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Antimicrobial Susceptibility Testing: An Updated Primer for Clinicians in the Era of Antimicrobial Resistance: Insights from the Society of Infectious Diseases Pharmacists

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ABSTRACT

Antimicrobial susceptibility testing (AST) is a critical function of the clinical microbiology laboratory and is essential for optimizing care of patients with infectious diseases, monitoring antimicrobial resistance (AMR) trends, and informing public health initiatives. Several methods are available for performing AST including broth microdilution, agar dilution, and disk diffusion. Technological advances such as the development of commercial automated susceptibility testing platforms and the advent of rapid diagnostic tests have improved the rapidity, robustness, and clinical application of AST. Numerous accrediting and regulatory agencies are involved in the process of AST and setting and revising breakpoints, including the U.S. Food and Drug Administration and the Clinical and Laboratory Standards Institute. Challenges to optimizing AST include the emergence of new resistance mechanisms, the development of new antimicrobial agents, and generation of new data requiring updates and revisions to established methods and breakpoints. Together the challenges in AST methods and their interpretation create important opportunities for well-informed clinicians to improve patient outcomes and provide value to antimicrobial stewardship programs, especially in the setting of rapidly changing and increasing AMR. Addressing AST challenges will involve continued development of new technologies along with collaboration between clinicians and the laboratory to facilitate optimal antimicrobial use, combat the increasing burden of AMR, and inform the development of novel antimicrobials. This updated primer serves to reinforce important principles of AST, and to provide guidance on their implementation and optimization.

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INTRODUCTION

The importance of assessing the effect of an antimicrobial on the growth of bacteria was recognized long before antimicrobials were even considered therapeutic agents. Alexander Fleming developed the first liquid media dilution-based testing method and was a pioneer in the field of antimicrobial susceptibility testing (AST) more than a decade before the penicillin he discovered was available for clinical use (1). Since that time, AST has become a fundamental tenet of infectious diseases patient management, antimicrobial drug development, epidemiology, public health, and clinical microbiology. Still, many of the AST methods in use today were developed 50-100 years ago and are reliant on phenotypic (growth-based) testing, which requires 48 to 72 hours from the time of culture collection. This delay necessitates the use of empiric, untargeted antimicrobial therapy that further contributes to antimicrobial resistance (AMR) when overly broad (2). When too narrow, especially in patients infected with resistant pathogens, delays in AST result in delays in time to administration of effective antimicrobial therapy, leading to increased mortality (3-5). Fortunately, technological improvements have led to advanced assays, such as automated conventional phenotypic AST systems, genotypic (molecular), and phenotypic rapid diagnostic tests (RDTs). These systems are capable of decreasing the time to effective and optimal antimicrobial therapy, thereby concomitantly improving patient outcomes and antimicrobial use (6, 7). In addition to improved diagnostic capabilities, AMR has also spurred the development of novel antimicrobial agents with the ability to combat these resistant pathogens. While these agents are a welcomed addition to the therapeutic armamentarium, implementing AST for them can be especially challenging for clinical laboratories due to the need for manual methods such as disk diffusion, in-house verification of those methods, and development of new workflows for when these antimicrobials should be tested (8).

Battling AMR while improving patient outcomes and appropriate antimicrobial use requires a comprehensive approach built principally on robust clinical microbiology and

antimicrobial stewardship (AMS) foundations. The ability to contain and combat AMR is directly related to the capacity of the microbiology laboratory; with molecular typing, surveillance, resistance characterization, and AST capabilities demonstrating the most significant impact on AMR rates (9). These same capabilities are essential to clinicians fighting to decrease mortality and improve clinical and economic outcomes by reducing delays in time to effective and optimal antimicrobial therapy, especially among patients with resistant pathogens (3, 4, 10). While it is well recognized that strong collaboration between clinicians and laboratorians improves patient care, significant challenges exist within and between the laboratory and clinical environments that impede optimal care. Although these challenges are complex and multifactorial, many relate to an underappreciation of the importance of developing and implementing microbial diagnostics by healthcare authorities and impediments created by the regulatory landscape (11-14). Moreover, clinicians often struggle to properly interpret and/or apply laboratory results, especially related to microbiology and AST due to a lack of knowledge, experience, and/or confidence (15, 16). Addressing these challenges is especially pertinent currently as the prevalence and complexity of AMR continues to increase and diagnostic innovations further expand the landscape of infectious diseases testing. Therefore, the purpose of this work is to provide an update to the previously published 2009 version of this paper with a particular focus on bacterial AST in the era of AMR in order to improve clinicians' understanding of AST and how best to incorporate it into their practice (17).

AST METHODS

Phenotypic AST detects bacterial cell growth arrest or death in the presence of an antimicrobial therefore allowing true susceptibility testing and can be categorized into either minimal inhibitory concentration (MIC) or non-MIC methods. Quantitative MIC methods (e.g., broth dilution, agar dilution, agar gradient diffusion) express results numerically whereas qualitative non-MIC methods (e.g., disk diffusion) allow for broad categorization by interpretive criteria into susceptible, susceptible-dose dependent, intermediate, non-susceptible or resistant

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interpretive categories without an MIC value **(Figure 1)**. For a more detailed description of phenotypic methods displayed in Figure 1, the reader is directed to the review by Kuper et al which covers most methods in detail, with the exception of the broth disk elution method which is described in detail in the "Quantitative MIC Methods" section of this review (17). Genotypic AST identifies resistance genes via molecular methods and serves as a surrogate for AST although susceptibility must be confirmed phenotypically (11). Regardless of the specific method, the completion of traditional phenotypic AST represents the culmination of the time- and labor-intensive clinical microbiology culture, identification, and susceptibility testing process for bacteria. The median [interquartile range] time from specimen collection to Gram stain, organism identification, and AST is 19 [15-26] hours, 43 [32-59] hours, and 65 [59-72] hours, respectively, for the typical blood culture processing workflow using conventional methods in U.S. laboratories (18). As patients receive empiric antimicrobial therapy for the duration of this period, streamlining this process wherever possible is crucial to optimizing therapy. The most effective approach to date for shortening the time from blood culture collection to receipt of actionable microbial ID and/or AST results has been the introduction of RDTs, many of which can produce a result in under 24 hours and dramatically impact clinical, economic, and antimicrobial stewardship outcomes (19). The workflow and timing of the microbiology process with and without the use of RDTs is illustrated in **Figure 2**, and each of these methods is discussed below. Importantly, at present these RDTs are used to augment, not replace, the conventional microbiological process and the greatest clinical benefit is realized when they are used in conjunction to inform optimal antimicrobial use. For example, a conventional phenotypic AST result demonstrating a carbapenem-resistant *Klebsiella pneumoniae* would typically prompt further testing of novel agents with activity against this phenotype such as ceftazidime-avibactam, meropenem-vaborbactam, and/or cefiderocol, often adding up to 24 hours to the AST process and further delaying optimal therapy. Incorporating a molecular or phenotypic RDT capable of identifying the enzyme responsible for carbapenem resistance in this isolate (e.g.,

