M44-A2
Vol. 29 No. 17
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Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition

This document provides newly established methodology for disk diffusion testing of *Candida* spp., criteria for quality control testing, and interpretive criteria.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Abstract

Clinical and Laboratory Standards Institute (CLSI) broth dilution reference methods are available for susceptibility testing of yeasts (see CLSI document M27—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*) and moulds (see CLSI document M38—*Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*). There still remains, however, a need for an alternative simple, rapid, and cost-effective approach to determine susceptibility of fungal organisms to various classes of antifungal agents that would make antifungal susceptibility testing more readily available to the clinical microbiology laboratory. The CLSI Subcommittee on Antifungal Susceptibility Testing has therefore developed a disk diffusion method for testing yeasts. CLSI document M44-A2—*Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition* provides approved zone interpretive criteria (breakpoints) for *Candida* species for caspofungin, fluconazole, and voriconazole after 20 to 24 hours incubation, as well as quality control parameters for caspofungin, fluconazole, posaconazole, and voriconazole. There are currently more than 10 systemically active antifungal agents, and it is expected that this document will further encourage the development of disk diffusion testing for at least some of these additional agents and genera.

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Foreword

Owing to the increased incidence of systemic fungal infections and number of antifungal agents available for systemic administration, antifungal susceptibility testing has gained greater recognition. Today, antifungal susceptibility testing has come of age in guiding physicians in the selection of antifungal therapy. Broth macrodilution and microdilution reference methods are now available for susceptibility testing of both yeasts (see CLSI document M27[1]) and moulds (see CLSI document M38[2]). To make antifungal susceptibility testing more readily available to clinical microbiology laboratories, there still remains a need for alternative, simple, rapid, and cost-effective approaches. Disk diffusion testing has served as such an example for antibacterial testing (see CLSI document M02[3]), and therefore, the CLSI Subcommittee on Antifungal Susceptibility Tests has developed recommendations for disk diffusion testing for antifungal agents.

A disk diffusion method for testing yeasts has been developed. At present, this method is validated only for *Candida* spp. tested vs various azoles and echinocandins. This method provides qualitative results after 20 to 24 hours incubation. In addition, the use of supplemented Mueller-Hinton agar in lieu of RPMI 1640 medium should make antifungal susceptibility testing more readily available to at least some clinical laboratories, and at reduced cost. Zone interpretive criteria (breakpoints) for caspofungin, fluconazole, and voriconazole, and quality control parameters for caspofungin, fluconazole, posaconazole, and voriconazole have been established according to standard CLSI procedures. CLSI expects that this document will encourage the development of disk diffusion testing for other antifungal agents and fungal genera.

Key Words

Antifungal, antimicrobial, disk, disk diffusion, Kirby-Bauer method, susceptibility testing

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1 Scope

With a need to make antifungal susceptibility testing more readily available to the clinical laboratory, CLSI document M44-A2 provides an established methodology for disk diffusion testing of *Candida* spp.; zone interpretive criteria for caspofungin, fluconazole, and voriconazole; and recommended quality control ranges for caspofungin, fluconazole, posaconazole, and voriconazole.

The method described in this document is intended for testing *Candida* spp. This method does not currently encompass any other genera and has not been used in studies of the yeast form of dimorphic fungi, such as *Blastomyces dermatitidis* or *Histoplasma capsulatum.* Moreover, testing of filamentous fungi (ie, moulds) is not addressed in the current procedure.

The method described herein must be followed exactly to obtain reproducible results. When new problems are recognized or improvements in these criteria are developed, changes will be incorporated into future editions of this guideline and also distributed in periodic informational supplements.

This guideline is intended for use by, but not limited to, health care, academic, government, industry, or independent research organizations that perform antifungal susceptibility testing of yeasts.

2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[4] For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious diseases, refer to CLSI document M29.[5]

3 Terminology

3.1 Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards focuses on harmonization of terms to facilitate the global application of standards.

Of particular note in CLSI document M44-A2 are two terms whereby CLSI intends to eliminate confusion, over time, through its commitment to harmonization. For the most part, in this guideline, the term "accuracy" in its metrological sense, refers to the closeness of agreement between a measured

quantity value and a true quantity value of a measurand, thus comprising both random and systematic effects. The term trueness, usually used to replace the term accuracy when referring to the closeness of agreement, does not apply in M44, because it refers to the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

3.2 Definitions

accuracy (measurement) – closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO/IEC Guide 99).[6]

antibiogram – overall profile of antimicrobial susceptibility results of a microbial species to a battery of antimicrobial agents.

antimicrobial susceptibility test interpretive category – **1)** a classification based on an *in vitro* response of an organism to an antimicrobial agent at levels of that agent corresponding to blood or tissue levels attainable with usually prescribed doses of that agent; **2) susceptible antimicrobial susceptibility test interpretive category** – a category that implies that an infection due to the isolate may be appropriately treated with the dosage of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated; **3) susceptible-dose dependent (S-DD) antimicrobial susceptibility test interpretive category** – a category that includes isolates with antimicrobial agent minimal inhibitory concentrations (MICs) that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates; **4) intermediate (I) antimicrobial susceptibility test interpretive category** – a category that includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates and/or available data do not permit them to be clearly categorized as either "susceptible" or "resistant"; **NOTE:** This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations; **5) resistant antimicrobial susceptibility test interpretive category** – resistant isolates that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or where clinical efficacy has not been reliable in treatment studies; **6) nonsusceptible (NS) test interpretive category** – a category used for organisms that currently have only a susceptible interpretive category, but not susceptible-dose dependent, intermediate, or resistant interpretive categories (ie, susceptible-only interpretive category); **NOTE:** This category is often given to new antimicrobial agents for which no resistant isolates have yet been encountered.

