Global Antimicrobial Resistance Surveillance System (GLASS)

Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System

World Health Organization

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WHO/WSI/AMR/2019.1

Suggested citation. Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System. Geneva: World Health Organization; 2019 (WHO/WSI/AMR/2019.1). Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at http://apps.who.int/iris.

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Disclaimer

The information on individual diagnostic tests in this document is publicly available and was obtained by searching PubMed for relevant publications on molecular AMR diagnostics for GLASS priority pathogens and Google searches for diagnostic companies that offer molecular AMR tests (see Annex 1 for search terms). All searches were conducted between 13 October 2017 and 4 December 2017.

WHO has not validated and does not endorse the use of any of the commercial tests mentioned in this document. The molecular diagnostic tests listed in Table A1.1 are approved by the United States Food and Drug Administration or marked conformité Européenne (conforming to the standards of the European Union and European Economic Area). As more molecular diagnostic tests are developed and validated, the table will be updated to include validated tests approved by other regulatory agencies.

Contents

Executive summary

Antimicrobial resistance (AMR) is a serious threat to global public health. In 2015, WHO launched the Global Antimicrobial Resistance Surveillance System (GLASS) in order to standardize the collection of data on AMR in Member States, for planning, prevention and intervention programmes. Reports to GLASS currently rely on detection of phenotypic resistance, which requires bacteria to be cultured and tested for growth in the presence of antimicrobial agents. In future, GLASS may incorporate the results of molecular testing for AMR detection by appropriate methods. Molecular diagnostic methods can be used at the same time as phenotypic testing to yield additional information, such as the exact gene or mutation underlying a resistance phenotype. This information can be used to interpret AMR profiles at surveillance sites and better understand the global occurrence of certain resistance mechanisms.

Different laboratory settings have different requirements for molecular methods for AMR diagnostics. This technical note addresses three generic laboratory settings with different capacity for molecular AMR testing: those with no prior experience in molecular AMR surveillance; newly established national reference laboratories (NRLs) with some experience in molecular methods; and fully established NRLs with experience in molecular AMR surveillance. Molecular diagnostic methods are graded according to their complexity of use, setup cost and cost per tested specimen. This technical note provides guidance to people involved at various levels of AMR surveillance in choosing the most appropriate molecular AMR test for their setting, including clinical and reference laboratories. The document also provides a review of available methods and how they could be used in national surveillance.

Of the available molecular methods, fully automated, integrated, cartridge-operated polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) devices and lateral flow assays are the most suitable for laboratories with no previous experience in molecular testing in AMR surveillance. It is difficult to determine the cost per tested specimen, especially for low- and middle- income countries (LMICs), because pricing models are specific to regions and suppliers; uncertainty about the cost of molecular testing is a major barrier to its use for AMR surveillance in LMICs. The WHO catalogue ordering system, which includes pre-negotiation of prices and optimizing the flow of supplies, could lower the cost per test. Moreover, harmonization and standardization of clinical laboratory testing within national laboratory systems in LMICs could result in pooled procurement, which would be useful for negotiating prices with manufacturers.

Although molecular AMR diagnostics for known resistance markers are highly sensitive, there is no firm evidence of their cost-effectiveness or affordability in all settings. Poor understanding of resistance mechanisms may impede use of effective molecular diagnostics for some disease organisms. For example, there is currently no validated molecular diagnostic test for AMR in gonorrhoea or pneumococcal infections. Nevertheless, as costs for molecular diagnostics fall and knowledge about the genetic mechanisms of AMR increases, molecular tests are likely to become valuable tools available for AMR surveillance in all settings. Proof-of-principle studies can be conducted to demonstrate the added value of molecular AMR diagnostics to supplement phenotypic testing. A future document will outline plans and guidance on conducting proof-of-principle studies.

Acknowledgements

WHO thanks the following authors and contributors: David Aanensen (Centre for Genomic Pathogen Surveillance at the Wellcome Sanger Institute), Roman Kozlov (WHO Collaborating Centre for Capacity Building on Antimicrobial Resistance Surveillance and Research, Russia), Monica Lahra (WHO Collaborating Centre for Sexually Transmitted Diseases, Australia), Carolin Vegvari (Centre for Genomic Pathogen Surveillance at the Wellcome Sanger Institute), Neil Woodford (WHO Collaborating Centre for Reference and Research on Antimicrobial Resistance and Healthcare Associated Infections, UK), and the WHO AMR Surveillance and Quality Assessment Collaborating Centers Network.

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External reviewers: Till Bachmann (Edinburgh University), Celia Carlos, Alejandra Corso, Diego Faccone (Laboratorio Nacional de Referencia en Resistencia a los Antimicrobianos MSAL, Argentina), Pilar Donado-Godoy, John Ndemi Kiiru (Centre for Microbiology Research, Kenya Medical Research Institute, Kenya), Roberto Melano (University of Toronto, Canada), Mario Fabian Martinez Mora (Laboratorio Central de Salud Pública, Paraguay), Jose E. Moreno (LCRSP-ICGES, Comisión RAM Panamá), Iruka Okeke, Alejandro Petroni, (Laboratorio Nacional de Referencia en Resistencia a los Antimicrobianos MSAL, Argentina), K.L. Ravikumar Soledad Ulloa (Instituto de Salud Pública, Chile), David Whiley (The University of Queensland Brisbane, Australia), Tomoyuki Yamaguchi (Hokkaido University, Japan).

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Financial support: The Government of the United States of America. The Government of Republic of Korea through the Korea International Cooperation Agency (KOICA).

Acronyms and abbreviations

AMR	antimicrobial resistance
AST	antimicrobial susceptibility testing
CC	collaborating centre
CE	conformité Européenne
CLIA	Clinical Laboratory Improvement Amendments
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EQA	External Quality Assessment
ESBL	extended-spectrum β-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EUSCAPE	European Survey on Carbapenemase-Producing Enterobacteriaceae
FISH	fluorescent in situ hybridization
FQ	fluoroquinolone drug
GLASS	Global Antimicrobial Resistance Surveillance System
LAMP	loop-mediated isothermal amplification
LED	light-emitting diode
LFIA	lateral flow immunoassay
LMIC	low- or middle-income country
LPA	line probe assay
MDR	multi-drug resistance
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
NLFA	nucleic acid lateral flow assay
NGS	next-generation sequencing
NRL	national reference laboratory
PCR	polymerase chain reaction
POC	point-of-care
QC	quality control
qPCR	quantitative polymerase chain reaction
rtPCR	real-time polymerase chain reaction
3GC R	third generation cephalosporins-resistant
TTR	time to result (from sample collection to results)
USFDA	Food and Drug Administration (USA)
WGS	whole-genome sequencing
WHO	World Health Organization

1. Introduction

The rising prevalence of AMR is a global threat to public health and was recognized by WHO Member States with the World Health Assembly resolution WHA 68.7 *(1)*. Data on antimicrobial-resistant pathogens is essential to inform policy and to monitor the effectiveness of interventions. As per the request of Member States, in 2015, WHO launched GLASS in order to standardize data collection on AMR , from sampling strategy to reporting of results, to facilitate evidence-based planning of prevention and intervention programmes *(2)*.

Currently, data reported to GLASS is based on detection of phenotypic resistance, in which bacteria are tested for growth in the presence of antimicrobial agents. These methods allow determination of the degree to which an isolated pathogen is resistant to a given antimicrobial, by measuring either the minimum inhibitory concentration (MIC) which is the gold standard, or a zone diameter in disk diffusion testing. These methods provide information for clinical management and for surveillance but not direct information about the mechanism(s) of resistance to the agent. Methods for detecting phenotypic resistance may be generally lengthy (from hours at their fastest to days). It is therefore worth considering supplementing these methods with molecular diagnostics for AMR to confirm and track resistance mechanisms *(3)*.

Molecular AMR diagnostics detect resistance-coding genes or resistance-associated mutations in DNA extracted from purified bacterial isolates or directly from clinical samples. These methods can give much faster results than phenotypic methods, in some cases in a matter of minutes if a cultured bacterial colony is tested directly or within a few hours for clinical specimens *(4, 5)*. Direct rapid testing of patient samples can be particularly advantageous in clinical settings when AMR diagnostic results are required for evidence-based prescription of antimicrobials *(6)*. If a molecular test requires that a new bacterial culture be set up, however, the time taken is similar to that of phenotypic testing. Molecular AMR diagnostics can be useful for confirming phenotypic testing and in surveillance, for example, for confirming the mechanisms responsible for certain resistance.

Various molecular tests are available commercially to detect specific resistance genes for both clinical and surveillance purposes. For instance, in a clinical setting, suspected methicillin-resistant *Staphylococcus aureus* (MRSA) isolates can be confirmed with a test for *mecA* and *mecC*, the genes that code for resistance to β-lactamase-stable penicillins *(7, 8)*. If an isolate of *E. coli* or *K. pneumoniae* is shown to be resistant to third-generation cephalosporins, tests for different extended-spectrum β-lactamases (ESBLs) are available to characterize the gene that codes for the resistance. Characterization of resistance genes provides important information for a better understanding of AMR molecular epidemiology. Similarly, many products are available to detect carbapenemase genes in carbapenem-resistant Enterobacteriaceae.

While molecular tests provide important, clinically relevant information, they have limits, moreover, molecular tests detect only known resistance genes or mutations, and phenotypic resistance testing is always necessary in surveillance to ensure correct classification of bacterial isolates. Molecular and phenotypic test results are not always well correlated. In particular, molecular diagnostic tests based on DNA amplification may give false-negative results because the gene responsible for the resistance phenotype was not tested; the gene was tested, but a mutation affecting primer annealing prevented amplification; or the resistance phenotype is due to a new, not yet characterized mechanism. Similarly, false-positives may be seen due to DNA contamination *(5)*.

This technical note describes the utility and applicability of established molecular diagnostics for AMR for identifying GLASS priority pathogens and types of resistance (Table 1) and their suitability for laboratories with different clinical and surveillance capability. “Established” tests are those that

have been validated and standardized. Many research laboratories develop their own diagnostic tests for in-house use, which are not necessarily standardized or quality-assured. Standardized testing eliminates variation in results due to the use of different assays and is preferable in laboratories that are participating in national surveillance programmes. Capability for molecular testing is, however, evolving rapidly, and new diagnostic methods are expected to be validated and become available within months of publication of this document.

Table 1. Pathogen–antimicrobial resistance combinations monitored in GLASS

Pathogen	Antimicrobial class	Suggested resistance mechanism to be monitored
Escherichia coli	Sulfonamides and trimethoprim Fluoroquinolones Extended-spectrum cephalosporins Carbapenems Polymyxins Penicillins	Fluoroquinolone R ESBL Carbapenemases Colistin R
Klebsiella pneumoniae	Sulfonamides and trimethoprim Fluoroquinolones Extended-spectrum cephalosporins Carbapenems Polymyxins	Fluoroquinolone R ESBL Carbapenemases Colistin R
***Acinetobacter* spp.**	Tetracyclines Aminoglycosides Carbapenems Polymyxins	 Carbapenemases Colistin R
Staphylococcus aureus	Penicillinase-stable β-lactams	*mecA, mecC*
Streptococcus pneumoniae	Penicillins Sulfonamides and trimethoprim Extended-spectrum cephalosporins	
***Salmonella* spp.**	Fluoroquinolones Extended-spectrum cephalosporins Carbapenems Macrolides	Fluoroquinolone R ESBL Carbapenemases Colistin R
***Shigella* spp.**	Fluoroquinolones Extended-spectrum cephalosporins Macrolides	Fluoroquinolone R ESBL Carbapenemases Colistin R
Neisseria gonorrhoeae	Extended-spectrum cephalosporins Macrolides Fluoroquinolones Aminoglycosides	 3GC R

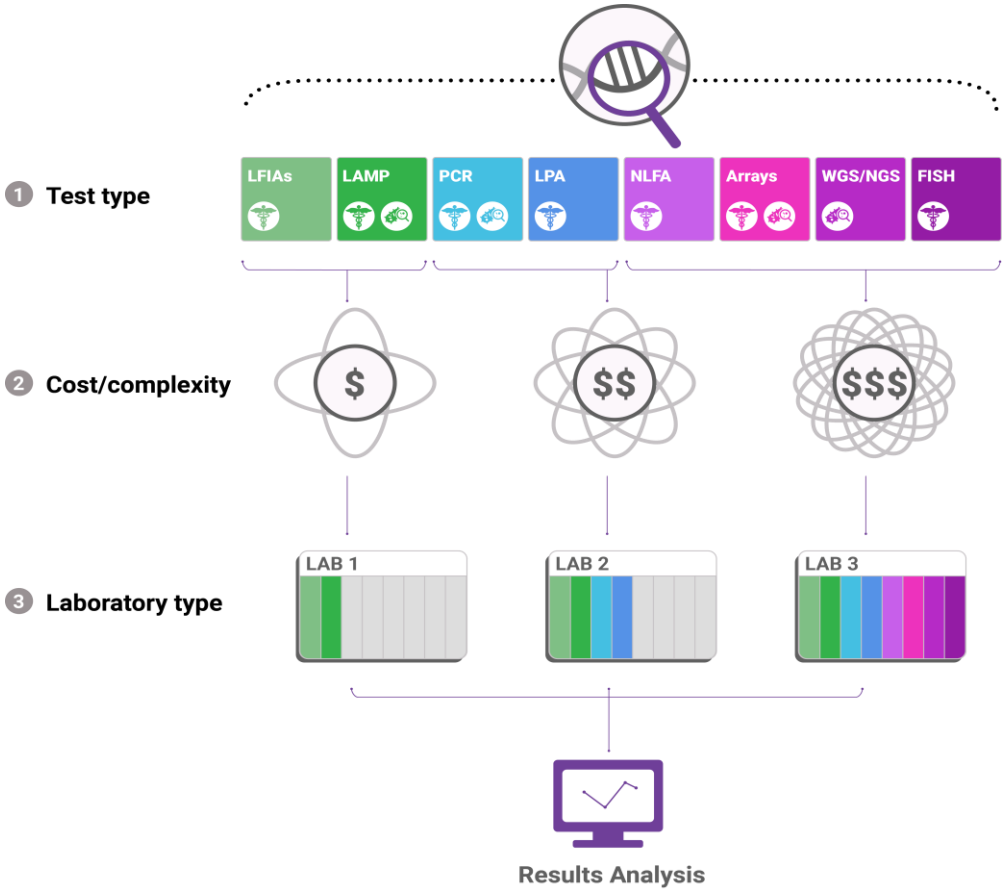
Fluoroquinolone R, Fluoroquinolone resistant; ESBL extended-spectrum β-lactamase; Colistin R, colistin resistant; 3GC R, third generation cephalosporins-resistant; *mecA, mecC* genes

2. How to use this technical note

This technical note gives an overview of the benefits, costs, limitations and challenges of the molecular diagnostics considered and of molecular AMR diagnostics that could be used for surveillance in non-reference laboratories and clinical settings. The document also indicates what the data generated by molecular diagnostic testing add to our understanding of AMR profiles at surveillance sites and the implications of the results for policy and clinical practice. It is intended to indicate to study site managers the position of their laboratory in the spectrum of capability for AST testing and, consequently, what molecular AMR diagnostics to choose and how to use them clinically and for surveillance (Fig. 1). More detailed information on the molecular diagnostics described in this report and their operational complexity is given in Annex 2.