NDM) into the lab workflow could improve time to appropriate antibiotic therapy and avoid unnecessary additional phenotypic AST testing for agents without activity against metallo-β-lactamases (20, 21).

Quantitative MIC methods

Manual broth microdilution (BMD) is one of the gold standard reference methods per the Clinical and Laboratory Standards Institute (CLSI) and other breakpoint-setting organizations, such as the U.S. Food and Drug Administration (FDA) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). It involves the use of microtiter trays and allows for multiple antibiotics to be tested in a range of 2-fold/$\log_2$ serial dilutions (i.e., 2, 4, 8 µg/mL) **(Figure 1A)** (22, 23). These microtiter trays are often prepared using prespecified aliquots of antibiotics and some pre-made versions are commercially available (22, 23). A bacterial suspension of the test organism in liquid media at standard inoculum is then added to the tray with the drug and incubated overnight, typically for 16-20 hours as is standard for most phenotypic AST methods. BMD has the advantage of providing an MIC that is accurate and reproducible; however, newly approved antimicrobials are often not initially included in commercial BMD panels (22, 23). As the time and labor required for manual BMD is not feasible for routine AST, most (>95%) clinical microbiology laboratories rely on automated conventional phenotypic AST platforms, which utilize a miniaturized BMD-based method and automated pathogen identification, AST, and interpretation within a single system (24, 25). There are currently four FDA-cleared automated conventional phenotypic AST systems for bacterial isolate testing: MicroScan WalkAway (Beckman Coulter Inc., Brea, CA), Phoenix™ (Becton, Dickinson and Company, Franklin Lakes, NJ), Sensititre™ (ThermoFisher, Waltham, MA), and Vitek2® (bioMérieux, Marcy-L'Etoile, France). Each of these four systems is capable of full AST automation from inoculation to interpretation, albeit by different methodologies resulting in varying time to result. The MicroScan, Phoenix, and Sensititre systems measure MICs directly by assessing bacterial growth in the presence of the antimicrobial included in each well of the

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respective panel. The Vitek system provides calculated, rather than measured, MICs by comparing growth of the test isolate in the presence of a given agent to growth of reference isolate with a known MIC using a limited number of antimicrobial drug dilutions. The position and functionality of automated conventional phenotypic AST systems within the microbiology workflow are depicted in **Figure 2.**

Agar dilution, another gold standard reference method for some organism-antimicrobial combinations, involves the addition of the antibiotic to the agar medium at 2-fold serial concentrations, inoculation with the test organism, and overnight incubation and reading **(Figure 1B)** (26). Agar dilution is also a labor- and time-intensive method and is not a feasible option for many clinical microbiology laboratories although commercial agar dilution panels have recently become available for certain organism-antimicrobial combinations (e.g., Liofilchem®, Waltham, MA).

Agar gradient diffusion strips, such as the Etest (bioMérieux, Durham, NC) or MIC test strip (MTS; Liofilchem®), use an established antimicrobial concentration gradient impregnated within a gradient strip, as a method of establishing an MIC [13]. The antimicrobial diffuses from the strip across the surface of the agar, inhibiting microorganism growth by the drug. The MIC value is then usually read after 16-20 hours of incubation at the inhibition of bacterial growth where the pointed end of the ellipse intersects the diffusion strip **(Figure 1C)**. In this case, unlike BMD, an “exact” MIC falling between two traditional $\log_2$ dilutions can be read and reported (e.g. 12 µg/mL) although rounding the MIC to the next highest $\log_2$ dilution for reporting may be recommended by the manufacturer. Manufacturer recommendations must be followed for FDA-cleared assays, unless a modification of the FDA clearance has been verified by the laboratory. The disadvantages to agar gradient diffusion strips, however, are that they are manual, are not reference methods, are relatively more expensive ($3-9/strip), and are subject to inter-reader variability in interpretation (23).

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The broth disk elution (BDE) method is an older AST method recently reintroduced for the testing of historically problematic agents like the polymyxins. Colistin AST has been challenging for multiple reasons, including the physiochemical properties of the drug leading to poor diffusion through agar-based media, adsorption to negatively charged plastics (e.g., pipette tips and polystyrene tubes and plates), and unacceptably high error rates which preclude disk or agar gradient diffusion methods (27). These issues are mitigated using the BDE method in which colistin disks are added to liquid Mueller-Hinton broth in tubes such that the final drug concentration in each tube approximates the serial $\log_2$ dilutions used in BMD testing **(Figure 1D)**. After colistin elutes from the disks into the broth, a standard inoculum of bacteria is added to the tubes and, after appropriate incubation, the MIC is read as the lowest concentration inhibiting growth of the test isolate. This method has yet to be formally evaluated for other antimicrobials (28).

Qualitative non-MIC methods

Disk diffusion is a standard method and results in a zone of inhibition of bacterial growth that is then measured in millimeters and translated into an interpretive category based on the breakpoint. For this method, a disk containing a fixed concentration of antimicrobial is placed onto an agar plate after it has been streaked with a standardized bacterial inoculum **(Figure 1E)** [7]. After 16-20 hours of incubation, the diameter of the zone of inhibition of bacterial growth is measured. Disk diffusion is often the first AST method commercially available for testing bacteria against new antimicrobial agents. It is an accurate method, although with the downside of not providing an MIC, which is crucial for dose optimization in certain situations. Therefore, disk zones of inhibition must be correlated with MIC values generated via BMD when setting and revising clinical breakpoints with the goal of minimizing categorical errors as discussed further below (29).