precision (measurement) – closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (ISO/IEC Guide 99).[6]

quality control – part of quality management focused on fulfilling quality requirements (ISO 9000)[7]; **NOTE:** This includes operational techniques and activities used to fulfill these requirements.

repeatability (measurement) – measurement precision under a set of repeatability conditions of measurement (ISO/IEC Guide 99).[6]

repeatability condition (of measurement) – condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time (ISO/IEC Guide 99).[6]

3.3 Abbreviations and Acronyms

GMB	2% glucose and 0.5 µg/mL methylene blue dye
MIC	minimal inhibitory concentration

4 Selection of Antimicrobial Agent Disks for Routine Testing and Reporting

Although interpretive criteria are now available for a limited number of organism-drug combinations, routine testing is not generally recommended. However, some institutions may find it useful to systematically test selected drug-organism combinations (eg, fluconazole vs *Candida* from sterile sites). At each institution, the decision to perform testing of yeast isolates is best made as a collaborative effort among infectious disease practitioners, the pharmacy and therapeutics committee, clinical microbiology personnel, and the infection control committee.

4.1 Use of Nonproprietary or Generic Names

To minimize confusion, all antifungal agents should be referred to by international nonproprietary (eg, generic) names.

4.2 Number of Agents Tested

To make routine susceptibility tests relevant and practical, the number of antimicrobial agents tested should be limited. Although this is not an immediate issue for antifungal agents, the same principle applies.

4.3 Suggested Guidelines for Selective Reporting

Testing may be warranted under certain selected circumstances such as: 1) part of periodic batch surveys that establish antibiograms for collections of pathogenic isolates obtained from within an institution; 2) to aid in the management of refractory infections due to *Candida* spp. in patients experiencing therapeutic failure with the standard agent at the standard dose; and 3) to aid in the management of invasive infections due to *Candida* spp. Disk diffusion interpretive criteria are available only for caspofungin, fluconazole, and voriconazole vs *Candida* spp., and the clinical relevance of testing any other drug-organism combination remains uncertain.

5 Equipment/Materials

The following equipment is recommended for performance of antifungal disk diffusion susceptibility testing:

- incubator set at 35 °C (±2 °C) with ambient air;
- McFarland 0.5 turbidity standard;
- sterile cotton (not synthetic polyester fiber) swabs;
- sterile physiological (8.5 g/L NaCl; 0.85%) saline; and
- spectrophotometer.

6 Reagents for the Disk Diffusion Test

6.1 Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium (see Appendix A)

Of the many agar media available, the subcommittee considers supplemented Mueller-Hinton agar to be a good choice for routine susceptibility testing of yeasts for the following reasons:

- It is readily available.
- It shows acceptable batch-to-batch reproducibility.[8,9]
- When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue dye to a final concentration of 0.5 µg/mL enhances zone edge definition.
- The base medium can easily be supplemented either pre- or postproduction to contain the final concentration of 2% glucose and 0.5 µg/mL methylene blue dye.

Although Mueller-Hinton agar is generally reliable for susceptibility testing, results obtained with some brands and batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test usually are larger than expected and may exceed the acceptable quality control limits. Only Mueller-Hinton medium formulations that have been tested and meet the acceptance limits described in CLSI document M06[10] should be used.

6.1.1 pH of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye Medium

The pH of each batch of prepared Mueller-Hinton agar should be checked. The method used largely depends on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. The pH can be checked by one of the following means:

- Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
- Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
- Use a properly calibrated surface electrode.

6.1.2 Moisture on Agar Surface

If excess surface moisture is present, the agar plates should be dried in an incubator or laminar flow hood with the lids ajar until the excess moisture has evaporated (usually 10 to 30 minutes). The surface should be moist, but with no droplets on the agar surface or the Petri dish cover.

6.2 Storage of Antimicrobial Disks

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Disks should be stored as follows:

- Refrigerate the containers at 8 °C or below, or freeze at −14 °C or below, in a nonfrost-free freezer until needed. The disks may retain greater stability if stored frozen until the day of use. Always refer to instructions in the product insert.
- The disk containers should be removed from the refrigerator or freezer 30 to 60 minutes before use so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold disks.
- Once a cartridge of disks is removed from its sealed packaging, it should be placed in a tightly sealed, desiccated container. Aseptic technique should be employed when handling individual disks.

- A disk-dispensing apparatus should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. The desiccant should be replaced when the indicator changes color.

- When not in use, the dispensing apparatus containing the disks should always be refrigerated.

- Only disks within their valid shelf life may be used. Disks should be discarded on the expiration date.

6.3 Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ suspension with a turbidity equivalent to a 0.5 McFarland standard or its optical equivalent (eg, latex particle suspension) should be used. See Appendix B for instructions on preparing a $BaSO_4$ turbidity standard.