We evaluated the applicability of current methods in laboratories with different capacity and capability for molecular AMR testing and surveillance. For simplicity, we categorized laboratories according to their capability in using molecular methods and in AMR surveillance as: type 1, with no prior experience in AMR surveillance or molecular methods; type 2, with prior experience in antimicrobial susceptibility testing (AST) but little or no prior experience in use of molecular methods (for example, a newly established NRL); and type 3, with extensive experience in both AST testing and applying molecular methods in AMR surveillance (for example, a fully established NRL).

Fig. 1. Example of molecular diagnostic test types suitable for laboratories with different capability in routine clinical use and surveillance.



LFIA, lateral flow immunoassay; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; LPA, line probe assay; WGS, whole-genome sequencing; NGS, next-generation sequencing; FISH, fluorescence in situ hybridization.

Tests suitable for clinical settings are indicated with the symbol of the staff and those suitable for surveillance are indicated with the symbol of a microbe.

The cost and complexity of molecular tests increases from left to right and along the colour gradient from green to purple. The three categories of complexity are indicated by an increasing number of elliptic orbits. The dollar signs indicate overall cost categories (setup and test costs). "LAB 1" in the figure refers to a laboratory with no prior experience in molecular testing, "LAB 2" to laboratories with some prior experience and "LAB 3" to laboratories with established expertise in molecular methods.

In routine clinical settings, the time to results (TTR) of a test is more important, whereas in surveillance settings high-throughput tests may be advantageous. Test types that are suitable for clinical and surveillance settings are marked in Fig. 1 (clinical indicated with the symbol of the staff, and surveillance indicated with the symbol of a microbe). Which test is suitable for which laboratory type depends on the complexity and cost of the test and the experience, budget, infrastructure of the laboratory and the AMR surveillance objectives. Low-complexity and low-cost tests may be suitable for most types of laboratory, whereas high-complexity and high-cost tests may be suitable only for established NRLs with a sufficient budget. Laboratories with no prior experience in molecular testing may consider low-complexity, low-cost tests, such as lateral flow immunoassays (LFIAs) and LAMP-based tests. Newly established NRLs may also consider PCR-based tests and line-probe assays (LPAs). Experienced, well-resourced NRLs can consider use of any of the available test types. Some more complex, expensive tests become more cost-efficient with increasing numbers of samples processed or resistance genes tested for. The methods for analysing results depend on the complexity of the test. For LFIAs and LAMP-based assays, visual inspection of the assay is sufficient to determine whether genes associated with resistance have been detected. For more complex diagnostic assays, such as arrays and WGS, complex analyses are necessary to interpret the raw data.

What are molecular AMR diagnostics?

- Phenotypic AMR diagnostics can be used to test the ability of bacteria to grow in the presence of a specific antimicrobial agent (i.e. detect susceptibility).
- In contrast, molecular diagnostic tests for AMR are used to detect the acquired genes or mutations in the genomes of bacterial pathogens that make them resistant to one or more agents of a given class of antimicrobial (i.e. detect genes associated with the mechanism of resistance).
- Molecular and phenotypic AMR diagnostics complement each other to improve understanding of both the extent of resistance in a given setting and the underlying mechanisms responsible for resistance

Benefits of molecular AMR diagnostic tests

- Potentially, faster results can be obtained with cartridge tests than with culture-based tests, which might be an advantage clinically.
- Cartridge tests are available that require minimal laboratory capacity and training and in which samples can be tested directly, without a culture step.
- Molecular tests may be more sensitive than phenotypic tests for detecting known resistance markers; however, the presence of a resistance marker does not always reliably predict phenotypic resistance.
- Molecular tests can be used to confirm resistance mechanisms in isolates with relevant phenotypic resistance.
- Data generated by molecular AMR diagnostics can contribute additional surveillance data and inform interventions.

3. Molecular methods for AMR diagnostics

Currently, most validated molecular diagnostic tests for AMR fall into one of four categories: those based on sequence, hybridization, amplification methods or immunoassays. In sequence-based tests, genome sequences are analysed to detect resistance genes; in hybridization-based tests, hybridized nucleic acid probes target gene sequences allowing detection; in amplification-based tests, the number of copies of a target gene sequence is amplified to allow detection; and immunoassays are based on binding of antibody to target genes or their products allowing detection. Illustrated explanations of how different molecular diagnostic tests work are given in Annex 2. Although novel mechanisms that result from minor changes in known resistance genes may be detectable with existing molecular AMR diagnostics, molecular methods cannot detect completely novel resistance mechanisms, as their design relies on prior knowledge of the responsible DNA sequences.

Molecular methods for AMR diagnostics

Molecular methods for AMR diagnosis are of varying complexity and require different laboratory capacity and training. Four categories of test are available, according to the mechanism of detection:

- amplification tests, such as PCR and LAMP;
- hybridization tests, such as arrays, fluorescent in situ hybridization (FISH) and LPAs;
- immunoassays, to detect AMR gene products by binding to specific antibodies, such as LFIAs and nucleic acid lateral flow assays; and
- sequencing tests, such as WGS and Nanopore.

4. Overview of AMR diagnostic tests

In order to identify molecular diagnostic tests that might be suitable for supplementing phenotypic methods for AMR surveillance of GLASS priority pathogens, we conducted a literature search on PubMed and searched Google for companies that sell AMR diagnostic tests. For search terms, period of search and a full summary of all the available, validated tests (as of January 2017), see Annex 1. We found 62 molecular AMR diagnostic tests for detecting resistance markers (genes or mutations) that are relevant to one or more of the GLASS priority pathogens (some tests are combined in larger testing panels). Of these, 33 tests are for carbapenem-resistant and extended-spectrum cephalosporin-resistant Enterobacteriaceae (including *E. coli*, *K. pneumoniae*, *Salmonella* spp. and *Shigella* spp.). Some of the same tests are also suitable for detecting genes that code for carbapenem resistance and β-lactam resistance in *A. baumannii* and other Gram-negative bacteria. A total of 29 tests can be used to detect methicillin-resistant *S. aureus.* We found no validated molecular diagnostic tests for penicillin or cephalosporin resistance in *S. pneumoniae* or *N. gonorrhoeae* (Table 2).

Table 2. Numbers of validated molecular AMR diagnostic tests according to pathogen–antimicrobial combination, test complexity and cost as of January 2018 (set up and test costs are separate).

Pathogen[Δ]	Antimicrobial class†	Total[‡]	Number of molecular AMR tests in Complexity category
Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, Salmonella spp., Shigella spp.), Acinetobacter spp.	Carbapenems Penicillins Extended-spectrum cephalosporins	33	1: 8 2: 8 3: 17
Staphylococcus aureus	Penicillinase–stable beta-lactams	29	1: 6 2: 10 3: 13
Streptococcus pneumoniae	Penicillin Sulphonamides and trimethoprim Extended-spectrum cephalosporins	0	1: 0 2: 0 3: 0
Neisseria gonorrhoeae	Extended-spectrum cephalosporins Fluoroquinolones Macrolides Aminoglycosides	0	1: 0 2: 0 3: 0

[Δ] only pathogens on which GLASS collects data are listed
*all molecular diagnostic tests found are for Enterobacteriaceae, and not for specific genera or species
† antimicrobial classes listed in this table are only those for which stand-alone tests are available, antimicrobial classes on the GLASS list for which there is currently no standardised, validated molecular test are not included in this table
‡ only formally validated tests have been included, since new molecular diagnostics are in development, the total number of tests will increase and will be updated in future versions of this document

Table 2 lists the number of molecular diagnostic tests for each pathogen–antimicrobial class combination in each complexity and cost category. Molecular tests of all three classes of complexity are available for all GLASS priority pathogens, except for *S. pneumoniae* and *N. gonorrhoeae*.

There are therefore molecular tests for detecting at least some AMR mechanisms in most GLASS priority pathogens, such as for ESBL genes in *E. coli*, *Klebsiella*, *Acinetobacter* and *Salmonella*. A comparison of tables 1 and 2, however, shows that the available tests do not detect all the pathogen–antimicrobial combinations of interest to GLASS.

Validated molecular diagnostic tests for AMR

- 62 validated molecular AMR diagnostic tests USFDA-cleared or marked as conforming to the standards of the European Union and European Economic Area (CE) are included in this technical note.
- 29 tests are for detecting MRSA.
- 33 tests are for detecting carbapenemase- or ESBL-producing isolates.
- Currently, there are no validated molecular AMR diagnostics for *S. pneumoniae* or *N. gonorrhoeae*.

5. Complexity and cost of molecular AMR diagnostics

In order to assist decision-makers in choosing diagnostic tests suitable for their setting, molecular diagnostic tests have been categorized according to their complexity in terms of the technology and technical expertise required, for example, in pipetting, handling reagents and avoiding contamination. Some molecular diagnostic tests are more suitable for use in clinical settings, such as those with rapid results but which do not have high throughput, whereas others may be more useful in laboratories for surveillance activities, such as high-throughput tests and those that provide the sequences of resistance genes. The three categories of test complexity are:

- **Low**: These comprise all-in-one devices that perform all processes, from the extraction of DNA from a sample to automatic display of results and documentation. The systems are contained in order to avoid contamination of the samples or the environment. They are usually small (hand-held or portable) and are operated with cartridges that contain all the reagents required for the molecular assay, so that users have only to load samples into the cartridge and the cartridge into the device. They do not include assays in which a sample is washed off a swab and then transferred to the cartridge. Test results are easy to interpret, and minimal training is required, so such devices should be suitable for settings with no prior molecular laboratory experience. Some of the devices also run on rechargeable batteries, so that power disruptions do not stop them from working immediately. The drawback is that test cartridges tend to be expensive, making them unaffordable for some LMICs.
- **Medium**: Tests in this category involve more than one device or off-cartridge sample handling steps; however, sample and DNA processing are automated, in a closed system. Some training may be required for sample handling and/or interpretation of results. The devices may not be portable and might require an uninterrupted energy supply.
- **High**: Such tests require substantial training and a fully equipped molecular laboratory. One or more processing steps are conducted manually, outside a closed system. Additional reagents and devices may be required that are not part of the assay kit provided by the manufacturer. For example, a separate PCR amplification step may be necessary before the amplified sample is loaded onto an array.

Tests can be categorized not only by complexity categories but also cost. Set-up costs include those of diagnostic devices specific to the test and, if applicable, additional devices and laboratory equipment necessary to run the test but that are not provided by the test manufacturer. The costs per test include the cost of single-use tests.

Many LMICs do not have the laboratory infrastructure required to use any of these tests. For molecular testing, therefore, isolates or specimens can be shipped to well-established reference laboratories that conduct molecular AMR testing. Hence, the cost of molecular AMR testing would be the same as in these laboratories plus shipping costs, which can be high, especially if a cold chain (for example, on dry ice) is required. However it is expected that laboratories that already have devices such as PCR machines will have a lower initial investment in equipment and training.

The costs of molecular diagnostic tests depend on the test complexity, the equipment required and the country or region, as laboratory devices and reagents are usually distributed by specialized suppliers, who usually set prices autonomously from those recommended by the manufacturer. This leads to a paradoxical situation, in which low-income countries may be charged higher prices for the same equipment and tests than higher-income countries. The current supplier-dependent pricing strategy is therefore a major barrier to widespread molecular surveillance of AMR.

Some manufacturers supply devices free of charge and recuperate their cost by sales of assay kits and obligatory maintenance fees. In some LMICs, suppliers use this strategy to sell equipment at affordable prices; however, this model is not available for all countries. More expensive devices that allow batch processing of samples may become more cost-efficient as the number of processed samples increases. For a laboratory with a low volume of samples to be screened, however, maintaining high-throughput devices, for example for WGS, may be less cost-effective than sending samples off-site to be sequenced commercially.

Complexity and cost of molecular AMR diagnostic methods *

Molecular AMR diagnostics were categorized as low, medium and high complexity
•Low-complexity tests require no or little prior molecular laboratory experience.
•High-complexity tests require substantial laboratory capacity and experience.
•Cartridge-based PCR methods, LAMP-based methods and lateral flow assays are currently available low- to medium-complexity molecular diagnostic tests
•Setup costs for molecular diagnostic devices are high in terms of infrastructure required and staff training

*These categories are not equivalent to the standard categories of "simple" or "waived", "moderate complexity" or "high complexity" of the US Clinical Laboratory Improvement Amendments (CLIA), which set out regulatory standards for clinical laboratory testing to ensure the accuracy and reliability of test results. There are currently no CLIA simple or waived molecular tests for AMR diagnostics. (See Annex 3 for web links to the USFDA CLIA guidelines on diagnostic devices.)

