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The Continued Value of Disk Diffusion for Assessing Antimicrobial Susceptibility in Clinical Laboratories: Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group

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ABSTRACT Expedited pathways to antimicrobial agent approval by the U.S. Food and Drug Administration (FDA) have led to increased delays between drug approval and the availability of FDA-cleared antimicrobial susceptibility testing (AST) devices. Antimicrobial disks for use with disk diffusion testing are among the first AST devices available to clinical laboratories. However, many laboratories are reluctant to implement disk diffusion testing for a variety of reasons, including dwindling proficiency with this method, interruptions of the laboratory workflow, uncertainty surrounding the quality and reliability of disk diffusion tests, and a perceived need to report MIC values to clinicians. This minireview provides a report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group on the current standards and clinical utility of disk diffusion testing.

KEYWORDS antimicrobial susceptibility testing, CLSI, disk diffusion, EUCAST, FDA

All antimicrobial susceptibility testing (AST) devices marketed in the United States are regulated by the Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH). In contrast, antimicrobials are regulated by a separate branch of the FDA, the Center for Drug Evaluation and Research (CDER). AST devices used by clinical laboratories can be broadly categorized into automated and manual methods. Automated AST devices available in the United States include the Vitek II (bioMérieux, Durham, NC), MicroScan (Beckman-Coulter, West Sacramento, CA), Phoenix (Becton, Dickinson and Company, Sparks, MD), Sensititre (ThermoFisher Scientific, Lenexa, KS), and PhenoTest (Accelerate Diagnostics, Tucson, AZ) systems. Manual AST devices include disks, gradient diffusion strips (i.e., Etest [bioMérieux] and MIC test strips [MTS] [Liofilchem, Italy]), and lyophilized MIC panels (Sensititre manual panels). Automated AST devices involve sophisticated instrumentation and software, which provide both improved testing standardization (e.g., objective reading of endpoints and closed-system incubation) and generally less hands-on technologist time than is required by manual methods. When performed according to the manufacturer's instructions, some automated AST devices yield results more rapidly than do manual methods (1–3). Additionally, “expert rule” software is typically provided along with automated AST devices, which helps mitigate the risk of the laboratory reporting erroneous susceptibility results and helps ensure compliance with regulatory guidelines

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TABLE 1 Summary of antimicrobial drugs approved since 2010 and times to AST devices

Antimicrobial agent	Month/year approved by FDA	Time (mo) ^a to:		Manual MIC test (Sensititre) clearance	Rapid automated AST device clearance
		First disk clearance ^b	First gradient diffusion strip clearance		
Delafloxacin	6/2017	2	2	2	NA
Meropenem-vaborbactam	8/2017	2	4	4	6
Ceftazidime-avibactam	2/2015	8	7	10	25
Ceftolozane-tazobactam	12/2014	11	19	8	36
Dalbavancin	5/2014	ND	25	14	NA
Oritavancin	8/2014	ND	NA	7	NA
Tedizolid	6/2014	NA	36	15	NA
Ceftaroline	5/2010	7	29	14	34

^aTimes were rounded to the nearest month. NA, not available; ND, no disk test is possible due to drug characteristics.

^bThe average time to clearance, from the time the 510(k) data package is submitted to the FDA, is 42 days for the disk manufacturers that participated in the sponsor's clinical trials.

and standards, such as those published by the Clinical and Laboratory Standards Institute (CLSI) (4). For these reasons, most U.S. clinical laboratories have converted to automated AST devices for the bulk of, if not all, their AST needs. By way of example, a 2016 survey of clinical laboratories in California reported that 84 (94.4%) of 89 surveyed laboratories performed AST exclusively with one of the automated systems listed above (5). With this reliance on automated methods, laboratory competency and comfort with manual AST devices have dwindled. In recent years, a dilemma has emerged with this scenario, as the only AST devices available for recently approved antimicrobial drugs are manual tests (Table 1).

The process for developing an AST device for a new antimicrobial drug is complex, expensive, and time-consuming. Development of automated AST devices to accommodate new drugs is generally a lengthier process than development of manual tests, because not only the test itself but also the instrumentation and software must be updated. As a result, new drugs are often not available on automated AST devices until many years after the new drug application (NDA) for an antimicrobial is approved by the CDER (Table 1) (5, 6). In contrast, manual AST devices are typically available prior to or soon after NDA approval. These manual tests may be distributed as research use only (RUO) products pending the availability of *in vitro* diagnostic (IVD) labeled devices, which are usually cleared by the CDRH within 6 months after NDA approval. New pathways that facilitate antimicrobial drug approvals, such as expedited development programs, shortened regulatory review, and 505(b) NDA approvals (2), have brought antimicrobial agents to market faster than ever before. While this is wonderful progress, the challenge for clinical laboratories is that these expedited pathways do not have any means to address the gap between drug approval and AST device clearance, even for manual tests, in part due to the number of new antimicrobials entering the marketplace. Since 2010, eight antibacterial agents that require AST have been approved by the FDA (Table 1). This demand for AST device development is compounded by ongoing breakpoint revisions, which also must be addressed by AST device manufacturers. For instance, the CLSI has revised 28 breakpoints in the M100 standard since 2010 (4). Addressing these updates may require new development or reformulation of the antimicrobials in question, new clinical trials, and software updates by AST device manufacturers. In combination, these advancements have put tremendous resource and financial strains on AST device manufacturers, which may further extend the time to automated AST device availability. These dilemmas have not gone unnoticed by the FDA. In September 2016, the FDA published a draft guidance (7) and held a public workshop that discussed pathways for pharmaceutical companies and device manufacturers to coordinate efforts to achieve device clearance coinciding with (or occurring soon after) drug approval. Recent AST device clearances demonstrate success with this approach. Three manual devices for testing delafloxacin, which obtained FDA approval on 19 June 2017, were cleared by the CDRH within 44 days after approval of the drug

