Enterococcus</i> ) and gram-negative bacteria (15 different genera, including <i>Acinetobacter</i> , <i>Enterobacter</i> , <i>Pseudomonas</i> , <i>Salmonella</i> and <i>Shigella</i> )
Beckman Coulter	MicroScan systems	Level III/IV	Automated phenotypic biochemical methods	Gram-positive ( <i>Staphylococcus</i> and related genera and <i>Streptococcaceae</i> ) and gram-negative glucose fermenting as well as glucose nonfermenting bacteria
Thermo Fisher Scientific	Sensititre™ ARIS™ 2X	Level III/IV	Automated phenotypic biochemical methods using fluorescence measurement	Nonfastidious gram-negative isolates ( <i>Enterobacteriaceae</i> , <i>Pseudomonas aeruginosa</i> and other non- <i>Enterobacteriaceae</i> ) and of nonfastidious gram-positive isolates ( <i>Staphylococcus</i> spp., <i>Enterococcus</i> spp. and beta-haemolytic streptococci other than <i>S. pneumoniae</i> ); additional testing capabilities are for yeasts ( <i>Candida</i> spp.) and MTB
Imaging-based ID/AST or AST only				
Accelerate Diagnostics	Accelerate Pheno™ system	Level III/IV	FISH probes targeting organism-specific rRNA and quantitative morphokinetic cellular analysis using time-lapse imaging for AST	BSIs: Gram-positive bacteria (CNS, <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus lugdunensis</i> and <i>Streptococcus</i> spp.); gram-negative bacteria ( <i>Acinetobacter baumannii</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Pseudomonas aeruginosa</i> and <i>Serratia marcescens</i> ); <i>Candida</i> species ( <i>C. albicans</i> and <i>C. glabrata</i> )
BioSense Solutions ApS	oCelloScope	Level III/IV	Automated microscopy using digital time-lapse technology	BSIs: ID and AST NOT COMMERCIALY AVAILABLE
Alifax SPA	Sidecar, Alfred 60AST and HB&L Systems	Level III/IV	Bacterial culture in real time using light-scattering technology	Urine screening (culture); susceptibility test in urine; susceptibility test in blood culture; RAA; human biological liquids bacterial culture  Pipeline: multidrug-resistant organisms (MRSA, ESBL/AmpC, carbapenem and VRE screening kit)

(Appendix II, continued)

Nonimaging AST only				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Affinity Biosensors	LifeScale®	Level III/IV	MEMS	AST for gram-negative bacteria; RUO
bioMérieux	RAPIDEC® CARBA NP	Level III/IV	Colorimetric assay from suspensions of isolates on agar plates	Detection of carbapenemase enzymes in <i>Enterobacteriaceae</i> and <i>Pseudomonas aeruginosa</i> colonies
Rosco Diagnostica	Rapid CARB Blue kit	Level III/IV	Colorimetric assay from suspensions of isolates on agar plates	Detection of carbapenemase enzymes in <i>Acinetobacter</i> spp., <i>Enterobacteriaceae</i> and <i>Pseudomonas aeruginosa</i> colonies; Rapid ESBL Screen kit; Neo-Rapid CARB kit
Pipeline AST technologies				
QuantaMatrix	dRAST™	Level III/IV (culture)	MAC technology	Detection of SIRS
Gradientech	QuickMIC™	Level III/IV (culture)	Microfluidics	Panels for gram-negative and gram-positive bacteria will be available; universal AST solution
Astrego	Captiver™ system	Level II/Level I (?)	Microfluidics and imaging	AST for UTIs
Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance				
Molecular platforms for identifying and characterizing bacterial resistance from blood culture				
Nanosphere/Luminex	Verigene®	Level III/IV	Target DNA hybridization to microarray-based oligonucleotides with visualization based on gold nanoparticle oligonucleotide probes	Gram-positive blood culture nucleic acid test and gram-negative blood culture nucleic acid test for ID and resistance; respiratory panel; enterics panel; <i>Clostridium difficile</i>
bioMérieux	BioFire® FilmArray®	Level III/IV; Level II for panels other than BCID and pneumonia (?)	Nested PCR and real-time PCR; detection using fluorescent intercalation dye within separate array module (1 target per well within array)	Multiple panels: blood culture ID, gastrointestinal, respiratory, meningitis/encephalitis, pneumonia
iCubate, Inc.	iC-system™	Level III/IV	PCR and microarray hybridization	Gram-positive bacteria panel: <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>Streptococcus pneumoniae</i> , <i>Enterococcus faecalis</i> and <i>E. faecium</i> Resistance markers: <i>mecA</i> resistance as well as <i>vanA</i> and <i>vanB</i>  Pipeline: gram-negative bacteria ( <i>Acinetobacter baumannii</i> complex, <i>Enterobacter cloacae</i> complex, <i>Escherichia coli</i> , <i>Klebsiella oxytoca</i> , <i>K. pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus</i> spp. and <i>Serratia marcescens</i> ); <i>Mycobacterium</i> , gastrointestinal and respiratory tests