7 Procedure for Performing the Disk Diffusion Test

7.1 Inoculum Preparation: Direct Colony Suspension Method

Steps for preparation of the inoculum are as follows:

(1) All organisms need to be subcultured onto blood agar or Sabouraud dextrose agar to ensure purity and viability. The incubation temperature throughout must be 35 °C (±2 °C).

(2) Inoculum is prepared by selecting five distinct colonies of approximately 1 mm in diameter from a 24-hour-old culture of *Candida* spp. Colonies are suspended in 5 mL of sterile 0.145 mol/L saline (8.5 g/L NaCl; 0.85% saline).

(3) The resulting suspension is vortexed for 15 seconds, and its turbidity is adjusted either visually or with a spectrophotometer by adding sufficient sterile saline or more colonies to adjust the transmittance to that produced by a 0.5 McFarland standard (see Appendix B) at 530 nm wavelength. This procedure yields a yeast stock suspension of 1×10^6 to 5×10^6 cells per mL, and should produce semiconfluent growth with most *Candida* spp. isolates.

7.2 Inoculation of Test Plates

(1) Optimally, inoculation of test plates occurs within 15 minutes after adjusting the turbidity of the inoculum suspension. If it is not technically feasible to inoculate test plates within 15 minutes, the adjusted inoculum suspension may be stored in the refrigerator for up to two hours. To inoculate test plates, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly against the inside wall of the tube above the fluid level. This removes excess fluid from the swab.

(2) The dried surface of a sterile Mueller-Hinton + GMB agar plate is inoculated by evenly streaking the swab over the entire agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. The swab may be reintroduced into the inoculum before the second and/or third streaks to ensure that a sufficient lawn of yeast (semiconfluent growth) is created. This may vary based on the *Candida* spp. and the type of swab used (swabs with loose fibers may not require redipping, whereas those with tight fibers may require redipping). As a final step, the rim of the agar is swabbed.

(3) The lid may be left ajar for three to five minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks.

NOTE: Variations in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

7.3 Application of Disks to Inoculated Agar Plates

(1) Antimicrobial disks are dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure its complete contact with the agar surface. Whether the disks are placed individually or with a dispensing apparatus, they must be distributed evenly so they are no closer than 24 mm from center to center. Ordinarily, no more than 12 disks should be placed on a 150-mm plate, or more than five disks on a 100-mm plate. Because the drug diffuses almost instantaneously, a disk should not be moved once it has come into contact with the agar surface. Instead, a new disk is placed in another location on the agar. Disks should be placed no less than 10 mm from the edge of the Petri dish.

(2) The plates are inverted and placed in an incubator set to 35 °C (± 2 °C) within 15 minutes after the disks are applied.

7.4 Reading Plates and Interpreting Results

Each plate is examined after 20 to 24 hours of incubation. If the plate was satisfactorily streaked and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a semiconfluent lawn of growth. The plate is held a few inches above a black, nonreflecting background illuminated with reflected light. The zone diameter is measured to the nearest whole millimeter at the point at which there is a prominent reduction in growth. This process is highly subjective, and experience results in greater accuracy. Pinpoint microcolonies at the zone edge or large colonies within a zone are encountered frequently and should be ignored. If these colonies are subcultured and retested, identical results are usually obtained, ie, a clear zone with microcolonies at the zone edge or large colonies within the zone.[11] Read at 48 hours only when insufficient growth is observed after 24 hours of incubation.

8 Interpretation of Disk Diffusion Test Results

8.1 Zone Diameter Interpretive Standards

Table 1 (see M44 Informational Supplement) provides zone diameter interpretive criteria to categorize accurately the levels of susceptibility of organisms to caspofungin, fluconazole, and voriconazole. These categories were developed by first comparing zone diameters to MICs for a large number of isolates, including those with known mechanisms of resistance relevant to the particular drug. MICs and correlated zone sizes were analyzed in relation to the pharmacokinetics of the drug from normal dosing regimens. Whenever possible, the tentative *in vitro* interpretive criteria were analyzed in relation to studies of clinical efficacy in the treatment of specific pathogens.[12-17] (See CLSI documents M23[18] and M27.[1])

Of note, interpretive breakpoints for fluconazole are not applicable to *Candida krusei;* thus, identification to the species level is required.

8.2 Interpretive Categories

8.2.1 Susceptible

The susceptible (S) category implies that an infection due to the strain may be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

8.2.2 Susceptible-Dose Dependent

The susceptible-dose dependent (S-DD) category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. Susceptibility is dependent on achieving the maximal possible blood level. This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

8.2.3 Intermediate

The intermediate (I) category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates and/or available data do not permit them to be clearly categorized as either "susceptible" or "resistant." This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.

8.2.4 Resistant

Resistant (R) strains are those that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or when zone diameters have been in a range where clinical efficacy has not been reliable in treatment studies.

8.2.5 Nonsusceptible

The nonsusceptible (NS) category includes organisms that currently have only a susceptible interpretive category, but not intermediate, susceptible-dose dependent, or resistant interpretive categories. This category is often given to new antimicrobial agents for which no resistant isolates have yet been encountered.

8.3 Zone Diameter Interpretive Criteria

Disk diffusion zone diameters correlate inversely with MICs from standard dilution tests. Table 1 (see M44 Informational Supplement) lists the zone diameter interpretive criteria. These criteria are based on zone diameter vs MIC comparisons for the MIC interpretive criteria defined in CLSI document M27.[1]

9 Quality Control Procedures

9.1 Purpose

The goals of a quality control program are to monitor the following:

- the precision (repeatability) and accuracy of the susceptibility test procedure;
- the performance of reagents used in the test; and
- the performance of persons who carry out the tests and read results.