by the CDER (data publicly available by searching the FDA website). Similarly, meropenem-vaborbactam AST devices were cleared by the CDRH within 112 days after NDA approval of this drug on 29 August 2017; disks were cleared 35 days after NDA approval. Such expedient development and clearance of AST devices are far more feasible for manual AST devices than for automated AST devices (Table 1), and it is likely that manual tests will remain the only AST devices available for new drugs in the near future. Furthermore, even when an automated AST device is cleared, it may be several years before the manufacturer markets the test, largely due to software update requirements for the instruments used by laboratories.

Many laboratories are unfortunately hesitant to introduce manual AST devices into their routine clinical practice. The reasons for this reluctance are complex but may include the labor associated with performing manual tests, dwindling staff proficient in performing manual tests, and purchasing contract restrictions (e.g., if a disk is available only from one manufacturer and the laboratory has a purchasing contract with a different manufacturer of disks). Laboratories have also expressed concern regarding disk tests specifically, due to reported issues with disk quality (http://www.eucast.org/ast_of_bacteria/warnings/#c13111), performance issues (8–10), or the perception that MIC values are needed to guide patient therapy. Nonetheless, laboratories must develop a strategy for testing new antimicrobial drugs. Such drugs are often the only drugs available to treat multidrug-resistant Gram-negative bacteria, and delays in implementing testing for these antimicrobials limit the ability of physicians to treat life-threatening infections (11, 12).

In recognition of the challenges faced by laboratories in performing timely AST of new antimicrobial drugs, the CLSI AST Subcommittee has formed an *ad hoc* working group (ahWG) to address the challenges surrounding the coordinated development of new antimicrobial drugs and AST devices and testing of such drugs by clinical laboratories. One primary objective of the ahWG is to provide context regarding the current standards for disk diffusion testing, given that this methodology is one of the few AST methods available for new drugs. This minireview was developed by the ahWG to illuminate the process of antimicrobial disk development, to describe how disk breakpoints are established, to review the established controls for disk quality, and to comment on the clinical value of disk diffusion results.

DEVELOPMENT PROCESS FOR DISKS

Determination of disk mass. Prior to an antimicrobial drug entering human clinical trials, the sponsoring pharmaceutical company (sponsor) collaborates with device manufacturers to develop disks for testing the activity of the drug against individual clinical isolates. The sponsor generally works with one or more reference laboratories that maintain large collections of recent clinical bacterial isolates. The reference laboratory determines the optimal disk mass (the concentration of antibiotic within the disk) by evaluating disk diffusion and reference broth microdilution (BMD) tests in parallel against an organism set, ideally using the same inoculum to minimize variability. The studies typically include >100 isolates, representing the genera and species that are associated with the infections of interest for the antimicrobial. It is critical that the study include isolates with MICs that span the full range of the MIC distribution, to ensure that the test can discriminate susceptible from tentatively resistant isolates, thus minimizing very major errors (VMEs) (i.e., isolates resistant by BMD testing but susceptible by disk diffusion testing) and also major errors (MEs) (i.e., isolates susceptible by BMD testing but resistant by disk diffusion testing). The disk mass for antibiotic compounds in the same drug class is taken into consideration as a starting point for this process. However, the performance characteristics of multiple disk mass options that extend below the expected mass are also studied, with the goal of defining the minimal disk mass that meets defined performance criteria. This approach yields smaller zone sizes that are easier to measure, may be less prone to error, and allow testing of multiple disks on the same plate. At this early stage of development, the reference

laboratory often manufactures the disks in-house, by manually aliquoting the antibiotic onto blank, sterile, paper disks.

The data obtained during these studies are analyzed using scattergrams of zone size versus MIC, as described in CLSI document M23, section 2.3 (13). Historically, if no significant difference in performance was noted between disk masses, then the mass that most closely matched the one used for other antimicrobial drugs of the same class was selected. However, there is now an effort to choose the lower disk mass, which facilitates global harmonization. If significant performance differences between disk masses are seen, then the mass that most closely meets the susceptible criterion of 15 to 35 mm, as also described in CLSI document M23, section 2.3 (13), is chosen, to maximize the number of disks that can be tested on a single plate. To date, disk masses defined by CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards have not always been harmonized. In 2017, however, a joint working group on disk testing was formed between CLSI and EUCAST, which aims to facilitate standardization of disk masses between the two organizations. To aid in this process and to ensure an expedient review of disk quality control (QC) ranges, drug sponsors are encouraged to engage the joint working group early in the development process. Early harmonization can simplify the disk development process, ensure that disk zone data collected from antimicrobial drug clinical trials use a disk that satisfies the regulatory approval requirements of both the FDA and the European Medicines Agency (EMA), and allow comparison of disk test results across geographically distinct studies that might otherwise have used different disks.