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Master Diagnóstica	Sepsis Flow Chip	Level III/IV	Multiplex PCR using biotinylated primers followed by reverse dot-blot hybridization	40 bloodstream pathogens in the same assay, including gram-positive and gram-negative bacteria as well as fungi, and 20 antibiotic resistance genes, including MRSA, <i>mecA</i> , <i>vanA</i> , <i>vanB</i> , ESBL and carbapenems
Curetis GmbH	Unyvero™ system	Level III/IV	Multiplex PCR followed by array hybridization	<p>BSI: 36 gram-positive and gram-negative analytes covering more than 50 pathogens; and 16 antibiotic resistance gene markers, including <i>mecC</i> (LGA251), <i>vanA</i>, <i>vanB</i>, CTX-M (<i>bla</i><sub>CTX-M</sub>), KPC (<i>bla</i><sub>KPC</sub>), IMP (<i>bla</i><sub>IMP</sub>), NDM (<i>bla</i><sub>NDM</sub>), OXA-23 (<i>bla</i><sub>OXA-23</sub>), OXA-24/40 (<i>bla</i><sub>OXA-24/40</sub>), OXA-48 (<i>bla</i><sub>OXA-48</sub>), OXA-58 (<i>bla</i><sub>OXA-58</sub>) and VIM (<i>bla</i><sub>VIM</sub>)</p> <p>LRT application: 19 bacteria and fungi, including <i>Acinetobacter</i> spp., <i>Escherichia coli</i>, <i>Haemophilus influenzae</i>, <i>Klebsiella pneumoniae</i>, <i>Pseudomonas aeruginosa</i>, <i>Staphylococcus aureus</i> and <i>Streptococcus pneumoniae</i>; and resistance markers <i>mecA</i>, CTX-M (<i>bla</i><sub>CTX-M</sub>), KPC (<i>bla</i><sub>KPC</sub>), NDM (<i>bla</i><sub>NDM</sub>), OXA-23 (<i>bla</i><sub>OXA-23</sub>), OXA-24/40 (<i>bla</i><sub>OXA-24/40</sub>), OXA-48 (<i>bla</i><sub>OXA-48</sub>) and VIM (<i>bla</i><sub>VIM</sub>)</p> <p>UTI panel: 88 pathogens and 15 resistance markers</p>
Cepheid	GeneXpert® system	Level III/IV; Level II for panels not requiring culture	Real-time PCR and fluorogenic target-specific hybridization	<p>BSI: MRSA/SA, MRSA/SA SSTI, MRSA Nasal Complete and MRSA NxG from swabs</p> <p>Bacterial resistance: <i>vanA</i> assay and Carba-R assay</p> <p><i>Clostridium difficile</i> assays; MTB/RIF assay; and CT/NG assay</p>
Hain Lifescience	GenoType assays and FluoroType® system	Level III/IV	LATE-PCR with fluorescence "lights on/lights off" probes	<p>BSI: Gram-negative – <i>Escherichia coli</i>, <i>Enterobacter</i> species (<i>E. aerogenes</i>, <i>E. cloacae</i> and <i>E. sakazakii</i>), <i>Klebsiella pneumoniae</i>, <i>Pseudomonas aeruginosa</i> and <i>Acinetobacter baumannii</i> – and gram-positive (<i>Staphylococcus aureus</i>, <i>Enterococcus faecium</i> and <i>Streptococcus pneumoniae</i>, along with the ID of <i>mecA</i> and <i>van</i> genes) test kits</p> <p>Other kits: MRSA, <i>Staphylococcus aureus</i>, <i>Helicobacter pylori</i>, <i>Clostridium difficile</i>, <i>Enterococcus</i> and assays for MTB complex</p>