6. Sustainability and flexibility of molecular AMR diagnostics

Other considerations in molecular surveillance of AMR are the flexibility and sustainability of the method. The "flexibility" of a diagnostic method refers to the possibility of analysing samples from different substrates, while its "sustainability" indicates that it should be readily adaptable to novel tests, in order to make a more favourable business case. The main hurdle to sustainability is budget constraints. The budget of limited-resource settings may allow use of molecular AMR diagnostic tests, but their continuous use requires continuous support, which may be difficult to sustain.

Most of the tests in this technical note are based on amplification. As even small amounts of genetic material can be amplified with these methods, they are the most flexible in terms of the sample types (swabs, fluids, culture, etc.) that can be analysed. The flexibility of LAMP and PCR methods is similar, but multiplex assays are more difficult with LAMP tests; i.e. it is more difficult to design a test that can amplify several genes at once. At the same time, LAMP-based methods are more robust to disturbance by inhibitors that may be present in complex samples (for example, haemoglobin or lactoferrin in blood samples) than PCR-based methods. Amplification occurs at a constant temperature, which can be maintained in a water bath, so that expensive thermocyclers are not necessary; however, many laboratories in limited-resource settings lack positive and negative controls for amplification-based tests.

Array-based methods allow testing for multiple genetic markers at the same time, although the full range of markers tested in large arrays may not be required in all circumstances. LPAs and LFIAs test for only one or a few resistance-associated genes or gene products, which may limit their utility if the gene or protein in the sample is not included. For LFIAs in particular, however, novel assay kits are relatively cheap. The principle of using FISH for detecting methicillin resistance markers in *S. aureus*, in which the genotype underlying phenotypic resistance is well understood, may not be

readily transferrable to other resistance markers for which the link between phenotypic resistance and genotype is less clear.

Automated cartridge-based amplification systems may be easy to operate but usually require test cartridges from the same manufacturer, which may limit the number of molecular AMR tests that can be conducted with a given device. Consequently, there may be a trade-off between test complexity and flexibility.

Currently, only one sequence-based test (WGS) for the identification of ESBL-producing Enterobacteriaceae has regulatory approval *(9)*. In view of the potential of WGS to detect essentially all known resistance genes present in an isolate for the same price as multiplex PCR, however, uptake of sequence-based methods may be expected to increase. A recent study of surveillance of invasive *S. aureus (10)* shows how WGS data can be integrated with epidemiological, geospatial and phenotypic data on resistance to identify high-risk clones, predict resistance profiles and trace transmission events. Table 3 lists the molecular diagnostic methods applicable to each laboratory type.

In many LMICs, lack of local bioinformatics expertise may be an additional limiting factor for the application of molecular methods for AMR surveillance. Microbiologists working on AMR may not have relevant training in bioinformatics, and bioinformaticians may lack knowledge of AMR and microbiology. Training and retention of qualified personnel is an important factor for applicability and sustainability.

Sustainability and flexibility of molecular AMR diagnostic methods

- Ideally, molecular AMR diagnostic methods are flexible and adaptable in terms of sample types and tests for novel AMR markers.
- PCR-based methods are the most flexible in terms of sample types and adaptability, followed by LAMP-based methods.
- There may be a trade-off between test complexity and flexibility.
- Sustainable funding models are required for molecular AMR testing in limited-resource settings

Table 3. Molecular diagnostic methods applicable to each laboratory type.

Test complexity is graded from 1 to 3, with 1 the simplest and 3 the most complex. See text for details on grading scheme and tests.

Molecular assay	Complexity/ Cost	Advantages	Disadvantages
PCR-based	Complexity: 1–3	• Fully automated, integrated devices run with single-use amplification test cartridges; can be used in type 1 laboratories. • Reagents for cartridges are freeze-dried and can be stored at room temperature. • Some devices come with rechargeable batteries(an advantage where there are power-cuts) • Multiplex PCR can be used to detect several resistance markers at once (for example, carbapenemase and ESBL genes can be detected simultaneously, facilitating identification of multi-drug-resistant organisms) • Quick turn-around time (one to a few hours). • Very reliable, known and trusted technology (invented in 1983, standard equipment in molecular biology laboratories).	• Stand-alone assays that require laboratory equipment and reagents not provided by the test supplier may be suitable only for type 3 laboratories (for example, DNA may have to be extracted manually with a separate kit before amplification). • Cartridges are expensive. • Devices other than complexity 1 (automated cartridge-based devices) require training in molecular laboratory techniques. • Consumables for devices other than complexity 1 may require dry ice (for example, to stop degradation of enzymes required for the reaction). • Devices other than complexity 1 may be sensitive to ambient temperature and require a constant energy supply to retain accuracy and function.
Whole genome sequencing	Complexity: 3	• Provides rich information. • Can detect many resistance markers at once, including those not commonly included in panels. • If multiple resistance-associated genes are to be tested, it may be as cost-effective as multiplex PCR.	• Requires dedicated sequencing machines and extensive training in bioinformatics. • The amount of information may be overwhelming and difficult to process. • All available WGS assays involve multiple manual steps and multiple devices. • Sequencers must be serviced regularly by

Molecular assay	Complexity/ Cost	Advantages	Disadvantages
		• As this is an emerging technology, funders, policy-makers and users are keen to acquire it.	trained personnel (frequently required to be those of the device manufacturer or supplier). • Interpretation of results requires training. • Bioinformatics support may be lacking in many countries. • Broadband Internet access is required for analysis of large amounts of data, typically on computing clusters that can be accessed only on the Internet. • Data storage costs can be high. • Quality control is challenging. • There is no random access. • Pooling of samples is usually required.
LAMP-based	Complexity: 1	• Fully automated, integrated devices run with single-use test cartridges can be used in type 1 laboratories. • Frequently faster and more robust than PCR. • Amplicon can be detected visually from increased turbidity in reaction container. • May be 10–100 times more sensitive than PCR (11). • Does not require expensive thermal cyclers or electrophoresis systems.	• Less versatile than PCR assays (the many primers involved may constrain target site selection). • Multiplexing (amplifying multiple genes in the same assay) is difficult. • Reaction volumes may be larger than in PCR, and more consumables may be required.
Array	Complexity: 3	• Many molecular resistance and species markers can be detected at the same time (for example, an array may contain markers for all the major pathogens that cause sepsis and for	• Labelled probe generation requires a PCR step (see potential disadvantages above). • May require other machines to read and interpret signal (e.g. laser and optical

Molecular assay	Complexity/ Cost	Advantages	Disadvantages
		resistance markers).	detector). • May require statistical correction for multiple testing (if many genes are tested at once). • Cost depends on the number of markers.
Line probe assay	Complexity: 3	• Can detect several resistance markers at once (for example, penicillinases and metallo-β-lactamases). • The test itself is relatively easy to use and quick (several hours to result).	• Requires equipment and reagents for sample pre-processing (PCR, see potential disadvantages above) and training to avoid contamination (open manual laboratory procedures).
Fluorescence in situ hybridization	Complexity: 3	• For direct detection of resistance markers in bacterial cells.	• Requires fluorescence laser microscope or, as cost-effective alternatives, mercury vapour bulb or light-emitting diodes. • Microscope requires purified water for lens care. • Fluorescence laser microscope requires regular servicing by trained personnel.
Lateral flow immunoassays	Complexity: 1	• Quick and easy to use • Cheap (< US$ 20) • Can detect several resistance markers at once (for example, multiple β-lactamase genes) • Does not require electricity.	• Detection of resistance only from bacterial culture. • Culture step required. • Commercially available tests detect gene products (proteins), not DNA sequences.
Nucleic acid lateral flow assays	Complexity: 3	• Can detect several resistance markers at once. • Test itself is relatively easy to use and quick.	• Requires equipment and reagents for sample pre-processing (PCR, see potential disadvantages above) and training to avoid contamination.

7. Which technology to choose for which laboratory?

Molecular test complexity and the required laboratory experience can be graded in parallel. Generally, low-complexity tests can be used in all types of laboratory, whereas medium-complexity tests may be more appropriate for laboratories with some experience in molecular methods, and high-complexity tests require the experience and equipment of established NRLs. NRLs should define the combinations of pathogens and AMR to be sent to them for confirmation and further testing.

According to the grading scale, LFIAs and fully automated, integrated PCR or LAMP-based assays are the most suitable for laboratories with no prior experience in molecular AMR surveillance; however, cartridge-based assays are expensive, and it may be difficult to sustain use on low budgets. Some high-complexity tests, such as LPAs, can be used in newly established reference laboratories. In general, the flexibility of PCR-based technologies allows the design of methods that are suitable for all laboratory types and possibly point-of-care tests.

More complex tests tend to be more expensive, in terms of the costs of both set-up and for testing a single specimen. They also tend to require a continuous power supply and additional infrastructure, such as to provide cooling or purified water. LFIAs and portable PCR-based technologies for use in the field are the least costly options; however, the costs depend on the number of tests run over a given time and on regional supplier pricing strategies. These factors should be considered when choosing a molecular AMR diagnostic method or diagnostic device for particular settings.

In national surveillance programmes, molecular AMR diagnostic tests are used to answer AMR surveillance questions. It may be that a particular surveillance question in a limited-resource setting, for example, the occurrence of an unusual isolate that appears to be resistant to a class of antimicrobials according to phenotypic testing results but no molecular mechanism can be identified, can be answered only with a complex or expensive test. In this case, samples could be shipped to a reference laboratory with more molecular laboratory capacity for extensive molecular testing, including WGS. In this example, molecular AMR diagnostics are used for confirmation of phenotypic AST.

Molecular diagnostic methods for each laboratory type

- Automated, integrated PCR- and LAMP-based methods and LFIAs can be used in all laboratory types, from those with no prior experience in AMR surveillance (although they should be able to culture bacteria from specimens) to established reference laboratories.
- Whether a test can be used in a setting also depends on resources and the availability of the test in the country.
- The most complex tests should be used only by reference laboratories with prior experience in molecular methods.
- Costs depend on scale (the number of tests run over a given time) and the regional supplier pricing strategy.

8. Limitations and challenges for molecular AMR diagnostics

Molecular methods for the detection of AMR markers are used in a number of surveillance programmes worldwide to provide additional data, and not as replacements for phenotypic AST. Commercially available molecular tests are, however, underused, even in high-income countries, partly because of their higher cost as compared with phenotypic methods. A recent analysis *(12)* indicated disparities between the analytical performance of many AMR diagnostics and evidence for their affordability and cost–effectiveness, their most suitable use in clinical management and how they fit into laboratory workflows (timing, hands-on requirements, equipment, infrastructure and ease of use).

The molecular diagnostics made currently by manufacturers are not necessarily those required by clinicians, and they vary in their ability to detect resistance mechanisms (in terms of both coverage and sensitivity). In at least three technology assessments undertaken in the United Kingdom of carbapenemase gene detection tests *(13–15)*, a positive recommendation could not be made because of lack of evidence for their clinical (as opposed to analytical) utility.

Aside from costs, skill, infrastructure and technical requirements, the correlation between molecular and phenotypic test results and/or clinical interpretation has significant limitations. Not all resistance gene-positive isolates will be clinically resistant to the relevant antimicrobials (e.g. carbapenemases and ESBLs). Therefore, a phenotypic method should always be used to verify the result of molecular testing. In contrast, interpretation of a negative molecular test result depends on the bacterial species and antibiotic being tested. Molecular tests detect only known resistance genes or mutations, and novel genetic mechanisms may not be detected unless they share a high level of similarity with a known gene. Resistance genes from settings that have not been well studied may not be included in databases. Therefore, resistance gene-negative isolates should not be reported as phenotypically susceptible. Molecular diagnostics can detect only resistance markers that are included in the test panel. (They cannot determine the presence or absence of resistance mechanisms that are not included.) Poor sensitivity and specificity of a molecular test may be due to incomplete understanding of the resistance mechanism to some drugs in some bacterial species.

Quality control of molecular assays is another challenge for many laboratories. The position of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is that current evidence does not support the use of WGS to guide clinical decision-making for most WHO priority pathogens *(16)*. WGS tests may still be useful in AMR surveillance by showing the relatedness of bacterial isolates and thus allowing tracing of the transmission chains and events that lead to the emergence of AMR.
Proof-of-principle studies may be required to test molecular AMR diagnostics for selected pathogens with quality-assured, standardized tests for surveillance purposes. This will reduce (but not eliminate) the confounding effects of variable assay performance in comparisons of results for external quality assessment and quality control among sentinel sites.

Limitations and challenges for molecular AMR diagnostics

- Molecular tests can detect only known resistance genes or mutations.
- There is insufficient evidence for the cost-effectiveness of use of molecular AMR tests in clinical management and laboratory workflows. This type of testing (with a few exceptions, such as for tuberculosis) will be used mainly for public health surveillance rather than clinical management.
- Tests vary in their ability to detect resistance mechanisms.
- Molecular tests impose additional costs.
- Sustainable funding models are required for molecular AMR testing in LMICs.
- The correlation of molecular test results with phenotypic test results and their clinical interpretation is imperfect and varies by bacterial species and antimicrobial class.
- Poor understanding of resistance mechanisms may lead to poor test sensitivity.
- Knowledge on molecular AMR mechanisms must be increased.
- Proof-of-principle studies may be required to test molecular AMR diagnostics for surveillance purposes.