Production-scale manufacturing. Once the optimal disk mass has been determined, the sponsor initiates development with commercial disk manufacturers, as larger scale lots are necessary to establish QC ranges, according to document M23 (13). To initiate the development process after all legal agreements have been completed, the sponsor provides the antibiotic powder, a certificate of analysis, and the material data sheet. In the early stages, the disk manufacturer prepares small-scale, benchtop preparations to confirm the solubility and potency. Batches of disks are prepared from filter paper cardstock of the required composition and thickness for standard disk diffusion testing. Standard disks have the ability to absorb 2.5 to 3.0 times their weight of distilled water. The cardstock must not exhibit inhibitory activity or contain residual material (e.g., oil and debris) that would affect the activity or pH of the antibiotic solution.

Using the sponsor's recommendations for mass, solvent system, and other defined conditions (e.g., preheating the filter paper cardstock), the cards are saturated with the antibiotic solution, excess moisture is removed, and the cards are oven dried, following each manufacturer's process. For this initial batch, the cards may be manually punched to produce the desired number of disks. The high-volume manufacturing process is mimicked as closely as possible. When the optimal preparation conditions have been determined through disk diffusion performance and/or high-performance liquid chromatography (HPLC) assessments of disk antimicrobial contents, a standardized manufacturing protocol (SMP) is drafted and prototype disks are manufactured. Manufacturing facilities that produce disks follow a quality management system that complies with ISO standards, i.e., ISO 9001, ISO 13485, and/or ISO 14001 (<https://www.iso.org/standards.html>).

Generally, a small pilot lot of disks are packaged into cartridges following the SMP. The disks are then tested to confirm accurate zone diameters and reproducibility. Analytical tests, such as an HPLC assay and/or a bioassay to verify the drug concentration, may also be performed, based on the sponsor's recommendations. Any failures from this testing require reevaluation of the production process, to determine whether and where changes are needed.

After a pilot lot is produced with acceptable performance, subsequent lots are produced by stacking cards and feeding them through an automated process to be punched into single cartridges with a desiccant, blister sealed, and labeled with

information specifically defined by the sponsor. The disk manufacturer continues to monitor the performance of multiple lots to establish stability and expiration dating; this is accomplished through both accelerated and real-time stability testing. Accelerated testing may include storage of the disks at 25°C and 35°C, with testing at various intervals. Depending on the antibiotic, real-time stability testing is conducted for a period of up to 27 months when disks are stored at the recommended storage temperature (e.g., 2°C to 8°C). The disks may be provided back to the sponsor for formal QC testing after accelerated stability testing, if performance is acceptable.

Establishing QC ranges. The sponsor initiates formal QC studies by working with a reference laboratory to test a large number of organisms and predefined QC isolates. In these studies, at least one disk lot from two manufacturers or two disk lots from the same manufacturer (if only one disk manufacturer collaborated with the sponsor to develop disks) and one lot of Mueller-Hinton agar from three different manufacturers are used (13). The data are then compiled, reviewed, and proposed to the CLSI AST subcommittee for approval. The subcommittee determines whether the data are sufficient for the establishment of QC zone sizes, and it may recommend further testing. Additional QC isolates that are specific to the new drug (e.g., an organism that can properly measure both components of a β -lactam- β -lactamase inhibitor combination) may be added. Once the proposed QC zones are approved, they are published in the QC table of the M100 document, which is updated every January.

Establishing disk breakpoints. Following the establishment of QC ranges, the drug sponsor progresses to testing contemporary clinical isolates, such as those collected during prospective surveillance studies and those isolated during the sponsor's phase III clinical trials. These clinical studies involve collecting specimens from hundreds of patients, isolating the infecting bacterium, and testing the susceptibility of each recovered isolate with the disk and reference BMD methods, which are performed in parallel, using the same inoculum. Multiple specimens are collected from each patient enrolled in the trial, i.e., one prior to administration of the antimicrobial drug (baseline) and additional specimens as clinically indicated if the infection persists. For some indications, such as urinary tract infections or complicated skin and skin structure infections, additional specimens may be taken daily, using noninvasive procedures, after the antimicrobial therapy commences. QC testing is performed on each day of clinical isolate testing. As a result, every isolate has a measured disk zone and MIC value, as well as QC results from that day of testing. These tests are repeated if QC values are out of range. QC data are collected and analyzed at the end of a clinical study for inclusion in the NDA submitted to the FDA. Disk zone diameter interpretive criteria (breakpoints) for each target organism are determined based on correlations with the MIC data collected during the phase III clinical studies, with the goal of minimizing categorical errors. The drug sponsor also presents these data to CLSI and EUCAST, and the breakpoints are added to the appropriate tables in CLSI document M100 and in the EUCAST breakpoint documents.