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
GenMark Diagnostics	ePlex® system	Level III/IV	Competitive nucleic acid hybridization and electrochemical detection of nucleic acids on a microchip	BSI: Gram-positive panel – <i>Enterococcus</i> , <i>E. faecium</i> , <i>Staphylococcus</i> and <i>S. aureus</i> , as well as resistance markers <i>mecA</i> , <i>mecC</i> , <i>vanA</i> and <i>vanB</i> . Gram-negative panel – <i>Acinetobacter baumannii</i> , <i>Enterobacter</i> (non- <i>cloacae</i> complex and <i>cloacae</i> complex), <i>Haemophilus influenzae</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> and <i>Salmonella</i> , as well as resistance markers including CPE ( <i>bla<sub>KPC</sub></i> , <i>bla<sub>VIM</sub></i> , <i>bla<sub>NDM</sub></i> , <i>bla<sub>IMP</sub></i> and <i>bla<sub>OXA</sub></i> ) and ESBL ( <i>bla<sub>CTX-M</sub></i> ) Blood culture assay for fungus
<b>Molecular platforms for ID and resistance from whole blood and other matrices</b>				
Seegene	Seeplex™, Allplex™, Anyplex™, and MagicPlex™	Level III/IV	Real-time PCR on validated systems not provided by Seegene	MagicPlex™ Sepsis panel: ID of 27 gram-positive and gram-negative pathogens at the species level from whole blood, including <i>Streptococcus pneumoniae</i> , <i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Acinetobacter baumannii</i> , <i>Salmonella typhi</i> , <i>Klebsiella pneumoniae</i> and <i>Escherichia coli</i> . Resistance markers include <i>vanA</i> , <i>vanB</i> and <i>mecA</i> Variety of Seeplex™, Anyplex™ and Allplex™ assays, including drug-resistance tests
ELITechGroup Solutions	ELITe MGB® kits and panels	Level II (?), Level III/IV	Real-time PCR on ELITe InGenius® platform	ID: <i>Clostridium difficile</i> kit; STI panel (CT/NG and MG); <i>Staphylococcus aureus</i> and MRSA Resistance: carbapenem resistance kit; ESBL gene kit; and colistin resistance kit
Mobidiag	Amplidiag® system	Level III/IV	Real-time PCR on nonintegrated instruments	ID and resistance testing for gastrointestinal bacteria (parasites and viruses) and antibiotic resistance Bacterial gastroenteritis panel ( <i>Campylobacter</i> , <i>Salmonella</i> and <i>Shigella</i> /EIEC, among others); <i>C. difficile</i> +027 panel; <i>H. pylori</i> +ClariR ( <i>Helicobacter pylori</i> and clarithromycin resistance); CarbaR+VRE ( <i>bla<sub>KPC</sub></i> , <i>bla<sub>NDM</sub></i> , <i>bla<sub>VIM</sub></i> , <i>bla<sub>IMP</sub></i> , <i>bla<sub>OXA-48</sub></i> , <i>bla<sub>OXA-181</sub></i> , <i>Acinetobacter bla<sub>OXA</sub></i> , <i>vanA</i> and <i>vanB</i> ); CarbaR + MCR ( <i>bla<sub>KPC</sub></i> , <i>bla<sub>NDM</sub></i> , <i>bla<sub>VIM</sub></i> , <i>bla<sub>IMP</sub></i> , <i>bla<sub>OXA-48</sub></i> , <i>bla<sub>OXA-181</sub></i> , <i>Acinetobacter bla<sub>OXA</sub></i> , <i>mcr</i> and <i>bla<sub>GES</sub></i> )
QIAGEN N.V.	QIASymphony® SP/AS	Level III/IV	qPCR and real-time qPCR amplification of nucleic acids; detection using fluorescence-labelled oligonucleotide probes	Kits for <i>Clostridium difficile</i> ; CT/NG; <i>vanR</i> for detecting <i>vanA</i> and <i>vanB</i> vancomycin-resistance genes