9. Data-sharing and analysis

A major activity in national AMR surveillance is compiling data from different surveillance sites in different contexts in a centralized system and analysing them to obtain an overview of what resistance has developed and spread, where and when. This information can then be fed back to surveillance sites to initiate or support interventions. Several national and regional AMR surveillance networks already use online platforms to share and manage AMR data derived from AST. For molecular AMR surveillance, these platforms could be augmented with information on resistance genes and/or WGS data. WHO offers WHONET, software for the management and analysis of AMR data based on AST data, which is used in countries in all six WHO regions. WHONET supports export of data to the GLASS data format and could be adapted to include molecular results. GLASS national data management platforms cover all regions.

Examples of national and regional surveillance databases are TESSy (The European Surveillance System), which is the European Centre for Disease Prevention and Control database for AST; Svebar, an automated system for collecting culture and AST data of the Public Health Agency of Sweden; and JANIS, the Japan Nosocomial Infection Surveillance, an automated programme for compiling, analysing and publishing data on AMR in hospitals; *(17)*. None of these platforms currently collects molecular AMR data. Another example is the ResFinder database at the Centre of Genomic Epidemiology at Denmark Technical University *(18)* (https://cge.cbs.dtu.dk/services/ResFinder/).

Ideally, AMR surveillance databases augmented by the results of molecular testing would combine features of surveillance databases and genetic reference databases and would facilitate common interpretation of both phenotypic and molecular data.

Data-sharing and analysis

- Effective AMR surveillance requires online tools to compile, share and analyze data from different surveillance sites.
- WHO offers WHONET, a software for managing and analyzing AST data.
- Database systems based on phenotypic AST can be augmented by molecular testing results.

10. Conclusions and outlook

Molecular diagnostic tests for AMR could provide valuable additional information to supplement phenotypic AMR surveillance. Molecular AMR diagnostics suitable for all three categories of laboratory are available for most GLASS priority pathogens. For example, PCR- or LAMP-based tests could be used to detect *mecA/C* genes that identify MRSA.

Low-complexity (cartridge-based portable or table-top devices) and low-cost molecular tests are available that are also suitable for LMICs. For molecular surveillance of ESBL and/or carbapenemase-producing organisms, lateral flow assays are the most cost–efficient method. In addition, PCR- and LAMP-based diagnostic tests for ESBLs and carbapenemases are suitable for widespread use, including in LMICs.

The cost of molecular diagnostic methods has decreased substantially in recent years and is likely to decrease further. Consequently, it can be expected that molecular diagnostics will soon become more affordable for a broader range of laboratories. EUCAST has indicated that data from WGS may significantly contribute to surveillance of resistance in the near future. The European Centre for Disease Prevention and Control has also indicated that WGS-based detection represents the future of AMR surveillance *(19)*.

The implementation of molecular epidemiological surveillance projects involves many facets which need to be developed, including use of data from WGS and shared databases and application of various analysis tools. .

As a global surveillance system, GLASS is well placed to develop and build further evidence for the potential role of WGS and other molecular methods in AMR surveillance.

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Annex 1.

Literature and online survey of validated, commercially available molecular diagnostic tests for AMR

Search strategy

We searched PubMed for relevant publications on molecular AMR diagnostics for GLASS priority pathogens, using the search terms: (((((((((antimicrobial resistance) OR (antibiotic resistance)) AND ((molecular diagnostic OR test) OR (point-of-care test))) AND (bacterial infection)))) AND ("2000"[Date - Publication]: "3000"[Date - Publication]))) AND humans) AND Name of GLASS priority pathogen.

As this search yielded mainly publications on tests that were neither standardized nor validated (in most studies, their own laboratory assays were used), we also searched Google for companies that offer molecular AMR diagnostic tests. The search terms were: molecular antimicrobial resistance diagnostic test; molecular AMR diagnostic test; molecular antibiotic resistance diagnostic test; and point-of-care diagnostic test for antimicrobial resistance and variations on those terms in combination with the species of GLASS priority pathogens. All searches were conducted between 13 October and 4 December 2017.

Table A1.1. Validated, standardized, quality-assured, commercially available molecular diagnostic tests for AMR in GLASS priority pathogens.

Table A1.1 shows the results of the search in identifying validated, standardized, quality-assured, commercially available molecular diagnostic tests for AMR in GLASS priority pathogens. It should be noted that **WHO has not validated and does not endorse the use of any of the tests listed in this table**[1]. The first column lists GLASS priority pathogens. Complexity is indicated as defined in the text: 1, low; 2, moderate; and 3, high. Tests that are suitable for use in LMICs are identified in the column headed "Details". The suitability of tests for LMICs is based on the complexity of the test and on information on the manufacturer's website on the setting for its intended use with regard to e.g. portability and power supply. These tests are a snapshot of those available at the time of writing, January 2018. As the field of molecular diagnostics is developing rapidly, more, different tests may soon be available.

Pathogen:	***Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)***		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
Unyvero ITI Application Curetis N.V./Curetis GmbH, Holzgerlingen, Germany	Complexity: 2	29 pathogens, 17 AMR genes (see panel on weblink) Hybridization-based test (array) Integrates DNA purification, multiplex PCR amplification Various sample types > 100 analytes possible Requires sample preparation with high DNA yield TTR, 4-5 h (excluding culture step) Laboratory or POC	http://www.unyvero.com/en/
Unyvero Pneumonia Application Curetis N.V./Curetis GmbH, Holzgerlingen, Germany	Complexity: 2	20 pathogens, 17 AMR genes (39 markers) (see panel on weblink) Hybridization-based test (array) Integrates DNA purification, multiplex PCR amplification, arrays Various sample types > 100 analytes possible Requires sample preparation with high DNA yield TTR, 4–5 h (excluding culture step) Laboratory or POC	http://www.unyvero.com/en/ *(1)* https://www.ncbi.nlm.nih.gov/pubmed/2029021968
Unyvero BCU Application Curetis N.V./Curetis GmbH, Holzgerlingen, Germany	Complexity: 2	34 pathogens, 16 resistance genes (see panel on weblink) Hybridization-based test (array) Integrates DNA purification, multiplex PCR amplification, arrays	http://www.unyvero.com/en/

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
		Various sample types > 100 analytes possible Requires sample preparation with high DNA yield TTR, 4–5 h (excluding culture step) Laboratory or POC	
Unyvero IAI Application Curetis N.V./Curetis GmbH, Holzgerlingen, Germany	Complexity: 2	26 pathogens, 22 resistance genes, 2 toxin markers Hybridization-based test (array) Integrates DNA purification, multiplex PCR amplification, arrays Various sample types > 100 analytes possible Requires sample preparation with high DNA yield TTR, 4–5 h (excluding culture step) Laboratory or POC	http://www.unyvero.com/en/
GeneXpert Carba-R Assay Cepheid Corp., now a Danaher company, Sunnyvale, CA, USA	Complexity: 2	Gram-negative organisms, including *Acinetobacter* spp., *P. aeruginosa,* Enterobacteriaceae KPC, NDM, VIM, OXA-48, IMP Amplification-based test Automated integrated sample preparation, amplification, detection Sensitivity: KPC: 100% OXA-48-like: 83% NDM: 100% VIM: 100% IMP: 71% Specificity: 100% TTR, 48 min (excluding culture step) Laboratory or POC	http://www.cepheid.com/us/ *(2)* https://www.ncbi.nlm.nih.gov/pubmed/25630646 NICE MedTech Innovation Briefing MIB52 (https://www.nice.org.uk/advice/mib52)

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
TRAPIST V6 **Coris BioConcept, Gembloux, Belgium**	Complexity: 3	Sepsis-associated Gram-positive and Gram-negative bacterial pathogens β-Lactamases: KPC, IMP, VIM, NDM, OXA-48-like, OXA-23, OXA-24, OXA-58, CTX-M-1, CTX-M-2, CTX-M-9 Hybridization-based test Portable device, PCR-amplification and hybridization performed on same chip TTR, < 1 h (excluding culture step) Intended for use in clinical laboratory Suitable for use in LMICs	www.corisbio.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420
ePlex System **Blood Culture Gram-negative Identification Panel** **GenMark Diagnostics Inc., Carlsbad, CA, USA**	Complexity: 1	21 Gram-negative pathogens including *Acinetobacter baumannii, Pseudomonas aeruginosa* 6 resistance genes (CTX-M, KPC, NDM, VIM, IMP, OXA) Hybridization-based test PCR amplification and hybridization in one cartridge, electrochemical technology (eSensor) Sensitivity: 97.5% Specificity: 99.5% for 199 clinical samples tested TTR, 90 min (excluding sample step) Intended for use in clinical laboratory	www.genmarkdx.com *(3, 4)* https://www.ncbi.nlm.nih.gov/pubmed/28343420 https://www.genomeweb.com/regulatory-news/fda-clearance-genmarks-eplex-intensifies-competition-highly-plexed-mdx-test-market*(5)* https://genmarkdx.com/wp-content/uploads/2018/05/ECCMID-2018-BCID-GN-Poster_DRAFT_FINAL-Landscape.pdf
Nanosphere/Verigene Bloodstream infection test **Luminex Corp., Austin, TX, USA**	Complexity: 2	Gram-negative and Gram-positive panels Gram-negative panel includes tests for: IMP, KPC, NDM, OXA, VIM, CTX-M Hybridization-based test Amplification and hybridization in same cartridge Blood culture sample OXA:	https://www.luminexcorp.com *(6, 7)* https://www.ncbi.nlm.nih.gov/pubmed/25994165 *(8)* https://www.ncbi.nlm.nih.gov/pubmed/25122857

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
		Sensitivity: 95.3% (95% CI: 86.9–99.0%) Specificity: 99.9% (95% CI: 99.5–100%) CTX-M: Sensitivity: 98.7% (95% CI: 95.4–99.8%) Specificity: 99.9% (95% CI: 99.5-100%) KPC: Sensitivity: 100% (95% CI: 93.1-100%) Specificity: 100% (95% CI: 99.7-100%) NDM: Sensitivity: 100% (95% CI: 91.4-100%) Specificity: 100% (95% CI: 99.7-100%) IMP: Sensitivity: 100% (95% CI: 92.6-100%) Specificity: 100% (95% CI: 99.7-100%) VIM: Sensitivity: 100% (95% CI: 91.4-100%) Specificity: 100% (95% CI: 99.7-100%) Positive predictive agreement compared with culture for *Acinetobacter* spp. 98.4% TTR, < 2 h (excluding culture step) Laboratory or POC	
IRIDICA BAC BSI **Ibis Biosciences, an Abbott Company, Carlsbad, CA**	Complexity: 3	Panel of 780 bacterial and fungal pathogens Detects resistance to KPC Amplification-based test (PCR) Blood samples Sensitivity: 88% Specificity: 63% TTR, 8 h Laboratory	*(9)* https://www.ncbi.nlm.nih.gov/pubmed/27384540

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
eazyplex SuperBug complete A kit **eazyplex SuperBug complete B kit** **Amplex, Gießen, Germany**	Complexity: 3	Detection of carbapenemase-producing bacteria Amplification-based test (LAMP) Portable Sensitivity: 95.5% Specificity: 100% TTR, 20–30 min from swab or culture Laboratory or POC Suitable for use in LMICs	www.eazyplex.com NICE MedTech Innovation Briefing MIB94 https://www.nice.org.uk/advice/mib94 *(2)* https://www.ncbi.nlm.nih.gov/pubmed/25630646
eazyplex SuperBug CRE **Amplex, Gießen, Germany**	Complexity: 3	Detects carbapenemase-producing Enterobacteriaceae: KPC, NDM, OXA-48, OXA-181, VIM ESBL genes of the CTX-M-1 and CTX-M-9 groups Amplification-based test (LAMP) Portable Sensitivity: 100% Specificity: 97.9% TTR, 15 min from swab or culture Laboratory or POC Suitable for use in LMICs	www.eazyplex.com
Amplidiag CarbaR+VRE **Mobidiag Ltd, Espoo, Finland**	Complexity: 3	Detects carbapenemase-producing organisms and vancomycin-resistant enterococci Carbapenemase groups: KPC, NDM, VIM, OXA-48, OXA-181, IMP, ISAbal-OXA-51, OXA-23, OXA-40, OXA-58 Amplification-based test (rtPCR) Sensitivity: 100% Specificity: 99% TTR, 2 h (excluding culture step) Laboratory, suitable for high-throughput screening	mobidiag.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420 *(10)* http://jcm.asm.org/content/56/3/e01092-17.abstract

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
Amplidiag CarbaR+MCR **Mobidiag Ltd, Espoo, Finland** **mobidiag.com/products/novodiag**	Complexity: 3	Identifies carbapenemase-producing organisms and colistin-resistant Enterobacteriaceae with selected *mcr* genes Carbapenemases: KPC, NDM, IMP, OXA-48, OXA-181, Acinetobacter OXA (ISAbal-OXA-51, OXA-23, OXA-40, OXA-58) In addition: MCR (MCR-1, MCR-2), GES Amplification-based test (rtPCR) TTR, 2 h (excluding culture step) Laboratory, suitable for high-throughput screening	mobidiag.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420
Novodiag **Mobidiag Ltd, Espoo, Finland**	Complexity: 1	Carbapenemase-producing organisms Hybridization-based test Amplification (PCR) and hybridisation in same cartridge, benchtop, portable TTR, 1 h Laboratory or POC	mobidiag.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420
NucliSENS easyQ® KPC Assay **bioMérieux, Marcy-l'Étoile, France**	Complexity: 3	Detects KPC Hybridization-based test Manual sample preparation, PCR amplification and probe detection KPC: Sensitivity: 93.3% (95% CI: 77.9–99.2%) Specificity: 99.9% (95% CI: 98.0–99.6%) TTR, 3–4 h Intended for use in high-complexity central laboratory	http://www.biomerieux-diagnostics.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf *(11)* https://www.ncbi.nlm.nih.gov/pubmed/ 22622445
FILMARRAY **Blood culture panel** **bioMérieux, Marcy-l'Étoile, France**	Complexity: 1	Detects KPC and vanA/B, *mecA* Hybridization-based test Amplification (PCR) and hybridization (array) in same cartridge Enterobacteriaceae KPC: Sensitivity: 100% (95% CI: 91.1–100%) Specificity: 100% (95% CI: 99.3–100%)	http://www.biomerieux-diagnostics.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf *(12)*