Special media requirements for AST

Media requirements for AST vary according to the organism and/or antimicrobial agent being tested. Certain fastidious organisms require extra nutrients added to the media for growth, such as *Streptococcus pneumoniae*, β-hemolytic streptococci, viridans group streptococci, and *Neisseria meningitidis* which all require lysed horse blood. Testing of daptomycin requires addition of calcium within either the medium or the test strip for adequate MIC determination due to daptomycin's calcium-dependent activity. The siderophore cephalosporin cefiderocol accesses the bacterial periplasmic space through active transport binding of iron. Given this mode of action, iron-depleted conditions are required for broth microdilution testing of cefiderocol since iron transporters are upregulated under these conditions, leading to uptake of cefiderocol to exert its action. Other media requirements such as cation adjustment and thymidine content must be tightly controlled and are covered in depth in CLSI AST methods document M02 and M07 (26, 30).

Rapid methods

Significant technological advances have been made to provide clinicians and microbiologists with more rapid organism identification and/or detection of antibiotic resistance determinants harbored in pathogens of interest. Currently, there are several commercially available RDTs capable of identifying pathogens and/or detecting antibiotic resistance from various specimen samples such as blood **(Table 1),** sputum, urine, bronchoalveolar lavage fluid, cerebrospinal fluid, and the gastrointestinal tract (31-34). Compared with the conventional microbiology process, these RDTs have significantly reduced the turnaround time for bacterial identification and susceptibility results from several days to a few hours allowing for more rapid optimization of therapy. The capabilities and timing of these different RDTs in the microbiological workflow are shown in **Figure 2**. Currently, only two assays, the T2Bacteria® and T2Candida® panels (T2 Biosystems, Lexington, MA), are FDA-cleared to be performed directly on the index blood culture (i.e., before the blood culture bottle flags positive for growth), while most are run after the blood culture bottle flags positive for growth and the Gram stain is

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performed (**Table 1).** Several of these systems also allow users to run tests on an as-needed basis and include scalable modules to allow laboratories to customize tests based on their capacity. The costs of these devices are significant; however, well-designed studies have demonstrated their cost-effectiveness stemming largely from improved antimicrobial usage and reductions in hospital length of stay (6, 35). Ultimately, the full benefit of these devices on patient outcomes is seen when they are integrated with AMS programs and infection control policies (36).

Although genotypic RDTs have demonstrated significant improvements in clinical and economic outcomes when combined with ASP intervention, especially in high-risk patients such as those with bacteremia, optimal use of these assays is labor- and time-consuming as the results require interpretation into treatment recommendations before they can be applied. Additionally, there is often a hesitancy among clinicians to de-escalate antimicrobial therapy based on the result of a genotypic RDT alone given the potential for a mismatch between genotype and phenotype (37). Therefore, there is a need to improve the rapidity of phenotypic AST results and pair them with genotypic AST results when possible to allow for more widespread use, reduced workload on ASPs, and to mitigate concerns regarding genotype-phenotype mismatches. One successful approach endorsed for a number of pathogen-antibiotic combinations is the disk diffusion method performed directly from positive blood culture broth (38, 39). Direct disk diffusion testing from blood demonstrated acceptable agreement when compared to reference disk diffusion, in as little as 6 hours, but there has been significant heterogeneity in methods used throughout the literature. Thus, this technique in the past was heavily impacted by experimental variables including the size of the bacterial inoculum after incubation, the reader, and drug being tested if standard methods were not followed (39, 40) **(Table 2).** In an early CLSI pilot study using a panel of 20 isolates of Enterobacterales, *A. baumannii*, and *P. aeruginosa* with resistant phenotypes including carbapenem resistance, direct disk diffusion from blood performed well with a categorical agreement of 87.4% at the 18-

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hour time point (41). After additional multi-center studies of more isolates using standardized methodology supported this method, CLSI approved several antimicrobial agents for direct disk diffusion from blood cultures positive for Enterobacterales or *P. aeruginosa*. CLSI-approved pathogen-antibiotic combinations for direct disk diffusion are displayed in **Table 2,** stratified by appropriate read times based on meeting CLSI acceptance criteria for one or more time points.

Currently, the only FDA-cleared phenotypic RDT for blood culture samples is the PhenoTest® BC system (Accelerate Diagnostics, Inc., Tucson, AZ). It capitalizes on some of the limitations of genotypic-based systems by providing a user-friendly output in the form of an AST report, similar to those clinicians are used to seeing from automated conventional phenotypic AST systems, within ~7 hours from culture positivity. In addition to demonstrating improved clinical and ASP outcomes, the PhenoTest® BC system may reduce or eliminate the need for additional real time ASP intervention as suggested for genotypic RDTs, promote rapid de-escalation, and potentially lead to shorter length of stay and costs (42-45).

IMPLEMENTING AND OPTIMIZING AST

It is critical that the emphasis on refining AST methods is complemented by the effort to use AST results optimally with a thorough understanding of the pertinent strengths and deficiencies. The most notable and influential limitation of all phenotypic AST methods is the inherent imprecision due primarily to significant inter-strain and inter-laboratory variability (46). Phenotypic MIC methods are accurate to within ± 1-$\log_2$ dilution, creating challenges for the laboratory when performing AST (especially during validation or verification studies) and when reporting results to clinicians. This accuracy is judged primarily as categorical agreement (CA) by determining the rates of false susceptibility (very major error (VME)) or false resistance (major error (ME)) reported by the test method compared to the reference method (47). This variability is particularly problematic for antimicrobials with MIC distributions clustered around the clinical breakpoints, for drugs with narrow therapeutic ranges, and when using commercial AST methods rather than CLSI reference methods. For example, the revised CLSI breakpoints