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
GeneFluidics	UtiMax™/BsiMax®	Level II (?); Level III/IV	Electrochemical-based sandwich hybridization method to measure bacterial 16S rRNA	UtiMax™: detection and AST of uropathogens from urine Pipeline: BsiMax®: (detection of BSIs from whole blood); ID panel ( <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter</i> spp., MRSA, MSSA and <i>Enterococcus</i> , among others); AST antibiotics (gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam)
<b>Nonphenotypic methods of detecting antibiotic resistance</b>				
<b>Molecular methods of detecting antibiotic resistance</b>				
Check-Points	Check-Direct and Check-MDR assays	Level III/IV	Assays only for use on BD MAX™ real-time PCR system and other systems	For BD MAX™: CPE kit (carbapenemases – <i>bka</i> <sub>KPC</sub> , <i>bla</i> <sub>OXA-48</sub> , <i>bla</i> <sub>VIM</sub> and <i>bla</i> <sub>NDM</sub> ); CPO kit for BD MAX™ (carbapenemase genes – <i>bka</i> <sub>KPC</sub> , <i>bla</i> <sub>OXA-48</sub> , <i>bla</i> <sub>VIM</sub> , <i>bla</i> <sub>NDM</sub> and <i>bla</i> <sub>IMP</sub> ); ESBL screen kit (ESBL genes: <i>bla</i> <sub>CTX-M-1</sub> group, <i>bla</i> <sub>CTX-M-2</sub> group, <i>bla</i> <sub>CTX-M-9</sub> group, <i>bla</i> <sub>SHV</sub> ESBL) For use on other instruments: CPE kit for carbapenemases ( <i>bka</i> <sub>KPC</sub> , <i>bla</i> <sub>OXA-48</sub> , <i>bla</i> <sub>VIM</sub> and <i>bla</i> <sub>NDM</sub> ) Microarray assays for epidemiology and confirmation
AmplexDiagnostics GmbH	eazyplex®	Level II (assays not requiring culture), Level III/IV	iNAAT on Genie® II or Gene® III platform	SuperBug® complete A: carbapenemases – KPC ( <i>bla</i> <sub>KPC</sub> ), NDM ( <i>bla</i> <sub>NDM</sub> ), VIM ( <i>bla</i> <sub>VIM</sub> ) and OXA ( <i>bla</i> <sub>OXA-23,40,48,58</sub> ) SuperBug® complete B: carbapenemases – KPC ( <i>bla</i> <sub>KPC</sub> ), NDM ( <i>bla</i> <sub>NDM</sub> ), VIM ( <i>bla</i> <sub>VIM</sub> ) and OXA ( <i>bla</i> <sub>OXA-23,40,48,181</sub> ) SuperBug® CRE: KPC ( <i>bla</i> <sub>KPC</sub> ), NDM ( <i>bla</i> <sub>NDM</sub> ), VIM ( <i>bla</i> <sub>VIM</sub> ) and OXA ( <i>bla</i> <sub>OXA48,181</sub> ) SuperBug® mcr-1: confirmation of <i>mcr-1</i> gene (colistin resistance) SuperBug® AmpC: confirmation of AmpC beta-lactamases eazyplex® VRE: <i>vanA</i> and <i>vanB</i> eazyplex® VRE basic: <i>vanA</i> or <i>vanB</i> from positive blood culture
Bruker	Carbaplex® IVD PCR	Level III/IV	Multiplex real-time PCR on validated platforms not from Bruker	CPE: carbapenemases – KPC ( <i>bla</i> <sub>KPC</sub> ), NDM ( <i>bla</i> <sub>NDM</sub> ), VIM ( <i>bla</i> <sub>VIM</sub> ), OXA ( <i>bla</i> <sub>OXA48,181</sub> ) and IMP ( <i>bla</i> <sub>IMP</sub> )
AUTOIMMUN DIAGNOSTIKA	Antibiotic resistance line probe assays	Level III/IV	LPA on end-point PCR equipment not provided by AUTOIMMUN	AID ESBL: ESBL genes ( <i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>CTX-M</sub> , <i>bla</i> <sub>SHV</sub> and <i>bla</i> <sub>KPC</sub> ) AID Carbapenemase: 13 different carbapenem resistances, including <i>bla</i> <sub>KPC</sub> , <i>bla</i> <sub>VIM</sub> , <i>bla</i> <sub>NDM</sub> and <i>bla</i> <sub>OXA-48</sub> AID MRSA combi: <i>mecA</i> and <i>mec</i> , and differentiation of <i>Staphylococcus aureus</i> and CNS

(Appendix II, continued)