These goals are best achieved by, but not limited to, the testing of quality control strains with known susceptibility to the antimicrobial agents tested.

9.2 Reference Strains for Quality Control

To control the precision (repeatability) and accuracy of the results obtained with the disk diffusion test procedure, several quality control strains should be obtained from a reliable source. The recommended quality control strains include

- *Candida albicans* ATCC®[a] 90028;
- *Candida parapsilosis* ATCC® 22019;
- *Candida tropicalis* ATCC® 750; and
- *Candida krusei* ATCC® 6258.

9.3 Storing Quality Control Strains

- The quality control strains should be tested by the standard disk diffusion test procedure described herein using the same materials and methods that are used to test clinical isolates.

- Quality control strains are stored in a way that minimizes the possibility of mutation in the organism.

- There are several methods for prolonged storage of reference strains. For example, yeasts may be grown on slants of potato dextrose agar and then frozen at −70 °C, as described by Pasarell and McGinnis.[19] Alternatively, strains can be preserved by suspending yeasts into vials containing 10% glycerol solution for freezing and storing at −70 °C.[20] Commercial storage systems that use a cryogenic solution containing porous beads and that have been demonstrated by the manufacturer to preserve fungi are also available.[21]

- Working quality control cultures are stored on blood agar or Sabouraud dextrose agar at 2 °C to 8 °C and subcultured each week for no more than three successive weeks. New working cultures should be prepared at least monthly from frozen, freeze-dried, or commercial cultures.

- Frozen or freeze-dried cultures should be subcultured at least twice before testing.

- A quality control strain can be used to monitor the precision (repeatability) and accuracy of the disk test, as long as there is no significant change in the mean zone diameter that cannot be attributed to a faulty methodology. If an unexplained result suggests a change in the organism's inherent susceptibility, a fresh new stock culture of the control strain should be obtained.

9.4 Zone Diameter Quality Control Limits

Acceptable zone diameter quality control limits for quality control strains are listed in Table 2[12,16,22,23] (see M44 Informational Supplement). The overall performance of the test system should be monitored using these ranges by testing the appropriate control strains each day the test is performed or, if satisfactory performance is documented, testing may be done weekly (see Section 9.5.2).

9.5 Frequency of Quality Control Testing

9.5.1 Daily Testing

When testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range (based on 95% confidence limits, 1 out of 20

[a] ATCC is a registered trademark of the American Type Culture Collection.

random results may be out of control). Any more than 1 out-of-control result in 20 consecutive tests requires corrective action (see Section 9.6).

9.5.2 Demonstrating Satisfactory Performance for Conversion From Daily to Weekly Quality Control Testing

- All applicable control strains are tested for 20 consecutive test days, and results are documented.
- To convert from daily to weekly quality control testing, no more than 1 out of 20 zone diameters for each antimicrobial agent/organism combination may be outside the acceptable zone diameter limits in Table 2 (see M44 Informational Supplement).

9.5.3 Implementing Weekly Quality Control Testing

- Weekly quality control testing may be implemented once satisfactory performance is documented (see Section 9.5.2).
- Quality control testing is performed once per week and whenever any reagent component of the test (eg, a new lot of agar plates or a new lot of disks from the same or a different manufacturer) is changed.
- If any of the weekly quality control results are out of the acceptable range, corrective action is required (see Section 9.6).
- If a new antimicrobial agent is added, it must be tested for 20 consecutive test days and satisfactory performance documented before converting to a weekly schedule. In addition, 20 days of consecutive testing are required if there is a major change in the method of reading test results, such as conversion from manual zone measurements to an automated zone reader.

9.6 Corrective Action

9.6.1 Out-of-Control Result Due to an Obvious Error

Obvious reasons for out-of-control results include

- use of the wrong disk;
- use of the wrong control strain;
- obvious contamination of the strain; or
- inadvertent use of the wrong incubation temperature or conditions.

In such cases, the reason is documented and the strain is retested on the day the error is observed. If the repeated result is within range, no further corrective action is required.

9.6.2 Out-of-Control Result Not Due to an Obvious Error

9.6.2.1 Immediate Corrective Action

If there is no obvious reason for an out-of-control result, immediate corrective action is required.

- The antimicrobial agent/organism combination is tested for a total of five consecutive test days. All results in question are documented.

- If all five zone diameter measurements for the antimicrobial agent/organism combination are within acceptable ranges, as defined in Table 2 (see M44 Informational Supplement), no additional corrective action is necessary.

- If any of the five zone diameter measurements are outside the acceptable range, additional corrective action is required (see Section 9.6.2.2).

- Daily control tests must be continued until final resolution of the problem can be achieved.

9.6.2.2 Additional Corrective Action

When immediate corrective action does not resolve the problem, it is likely due to a system error vs a random error. The following common sources of error should be investigated:

- Zone diameters were measured and transcribed correctly.

- The turbidity standard has not expired, is stored properly, meets performance requirements (see Section 6.3 and Appendix B), and was adequately mixed before use.

- All materials used were within their expiration date and stored at the proper temperature.

- The incubator is at the proper temperature and atmosphere.

- Other equipment used (eg, pipettors) are functioning properly.