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
		TTR, 1 h Laboratory or POC	https://www.ncbi.nlm.nih.gov/pubmed/26311866
Check-multi-drug resistant CT101 **Check-Points, Wageningen, Netherlands**	Complexity: 3	Carbapenemase-producing Enterobacteriaceae: KPC, NDM ESBL vs non-ESBL Mobile AmpCs Hybridization-based test Manual, microarray KPC Sensitivity: 100% Specificity: 96.8% TTR, < 7 h Laboratory use	http://www.check-points.eu/ *(13)* http://www.check-points.eu/downloads/Roberts_et_al_poster_ASM_2011.pdf *(14)* https://www.ncbi.nlm.nih.gov/pubmed/26888905
Check-multi-drug resistant CT102 **Check-Points, Wageningen, Netherlands**	Complexity: 3	Carbapenemase-producing and ESBL-producing Enterobacteriaceae: KPC, NDM, VIM, IMP, OXA-48-like CTX-M-1 group, CTX-M-2 group, CTX-M-8 and -25 group, CTX-M-9 group TEM wt, TEM E104K, TEM R164S, TEM R164H, TEM G238S SHV wt, SHV G238S, SHV G238A, SHV E240K Hybridization-based test Manual, microarray Sensitivity and specificity close to 100% for most targeted genes TTR, < 7 h Laboratory use	http://www.check-points.eu/ *(15)* https://www.ncbi.nlm.nih.gov/pubmed/21325547
Check-multi-drug resistant CT103 XL **Check-Points, Wageningen, Netherlands**	Complexity: 3	As CT102, but additional carbapenemases, ESBLs, AmpCs Hybridization-based test Manual, microarray TTR, < 7 h Laboratory use	http://www.check-points.eu/

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
Check-Direct CPE **Check-Points, Wageningen, Netherlands** **Automated version in collaboration with BD MAX**	Complexity: 2 (BD MAX version)	Detects KPC, OXA-48, VIM, NDM in Enterobacteriaceae Amplification-based test Multiplex rtPCR, swabs or cultures Sensitivity: 100% Specificity: 88% TTR, 2 h Laboratory or POC	http://www.check-points.eu/ *(2, 16)* https://www.ncbi.nlm.nih.gov/pubmed/24135412 https://www.ncbi.nlm.nih.gov/pubmed/25630646 *(17)* https://www.ncbi.nlm.nih.gov/pubmed/26338860
Check-Direct ESBL Screen for BD MAX **Check-Points, Wageningen, Netherlands**	Complexity: 2	Enterobacteriaceae: CTX-M-1 group, CTX-M-2 group, CTX-M-9 group, SHV-ESBL Amplification-based test (rtPCR) Sensitivity: 95.2% Specificity: 97.6% TTR, 2 h Laboratory or POC	http://www.check-points.eu/ *(18)* https://www.ncbi.nlm.nih.gov/pubmed/28633496
QIAsymphony **QIAGEN, Hilden, Germany**	Complexity: 3	Sequencing kits for ≥ 30 β-lactam resistance genes, including carbapenemases and ESBL Amplification-based test (PCR) Sensitivity: 64.5% Specificity: 76.1% TTR, 3.5 h Laboratory	https://b2b.qiagen.com/ca// *(19)* https://www.escmid.org/escmid_publications/escmid_elibrary/?tx_solr%5Bfilter%5D%5B0%5D=main_category%253ADiagnostic%2BBacteriology%2B%2526%2BGeneral%2BMicrobiology&tx_solr%5Bfilter%5D%5B1%5D=entry_type%253APoster&tx_solr%5Bfilter%5D%5B2%5D=author%253AGeorg%2BPlum&tx_solr%5Bsort%5D=created_desc%20DESC

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
HAI BioDetection System **Pathogenica, Boston, MA, USA**	Complexity: 3	ESBL-producing Enterobacteriaceae Sequence-based test (next-generation sequencing) Comment: 98% sensitivity, but does not discriminate between ESBL and non-ESBL TEM and SHV □-lactamases or specify CTX-M genes by group TTR (from DNA isolation), 12 h Laboratory, for surveillance	*(20)* https://www.ncbi.nlm.nih.gov/pubmed/24789184
Master Diagnostica AMR Direct Flow Chip **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	Detects panel of 20 antimicrobial resistance genes found in Gram-positive and Gram-negative bacteria Hybridization-based test Multiplex PCR and macroarray chip; no DNA extraction or purification necessary TTR, several hours Laboratory	http://www.oxfordbiosystems.com/
Master Diagnostica Sepsis Flow Chip **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	Detects 40 pathogens responsible for sepsis and 20 AMR genes Hybridization-based test Multiplex PCR and macroarray chip, no DNA extraction or purification necessary TTR, several hours Laboratory	http://www.oxfordbiosystems.com/
AID Diagnostica Line Probe Assay ESBL **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	Detection of the most important mutations in TEM and SHV, detection of CTX-M, KPC Hybridization-based test (LPA) Validated for use with bacterial cultures and clinical samples Accuracy: 100% (sample size, 424) TTR, 5 h Laboratory	http://www.oxfordbiosystems.com/ *(21)* https://www.ncbi.nlm.nih.gov/pubmed/24004861

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
AID Diagnostika Line Probe Assay Carbapenemases Oxford Biosystems, Oxford, United Kingdom	Complexity: 3	Penicillinases (Class A): KPC, IMI, NMC-A, BIC Metallo-β-lactamases (class B): IMP, VIM, NDM, AIM, DIM, GIM, SIM, SPM Oxacillinases (Class D): OXA-48 Hybridization-based test (LPA) TTR, 5 h Laboratory	
RESIST KPC K-SeT Coris BioConcept, Gembloux, Belgium	Complexity: 1	Detection of KPC in bacterial culture Immunoassay: detects KPC enzyme epitopes Sensitivity: 100% (95% CI: 87.0–100%) Specificity: 100% (95% CI: 98.5–100%) TTR, 20 min (5 min hands-on, 15-min reaction time) Laboratory or POC Suitable for use in LMICs	http://www.corisbio.com *(22)* https://www.ncbi.nlm.nih.gov/pubmed/28844719 *(23)* https://www.ncbi.nlm.nih.gov/pubmed/29126269
RESIST OXA-48 K-SeT Coris BioConcept, Gembloux, Belgium	Complexity: 1	Detection of OXA-48 K-SeT in bacterial culture Immunoassay: detects OXA-48 enzyme epitopes Sensitivity: 100% (95% CI: 96.4–100%) Specificity: 100% (95% CI: 97.8–100%) TTR, 20 min (5 min hands-on, 15-min reaction time) Laboratory or POC Suitable for use in LMICs	http://www.corisbio.com *(24)* https://www.ncbi.nlm.nih.gov/pubmed/28483549
RESIST-3 O.O.K. RESIST-3 O.K.N. Coris BioConcept, Gembloux, Belgium	Complexity: 1	Detection of OXA-48, OXA-163, KPC (O.O.K.) or OXA-48, KPC, NDM (O.K.N.) in bacterial culture Immunoassay: detects enzyme epitopes Sensitivity and specificity: 100% TTR, 20 min Laboratory or POC Suitable for use in LMICs	http://www.corisbio.com/ *(25)* https://www.ncbi.nlm.nih.gov/pubmed/27535687 *(26)* https://www.ncbi.nlm.nih.gov/pubmed/28151407

Pathogen:	*Acinetobacter baumannii,* Enterobacteriaceae *(Escherichia coli, Klebsiella, Shigella, Salmonella)*		
Drug Resistance:	**Carbapenems ESBL-producing polymyxins**		
Test and provider	**Complexity**	**Details**	**Reference**
NG-Test CTX-M **Next Generation Biotech, Guipry, France**	Complexity: 1	Immunoassay: detects CTX-M enzyme Sensitivity: 100% (95% CI 94.8–100%) Specificity: 100% (95% CI 96.5%–100%) TTR, 15 min Laboratory or POC Suitable for use in LMICs	http://ngbiotech.com/
NG-Test CARBA 5 **Next Generation Biotech, Guipry, France**	Complexity: 1	Immunoassay: detects NDM, IMP, VIM, OXA, KPC enzymes Sensitivity: 100% (95% CI 94.8%–100%) Specificity: 100% (95% CI 96.5%–100%) TTR, 15 min (excluding culture step) Laboratory or POC Suitable for use in LMICs	http://ngbiotech.com/.
Carbaplex assay **Bruker Daltonik GmbH, Bremen, Germany**	Complexity: 3	Detects KPC, NDM, VIM, IMP and OXA-48-like carbapenemases Amplification-based test (rtPCR) Sample types: rectal swabs or culture Sensitivity: 96.3% Specificity: 99.5% TTR, 3 h (excluding culture step) Laboratory	https://www.bruker.com

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
Unyvero ITI Application **Curetis N.V./Curetis GmbH, Holzgerlingen, Germany**	Complexity: 2	For details, see above	http://www.unyvero.com/en/
Unyvero BCU Application **Curetis N.V./Curetis GmbH, Holzgerlingen, Germany**	Complexity: 2	For details, see above	http://www.unyvero.com/en/
Unyvero IAI Application **Curetis N.V./Curetis GmbH, Holzgerlingen, Germany**	Complexity: 2	For details, see above	http://www.unyvero.com/en/
iC-Cassette **Gram-positive cassette** **iCubate Inc., Huntsville, AL, USA**	Complexity: 2	5 Gram-positive pathogens, 3 resistance genes: *mecA* Hybridization-based test Portable, one-step multiplex PCR amplification and detection by probe Sensitivity: 93.8% Specificity: 98.7% TTR, 4.5 h Laboratory or POC	http://icubate.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420 *(27)* https://www.ncbi.nlm.nih.gov/pubmed/26468498
TRAPIST V6 **Coris BioConcept, Gembloux, Belgium**	Complexity: 3	Sepsis-associated Gram-positive and Gram-negative bacterial pathogens *mecA, mecC* Hybridization-based test Portable device, PCR-amplification and hybridization performed	www.corisbio.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
		on same chip TTR, < 1 h Suitable for use in LMICs	
ePlex System **GenMark Diagnostics Inc., Carlsbad, CA, USA**	Complexity: 1	> 20 Gram-positive bacteria > 25 Gram-negative bacteria 4 antibiotic resistance genes (*mecA, mecC)* Hybridization-based test PCR amplification and hybridization in one cartridge, electrochemical technology (eSensor) TTR, 90 min (excluding sample step) Intended for use in clinical laboratory	www.genmarkdx.com *(3, 4)* https://www.ncbi.nlm.nih.gov/pubmed/28343420 https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=26256
FILMARRAY **Blood culture panel** **bioMérieux, Marcy-l'Étoile, France**	Complexity: 1	Detects *mecA*, KPC Hybridization-based test Amplification (PCR) and hybridization (array) in same cartridge *mecA*: Sensitivity: 98.4% Specificity: 98.3% TTR, 1 h Laboratory or POC	http://www.biomerieux-diagnostics.com/ *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf *(28)* https://www.ncbi.nlm.nih.gov/pubmed/26739158

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
Nanosphere/Verigene **Bloodstream infection test** **Luminex Corporation, Austin, TX, USA**	Complexity: 2	Gram-positive and Gram-negative pathogens *mecA* Hybridization-based test Amplification and hybridization in same cartridge Blood culture sample *mecA*: Sensitivity: 94.2% (95% CI: 91.5–96.3%) Specificity: 98.2% (95% CI: 97.1–98.9%) TTR, < 2 h Laboratory or POC	https://www.luminexcorp.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf *(8)* https://www.ncbi.nlm.nih.gov/pubmed/25122857
NucliSENS easyQ® MRSA Assay **bioMérieux, Marcy-l'Étoile, France**	Complexity: 3	*mecA* Hybridization-based test Manual sample preparation, PCR amplification and probe detection Sensitivity: 95.8% (95% CI: 91.1–98.4%) Specificity: 96.8% (95% CI: 95.5–97.7%) TTR, 3–4 h Intended for use in high-complexity central laboratory	http://www.biomerieux-diagnostics.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf
Light Cycler **MRSA Advanced Test** **Roche Molecular Diagnostics**	Complexity: 3	MRSA *mecA* Amplification-based test (rtPCR) Automated or manual, nasal or perianal swab	https://molecular.roche.com *(7)* https://www.gardp.org/wp-

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
		Sensitivity: 95.2% (95% CI: 91.1–97.8%) Specificity: 96.4% (95% CI: 95.2–97.4%) TTR 2-3h Intended for use in high-complexity central laboratory	content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf https://www.gsaadvantage.gov/ref_text/V797P7037A/0OME86.372DCQ_V797P-7037A_V797P7037A.PDF
Light Cycler **SeptiFast MecA Test MGRADE** **Roche Molecular Diagnostics**	Complexity: 3	MRSA *mecA* Amplification-based test (rtPCR) Automated or manual, optional *mecA* gene detection when samples test positive for *S. aureus* Sensitivity: 60–95% Specificity: 74–99% depending on pathogen TTR, < 6 h Intended for use in clinical laboratory	https://molecular.roche.com https://www.nice.org.uk/guidance/dg20/chapter/3-The-diagnostic-tests https://www.gsaadvantage.gov/ref_text/V797P7037A/0OME86.372DCQ_V797P-7037A_V797P7037A.PDF *(29)* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3580598/
Cobas MRSA/SA **Roche Molecular Diagnostics**	Complexity: 2	Amplification-based test (rtPCR) Automated, partially integrated, transfer-prepared PCR plate to thermal cycler, nasal swab Sensitivity: 93.1% (95% CI: 88.1–96.1%) Specificity: 97.5% (95% CI: 96.8–98.0%) TTR, 2–3 h	https://molecular.roche.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf https://pim-eservices.roche.com/eLD_SF/gb/e