Immunoassays and other methods for detecting antibacterial resistance				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
NG Biotech	Antimicrobial lateral flow immunoassays	Level III/IV (culture required)	LFIA using colloidal gold-labelled antibodies; no equipment required	Detection/confirmation of resistance genes from culture: CARBA 5 – carbapenemases: NDM ( <i>bla<sub>NDM</sub></i> ), KPC ( <i>bla<sub>KPC</sub></i> ), NDM ( <i>bla<sub>NDM</sub></i> ), VIM ( <i>bla<sub>VIM</sub></i> ) and OXA-48 ( <i>bla<sub>OXA-48</sub></i> ) CTX-M – ESBL ( <i>bla<sub>CTX-M</sub></i> ) MCR-1 – <i>mcr-1</i> gene
Coris BioConcept	RESIST assays	Level III/IV (where culture required); Level II/I for identifying pathogens from stool on LFIA	LFIA; no equipment required Pipeline: TRAPIST V6: microfluidic chip technology using both molecular and immunoassay modalities	RESIST assays: carbapenemases – OXA-48 K-SeT for detecting <i>bla<sub>OXA-48</sub></i> ; KPC K-SeT for detecting <i>bla<sub>KPC</sub></i> only; O.K.N. K-SeT for detecting <i>bla<sub>OXA-48-like</sub></i> , <i>bla<sub>KPC</sub></i> and <i>bla<sub>NDM</sub></i> ; O.O.K. K-SeT for detecting <i>bla<sub>OXA-48-like</sub></i> , <i>bla<sub>KPC</sub></i> and <i>bla<sub>OXA-163</sub></i> ; and O.K.N.V. for detecting <i>bla<sub>OXA-48-like</sub></i> , <i>bla<sub>KPC</sub></i> , <i>bla<sub>NDM</sub></i> and <i>bla<sub>VIM</sub></i> Assays for bacterial pathogen ID from stool: <i>Helicobacter pylori</i> , <i>Escherichia coli</i> and <i>Clostridium difficile</i> TRAPIST pipeline: sepsis panels – multiplex gram-positive cassette (e.g., <i>Staphylococcus aureus</i> ); multiplex gram-negative cassette (e.g., <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i> ); and resistance markers (e.g., <i>vanA</i> , <i>vanB</i> , <i>mecA</i> and <i>mecC</i> for gram-positive bacteria)
Pipeline molecular technologies for identifying pathogens and/or detecting antibiotic resistance				
SpinDiag	LabDisk	Level II/Level I (?)	Nested PCR; microfluidics with disc-based test cartridge	Test 25 drug-resistant bacterial strains. Prototype tests: MRSA from nasal swabs and VRE from rectal swabs. Additional assays planned for RTIs and STIs.
ExcitePCR (Positive ID)	FireflyDx™	Level II/Level I (?)	Real-time PCR and single-use disposable cartridges	Plan to process whole blood, nasal swabs and urine samples, among others. Have tested MRSA, MSSA and <i>Clostridium difficile</i> on prototype system. Resistance assays planned.
DxNA, LLC	GeneSTAT® analyser system	Level III/IV	Real-time PCR	Pipeline test: identify and differentiate <i>Staphylococcus aureus</i> and CNS
Q-linea	ASTar™, ASTrID®	Level IV (when combined with MALDI-TOF MS); possibly Level III for ASTrID®	ASTar™: automated microdilution for AST ASTrID®: padlock probe technology and amplification via RCA and subsequent C <sub>2</sub> CA; RCA products labelled with fluorescence probes and detected on a microarray	ASTar™: phenotypic AST from culture after ID (e.g., via MALDI-TOF MS). Currently in prototype form. ASTrID®: will enable ID of more than 50 sepsis pathogens and select resistance genes as well as phenotypic AST in 10 hours directly from whole blood.
Specific Diagnostics	Reveal AST™/ID	Level III/IV (culture required)	SMS arrays that detect low concentrations of VOCs	Bacterial pathogen ID from blood culture; AST, including phenotypic MICs, from blood culture or from isolate dilutions
Cepheid	GeneXpert® Omni	Level II for tests not requiring culture	Real-time PCR and fluorogenic target-specific hybridization	Xpert® CT/NG and eventually other relevant assays available on the larger GeneXpert® platforms (e.g., MRSA)

(Appendix II, continued)

Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Binx Health	io <sup>®</sup> diagnostic system	Level I/II	iNAAT using electrochemical DNA-detection technology based on differential pulse voltammetry	STIs: CT/NG assay and possible ciprofloxacin-sensitive NG resistance test
QuantuMDx Group	Q-POC™	Level I/II	End-point and qPCR chemistries and microarray after amplification	STIs: CT/NG/TV and possible NG antimicrobial resistance to accompany this assay
DNA Electronics (DNAe)	LiDia <sup>®</sup>	Level I/II	Semiconductor genomic analysis and multiplexed PCR and nested PCR	Sepsis assay from whole blood
Roche	Smarticles™	Level II, Level I (?)	Molecular technology using “Smarticles™”, nonreplicative transduction phages that bind to bacteria	MRSA; CRE and VRE in development

### Host response and biomarker detection assays

#### CRP

##### Qualitative or semi-quantitative tests

Creative Diagnostics, Inc.	DTS233	N/A	Colloidal gold immunochromatography	Qualitative CRP detection; RUO
Assure Tech	WD-23	Level I	Colloidal gold conjugate and anti-CRP antibodies	Semi-quantitative CRP concentration ranges
bioMérieux	bioNexia <sup>®</sup> CRPplus	Level I		Semi-quantitative CRP concentration ranges
Medix Biochemica	Actim <sup>®</sup> CRP	Level I		Semi-quantitative CRP concentration ranges

##### Quantitative tests

Abbott	Alere Afinion™ CRP	Level I/II	Solid-phase immunochemical assay	Quantitative determination of CRP levels
Orion Diagnostica Oy	QuikRead go and QuikRead 101 instruments	Level I/II	Particle-enhanced immunoturbidimetric assay	Quantitative determination of CRP levels
Radiometer Medical ApS	AQT90 FLEX CRP	Level I/II	Time-resolved fluorescence using a europium chelate as the fluorescent label	Quantitative determination of CRP levels
Boditech Med	iChroma™ CRP	Level I/II	Fluorescence immunofluorescent assay	Quantitative determination of CRP levels
Abbott	NycoCard™	Level I/II	Immunochemical assay	Quantitative determination of CRP levels
Eurolyser Diagnostica GmbH	CRP test kit	Level I using CUBE-S; Level II	Immunoturbidimetric assay using photometric measurement	Quantitative determination of CRP levels
DiaSys Diagnostic Systems GmbH	CRP IS - InnovaStar <sup>®</sup>	Level I/II	Immunoturbidimetric assay	Quantitative determination of CRP levels
biosurfit	spinit <sup>®</sup>	Level I/II	Spectrophotometry	Quantitative determination of CRP levels