- Disks are stored desiccated and at the proper temperature.

- The control strain has not changed and is not contaminated.

- Inoculum suspensions were prepared and adjusted correctly.

- Inoculum for the test was prepared from a plate incubated for the correct length of time and in no case was more than 24 hours old.

It may be necessary to obtain a new quality control strain (either from freezer stock or a reliable source) and new lots of materials (including new turbidity standards), possibly from different manufacturers. If the problem appears to be related to a commercial product, the manufacturer should be contacted. It is also helpful to exchange quality control strains and test materials with another laboratory using the same method. Until the problem is resolved, an alternative test method should be used.

Once the problem is corrected, documentation of satisfactory performance for another 20 consecutive days is required before returning to weekly quality control testing (see Section 9.5.2).

9.7 Reporting Patient Results When Out-of-Range Tests Occur

Whenever an out-of-range result or corrective action is necessary, careful assessment of whether to report patient results should be made on an individual basis, taking into account if the source of error, when known, is likely to have affected relevant patient results. Options that may be considered include suppressing the results for an individual antimicrobial agent; retrospectively reviewing individual patient

or cumulative data for unusual patterns; and using an alternate test method or a reference laboratory until the problem is resolved.

10 Limitations of Disk Diffusion Methods

10.1 Application to Various Organism Groups

The disk diffusion method described in this document has been standardized for *Candida* spp. only. For other yeasts, consultation with an infectious disease specialist is recommended for guidance in determining the need for susceptibility testing and interpretation of results. Published reports in the medical literature and current consensus recommendations for therapy of uncommon microorganisms may obviate the need for testing. If necessary, a reference dilution method may be the most appropriate alternative testing method, and this may require submitting the organism to a reference laboratory.

10.2 Verification of Patient Results

Multiple test parameters are monitored by following the quality control recommendations described in this standard. However, acceptable results derived from testing quality control strains do not guarantee accurate results when testing patient isolates. It is important to review all of the results obtained from all drugs tested on a patient's isolates before reporting the results.

Unusual or inconsistent results should be verified by checking for the following: 1) transcription errors; 2) contamination of the test (recheck purity plates); and 3) previous results on the patient's isolates. If a reason for the unusual or inconsistent result cannot be ascertained, the susceptibility test is repeated, the species identity is verified, or a new clinical specimen is requested. Each laboratory must develop its own policies for verification of unusual or inconsistent antimicrobial susceptibility test results.

References

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3 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition.* CLSI document M02-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

4 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007. http://www.cdc.gov/ncidod/dhqp/gl_isolation.html. Accessed 27 October 2008.

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Appendix A. Preparation of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye

The medium can be prepared and poured as the complete media with supplements (A1), *or* the supplements can be added to commercially prepared Mueller-Hinton agar plates (A2). Using the latter technique enables the use of routine Mueller-Hinton agar plates from the bacteriology laboratory.

A1. Preparation of Supplemented Mueller-Hinton Agar:

(1) Prepare Mueller-Hinton agar from a commercially available dehydrated Mueller-Hinton agar base according to the manufacturer's instructions.

(2) Dissolve 0.1 g of methylene blue dye in 20 mL of distilled water and warm gently to dissolve. Do not overheat. Add 100 µL of this solution per liter of agar suspension.

(3) Add 20 g of glucose per liter of agar suspension.

(4) Autoclave as directed by the manufacturer's instructions.

(5) Immediately after autoclaving, allow the agar solution to cool in a 45 °C to 50 °C water bath.

(6) Pour the freshly prepared and cooled medium into plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 67 mL to 70 mL of medium for plates with diameters of 150 mm, and 28 mL to 30 mL for plates with a diameter of 100 mm.

(7) Allow the agar medium to cool to room temperature and, unless the plate is used on the same day of preparation, store at refrigerator temperature (2 °C to 8 °C). The agar medium should have a pH between 7.2 and 7.4 at room temperature (see CLSI document M07).[1]

(8) Use plates within seven days after preparation unless adequate precautions such as wrapping in plastic have been taken to minimize drying of the agar.

(9) Examine a representative sample of each batch of plates for sterility by incubating at 30 °C to 35 °C for 24 hours or longer. Plates should undergo quality control testing as per Section 9.

A2. Glucose-Methylene Blue (GMB) Supplementation of Commercially Prepared Mueller-Hinton Agar:

(1) Commercially prepared Mueller-Hinton agar plates can be obtained from several manufacturers. These are the same plates used by the bacteriology laboratory for performing Kirby-Bauer disk diffusion tests on bacteria.

(2) Dissolve 0.1 g of methylene blue dye to 20 mL of distilled water and warm gently to dissolve. Do not overheat.

(3) Prepare a 0.4 g/mL stock solution of glucose by dissolving 40 g of glucose in 100 mL of distilled water. Heat gently and mix to dissolve.

(4) Add 200 µL of the methylene blue dye stock solution (see [2] above) to 100 mL of the glucose stock solution (see [3] above) to make a GMB stock with a final concentration of 40% glucose and 10 µg/mL of methylene blue dye.

Appendix A. (Continued)

(5) Dispense GMB stock solution in bottles or vials containing 3.5-mL aliquots for 150-mm plates or 1.5-mL aliquots for 90- to 100-mm plates.

(6) Autoclave for 15 minutes at 121 °C followed by slow exhaust.