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for piperacillin-tazobactam (PTZ) against the Enterobacterales are susceptible (S) ≤8/4 µg/mL, susceptible-dose dependent (SDD) 16/4 µg/mL, and resistant (R) ≥32/4 µg/mL (48). Therefore, an *Escherichia coli* with a piperacillin-tazobactam MIC of 16/4 µg/mL interpreted as SDD on initial testing may yield an MIC of 8/4, 16/4, or 32/4 µg/mL on repeat testing, all of which are within the acceptable error of MIC testing but result in disparate categorical interpretations of S, SDD, and R for the same isolate. In a recent study including 284 Enterobacterales and 28 *P. aeruginosa* using the revised 2023 CLSI PTZ breakpoints for both species (49), rates of CA and VME, respectively, were 80% and 31% for MicroScan, 84% and 4% for Phoenix, and 94% and 6% for Vitek2 (50). These error rates can therefore be problematic for clinicians attempting to make treatment and/or dosing decisions based on these results; highlighting the importance of good communication between the laboratory, clinicians, and antimicrobial stewards. Although the accuracy of commercial AST systems typically improves over time as adjustments are made, the emergence of new resistance mechanisms may challenge the system's capabilities and require recalibration and/or release of updated panels which can take months to years. In addition to avoiding potential negative outcomes, strong collaboration between clinicians and the microbiology laboratory can help clinicians utilize AST results more effectively via a high-yield, low-cost antimicrobial stewardship intervention via cascade or selective reporting of AST results for example (51, 52). At the population level, increased synergy between clinicians and microbiologists through CLSI has helped produce more comprehensive breakpoint recommendations that have shifted clinical practice and positively impacted patient outcomes, such as the revised daptomycin breakpoints for enterococci (53-56). This cooperation will continue to be essential moving forward to ensure the technological strides made in areas such as whole genome sequencing and pharmacokinetic/pharmacodynamic (PK/PD) modeling can keep pace with the increasing complexity and prevalence of AMR and new drug development. On a global level, increased awareness and appreciation for the importance of AST through

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better communication should improve collaboration between key stakeholders and help identify solutions needed to maximize the availability and use of AST platforms.

Standards development organizations

The 21st Century Cures Act, signed into law in 2016, was intended to accelerate medical product development and bring new innovations and advances to patients more quickly and efficiently (12). The Cures Act created a system to expedite the recognition of antimicrobial susceptibility test interpretive criteria (STIC) and provide up-to-date information to the healthcare community in a more straightforward manner (57). This was achieved in part by allowing organizations outside the FDA to become recognized as standards development organizations (SDO) if the following criteria are met: 1) establishment and maintenance of procedures to address potential conflicts of interest and ensure transparent decision making; 2) holding of open meetings to ensure that there is an opportunity for public input and incorporation into decision making; and 3) permission of public availability of standards. Once the SDO designation is obtained, the FDA is then required to recognize or withdraw recognition of STIC proposed by the SDO in a timely fashion via an online database located at https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria. This process allows for the FDA to adopt new and revised breakpoints more rapidly than prior to the Cures Act, which required updating the drug labeling. Currently, CLSI is the only organization to acquire the SDO designation from the FDA. While the United States Committee on Antimicrobial Susceptibility Testing (USCAST) also generates, integrates, and interprets data to support STIC, they do not currently meet the criteria to be recognized as an SDO by the FDA. Outside the U.S., there are multiple organizations that set breakpoints and have established or published guidelines including EUCAST (58).

Setting and revising breakpoints

Once a request for setting new breakpoints or revision of current breakpoints has been deemed appropriate by the CLSI Breakpoint Working Group and AST Subcommittee, an ad hoc

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working group is formed for the purpose of evaluating the breakpoints. For breakpoint reassessment and revision, requests may be made by any individual, a group, or a sponsor on the basis of new information (e.g., emergence of new resistance mechanisms or new PK/PD data). In order to set new or revise current breakpoints, the CLSI evaluates three types of data–microbiologic, PK/PD and clinical outcomes–as described in the procedures for establishing breakpoints. The CLSI AST Subcommittee meets twice annually–once in the winter and once in the summer—and meetings have free registration and are open to the public.

Data evaluation for new and/or revised breakpoints includes review of MIC distribution and evaluation of resistance mechanisms (59). A critical step in setting or revising breakpoints is to assess species-specific MIC distributions, evaluate the appropriate epidemiologic cutoff value (ECV), and determine where the proposed breakpoints would fall in relation to the ECV (60). The ECV is the MIC value for a specific antibiotic agent that purportedly separates the microbial populations with and without phenotypically detectable resistance (61). They differ from clinical breakpoints in that PK/PD and clinical outcomes data are not considered when determining the ECV which is set via visual inspection and/or statistical analysis to capture ≥97.5% of the wild-type population. Data used for determining the ECV are gathered from MIC distributions performed at multiple laboratories in order to generate a sufficient number of observations over a broad concentration range. It is recommended that clinical breakpoints not be set below the ECV to avoid bisecting the wild-type distribution, which could lead to unacceptably high error rates during validation and/or verification (62). Conversely, isolates with an MIC 1-2 dilutions above the ECV can typically be successfully treated using PK/PD-optimized antimicrobial dosing and therefore clinical breakpoints are often set higher than the ECV.

PK/PD analyses are vital tools in the process of setting and revising breakpoints and are included as part of the essential data requirements in the CLSI guideline for developing susceptibility testing criteria (59). For PK/PD analyses, a nonclinical cutoff should be determined, which is defined as the highest MIC value at which antibacterial activity and/or

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efficacy would be predicted. This prediction is based on the ability to achieve the PK/PD target using a human-simulated PK exposure in a nonclinical model system (e.g., mouse thigh or pneumonia infection, Monte Carlo simulation). PK/PD-based predictions may also vary by the target site of infection and therefore may require site-specific breakpoints, such as the urinary and meningitis-specific breakpoints provided by CLSI for certain β-lactam agents. Given the differences in PK/PD properties between antimicrobial agents, even within the same class, caution is advised when extrapolating breakpoints from one agent to another. A notable example is the use of urinary breakpoints for cefazolin as a surrogate for oral cephalosporins such as cefadroxil which has been shown to lead to unacceptably high VME rates (63). A clinical cutoff—which correlates clinical outcome to the MIC of an antimicrobial for the infecting pathogen(s)—should also be determined. Importantly, the dosage regimen should be considered when evaluating outcomes by MIC. Generally, no one source of data will be sufficient to set a breakpoint and it is unlikely that every request to review breakpoints will include all data elements described above.