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
		Laboratory or POC	n/Documents/GetDocument?documentId=cffe450d-f2bd-e411-a8af-00215a9b0ba8 https://www.gsaadvantage.gov/ref_text/V797P7037A/0OME86.372DCQ_V797P-7037A_V797P7037A.PDF
GenoType Assays **Gram-positive (with resistance detection) panels** **Hain Lifescience GmbH, Nehren, Germany**	Complexity: 3 Set-up cost: 3 Cost per test: 2	Gram-positive panel: *mecA* Hybridization-based test (LPA) Manual or automated, blood culture TTR, 4.5 h Laboratory	http://www.hain-lifescience.de/en *(30)* https://www.ncbi.nlm.nih.gov/pubmed/22170900
FluoroType MRSA **Hain Lifescience GmbH, Nehren, Germany**	Complexity: 3	MRSA Hybridization-based test Automated, integrated, PCR amplification and probe detection Swabs for MRSA Sensitivity: 100% Specificity: 96.1–99.2% TTR, 2.5 h Laboratory	http://www.hain-lifescience.de/en *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
GenoType/GenoQuick Assays MRSA **Hain Lifescience GmbH, Nehren, Germany**	Complexity: 3	MRSA, *mecA, mecC* Hybridization-based test (LPA) Manual or automated GenoQuick: Sensitivity: 57% Specificity: 100% TTR, 2.5 h GenoType: Sensitivity: 94.59% Specificity: 98.73% TTR, 4 h Laboratory	http://www.hain-lifescience.de/en http://www.hain-lifescience.de/en/products/microbiology/mrsa/genoquick-mrsa.html *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf
IRIDICA BAC BSI **Ibis Biosciences, an Abbott Company, Carlsbad, CA, USA**	Complexity: 3	Panel of 780 bacterial and fungal pathogens *mecA* Amplification-based test (PCR) Blood samples Sensitivity: 88% Specificity: 63% TTR, 8 h Intended for use in clinical laboratory	*(9)* https://www.ncbi.nlm.nih.gov/pubmed/27384540

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
eazyplex MRSA **eazyplex MRSA plus** **Amplex, Giessen, Germany**	Complexity: 3	MRSA *S. aureus, S. epidermidis* *mecA, mecC* Amplification-based test (LAMP) Portable, integrated MRSA detection in pleural and synovial fluids: Sensitivity: 83.3% Specificity: 97.8% TTR, 30 min Laboratory or POC Suitable for use in LMICs	www.eazyplex.com *(31)* https://www.ncbi.nlm.nih.gov/pubmed/28450195
GeneXpert MRSA Assay **Cepheid Corp., now a Danaher company, Sunnyvale, CA, USA**	Complexity: 2	MRSA Amplification-based test (rtPCR) Automated, integrated sample preparation, amplification and detection Sensitivity: 86.9% Specificity: 97.9% TTR, < 66 min Use in Clinical Laboratory Improvement Amendments moderate-complexity laboratory POC potential	http://www.cepheid.com/us/ *(32)* https://www.ncbi.nlm.nih.gov/pubmed/21377748 *(33)* https://www.ncbi.nlm.nih.gov/pubmed/27995869 *(34)* https://www.ncbi.nlm.nih.gov/pubmed/28321579

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
GeneXpert MRSA NxG Assay **Cepheid Corp., now a Danaher company, Sunnyvale,** **CA, USA**	Complexity: 2	MRSA *orfX* SCC*mec* junction and *mecA, mecC* Amplification-based test (rtPCR) Automated integrated sample preparation, amplification and detection Sensitivity: 91.8% (95% CI: 87.4–94.8%) Specificity: 97.2% (95% CI: 96.3–97.9%) TTR, 70 min Use in Clinical Laboratory Improvement Amendments moderate-complexity laboratory	http://www.cepheid.com/us/ *(35)* https://www.ncbi.nlm.nih.gov/pubmed/29118165?dopt=Abstract
GeneSTAT System **DxNA LLC, St George, UT, USA**	Complexity: 1	MRSA Amplification-based test (rtPCR) Portable Also detects multi-drug resistant coagulase-negative *S. aureus* TTR, 60 min Laboratory or POC Suitable for use in LMICs	http://dxna.com https://www.genomeweb.com/pcrsample-prep/dxna-licenses-staph-assay-pathogene-use-genestat-system *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420
Portrait **Great Basin Scientific Inc., Salt Lake City, UT, USA**	Complexity: 1	*S. aureus, mecA* Hybridization-based test Portable, PCR amplification and probe detection in same cartridge	https://gbscience.com *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
		Percentage agreement for *mecA* detection compared with reference methods: Sensitivity: 94.4% (95% CI: 86.6–97.8%) Specificity: 98.8% (95% CI: 97.7–99.4%) TTR, < 3 h Laboratory or POC Suitable for use in LMICs	*(36)* https://www.ncbi.nlm.nih.gov/pubmed/28122871
BD MAX MRSA XT panel **BD MAX StaphSR panel** **BD, Franklin Lakes, NJ, USA**	Complexity: 1	MRSA, *S. aureus* Amplification-based test (rtPCR) Broadest available range of MRSA strains Automated, fully integrated (sample preparation, amplification and detection), nasal swab Sensitivity: 96.5% (95% CI: 92.0–98.5%) Specificity: 96.9% (95% CI: 96.1–97.6%) Moderate-complexity central laboratory	https://www.bd.com/en-us
GeneOhm Staph SF **GeneOhm MRSA ACP** **BD, Franklin Lakes, NJ, USA**	Complexity: 2	MRSA, *S. aureus, mecA* *mecA* and *OrfX* Hybridization-based test Manual sample preparation, no culture step, automated, integrated PCR amplification and detection by probe Blood culture, nasal swabs	*(32, 37)* https://www.ncbi.nlm.nih.gov/pubmed/21377748 https://www.ncbi.nlm.nih.gov/pubmed/2021508148

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
		Sensitivity: 92% Specificity: 94.6% TTR, 2 h Intended for use in Clinical Laboratory Improvement Amendments high-complexity central laboratory	
GenomEra CDX system **Abacus Diagnostica Oy, Turku, Finland**	Complexity: 1	MRSA Amplification-based test (rtPCR) Sensitivity: 100% Specificity: 99.8–100% TTR, 50 min Laboratory or POC Suitable for use in LMICs	www.abacusdiagnostica.com/ *(3)* https://www.ncbi.nlm.nih.gov/pubmed/28343420
MRSA ELITe MGB **ELITech**	Complexity: 2	MRSA Amplification-based test (rtPCR) Semi-automated, nasal swab Sensitivity: 92.3% (95% CI: 88.1–95.2%) Specificity: 95.2% (95% CI: 94.3–95.9%) TTR, 3–5 h Laboratory	https://www.elitechgroup.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
mecA Xpress FISH **OpGen**	Complexity: 3	Hybridization-based test Manual for blood cultures, fluorescence-labelled FISH probes Sensitivity: 98.7% (95% CI: 95.4–99.6%) Specificity: 99.5% (95% CI: 97.0–99.9%) TTR, 70 min Laboratory or POC	http://www.opgen.com *(7)* https://www.gardp.org/wp-content/uploads/2017/05/AMR_Tech_Landscape_Analysis.pdf
Master Diagnostica AMR Direct Flow Chip **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	Detects panel of 20 antimicrobial resistance genes in Gram-positive and Gram-negative bacteria Hybridization-based test (array) Based on multiplex PCR and macroarray chip; no DNA extraction or purification necessary TTR. several hours Laboratory	http://www.oxfordbiosystems.com
Master Diagnostica Sepsis Flow Chip **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	Detects 40 pathogens responsible for sepsis and 20 AMR genes Hybridization-based test (array) Based on multiplex PCR and macroarray chip; no DNA extraction or purification necessary TTR, several hours Laboratory	http://www.oxfordbiosystems.com

Pathogen:	*Staphylococcus aureus*		
Drug Resistance:	**Methicillin**		
Test and provider	**Complexity**	**Details**	**Reference**
AID Diagnostika Line Probe Assay MRSA **Oxford Biosystems, Oxford, United Kingdom**	Complexity: 3	mecA, mecC, differentiation of *S. aureus* and coagulase negative staphylococci Hybridization-based test (LPA) TTR, 5 h Laboratory	http://www.oxfordbiosystems.com

Pathogen:	*Neisseria gonorrhoeae*
Drug Resistance:	**Extended-spectrum cephalosporins** **Macrolides** **Fluoroquinolones** **Aminoglycosides**
Currently no molecular diagnostic tests available	

Pathogen:	*Streptococcus pneumoniae*
Drug Resistance:	**Penicillins** **Sulfonamides and trimethoprim** **Extended-spectrum cephalosporins**
Currently no molecular diagnostic tests available	

[1]**Disclaimer: The molecular diagnostic tests listed in the table are marked as conforming to the standards of the European Union and European Economic Area (CE) or are approved by the USFDA. Other molecular diagnostic tests are being developed and validated, and future versions of the table will update the list of validated tests, including tests approved by other regulatory agencies.**

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Annex 2.

Description of molecular methods for AMR diagnostics

Amplification-based methods

Amplification tests produce many copies from a target sequence of DNA (amplification). This enables the detection of specific pieces of DNA, for example, antimicrobial resistance (AMR) genes, by incorporating fluorescent labels during amplification or subsequent electrophoresis.

Polymerase chain reaction

Polymerase chain reaction (PCR) assays are highly sensitive and specific and can be used to detect the presence of a number of genes associated with resistance simultaneously. They are relatively easy to use and may be available as portable, automated, all-in-one devices that require minimal training. Some PCR assays are designed to detect a specific resistance gene in a specific pathogen, whereas multiplex PCR assays can detect multiple resistance genes and/or pathogens at the same time. Moreover, quantitative PCR (qPCR) can be used to quantify the pathogen-burden in an infected individual, by incorporation of a fluorescent dye into amplified DNA segments and measurement of the ensuing fluorescent signal. The main advantage of qPCR for gene detection is its rapidity compared to PCR, as it can monitor DNA amplification in real time and does not require electrophoresis.

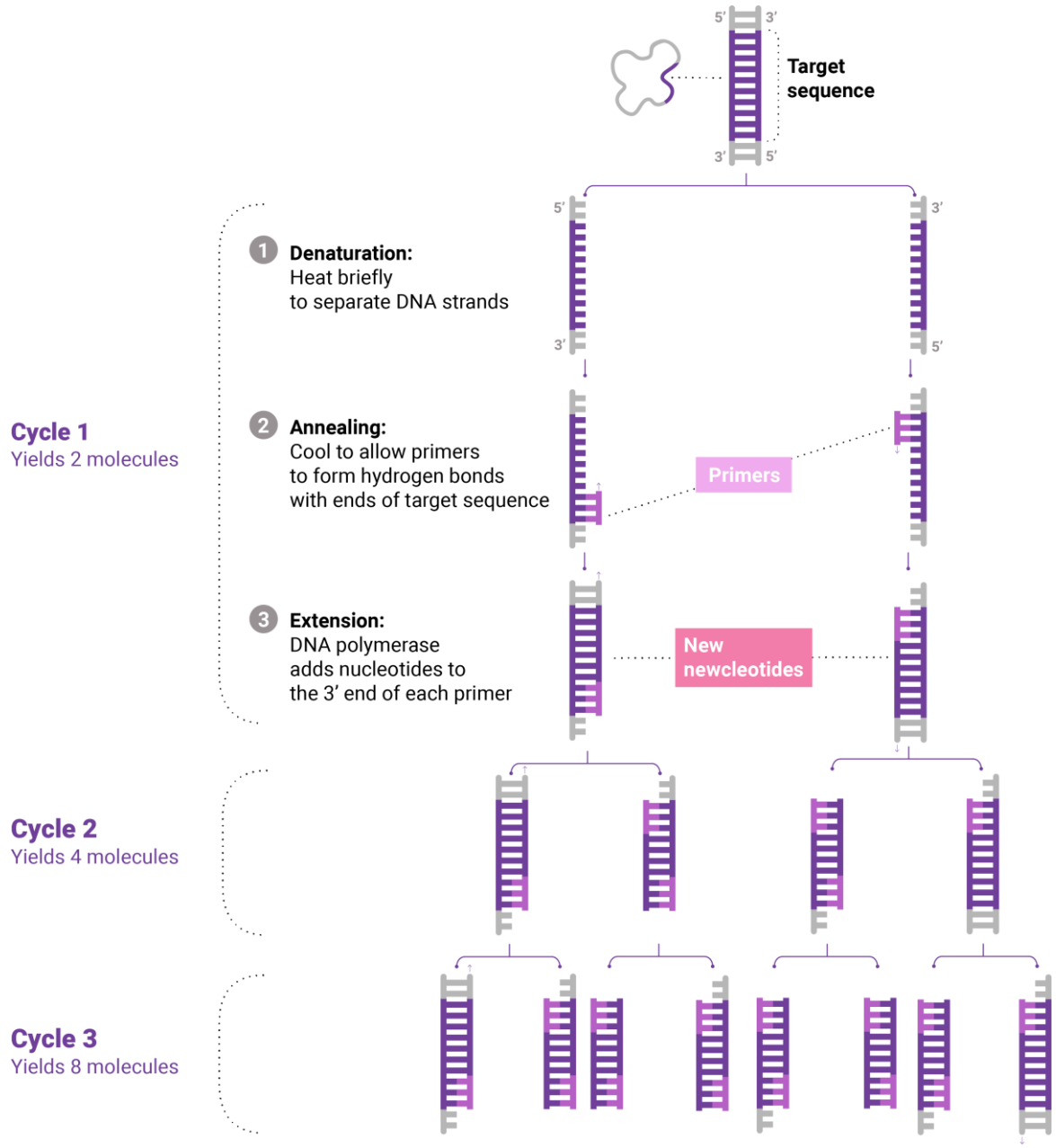
The principle of PCR is similar to that of natural DNA replication in cells, in which each of the two complementary DNA strands can act as a template for the synthesis of a new strand. The enzyme that synthesizes the new strand along the template strand is called “DNA polymerase” (a heat-resistant enzyme first found in the thermophilic bacterium *Thermus aquaticus*). Because of chemical constraints, DNA synthesis can occur in only one direction, from the 5´ to the 3´ end of the new strand. DNA polymerase requires at least a small starting segment (primer) of the new strand to start adding nucleotides, the building blocks of DNA. When the right primer sequence is chosen, only the sequences of interest are amplified.