(7) Store at room temperature and handle aseptically. Do not refrigerate, because this may cause precipitation. A one-year shelf life is generally assumed.

(8) Pour 3.5 mL of the GMB supplement onto the surface of a 150-mm plate with a 70-mL fill *or* 1.5 mL onto a 90- to 100-mm plate with a 30-mL fill. (The actual volume of GMB supplement added may vary slightly depending upon the volume of media plated by the manufacturer. Check with commercially prepared media manufacturers for the exact volume of media used.)

(9) Tilt the plate to spread the supplement evenly. A sterile spreader or sterile bent glass rod can be used to spread the solution evenly across the surface of the agar plate.

(10) Allow the GMB solution to *completely* absorb before inoculating the plate. This requires the plates to be held from 4 to 24 hours before use. The plates can be dried at room temperature, incubator temperature, or stored at refrigerated temperature until used. Plates should be used within seven days after preparation unless adequate precautions such as wrapping in plastic have been taken to minimize drying of the agar.

Reference for Appendix A

[1] CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition.* CLSI document M07-A8. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

Appendix B. McFarland 0.5 Barium Sulfate Turbidity Standard

To standardize the inoculum density, a $BaSO_4$ turbidity standard is used (0.5 McFarland Standard).

The procedure consists of the following steps:

(1) Prepare this turbidity standard by adding 0.5 mL of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot H_2O$) to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

(2) Verify the correct density of the turbidity standard by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.

(3) Distribute 4 mL to 6 mL into screw-cap tubes of the same size as those used in growing or diluting the broth culture inoculum.

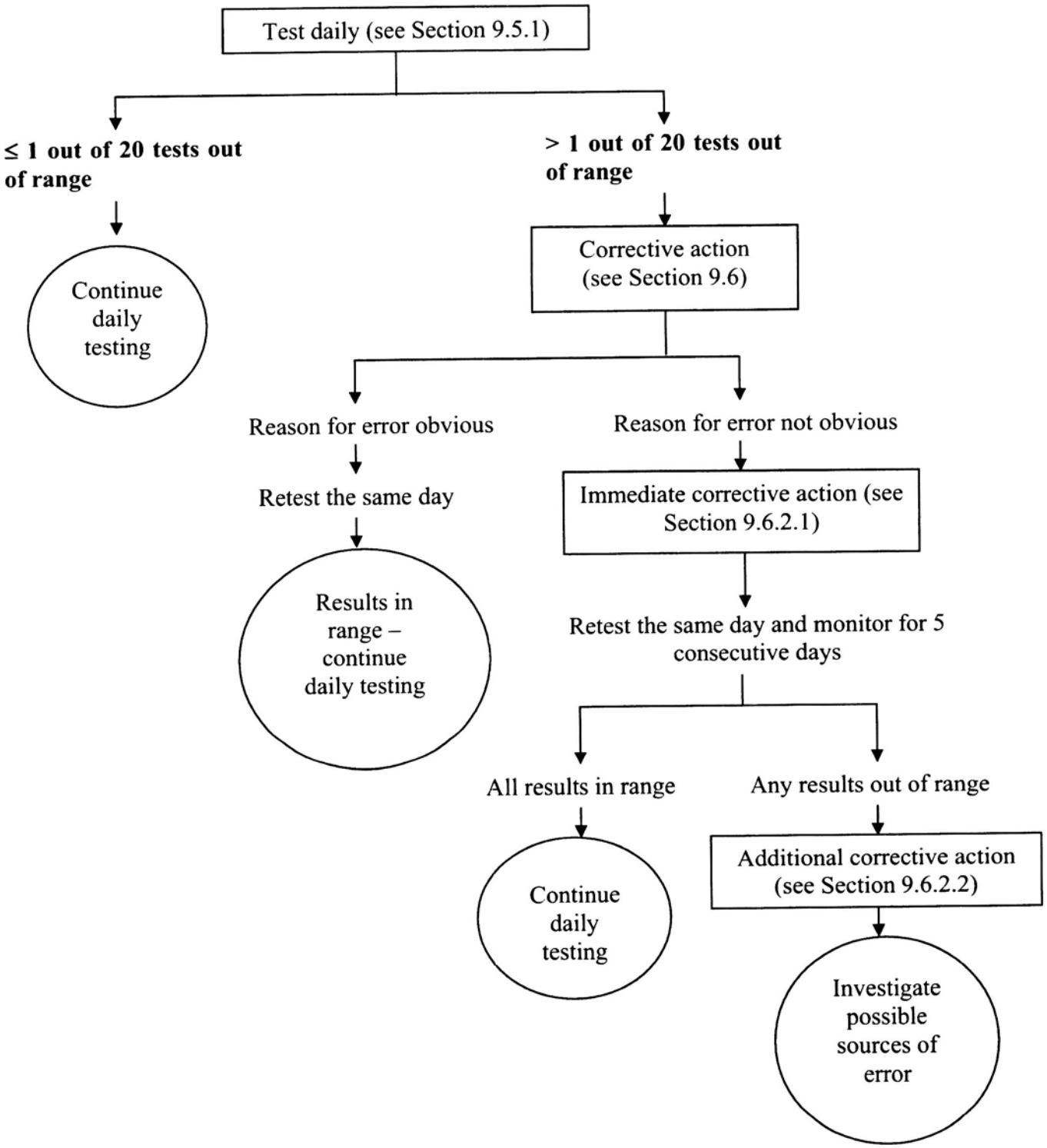
(4) Tightly seal these tubes and store them in the dark at room temperature.