Implementation of new methods and breakpoints

Before implementing an assay, laboratories are required to confirm (i.e., verify) or establish (i.e., validate) the performance characteristics of the assay. Assessment of assay performance varies according to whether the assay is FDA-cleared or not. Automated conventional phenotypic AST systems in the U.S. may receive FDA clearance as in vitro diagnostic devices after their performance is evaluated against reference BMD according to FDA acceptance criteria, as described in Kuper *et al.* (14, 17). The verification process of either a new AST method or addition of a new drug to an existing method is required and overseen primarily by the Clinical Laboratory Improvement Amendments (CLIA) with support from CLSI (8, 64). At present, the specific in-house verification process is at the discretion of the laboratory director, but typically involves testing against a group of 30 or more isolates that represent the target pathogen(s) against a particular antimicrobial. The isolates include those with known

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resistance mechanisms and/or those with MICs near the breakpoint (8, 64). While this task may seem daunting for laboratories, efforts have been made to assist the verification process. For example, the Centers for Disease Control and Prevention (CDC) & FDA Antibiotic Resistance Isolate Bank provides standardized panels of isolates for specific antimicrobials and/or resistance mechanisms (65). In addition to method verification, implementation of current breakpoints is imperative to ensure both optimal outcomes for the patients the laboratory serves and to address serious antimicrobial resistance issues, which threaten the public health. If the local laboratory applies obsolete breakpoints to AST results, clinicians should be made aware that such breakpoints may lead to incorrect test results for a given patient. Infection control and prevention staff must also be made aware that laboratory identification of antimicrobial-resistant bacteria may be suboptimal (25). Recent guidance by the College of American Pathologists (CAP) has stated that laboratories must update to use current breakpoints; this change will be impactful for many laboratories and will require coordination of efforts between many stakeholders including the laboratory, clinicians, and antimicrobial stewardship teams.

Clinical application of AST

Accurate interpretation of laboratory diagnostics, including AST, is key to successful clinical practice and antimicrobial stewardship. The guidelines for antimicrobial stewardship by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) highlight the importance of collaboration between antimicrobial stewardship and the microbiology laboratory (66). The strongest recommendation for this collaboration is the development of an antibiogram, which provides susceptibility data and therefore a foundation for empiric therapy recommendations. The antimicrobial stewardship program and microbiology laboratory should work together to review, collate, and design the reporting of the susceptibility data for the antibiogram following CLSI guidelines (67). Expanding the traditional antibiogram to include novel broad spectrum agents may further assist clinicians in selecting optimal empiric antimicrobial therapy regimens, depending on institutional pathogens and resistance rates (68).

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In addition to the antibiogram, decisions regarding AST technologies including adoption of new methods and/or breakpoints should involve collaboration between clinicians and the laboratory and may follow processes akin to diagnostic stewardship (DS). Antimicrobial stewardship programs should monitor and be aware of changes to existing and new AST methods or breakpoints and establish a process to facilitate communication and requests for changes with the microbiology laboratory and other stakeholders.

Diagnostic stewardship

The concept of stewardship as it applies to diagnostics emerged within the last two decades as the number of clinical laboratory tests available for patient care increased to over 3,000 (69). Due to the complexity and volume of tests available, errors of over-diagnosing (i.e., colonization vs infection) or under-diagnosing (potentially delaying care) may occur (70). To maximize the benefits of testing available for infectious diseases including AST, DS must be incorporated to optimize communication, education, and interpretation of AST results within the healthcare system. DS is defined as "coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions" (71) but can be simplified to "right test, right patient, right time" (72). DS is recommended by the 2016 IDSA and SHEA Stewardship Guidelines (66), CDC Core Elements of Hospital Antibiotic Stewardship Programs (73), and by the Society of Infectious Diseases Pharmacists (74) due to its positive impact on patient care. With DS, it is essential to evaluate the methods available, better integrate the selected methods into the healthcare system, and develop innovative and appropriate uses to improve patient outcomes. The needs and value at an individual institution must be balanced with considerations of laboratory and stewardship workflow, workload, and cost (74). An excellent framework and stepwise approach to justifying, implementing, optimizing, and tracking the impact of a new microbial diagnostic following the principles of DS is detailed in Ticcioni *et al*. (75)

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There are three phases of DS that should be incorporated when implementing any new diagnostic or AST technology: the pre-analytic, analytic, and post-analytic phase **(Table 3)** (70). For AST to work most efficiently, communication and education are paramount to apply the test results effectively and economically. Stakeholders involved in these decisions should be professionals from the departments of clinical microbiology, information technology, medical staff, and pharmacy (76). The goal of the pre-analytic phase is to determine which test or method would be best for the specific institution. This phase would include the evaluation of available testing methodologies and their potential for implementation for the individual institution. In order to effectively implement this phase, it is important to have baseline data on the problematic pathogens and resistance patterns seen at the institution, using resources such as antibiograms and RDTs (77). Once implemented at the institution level, this phase may also include decisions regarding when to order the test and how to interpret the results. These algorithms may describe criteria for use, criteria for specimen rejection, prior authorization, and cost information. Reflex and cascade approaches to diagnostics may also be beneficial. Finally, proper education and communication are essential in this phase (71). Evidence suggests that providers believe laboratory diagnostics are often implemented without sufficient education or evidence, leading to distrust and lack of use of new technologies including RDTs and AST (78). Each step of the laboratory workflow, from processing to resulting, is considered to be within the analytic phase (**Figure 2)**. Considerations include staffing hours, workload, physical space, training/skills required to run the test, and cost, as technologies vary in these requirements. As such, the analytic phase should involve substantial collaboration with representatives from the clinical microbiology laboratory. The post-analytic phase includes communication of results to clinicians and guidance on interpretation. In preparation for this phase, institutions should consider methods of communication such as phone alerts versus alerts reported in the electronic medical record and the impact of real-time clinical decision support surveillance software. Actions taken in the analytic phase, such as selective or cascade susceptibility result

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reporting and templated comments, may affect the post-analytic phase. Templated comments may include information about resistance to specific agents, recommendations for infection prevention precautions, and recommendations to consult infectious diseases. These templated comments are often added into the microbiology section of the patient's chart where the positive culture result is displayed. Each step within this process is essential for optimization of the use of advanced technologies to support patient care.