The sponsor prepares the final NDA submission and also provides the specifications to disk manufacturers, including updated zone breakpoint criteria and the indicated organism list. The drug sponsor maintains communication with disk manufacturers throughout the drug approval process, to ensure that the disk labeling for the disk brand that was evaluated during the drug approval process can be submitted for FDA clearance as soon as possible following NDA approval. At the present time, this involves a labeling review only for the disk brand that was evaluated as part of the drug approval process and met the acceptance criteria. However, to demonstrate that all disks provide equivalent performance, new testing requirements have recently been requested by the FDA for clearance of a disk brand not evaluated during the drug approval process. At the time of this writing, discussions between the FDA, disk manufacturers, and drug sponsors to define these testing requirements are under way. If this additional testing is required, it will extend the time from NDA approval to the availability of disks from manufacturers not evaluated during the drug approval pro-

TABLE 2 High-level overview of disk development

Step	Description
Drug sponsor engages reference laboratory	Small number of disks are made by hand Optimal disk mass is determined MIC correlates are evaluated
Drug sponsor works with disk manufacturers	CDA and development agreements are signed Powder, with certificate of analysis and material data sheet, is provided to manufacturer by sponsor Small-scale, benchtop preparations of disks are made and used by manufacturer to confirm solubility and potency Optimal preparation conditions are determined through biological and chemical checks by manufacturer Standardized manufacturing protocol is drafted
Disk manufacturer produces RUO disk lots	Manufacturer follows standardized manufacturing protocol to produce small prototype batch of disks in cartridges Prototype disks are tested to confirm accurate zone diameters and reproducibility Subsequent RUO lots are provided to sponsor by manufacturer, with labeling as defined by sponsor Disk performance continues to be monitored by manufacturer through stability studies
RUO lots are provided to reference laboratory	QC studies are performed following CLSI M23 guidelines QC data are presented by sponsor to CLSI AST subcommittee for approval QC range is established Reference laboratory tests RUO disks against isolates collected during clinical trials and establishes disk-MIC correlates (i.e., disk breakpoints)
Drug sponsor submits NDA to FDA	Data from clinical trials, including disk breakpoints, are compiled for NDA by sponsor and presented to CDER Data may also be presented by sponsor to CLSI and EUCAST for review and inclusion in breakpoint tables Drug sponsor provides final specifications included in approved NDA to all interested disk manufacturers
Post-NDA approval	Disk manufacturers involved in drug sponsor's clinical trial may submit disk labeling to CDRH Testing requirements for disk manufacturers not involved in drug sponsor's clinical trials have yet to be determined After 510(k) clearance, disks can be stocked for sale Disk manufacturer continues to perform QC testing for each new lot of disks

^aCDA, confidential disclosure agreement.

cess; this could limit the ability of laboratories to test new drugs if the laboratories do not have contracts with the initial manufacturer included in the drug approval process. This concern is particularly pressing for the ~12 new antimicrobial drugs that have recently been approved by the FDA, are awaiting FDA approval, or are midway through the development process.

Ongoing quality assessments. Once the disks are available for sale, the disk manufacturer performs stringent QC testing on each lot of disks produced. For each antimicrobial drug, QC testing minimally consists of the CLSI-recommended strains. If the manufacturer also makes media, then all recommended media are included in the testing. Additional QC strains and/or analytical tests may be used as well. Once the new lot passes QC testing, the lot of disks can be released for sale. A certificate of analysis, which typically includes the assay potency and range, accompanies each lot and is available on the disk manufacturer's website. Table 2 summarizes these steps in disk development. While not required, some degree of postmarketing performance assessment (i.e., MIC versus disk diffusion testing) is performed with selected disks and drugs. In general, however, stability data that support the shelf life and QC performance of each disk prior to release are used to ensure the quality of the product. In addition, the manufacturer monitors performance via the complaint feedback system. All complaints are investigated, retained samples of the specific batch or lot are tested with QC and clinical strains, and the root causes of any observed errors are determined, when possible.