(5) Vigorously agitate this turbidity standard on a mechanical vortex mixer just before use.

(6) Replace the barium sulfate standards or verify their densities monthly.

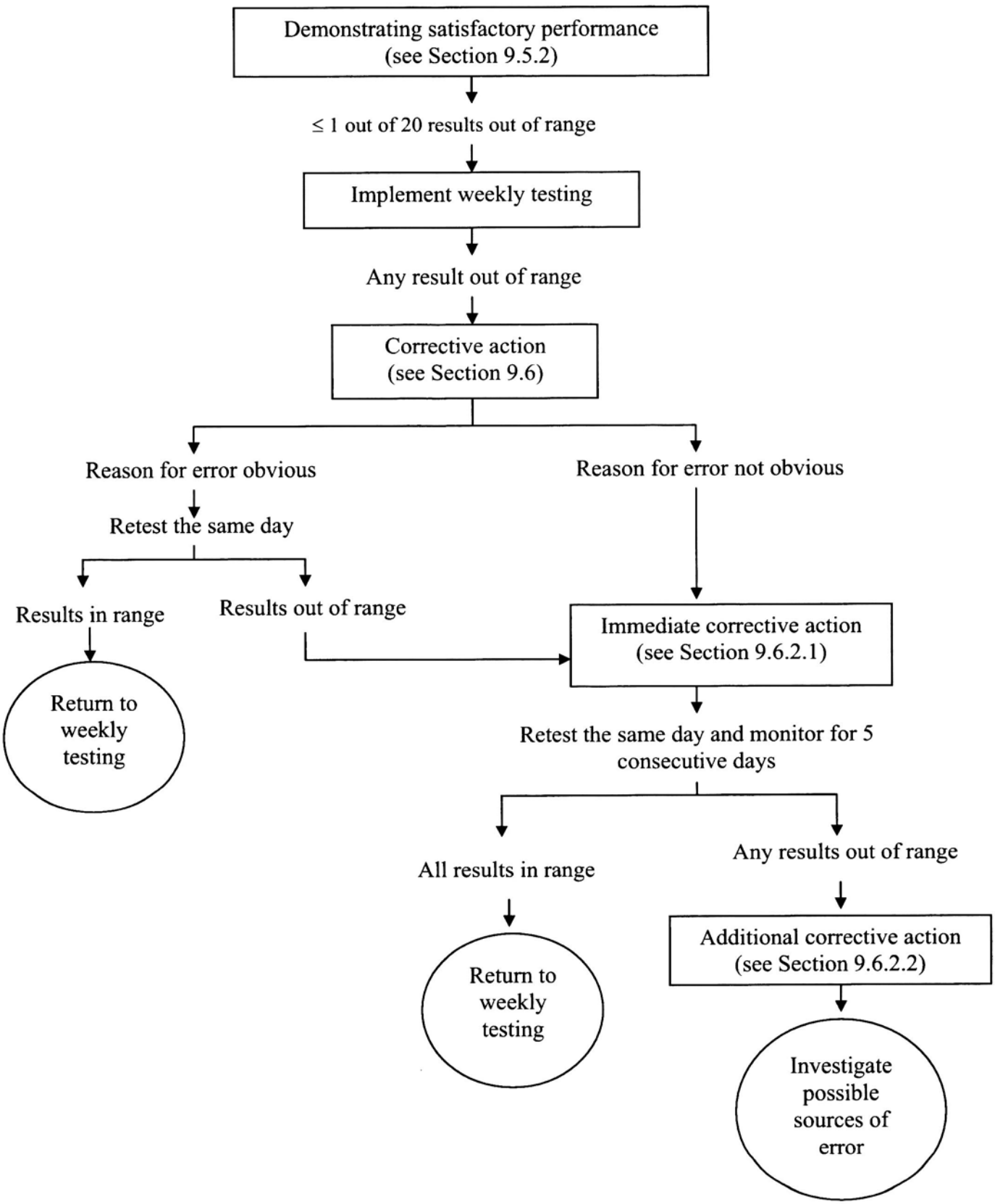
Appendix C. Quality Control Protocol Flow Charts

Disk Diffusion Daily Quality Control Testing Protocol



Appendix C. (Continued)

Disk Diffusion Weekly Quality Control Testing Protocol



Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Delegate Comments and Subcommittee Responses

M44-A2: *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline—Second Edition*

Section 3.1, Note on Terminology

1. First paragraph, second to the last line: The last sentence in the paragraph refers to "implementation of this policy," but no policy was identified. Identify the policy.

- **The policy in question is the CLSI Organization Policy on Harmonization. CLSI decided that the introductory paragraph in the "Note on Terminology" will be streamlined and revised to read, "CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards focuses on harmonization of terms to facilitate the global application of standards." To ease confusion, the reference to the policy was removed.**

Section 6.1, Mueller-Hinton Agar +2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium

2. Commercially prepared culture media are not readily available. Most laboratories do not prepare culture media anymore.