Leveraging antimicrobial and diagnostic stewardship

Development of novel rapid diagnostic tests is a goal of the Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria and is supported by IDSA (79). These organizations recommend that clinical value for future development of diagnostics should include improvement in identification of pathogens, time to results, time to appropriate antibiotic pharmacotherapy, reduction in adverse events and outcomes, and time to appropriate infection control precautions. As diagnostic technologies continue to advance, members of a multidisciplinary ASP will be responsible for interpreting the results of a test and understanding its application to patient care. As mentioned above in the section on rapid methods, these diagnostics may only have the ability to identify a pathogen, provide phenotypic results, detect resistance mechanisms, or provide a combination of these results. Clinicians may assist in interpreting these results by applying changes in breakpoints to clinical outcomes, recognizing forms of inducible resistance that may not be reflected with in vitro results, and contributing to templated results/comments based on available antibiotic formulary options (17). Alone, diagnostic tests are unable to determine whether a result represents colonization or infection. As organism recovery increases due to improved diagnostic sensitivity and assay inclusivity over conventional culture, ASPs will need to be even more diligent about investigating potential contaminants and determining appropriate therapy recommendations. Interpretation of the test result may incorporate how the result is communicated such as selective reporting based on formulary, language of a templated comment, and monitoring/providing feedback on selection of

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diagnostics. Utilization of technology for decision support and guidelines for diagnostics use can support appropriate selection of diagnostics. Similar to preauthorization and prospective audit and feedback in antimicrobial stewardship, novel or niche AST diagnostics can incorporate DS for appropriate use (72).

In addition to the diagnostic test result, in vivo considerations such as PK/PD, appropriate dosing, drug-drug interactions, and spectrum of activity (i.e. overly broad) must be incorporated as part of antimicrobial stewardship. The ASP and microbiology laboratory can collaborate to provide guidance and communication, particularly in settings in which in vitro and in vivo results may not align (i.e. dosing adjustments by indication or MIC). Ongoing feedback should be provided to clinicians for appropriate use of new diagnostics and interpretation of their results. Provider education may include a description of the test, comparison to other available tests at the institution, sensitivity and specificity, benefits and risks, turnaround time, and interpretation of results (80). Direct education can be provided via handshake stewardship, with in-person communication about a specific patient's care and diagnostic test selection. A 2020 study of a handshake stewardship program determined that 40% of interventions were related to diagnostic errors (81). The clinical value associated with advancing technology will rely on multidisciplinary collaboration for successful uptake and utilization (79). Aligning antimicrobial stewardship with appropriate use of innovative diagnostic technologies will contribute to advancements in patient care and improvement in outcomes.

CONCLUSIONS

AST has been the cornerstone of optimal antimicrobial therapy for more than a century and will continue to play a critical role in ensuring adequate therapy for patients, as well as tracking and monitoring the spread of AMR. Although many of the AST methods developed at the turn of the 20th century are still in routine use today, the past decade has seen an explosion in new technologies including molecular and phenotypic RDTs, and more frequent updates and revisions to clinical breakpoints. These rapid advances in the antimicrobial use process make

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strong collaborations between clinicians and microbiologists essential. Moving closer to the goal of immediate antimicrobial therapy optimization with little to no delay would benefit patients afflicted with an infectious disease syndrome. Finally, efforts of CAP, CLIA, CLSI, FDA, CDC, industry, and other key members to support the updating and use of appropriate methods and breakpoints is in the best interest of all clinicians. This will help ensure a concerted effort in improving our understanding of AST, PK/PD, and the application of breakpoints to improve patient outcomes and prolong the lifespan of our existing antimicrobials.

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Table 1. FDA-cleared rapid diagnostic tests for patients with bloodstream infections caused by bacterial pathogens

Test (manufacturer [reference])	Technology	Time to AST result from positive culture	Bacteria identified	Resistance genes detected
PhenoTest® BC Kit (Accelerate Diagnostics, Tucson, AZ (82))	Fully automated fluorescence in situ hybridization for identification and morphokinetic cellular analysis for phenotypic AST	7 hours	*S. aureus* *S. lugdunensis* CONS spp. *E. faecalis* *E. faecium* *Streptococcus* spp.	None as this is a phenotypic system. MICs are reported along with associated interpretive categories
			E. coli *Klebsiella* spp. *Enterobacter* spp. *Proteus* spp. *Citrobacter* spp. *S. marcescens* *P. aeruginosa* *A. baumannii*	

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BioFire® BCID2 (BioFire Diagnostics, Salt Lake City, UT (83))	Multiplex PCR	1 hour	*Enterococcus faecalis* *Enterococcus faecium* *Listeria monocytogenes* *Staphylococcus* spp. *S. aureus* *S. epidermidis* *S. lugdunensis* *Streptococcus* spp. *S. agalactiae* *S. pneumoniae* *S. pyogenes*	*mecA/C* MREJ *vanA/B*
			Acinetobacter calcoaceticus-baumannii complex *Bacteroides fragilis* *Enterobacter cloacae* complex *E. coli* *Klebsiella aerogenes*	IMP KPC OXA-48-like NDM VIM *mcr-1* CTX-M

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			K. oxytoca *K. pneumoniae* *Proteus* spp. *Salmonella* spp. *S. marcesens* *Haemophilus influenzae* *Neisseria meningitidis* *P. aeruginosa* *Stenotrophomonas maltophilia*	
ePlex® BCID (Roche, Indianapolis, IN (84))	Multiplex PCR and eSensor technology	1.5 hours	*Bacillus cereus* group *Bacillus subtilis* group *Corynebacterium* spp. *Cutibacterium acnes (Propionibacterium acnes)* *Enterococcus* spp. *Enterococcus faecalis* *Enterococcus faecium*	*mecA* *mecC* *vanA* vanB

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			Lactobacillus spp. *Listeria* spp. *Listeria monocytogenes* *Micrococcus* spp. *Staphylococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus* spp. *Streptococcus agalactiae* *Streptococcus anginosus* group *Streptococcus pneumoniae*	
			A. baumannii *B. fragilis* *Citrobacter* spp.	CTX-M IMP KPC