(Appendix II, continued)

PCT				
Manufacturer	Platform	Minimum laboratory level	Technology	Assays
Thermo Fisher Scientific	B-R-A-H-M-S PCT™ direct assay	Level II, Level I (?)	Immunochromatographic sandwich assay	Quantitative determination of PCT
	B-R-A-H-M-S PCT™ LIA	Level III/IV	Immunoluminescence sandwich assay	Quantitative determination of PCT
	B-R-A-H-M-S PCT-Q	Level II (requires serum)	Immunochromatographic sandwich assay with immunogold labelling	Semi-quantitative determination of PCT
Diazyme Laboratories	Diazyme PCT assay	Level III/IV	Latex particle enhanced immunoturbidimetric assay	Quantitative determination of PCT
Tests using novel biomarkers, a combination of host biomarkers, or combinations of protein biomarkers and gene classifiers				
MeMed BV	ImmunoXpert™	Level III/IV	Measures three human immune system biomarkers in serum	Qualitative assay to distinguish between bacterial and viral infection Pipeline: MeMed Sepsis™ and MeMed Neo™ (neonates); ImmunoPoc™ device
RPS Diagnostics	FebriDx®	Level I	Disposable immunoassay assay that detects CRP and MxA, an inflammatory protein	Qualitative assay to help differentiate a clinically significant immune response to viral and/or bacterial ARI
Immunexpress	SeptiCyte™	Level III/IV Level II for use on Idylla™	Real-time RT-PCR	Quantitative assay to differentiate infection-positive (sepsis) from infection-negative systemic inflammation
Abionic	abioSCOPE®	Depending on instrument, Level II, III, IV	Nanofluidic immunoassay technology; fluorescent molecular complexes are formed on a nanosensor	Quantitative determination of sepsis risk through measurement of pancreatic stone protein NOT COMMERCIALY AVAILABLE
Pipeline host biomarker tests				
Inflammatix	HostDx Sepsis and HostDx Fever	Level III/IV (HostDx Sepsis) Level II (HostDx Fever)	Quantitative multiplex gene expression using molecular, multiplex platform	Assay to quantitate acute infection (HostDx Sepsis) and assay to quantitate whether fever is caused by bacteria or virus (HostDx Fever)
Mologic	UTRiPLEX	Level I/II	Qualitative lateral flow assay detecting host biomarkers	Assay to rule out bacterial infections from urine

# Annex III – Diagnostic platforms suitable for Level I/Level II

Diagnostic platforms for combating ABR suitable for Level I and/or Level II				
Automated Gram staining				
Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
bioMérieux	PREVI® COLOR GRAM	I/II	Automated Gram staining via spray technology	Pan-bacteria
ALL.DIAG - Biosynex	MULTISTAINER®	I/II	Gram stain and fast staining	Pan-bacteria
ELITechGroup Solutions	Aerospray® Gram series 2	I/II	Gram stain	Pan-bacteria
Hardy Diagnostics	QuickSlide™ GramPRO 1™	I/II	Gram stain	Pan-bacteria
Automated specimen processing and inoculation of media				
None				
Phenotypic bacterial ID				
None				
Automated culture systems				
None				
Manual biochemical bacterial ID systems				
None				
Automated biochemical bacterial ID systems				
None				
Nonphenotypic methods of identifying bacteria				
Immunoassays				
Thermo Fisher Scientific	RAPID™ Hp StAR™	Level I	Immunochromatographic test	<i>Helicobacter pylori</i> from stool
Otsuka Pharmaceutical Co., Ltd.	RAPIRUN® <i>H. pylori</i> antibody detection kit	Level I	Immunochromatographic test	<i>Helicobacter pylori</i> from stool
Meridian Bioscience, Inc.	ImmunoCard STAT!® CAMPY	Level I	Immunochromatographic test	<i>Campylobacter</i> antigens ( <i>C. jejuni</i> and <i>C. coli</i> ) from stool
Abbott	<i>C. DIFF</i> QUIK CHEK COMPLETE®	Level I	Immunochromatographic test	<i>Clostridium difficile</i> (TcdA and TcdB) from stool