CLINICAL VALUE OF DISK DIFFUSION TESTING

One of the primary arguments against the use of disks is that disk diffusion testing does not generate MIC values. MIC values are being used with increasing frequency to optimize antibiotic therapy, through individualized pharmacokinetic/pharmacodynamic models designed to improve drug exposure for difficult-to-treat infections. However, these models assume that the MIC is an absolute value. As recently reviewed by Mouton and colleagues, the use of laboratory-measured MICs to determine individualized pharmacokinetic/pharmacodynamic targets is not advisable (14). The accuracy of MIC measurements with the reference BMD method is within 1 doubling dilution (i.e., MIC of 2 $\mu\text{g/ml}$ is precise within 1 to 4 $\mu\text{g/ml}$). An excellent example of this is the accepted vancomycin QC range for *Staphylococcus aureus* ATCC 25923, which is 0.5 to 2.0 $\mu\text{g/ml}$. This range is due to small uncontrolled variables in the test, such as minor differences in lots and brands of media and differences in inoculum preparation, incubation time, and incubation temperature (15). Medium and drug lot differences have specifically been shown to result in differences in vancomycin MICs, as have test methods. *S. aureus* vancomycin MICs differ consistently between Etest and BMD methods (16), and BMD MICs vary according to the source of the drug powder and Mueller-Hinton agar (17). Such variance may also be attributable to biological variability among the strains, including factors outside resistance mechanisms, such as division rates and metabolic status (14, 15). There are some advocates for the idea that a vancomycin MIC value of 2 $\mu\text{g/ml}$ for *S. aureus* may be an indication not to use vancomycin for treatment, even though the isolate is susceptible (18–20). This idea is problematic, given that even a QC strain demonstrating good reproducibility may yield test results of 0.5 $\mu\text{g/ml}$ or 2 $\mu\text{g/ml}$. In addition, if the specific MIC value is used for dosing calculations, then different results would be obtained if a value of 0.5 $\mu\text{g/ml}$ versus 2 $\mu\text{g/ml}$ was used for the calculations (21). For these reasons, pharmacodynamic targets published in the literature are established by replicate testing of strains in a single laboratory, using reference methods, and not by a single MIC measurement, as performed in clinical laboratories for routine patient testing. Overinterpretation of the precision of a specific MIC value may have unintended consequences for therapeutic decision-making and/or may contribute to overestimation of “MIC creep” in longitudinal studies (22–25). It should be noted that disk diffusion testing is also subject to variability. As an example, the CLSI M23 document accepts QC ranges of ≤ 4 dilutions for MICs and ≤ 12 mm for disk diffusion tests (13). In practice, the precision of these tests is generally ≤ 3 dilutions and ≤ 8 mm, respectively. While disk diffusion data are not subject to evaluation of essential agreement, because zone diameters are not reported, laboratories should be cognizant that variability also exists for disk testing, particularly when multiple brands and lots of media and disks are used, as is required by CLSI when QC ranges are being established.

The reporting of specific MIC results has the potential to be misinterpreted. The end user of the data may not be aware of the relative concentrations of the various antimicrobials in different body compartments, to place the data in a clinical context, and not all prescribers are aware of the relationship between MIC values and categorical interpretations for a specific antimicrobial agent (26). Numerical values may result in confusion, with some health care providers concluding that the best therapy is the one with the lowest numerical MIC, which frequently does not correlate with the most appropriate therapy for a given infection. For example, in the setting of urinary tract infections, nitrofurantoin may exhibit a much higher numerical MIC value than a β -lactam antibiotic, but nitrofurantoin is highly concentrated in the urine and often is an appropriate selection for the treatment of uncomplicated urinary tract infections (27, 28).

The predominant value of MIC-based dosing of antimicrobials is for isolates with MICs that approach the resistance breakpoint or are immediately above it. In general, such isolates represent a minority of the bacterial population, although there are instances in which breakpoints are found within the wild-type distribution (e.g., the cefazolin breakpoint for *Enterobacteriaceae*). Although it is not routinely used for this

purpose, disk testing can often provide more data on the relative “resistance” or “susceptibility” of an isolate than can MICs determined with commercial AST devices that test a truncated range of antimicrobial concentrations. For instance, an isolate for which no zone of growth inhibition is observed would be predicted to have a higher MIC than does an isolate with a zone of growth inhibition approaching the resistance breakpoint. Similarly, an isolate with a very large zone of growth inhibition would be predicted to be more susceptible than an isolate with a zone of inhibition at the susceptibility breakpoint. However, it should be cautioned that disk zones should never be reported to clinicians, because the correlation of disk zones and MICs may not be as good for MICs above or below the breakpoint. If the disk zone size suggests an isolate outside the wild-type distribution and clinicians desire a MIC value, then the MIC can be determined by reflex testing, if available.

Additional benefits of disk diffusion testing include low cost and ease of implementation. Disk tests can be performed with Mueller-Hinton agar, which is available from multiple manufacturers, and with little or no additional instrumentation (e.g., calipers or a ruler and an incubator). CLSI recommendations for verification studies to be performed prior to implementation of a new disk test in laboratories that already perform disk diffusion testing are minimal. Typically, only 5 clinical isolates are required to be tested in order to verify disk accuracy. Laboratories that are implementing disk diffusion testing as a new method for the laboratory should test at least 30 isolates (29). Challenge isolates, with reference BMD MIC data, are available free of charge from the Centers for Disease Control-FDA organism bank, for laboratories to use to verify the performance of AST devices for most new antimicrobial agents (<https://www.cdc.gov/drugresistance/resistance-bank/index.html>). An additional source of isolates may be the drug sponsor.