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			Cronobacter sakazakii *Enterobacter* (non-*cloacae* complex) *Enterobacter cloacae* complex *E. coli* *Fusobacterium necrophorum* *Fusobacterium nucleatum* *Haemophilus influenzae* *Klebsiella pneumoniae* *Morganella morganii* *Neisseria meningitidis* *Proteus* spp. *Proteus mirabilis* *P. aeruginosa* *Salmonella* spp. *Serratia* spp. *Serratia marcescens*	NDM OXA-48/-23 groups VIM

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			S. maltophilia	
T2Bacteria (T2 Biosystems®, Lexington, MA, (85))	Nuclear magnetic resonance and PCR directly from specimen	6 hours from collection	*E. faecium* *S. aureus* *K. pneumoniae* *P. aeruginosa* *E. coli*	*None*
Verigene® Blood Culture Gram-positive (BC-GP) and Gram-negative (BC-GN) (Luminex Corporation, Austin, TX, (86))	PCR and microarray hybridization	2.5 hours	*Staphylococcus* spp. *S. aureus* *S. epidermidis* *S. lugdunensis* *Streptococcus* spp. *S. anginosus* group *S. agalactiae* *S. pneumoniae* *S. pyogenes* *E. faecalis* *E. faecium* *Listeria* spp.	*mecA* MREJ *vanA* *vanB*

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			E. coli	CTX-M
			Klebsiella pneumoniae	IMP
			K. oxytoca	KPC
			P. aeruginosa	NDM
			Acinetobacter spp.	OXA
			Citrobacter spp.	VIM
			Enterobacter spp.	
			Proteus spp.	

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Table 2. Antimicrobial agents and appropriate read times endorsed by CLSI for direct disk diffusion from blood cultures positive for growth of Enterobacterales or *Pseudomonas aeruginosa.*

Antibiotic	Enterobacterales		*P. aeruginosa*	
	8-10 hour read	16-18 hour read	8-10 hour read	16-18 hour read
Ampicillin	X	X		
Aztreonam	X	X		
Ceftazidime	X	X		X
Ceftriaxone	X	X		
Ciprofloxacin	X	X	X[a]	X
Meropenem	X	X	X	X
Tobramycin	X	X	X	X
Trimethoprim-sulfamethoxazole		X		

[a]Direct disk diffusion breakpoint differs from standard CLSI disk diffusion breakpoints

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Table 3. Clinical and/or laboratory diagnostic stewardship considerations for AST across the phases of AST implementation and utilization. Adapted from (53, 65, 66)

	Pre-analytic	Analytic	Post-analytic
Clinical Considerations	Evaluation of clinical relevance of technology based on institution pathogens and resistance patterns Prevalence of disease state to optimize pretest probability Development of algorithms for appropriate use Sensitivity/specificity of laboratory assays	Provide recommendations/procedures to determine colonization vs infection Cascade/reflex testing	Tracking Education Feedback
Institution-Specific Considerations	Assessment of implementation potential Cost-effectiveness of technology		Evaluation of usage of local and send-out testing methodologies
Workflow Considerations	Optimize process for specimen collection to reduce contamination Clinical decision support and hard stops for ordering	Microbiology validation of diagnostics Processed in real-time or in batches Minimum processing requirements	Reporting of results Communication of results

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	Processes to prevent duplicate orders		
Considerations for Collaboration	Considerations for effects on infection control/prevention precautions and surveillance	Collaboration with microbiology laboratory	Collaboration with clinical microbiology, information technology, medical staff, and pharmacy

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Figure 1. Conventional phenotypic antimicrobial susceptibility testing methods. Broth microdilution is shown in (A) as antimicrobials in serial $\log_2$ dilutions across rows and columns, bacterial growth indicated by turbidity, and the MIC read as the lowest dilution of antimicrobial with no visible growth in the well. Agar dilution shown in (B) as antibiotic-impregnated Mueller-Hinton agar plate with various bacterial isolates spotted onto plate and the MIC read as the plate with the lowest antimicrobial concentration plate and no visible growth of the spotted isolate (India ink dot on upper right of plate aids in orientation). Agar gradient diffusion shown in (C) as an antibiotic-impregnated gradient strip plated on Mueller-Hinton agar streaked with a *Pseudomonas aeruginosa* isolate and the MIC read as the point at which visible bacterial growth intersects with the strip. Broth disk elution shown in (D) as tubes containing Mueller-Hinton broth inoculated with an *Escherichia coli* isolate and (from left to right) zero, one, two, or four disks containing 10 µg of colistin corresponding to concentrations of 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL. The MIC is read as the lowest dilution of antimicrobial with no visible growth in the tube. Agar disk diffusion shown in (E) as an antibiotic-impregnated disk plated on Mueller-Hinton agar streaked with a *Stenotrophomonas maltophilia* isolate and results read as the diameter of the zone of visible bacterial growth inhibition around the disk.

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Figure 2. Typical clinical microbiology laboratory workflow and timing from blood culture collection to bacterial identification and antimicrobial susceptibility testing (AST). The conventional process without rapid diagnostics consists of: (1) blood culture collection; (2) incubation in an automated blood culture system; (3) Gram staining, subculturing on solid agar, and incubation; (4) organism identification and phenotypic AST. Rapid diagnostics are capable of providing organism identification and/or genotypic or phenotypic AST directly from the blood culture bottle (A), from blood culture bottle positive for bacterial growth (B), or from colony growth on solid agar (C). The hourglass symbol represents approximate turnaround time. Image created with BioRender.com.