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Thermo Fisher Scientific	Xpect® <i>Clostridium difficile</i> Toxin A/B test	Level I	Immunochromatographic test	<i>Clostridium difficile</i> (TcdA and TcdB) from stool
Meridian Bioscience, Inc.	ImmunoCard Toxins® A&B	Level I	Horizontal flow enzyme immunoassay	<i>Clostridium difficile</i> (TcdA and TcdB) from stool
Thermo Fisher Scientific/ BioStar	BioStar® OIA GC	Level I	Optical immunoassay	NG from female endocervical swabs and male urine specimens
Abbott	BinaxNOW® <i>Streptococcus pneumoniae</i>	Level I	Immunochromatographic assay	<i>Streptococcus pneumoniae</i> from urine
BIOSYNEX	BIOSYNEX S. <i>pneumoniae</i>	Level I	Immunochromatographic test	<i>Streptococcus pneumoniae</i> from urine and CSF
Malaysian Biodiagnostic Research	Typhidot®	Level II, possibly Level I	ELISA	<i>Salmonella typhi</i> in serum
IDL Biotech	TUBEX®	Level I	IMBI	<i>Salmonella typhi</i> in serum
Thermo Fisher Scientific	Wellcogen™ <i>Haemophilus influenzae</i> b Rapid latex agglutination test	Level I	Latex diagnostic test	<i>Haemophilus influenzae</i> type b from CSF, serum, urine or blood cultures
Meridian Bioscience, Inc.	ImmunoCard STAT!® E. coli O157 Plus	Level I	Horizontal-flow enzyme immunoassay	<i>Escherichia coli</i> in stool or culture
<b>Molecular methods</b>				
Hybridization methods				
<b>None</b>				
<b>Amplification methods – integrated systems</b>				
Roche	cobas® Liat® system	Level II/ possibly Level I if cold chain	Real-time PCR	<i>Clostridium difficile</i>
Quidel Corporation	Solana® platform	Level II	HDA	<i>Clostridium difficile</i> ; Group A Strep, Group B Strep
	AmpliVue® platform	Level II	HDA and lateral flow	<i>Clostridium difficile</i> ; Group A Strep, Group B Strep
Luminex	ARIES® and ARIES® M1 systems	Level II	Real-time PCR	<i>Clostridium difficile</i> ; Group A Strep
Mobidiag	Novodiag® platform	Level II	Real-time qPCR and microarray	<i>Clostridium difficile</i> ; Bacterial GE+ assay
QIAGEN NV	QIAstat-Dx™	Level II	Real-time PCR with optical sensor	Respiratory panel; gastrointestinal panel; sepsis panel
<b>Amplification systems – not integrated</b>				
<b>None</b>				
<b>Pipeline molecular methods</b>				
Lucira Health	Disposable (no name)	Level I/II	RPA and LAMP	CT and NG, Strep (swabs)
Qvella	FAST-ID™	Level II	PCR	Sepsis pathogens (whole blood)

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
NanoDetection Technology	No name	Level II (?)/ Level III	Biochip	MRSA screening, MRSA/ MSSA dual screening, sepsis, influenza A and B, HCV, STIs and dengue
3iDx	Biospectrix	Level II/ Level I (?)	Microfluidic and nanotechnology	BSIs (whole blood)
<b>Sequencing methods</b>				
<b>None</b>				
MS				
<b>None</b>				
<b>Phenotypic methods of ID and AST</b>				
Semi-automated classical phenotypic methods				
<b>None</b>				
Automated classical phenotypic methods				
<b>None</b>				
Imaging-based ID/AST or AST only				
<b>None</b>				
Nonimaging AST only				
<b>None</b>				
<b>Pipeline AST technologies</b>				
Astrego	Captiver™ System	Level II/ Level I (?)	Microfluidics and imaging	AST for UTIs
<b>Nonphenotypic methods of identifying pathogens and detecting antibiotic resistance</b>				
<b>Molecular platforms for identifying and characterizing bacterial resistance from blood culture</b>				
bioMérieux	BioFire® FilmArray®	Level III/ IV; Level II for panels other than BCID and pneumonia (?)	Nested PCR and real-time PCR; detection using fluorescent intercalation dye within separate array module (one target per well within array)	Multiple panels: blood culture ID panel, gastrointestinal, respiratory, meningitis/encephalitis, pneumonia
Cepheid	GeneXpert® system	Level III/IV; Level II for panels nor requiring culture	Real-time PCR and fluorogenic target-specific hybridization	BSI: MRSA/SA; MRSA/SA SSTI, MRSA Nasal Complete and MRSA NxG from swabs Bacterial resistance: <i>vanA</i> assay and Carba-R assay <i>Clostridium difficile</i> assays; MTB/RIF assay; and CT/NG assay

(Annex III, continued)