Use of disk diffusion testing for new antimicrobials allows laboratories to customize the panel of new antimicrobials to be tested, to address local or institutional resistance issues and to support antimicrobial stewardship endeavors. In contrast, automated devices are typically available with predefined antimicrobial panels that cannot be changed. Similarly, in times of antimicrobial shortage, it is relatively easy for a laboratory to add an antimicrobial disk to the panel of disks routinely being tested. Both CLSI and EUCAST are developing direct-from-blood-culture disk diffusion tests, for which results may be read at earlier time points than the traditional 18 to 20 h (30, 31). Disadvantages of disk diffusion testing include the absence of a disk method for anaerobic bacteria and the fact that certain antimicrobials, such as daptomycin and colistin, cannot be tested with disks.

TOOLS TO AID IN STANDARDIZATION OF DISK DIFFUSION TESTING IN THE CLINICAL LABORATORY

Accurate performance of disk diffusion testing relies on proficient technologists. The major contributor to disk diffusion testing variability has been shown to be interoperator variability, including the methods used to prepare the inoculum and to streak the Mueller-Hinton agar plate (15). Several tools are now available to facilitate operator standardization, including both recently developed educational materials and next-generation instrumentation. The EUCAST website (http://www.eucast.org/ast_of_bacteria) includes several resources that outline the steps for performing and interpreting disk diffusion tests; these are available in many languages. The CLSI M02 document includes detailed instructions for performing disk diffusion, and new to the 2018 edition is a disk diffusion reading guide, with pictures that outline how to evaluate challenging disk zones (32).

Several instruments have been developed to automate the discrete steps of disk diffusion testing, including photometric devices to standardize inoculum preparation, automated plate streakers, and digital imaging systems to read disk diffusion zones of inhibition. In one study, automated streaking significantly reduced interoperator differences in disk diffusion zones of inhibition (33). Instrumentation that automates reading of disk diffusion test results includes the FDA-cleared BIOMIC system (Giles Scientific, Inc. Santa Barbara, CA), as well as several systems not yet available in the

United States (34). Such systems allow both objective and automated assessment of zones of inhibition, with application of expert rules software that laboratories have come to rely on with automated AST devices. The use of automated readers has been shown to significantly improve the precision of disk diffusion zone assessments (35). Furthermore, with the use of digital readers, early reading and interpretation of results for some antimicrobial agents and microorganisms may be possible. One study demonstrated that ME and VME rates were <1% after only 10 h of incubation, compared to a full 20 h of incubation, for a collection of 88 challenge isolates of *Enterobacteriaceae* (36). Finally, fully automated solutions to disk diffusion testing are emerging, including total laboratory automation systems. These will further simplify the process of disk diffusion testing by automating colony picking, inoculum preparation, incubation, and reading (37). The use of these fully automated disk diffusion solutions will expedite reporting and will fit into today's laboratory workflow.

SUMMARY

Antimicrobial disks are among the first AST devices available to clinical laboratories for new antimicrobial drugs. Testing of such agents is critical to optimize clinical outcomes, because resistance is not predictable. Disk development and quality are carefully monitored by both the drug sponsor during development and the manufacturer during marketing. Educational resources and new instrumentation are available to aid laboratories in performing disk diffusion testing. While MIC values can be helpful for interpreting susceptibility results, MIC methods are not always available for new drugs. Ultimately, if the option is a disk diffusion test result or no result at all for new antimicrobials, most clinicians would favor a disk diffusion result.

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REFERENCES

- Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, Granato PA, Harrington AT, Hernandez DR, Humphries RM, Jindra MR, Ledebore NA, Miller SA, Mochon AB, Morgan MA, Patel R, Schreckenberger PC, Stamper PD, Simner PJ, Tucci NE, Zimmerman C, Wolk DM. 2018. Multicenter evaluation of the Accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. *J Clin Microbiol* 56:e01329-17. <https://doi.org/10.1128/JCM.01329-17>.
- Barenfanger J, Drake C, Kacich G. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing. *J Clin Microbiol* 37:1415-1418.
- Doern GV, Vautour R, Gaudet M, Levy B. 1994. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. *J Clin Microbiol* 32:1757-1762.
- Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial susceptibility testing; approved standard—28th ed. M100. Clinical and Laboratory Standards Institute, Wayne, PA.
- Humphries RM, Hindler JA, Epton E, Horwich-Scholefield S, Miller LG, Mendez J, Martinez JB, Sinkowitz J, Sinkowitz D, Hershey C, Marquez P, Bhauria S, Moran M, Pandes L, Terashita D, McKinnell JA. 2018. Carbapenem-resistant *Enterobacteriaceae* (CRE) detection practices in California: what are we missing? *Clin Infect Dis* 66:1061-1067. <https://doi.org/10.1093/cid/cix942>.
- Humphries RM, Hindler JA. 2016. Emerging resistance, new antimicrobial agents, but no tests! The challenge of antimicrobial susceptibility testing in the current US regulatory landscape. *Clin Infect Dis* 63:83-88. <https://doi.org/10.1093/cid/ciw201>.
- Food and Drug Administration. 2016. Coordinated development of antimicrobial drugs and antimicrobial susceptibility test devices: draft guidance for industry and Food and Drug Administration staff. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM521421.pdf>.
- Flynt LK, Veve MP, Samuel LP, Tibbetts RJ. 2017. Comparison of Etest to broth microdilution for testing of susceptibility of *Pseudomonas aeruginosa* to ceftolozane-tazobactam. *J Clin Microbiol* 55:334-335. <https://doi.org/10.1128/JCM.01920-16>.
- Humphries RM, Hindler JA, Magnano P, Wong-Beringer A, Tibbetts R, Miller SA. 2018. Performance of ceftolozane-tazobactam Etest, MIC test strips, and disk diffusion compared to reference broth microdilution for β -lactam-resistant *Pseudomonas aeruginosa* isolates. *J Clin Microbiol* 56:e01633-17. <https://doi.org/10.1128/JCM.01633-17>.
- Cohen D, Swift G. 2013. Laboratories and regulator misled over antibiotic susceptibility test discs. *BMJ* 346:f837. <https://doi.org/10.1136/bmj.f837>.
- Munita JM, Aitken SL, Miller WR, Perez F, Rosa R, Shimose LA, Lichtenberger PN, Abbo LM, Jain R, Nigo M, Wanger A, Araos R, Tran TT, Adachi J, Rakita R, Shelburne S, Bonomo RA, Arias CA. 2017. Multicenter evalu-