PCR consists of several subsequent reaction cycles in each of which the number of targeted DNA molecules is doubled, resulting in an exponential increase in the number of targeted DNA molecules. Each cycle consists of three steps (Fig. A2.1):

- denaturation, in which the reaction mix is heated to melt the DNA, i.e. to separate the complementary double strands;
- annealing, in which the reaction mix is cooled to allow the primers to bind to the regions flanking the target region; and
- extension, in which DNA polymerase adds nucleotides to the 3´ end of each primer.

In each PCR cycle, therefore, the number of targeted DNA sequences doubles. Starting from one DNA molecule, amplification cycle 1 yields two copies of the target sequence, cycle 2 produces four copies, cycle 3 eight copies and so on.

Fig. A2.1. Polymerase chain reaction.



Sequence-specific primers (light purple) can be used to amplify target DNA sequences (dark purple) such as those of antimicrobial resistance genes.

The amplified DNA can be subjected to electrophoresis for detection or used as part of a hybridization-based assay in the next step. In real-time PCR, the extension and detection steps are coupled, for example, by use of fluorescence-labelled nucleotides or intercalating dyes and detection of the fluorescent signal of the growing strand. An example of use of multiplex PCR assay is for detection of extended-spectrum □-lactamase (ESBL) in Enterobacteriaceae and in *Acinetobacter* spp.

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) assays are an alternative to PCR for amplifying DNA sequences. The principle of LAMP is similar to that of PCR but involves a DNA polymerase with high strand displacement activity (*Bst* DNA polymerase). Consequently, a DNA denaturation step is not required, and amplification can occur at constant temperature (60–65 °C); LAMP does not require a thermal cycler.

Four types of primer are used, two inner and two outer primers, which makes the assay very specific. Two loop primers can be added to accelerate the amplification reaction, so that 10^9 amplified sequences can be generated within half an hour *(1, 2)*. LAMP amplification includes one cyclic and one non-cyclic step. In the non-cyclic step, dumbbell-shaped DNA stem-loops are formed at each end of the DNA sequence, flanked by the primers. These structures serve as the starting material for the cyclic step. Most target amplification occurs in the cyclic step with internal primers. Amplified sequences are linked via primer sequences. The end products of LAMP are alternately inverted repeats of the target sequence on the same DNA strand, which form cauliflower-shaped structures.

LAMP is less vulnerable to inhibitors that may be present in complex samples (e.g. sample matrices), which makes it more suitable for low-resource settings and field conditions than PCR. Magnesium pyrophosphate, a by-product generated during amplification, increases the turbidity of the fluid in the reaction tube, so that successful amplification can be identified with the naked eye. Intercalating fluorescent dyes can be used for real-time detection, as with PCR.

An example of use of LAMP is a multiplex LAMP assay for the detection of carbapenemases in Enterobacteriaceae.

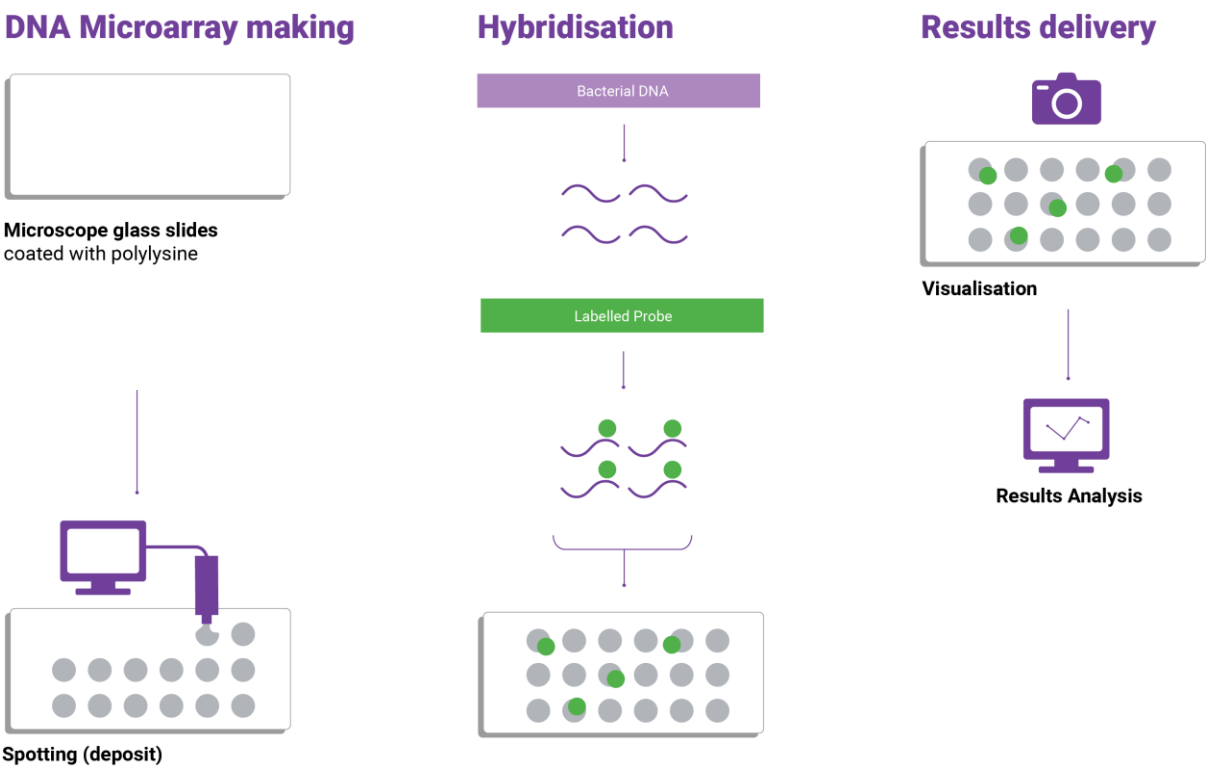
Hybridization-based methods

Hybridization assays are used to detect AMR genes by hybridizing them to labelled probes. The tests can be further subdivided into those based on arrays, line probe assays (LPAs) and fluorescent in situ hybridization (FISH). The common principle of these methods is detection of specific DNA (e.g. resistance genes) by labelled probes or target sequences. Arrays and LPAs are designed to detect several target sequences simultaneously. Hybridization is preceded by an amplification step, to ensure that enough DNA is present in the sample to be detected.

Array-based methods

DNA arrays can be used to detect resistance genes, some mutations and bacterial species markers as part of molecular diagnostic assays. An array consists of spots of the DNA sequences of interest attached to a solid surface in a known configuration (Fig. A2.2). The DNA in the spots is single-stranded, so that complementary DNA from a denatured sample can bind. In “denatured” DNA, the two strands have been separated, usually by heat treatment. The DNA in the sample is labelled with a fluorescent marker (and possibly amplified by PCR) and added to the array (hybridization). After unbound DNA is washed off, a laser scanner can detect the spots to which sample DNA has bound and identify the genes present in the sample. As arrays can potentially detect many different genes, they are most useful in AMR diagnostics when used as part of large panels to test for various pathogens and resistance gene combinations.
An example of their use is for identifying species of pathogens and AMR genes in bloodstream infections.

Fig. A2. Array-based assays for detection of genes



Cy3 and Cy5, green and red fluorescent dyes; PMT, photomultiplier tube for detection of weak light signals

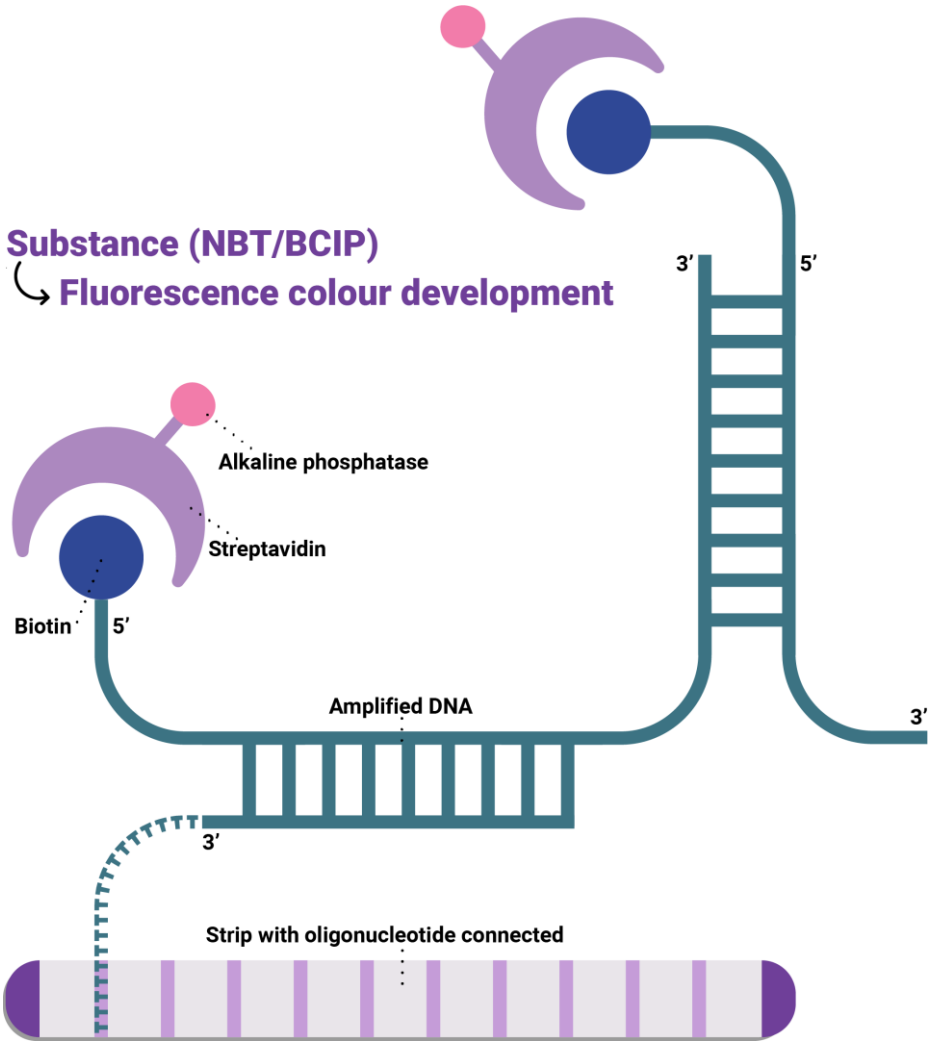
Line probe assays

In LPAs, PCR is followed by reverse hybridization on strips coated with DNA probes arranged in discrete bands (Fig. A2.3). LPAs require amplification of DNA extracted from a clinical sample, during which a biotin tag is added to each sample DNA sequence. The amplified DNA is denatured, and single-stranded DNA probes for resistance genes or bacterial species markers are attached to a strip, to which they will bind if they are complementary to the probe sequences. The location of each probe along the strip is known. Unbound sample DNA is washed off, and alkaline phosphatase with a streptavidin anchor is added to the strip, which binds to the biotin-tagged DNA sequences. Next, nitro blue tetrazolium (NCT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) are added, which cleave to alkaline phosphatase, turning it into a dark-blue dye, which stains the bands to which sample DNA has bound.

The presence of resistance genes can be inferred by comparing the pattern of coloured bands on the test strip to that on a template.

An example of use of LPA is to identify multi-resistant *Staphylococcus aureus* (MRSA) in blood cultures.