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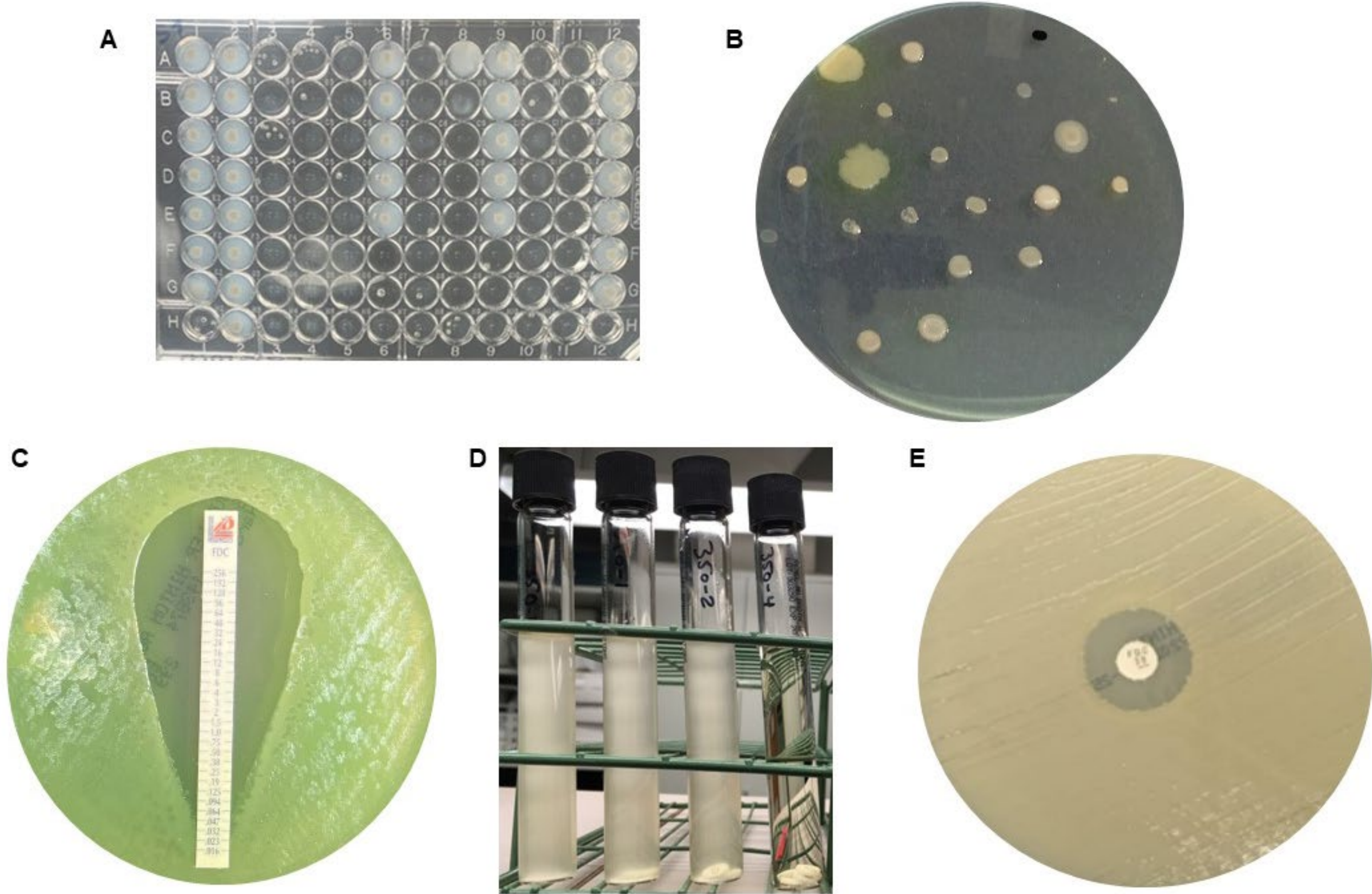

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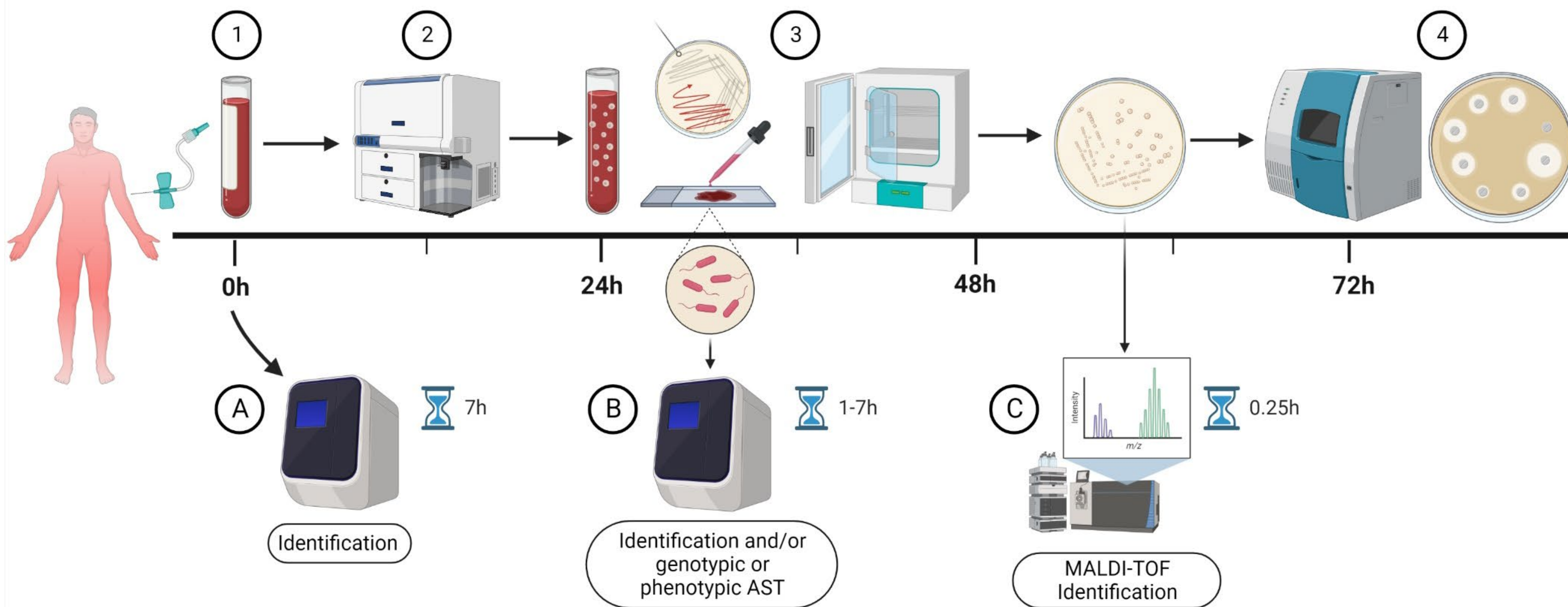

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