- ation of ceftolozane/tazobactam for serious infections caused by carbapenem-resistant *Pseudomonas aeruginosa*. Clin Infect Dis 65: 158–161. <https://doi.org/10.1093/cid/cix014>.
12. Wright H, Bonomo RA, Paterson DL. 2017. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect 23:704–712. <https://doi.org/10.1016/j.cmi.2017.09.001>.
 13. Clinical and Laboratory Standards Institute. 2016. Development of in vitro susceptibility testing criteria and quality control parameters, 4th ed. M23. Clinical and Laboratory Standards Institute, Wayne, PA.
 14. Mouton JW, Muller AE, Canton R, Giske CG, Kahlmeter G, Turnidge J. 2018. MIC-based dose adjustment: facts and fables. J Antimicrob Chemother 73:564–568. <https://doi.org/10.1093/jac/dkx427>.
 15. Hombach M, Ochoa C, Maurer FP, Pfiffner T, Bottger EC, Furrer R. 2016. Relative contribution of biological variation and technical variables to zone diameter variations of disc diffusion susceptibility testing. J Antimicrob Chemother 71:141–151. <https://doi.org/10.1093/jac/dkv309>.
 16. Prakash V, Lewis JS, II, Jorgensen JH. 2008. Vancomycin MICs for methicillin-resistant *Staphylococcus aureus* isolates differ based upon the susceptibility test method used. Antimicrob Agents Chemother 52:4528. <https://doi.org/10.1128/AAC.00904-08>.
 17. Charlton CL, Hindler JA, Turnidge J, Humphries RM. 2014. Precision of vancomycin and daptomycin MICs for methicillin-resistant *Staphylococcus aureus* and effect of subculture and storage. J Clin Microbiol 52: 3898–3905. <https://doi.org/10.1128/JCM.01571-14>.
 18. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, Stellrecht K. 2008. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. Antimicrob Agents Chemother 52: 3315–3320. <https://doi.org/10.1128/AAC.00113-08>.
 19. Martirosov DM, Bidell MR, Pai MP, Scheetz MH, Rosenkranz SL, Lodise TP. 2017. Relationship between vancomycin exposure and outcomes among patients with MRSA bloodstream infections with vancomycin Etest MIC values of 1.5mg/L: a pilot study. Diagn Microbiol Infect Dis 88:259–263. <https://doi.org/10.1016/j.diagmicrobio.2017.03.008>.
 20. Moise PA, Culshaw DL, Wong-Beringer A, Bensman J, Lamp KC, Smith WJ, Bauer K, Goff DA, Adamson R, Leuthner K, Virata MD, McKinnell JA, Chaudhry SB, Eskandarian R, Lodise T, Reyes K, Zervos MJ. 2016. Comparative effectiveness of vancomycin versus daptomycin for MRSA bacteremia with vancomycin MIC >1 mg/L: a multicenter evaluation. Clin Ther 38:16–30. <https://doi.org/10.1016/j.clinthera.2015.09.017>.
 21. Eum S, Bergsbaken RL, Harvey CL, Warren JB, Rotschafer JC. 2016. Discrepancy in vancomycin AUC/MIC ratio targeted attainment based upon the susceptibility testing in *Staphylococcus aureus*. Antibiotics (Basel) 5:34. <https://doi.org/10.3390/antibiotics5040034>.
 22. Castaneda X, Garcia-de la-Maria C, Gasch O, Pericas JM, Armero Y, Soy D, Garcia-Gonzalez J, Falces C, Ninot S, Almela M, Ambrosioni J, Quintana E, Vidal B, Fuster D, Llopis J, Soto S, Moreno A, Marco F, Miro JM. 2017. AUC/MIC pharmacodynamic target is not a good predictor of vancomycin efficacy in methicillin-resistant *Staphylococcus aureus* experimental endocarditis. Antimicrob Agents Chemother 61:e02486-16. <https://doi.org/10.1128/AAC.02486-16>.
 23. Diaz R, Ramalheira E, Afreixo V, Gago B. 2017. Evaluation of vancomycin MIC creep in *Staphylococcus aureus*. J Glob Antimicrob Resist 10:281–284. <https://doi.org/10.1016/j.jgar.2017.04.007>.