Fig. A2.3. Line probe assays

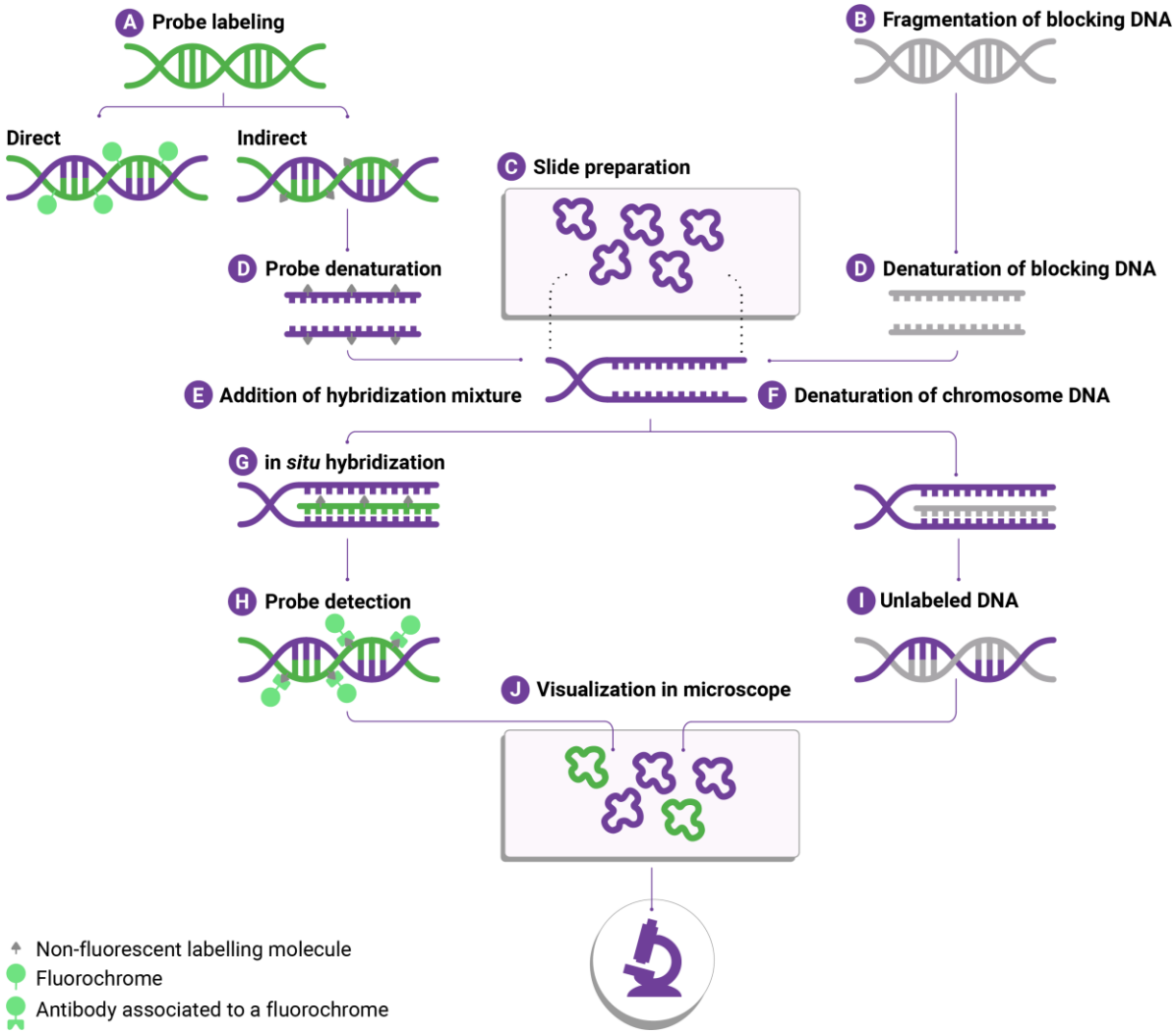


Blue circle, biotin tag; pink circle, alkaline phosphatase; purple crescent, streptavidin anchor.

Fluorescence in situ hybridization

In FISH, fluorescent DNA probes are used to detect the presence of resistance genes directly in bacterial cells isolated from patients (Fig. A2.4). FISH is commonly used to detect certain mutations in cancer cells and in developmental biology to detect mutations that can lead to birth defects. Cells from samples are smeared on microscope slides, and fluorescence-labelled probes are added. Both the probes and the bacterial genomic DNA must be denatured to allow the probe to bind to its target sequence in situ. After hybridization, all remaining probe is washed off, and a fluorescence laser microscope is used to visualize probes binding to target sequences and to detect the presence of resistance genes. Cheaper alternatives for visualization are mercury vapour bulbs and light-emitting diodes *(3)*. An example of use of FISH is to identify MRSA directly in a sample.

Fig. A2.4. Fluorescent in situ hybridization



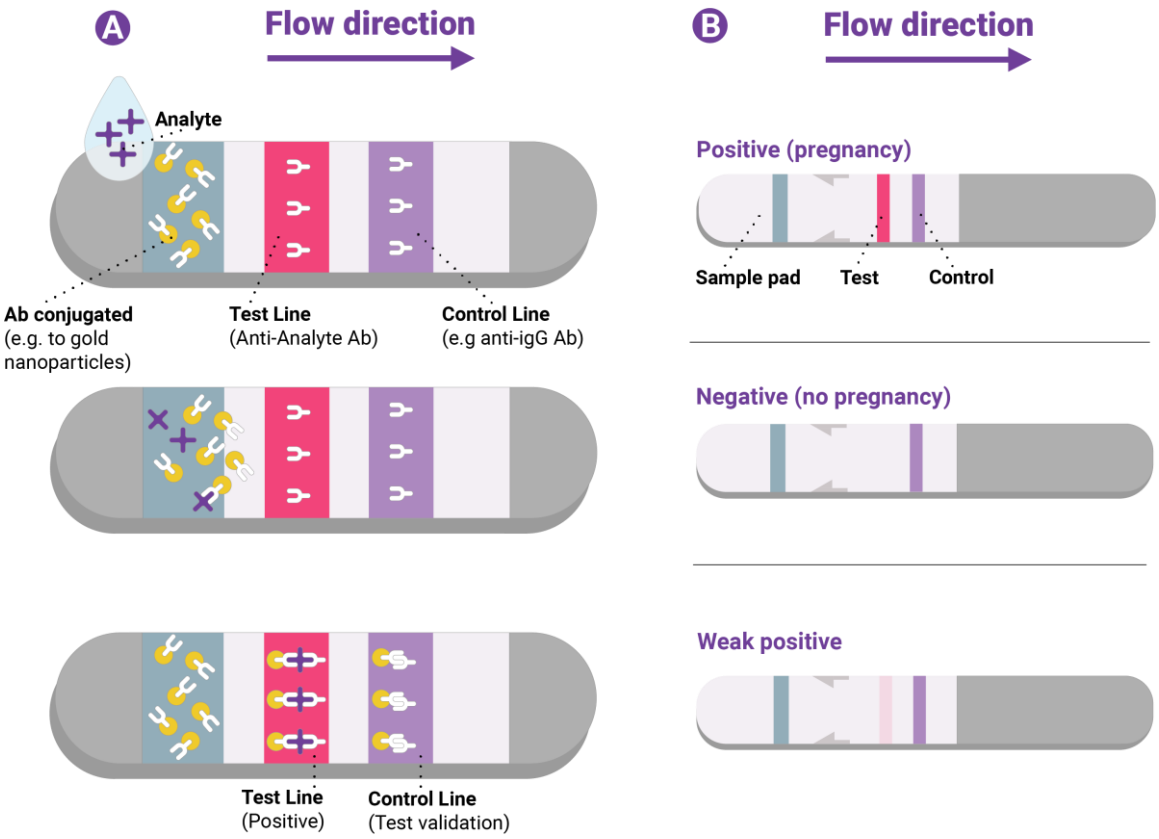
Immunoassays

Lateral flow assays (antibody-dependent)

Immunoassays for the detection of resistance markers are based on lateral flow chromatography. The format of the tests is often similar to that of pregnancy tests. Lateral flow immunoassays (LFIAs) include antibodies that recognize proteins encoded by resistance genes (Fig. A2.5). For example, although various carbapenemase genes can make bacteria resistant to carbapenem antimicrobials, the carbapenemase enzymes that they encode have different amino acid sequences and so can be distinguished by specific antibodies. As LFIAs can be applied directly to a liquid sample (e.g. a suspended bacterial colony) and do not require PCR amplification, they are rapid (results within 15 min) and easy to use. If the starting material is a bacterial colony, however, the test can be performed only 18–48 h after the specimen has been processed in a laboratory.

Nucleic acid lateral flow assays are similar to LPAs but are used to detect PCR-amplified DNA segments with antibodies rather than with hybridization probes. During PCR, antigenic labels are incorporated into DNA segments that can subsequently be recognized by antibodies in a lateral flow cell. A liquid sample is added to one end of a membrane device or strip that contains antibodies conjugated to coloured fluorescent or gold particles that recognize and bind specifically to the molecules of interest, e.g. resistance gene DNA sequences or mutant proteins. As the sample migrates through the membrane, it binds to the conjugated antibodies. Coloured conjugate markers accumulate in the detection zone, and a coloured line forms that signals the presence of resistance markers. If no resistance markers are present, no coloured line forms. A control line indicates proper liquid flow through the membrane. The colour signal may be of varying intensity and can be assessed by eye or with a reader *(4)*. An example of use of LFIA is for the detection of epitopes of *Klebsiella pneumoniae* carbapenemase in cultured isolates.

Fig. A2.5. Lateral flow immunoassays



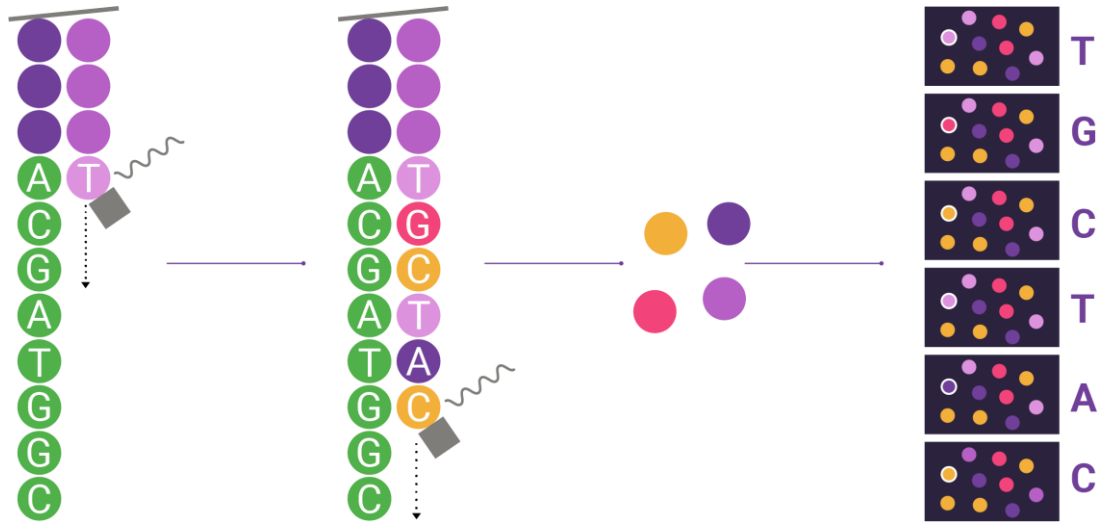
A. The liquid sample extract is added to one end of a porous membrane and migrates to the other end. The membrane contains antibodies conjugated to coloured fluorescent or gold particles that recognise and bind to the molecules of interest, e.g. in the detection zone (test line, red), immobilized antibodies bind to the complex of conjugate antibodies (Ab) and resistance markers. Coloured conjugate markers accumulate in the detection zone. The control line (purple) indicates that the sample is flowing properly through the membrane. B. Example of results on a test strip. The upper figure shows a positive result, the middle figure a negative result and the lower figure a weak positive result, e.g. little of the resistance-causing protein is expressed. To avoid reporting a false-positive result, a test showing a weak positive may be repeated.

Whole-genome sequencing

Whole-genome sequencing (WGS) provides information on the "entire" genome of a bacterial isolate. Several techniques exist for sequencing whole genomes. The most commonly used is Illumina dye sequencing, in which a "sequence by synthesis" approach is used (Fig. A2.6). Other methods are pyrosequencing and single molecule real-time sequencing. In a first step, genomic DNA is cut randomly into short segments to serve as templates for DNA synthesis and is attached to the inner surface of a flow cell, in which sequencing will take place. After several pre-processing steps, the main sequencing cycle begins. The principle of sequencing by synthesis is that the DNA sequence is inferred by using different fluorescent dyes for different nucleotide species. At each synthesis step, only one nucleotide is incorporated by attaching reversible terminators. When a fluorescence-labelled nucleotide is incorporated into the DNA sequence, it emits a characteristic wavelength that is recorded on a photodetector. The reversible terminator is then enzymatically cleaved to allow addition of the labelled nucleotide in the next elongation cycle. By recording which fluorescence-labelled nucleotide is incorporated into the growing DNA strand during each elongation cycle, the sequence of each DNA fragment can be determined.

The sequence of the whole genome is assembled by finding overlapping fragment sequences. The occurrence of mutations and the presence of known resistance genes and variations of these genes can be determined by comparing the sequence of a bacterial genome with reference databases. WGS provides a huge amount of information but involves complex technology, and training is necessary to analyse and interpret results. An example of use of WGS is for identification of the genes that cause resistance to ESBLs in Enterobacteriaceae.

Fig. A2.6. Example of whole-genome sequencing with Illumina dye sequencing.



Segments of cut genomic DNA are attached to the inside of a flow cell (left panels). The sequence of a DNA segment can be determined by adding fluorescence-labelled nucleotides into the newly synthesized strand. A different fluorescent dye is used for each nucleotide species (A, T, G, C), and, during each elongation cycle, only one nucleotide is added. A photodetector (grey box) records the wavelength emitted by the newly incorporated nucleotide (wavy line). Then, the next elongation cycle starts, another nucleotide is added and recorded and so on, until the entire segment is sequenced. Inside the flow cell, many DNA segments are attached to the surface next to each other and can be sequenced in parallel. The photodetector records which nucleotide is incorporated into each segment during each elongation cycle (right panel, white circle highlights one DNA segment followed through six subsequent elongation cycles during each of which one nucleotide is added).

References for Annex 2.

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Annex 3.

Weblinks to FDA and CE guidelines for molecular diagnostic devices

Requirements for marking of medical devices as conforming to the standards of the European Union and European Economic Area (CE):
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32017R0746

Food and Drug Administration Clinical Laboratory Improvement Amendments guidelines on clearance of molecular diagnostic devices:
https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm393229.htm

Requirements of procedure for applying for waiver from Clinical Laboratory Improvement Amendments:
https://www.fda.gov/MedicalDevices/ucm079632.htm