Molecular platforms for ID and resistance from whole blood and other matrices				
Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
ELITechGroup Solutions	ELITe MGB® kits and panels	Level II (?), Level III/IV	Real-time PCR on ELITe InGenius® platform	ID: <i>Clostridium difficile</i> kit, STI panel (CT/NG and MG); <i>Staphylococcus aureus</i> and MRSA Resistance: carbapenem resistance kit; ESBL gene kit and colistin resistance kit.
GeneFluidics	UtiMax™/BsiMax®	Level II (?); Level III/IV	Electrochemical-based sandwich hybridization method to measure bacterial 16S rRNA	UtiMax™: detection and AST of uropathogens from urine Pipeline: BsiMax® – detection of BSIs from whole blood. ID panel: <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Enterobacter</i> spp., MRSA, MSSA and <i>Enterococcus</i> , among others. AST antibiotics: gentamicin, ciprofloxacin, cefepime, meropenem, ceftriaxone, ampicillin and piperacillin-tazobactam
<b>Nonphenotypic methods of detecting antibiotic resistance</b>				
<b>Molecular methods of detecting antibiotic resistance</b>				
None				
<b>Immunoassays and other methods for detecting ABR</b>				
None				
<b>Pipeline molecular technologies for identifying pathogens and/or detecting antibiotic resistance</b>				
SpinDiag	LabDisk	Level II/ Level I (?)	Nested PCR; microfluidics with disc-based test cartridge	Tests 25 drug-resistant bacterial strains. Prototype tests: MRSA from nasal swabs and VRE from rectal swabs. Additional assays planned for RTIs and STIs.
ExcitePCR (Positive ID)	FireflyDx™	Level II/ Level I (?)	Real-time PCR and single-use disposable cartridges	Plan to process whole blood, nasal swabs and urine samples, among others. Have tested MRSA, MSSA and <i>Clostridium difficile</i> on prototype system. Resistance assays planned.
Binx Health	io® diagnostic system	Level I/II	iNAAT using electrochemical DNA detection technology based on differential pulse voltammetry	STIs: CT/NG assay and possible ciprofloxacin-sensitive NG resistance test
QuantuMDx Group	Q-POC™	Level I/II	End-point and qPCR chemistries and microarray after amplification	STIs: CT/NG/TV and possible NG antimicrobial resistance to accompany this assay
DNA Electronics (DNAe)	LiDia®	Level I/II	Semiconductor genomic analysis and multiplexed PCR and nested PCR	Sepsis assay from whole blood

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Roche	Smarticles™	Level II, Level I (?)	Molecular technology using "Smarticles™", nonreplicative transduction phages that bind to bacteria	MRSA; CRE and VRE in development
<b>Host response and biomarker detection assays</b>				
<b>CRP</b>				
<b>Qualitative or semi-quantitative tests</b>				
Assure Tech	WD-23	Level I	Colloidal gold conjugate and anti-CRP antibodies	Semi-quantitative CRP concentration ranges
bioMérieux	bioNexia® CRPplus	Level I		Semi-quantitative CRP concentration ranges
Medix Biochemica	Actim® CRP	Level I		Semi-quantitative CRP concentration ranges
<b>Quantitative tests</b>				
Abbott	Alere Afinion™ CRP	Level I/II	Solid-phase immunochemical assay	Quantitative determination of CRP levels
Orion Diagnostica Oy	QuikRead go and QuikRead 101 instruments	Level I/II	Particle-enhanced immunoturbidimetric assay	Quantitative determination of CRP levels
Radiometer Medical ApS	AQT90 FLEX CRP	Level I/II	Time-resolved fluorescence using a europium chelate as the fluorescent label	Quantitative determination of CRP levels
Boditech Med	iChroma™ CRP	Level I/II	Immunofluorescent assay	Quantitative determination of CRP levels
Abbott	NycoCard™	Level I/II	Immunochemical assay	Quantitative determination of CRP levels
Eurolyser Diagnostica GmbH	CRP test kit	Level I using CUBE-S; Level II	Immunoturbidimetric assay using photometric measurement	Quantitative determination of CRP levels
DiaSys Diagnostic Systems GmbH	CRP IS -InnovaStar®	Level I/II	Immunoturbidimetric assay	Quantitative determination of CRP levels
biosurfit	spinit®	Level I/II	Spectrophotometry	Quantitative determination of CRP levels
<b>PCT</b>				
Thermo Fisher Scientific	B-R-A-H-M-S PCT™ direct assay	Level II, Level I (?)	Immunochromatographic sandwich assay	Quantitative determination of PCT
	B-R-A-H-M-S PCT-Q	Level II (requires serum)	Immunochromatographic sandwich assay with immunogold labelling	Semi-quantitative determination of PCT
<b>Tests using novel biomarkers, a combination of host biomarkers or combinations of protein biomarkers and gene classifiers</b>				
RPS Diagnostics	FebriDx®	Level I	Disposable immunoassay that detects CRP and MxA, an inflammatory protein	Qualitative assay to help differentiate a clinically significant immune response to viral and/or bacterial ARI

(Annex III, continued)

Manufacturer	Platform	Minimum laboratory Level	Technology	Assays
Immunexpress	SeptiCyte™	Level II for use on Idylla	Real-time RT-PCR	Quantitative assay to differentiate infection-positive (sepsis) from infection-negative systemic inflammation
Pipeline host biomarker tests				
Mologic	UTRiPLEX	Level I/II	Qualitative lateral flow assay detecting host biomarkers	Assay to rule out bacterial infections from urine

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