 24. Ruiz-Ramos J, Vidal-Cortes P, Diaz-Lamas A, Reig-Valero R, Roche-Campo F, Del Valle-Ortiz M, Nuvials-Casals X, Ortiz-Piquer M, Andaluz-Ojeda D, Tamayo-Lomas L, Blasco-Navalpotro MA, Rodriguez-Aguirregabiria M, Aguado J, Ramirez P. 2017. Ventilator-associated pneumonia by methicillin-susceptible *Staphylococcus aureus*: do minimum inhibitory concentrations to vancomycin and daptomycin matter? Eur J Clin Microbiol Infect Dis 36:1569–1575. <https://doi.org/10.1007/s10096-017-2970-3>.
 25. Song KH, Kim M, Kim CJ, Cho JE, Choi YJ, Park JS, Ahn S, Jang HC, Park KH, Jung SI, Yoon N, Kim DM, Hwang JH, Lee CS, Lee JH, Kwak YG, Kim ES, Park SY, Park Y, Lee KS, Lee YS, Kim HB. 2017. Impact of vancomycin MIC on treatment outcomes in invasive *Staphylococcus aureus* infections. Antimicrob Agents Chemother 61:e01845-16. <https://doi.org/10.1128/AAC.01845-16>.
 26. Cunney RJ, Smyth EG. 2000. The impact of laboratory reporting practice on antibiotic utilisation. Int J Antimicrob Agents 14:13–19. [https://doi.org/10.1016/S0924-8579\(99\)00144-2](https://doi.org/10.1016/S0924-8579(99)00144-2).
 27. Huttner A, Verhaegh EM, Harbarth S, Muller AE, Theuretzbacher U, Mouton JW. 2015. Nitrofurantoin revisited: a systematic review and meta-analysis of controlled trials. J Antimicrob Chemother 70:2456–2464. <https://doi.org/10.1093/jac/dkv147>.
 28. Tan TY, McNulty C, Charlett A, Nessa N, Kelly C, Beswick T. 2003. Laboratory antibiotic susceptibility reporting and antibiotic prescribing in general practice. J Antimicrob Chemother 51:379–384. <https://doi.org/10.1093/jac/dkg032>.
 29. Clinical and Laboratory Standards Institute. 2016. Verification of commercial microbial identification and antimicrobial susceptibility testing systems. M52. Clinical and Laboratory Standards Institute, Wayne, PA.
 30. Chandrasekaran S, Abbott A, Campeau S, Zimmer BL, Weinstein M, Thrupp L, Hejna J, Walker L, Ammann T, Kirn T, Patel R, Humphries RM. 2018. Direct-from-blood culture disk diffusion to determine antimicrobial susceptibility of Gram-negative bacteria: preliminary report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. J Clin Microbiol 56:e01678-17. <https://doi.org/10.1128/JCM.01678-17>.
 31. Froding I, Vondracek M, Giske CG. 2017. Rapid EUCAST disc diffusion testing of MDR *Escherichia coli* and *Klebsiella pneumoniae*: inhibition zones for extended-spectrum cephalosporins can be reliably read after 6 h of incubation. J Antimicrob Chemother 72:1094–1102.
 32. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial disk susceptibility tests; approved standard—13th ed. M02. Clinical and Laboratory Standards Institute, Wayne, PA.
 33. Hombach M, Maurer FP, Pfiffner T, Bottger EC, Furrer R. 2015. Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. J Clin Microbiol 53:3864–3869. <https://doi.org/10.1128/JCM.02351-15>.
 34. Korgenski EK, Daly JA. 1998. Evaluation of the BIOMIC video reader system for determining interpretive categories of isolates on the basis of disk diffusion susceptibility results. J Clin Microbiol 36:302–304.
 35. Hombach M, Zbinden R, Bottger EC. 2013. Standardisation of disk diffusion results for antibiotic susceptibility testing using the Sirscan automated zone reader. BMC Microbiol 13:225. <https://doi.org/10.1186/1471-2180-13-225>.
 36. van den Bijlaardt W, Buiting AG, Mouton JW, Muller AE. 2017. Shortening the incubation time for antimicrobial susceptibility testing by disk diffusion for Enterobacteriaceae: how short can it be and are the results accurate? Int J Antimicrob Agents 49:631–637. <https://doi.org/10.1016/j.jantimicag.2016.12.019>.
 37. Hombach M, Jetter M, Blochliger N, Kolesnik-Goldmann N, Bottger EC. 2017. Fully automated disc diffusion for rapid antibiotic susceptibility test results: a proof-of-principle study. J Antimicrob Chemother 72: 1659–1668. <https://doi.org/10.1093/jac/dkx026>.