- **Mueller-Hinton agar is readily available and can easily be supplemented either pre- or postproduction to contain the final concentration of 2% glucose and 0.5 µg/mL of methylene blue dye. Therefore, no change was made to the document.**

Section 6.2, Storage of Antimicrobial Disks

3. Can the disks be handled with bare hands, or should gloves be worn to protect either the disk or the user? Add a sentence about the need or lack of need for gloves when handling disks.

- **To clarify this issue, the following was added to the third bullet of Section 6.2: "Aseptic technique should be employed when handling individual disks."**

4. Page 5 does not indicate if refreezing of unused disks is acceptable practice. Confirm if refreezing of unused disks is acceptable practice.

- **No change was made to the document. Storage conditions for disks should be followed as outlined in the product insert and may be different for each drug. Therefore, this is addressed in the first bullet under Section 6.2 as follows: "Always refer to instructions in the product insert."**

Section 7.2, Inoculation of Test Plates

5. Step 2. Is the swab re-"dipped" in the inoculum prior to the second and third streaks? Clarify whether the swab is reintroduced into the inoculum for the second and third streaks.

- **This was clarified as suggested. The following statement was added to Section 7.2 (2): "The swab may be reintroduced into the inoculum before the second and/or third streaks to ensure that a sufficient lawn of yeast (semiconfluent growth) is created. This may vary based on the *Candida* spp. and type of swab used (swabs with loose fibers may not require redipping, whereas those with tight fibers may require redipping)."**

Section 7.3, Application of Disks to Inoculated Agar Plates

6. Is a minimum distance required between the disk and the wall of the agar plate? Indicate whether the distance between the disk and the plate wall is important.

- **For clarity, the following statement was added to Section 7.3: "(1) Disks should be placed no less than 10 mm from the edge of the Petri dish."**

Section 7.4, Reading Plates and Interpreting Results

7. Is the zone of inhibition measured from the outer edge of the disk or from the center of the disk? It is not clear from the text. Clarify how the zone is measured.

- **No change was made to the document. As written, "the zone diameter is measured" indicates that the diameter of the entire zone of inhibition is measured (from one edge of inhibition to the opposite edge of inhibition) rather than the radius (from the disk edge/center to the edge of inhibition).**

Section 8.1, Zone Diameter Interpretive Standards

8. Should the last sentence read "in addition to zone determination" rather than MIC determination for this guideline?

- **The subcommittee agrees. The phrase "in addition to MIC determination" was deleted as suggested.**

Section 9.4, Zone Diameter Quality Control Limits

9. Last sentence: Currently, Section 9.5.2 contains only one subsection, 9.5.2.1. Therefore, referencing both numbers seems unnecessary. Delete "(see Section 9.5.2.1)."

- **The subcommittee agrees, and the text was revised as suggested.**

Section 9.5.2, Weekly Testing

10. Because there are no other subsections, I suggest eliminating "9.5.2.1," and changing the title of 9.5.2 to "Demonstrating Satisfactory Performance for Conversion From Daily to Weekly Quality Control Testing." Alternatively, Section 9.5.2.1 could be kept, and 9.5.3 changed to 9.5.2.2.

- **The subcommittee agrees. Because Section 9.5.2 contains only one subsection (9.5.2.1), referencing both numbers seems unnecessary. Therefore, as suggested, subsection 9.5.2.1 was deleted and the title of Section 9.5.2 was changed to "Demonstrating Satisfactory Performance for Conversion From Daily to Weekly Quality Control Testing."**

Appendix A. Preparation of Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye

11. A2. (2): Remove the phrase "Add 100 µL of this solution per liter of agar suspension." It does not apply in this section.

- **The subcommittee agrees. The phrase was deleted as suggested.**

M44-S3: *Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretative Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Third Informational Supplement*

Table 2. Recommended Quality Control Zone Diameter (mm) Ranges

1. Unclear why posaconazole is included in Table 2. It is not in Table 1, so why include it in Table 2? Delete posaconazole from Table 2.

- **Posaconazole quality control ranges have been established; therefore, quality control data for posaconazole are included in Table 2. Conversely, interpretive criteria for posaconazole have not yet been established; therefore, interpretive criteria for posaconazole are not included in Table 1. No change was made to Table 1.**

2. The † note under Table 1 is somewhat confusing. The caspofungin standards were adopted 26 January 2008, and are "tentative for one year." It is now April 2009, so one year has passed, or is just starting, depending on when the "tentative for one year" begins? Put in the actual date for the one-year comment period (M44-S2 verbiage was "one year from publication of this document.")

- **The note was changed to "tentative for one year from publication of this document" for clarity.**

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The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines that facilitates project management, defines a document structure via a template, and provides a process to identify needed documents. The approach is based on the model presented in CLSI document HS01—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are as follows:

Documents and Records	Equipment	Information Management	Process Improvement
Organization	Purchasing and Inventory	Occurrence Management	Customer Service
Personnel	Process Control	Assessments—External and Internal	Facilities and Safety

M44-A2 addresses the QSEs indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Documents and Records	Organization	Personnel	Equipment	Purchasing and Inventory	Process Control	Information Management	Occurrence Management	Assessments—External and Internal	Process Improvement	Customer Service	Facilities and Safety
M07					X M02 M06 M07 M23 M27 M29 M38						M29

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M44-A2 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

Preexamination				**Examination**			**Postexamination**	
Examination ordering	Sample collection	Sample transport	Sample receipt/processing	Examination	Results review and follow-up	Interpretation	Results reporting and archiving	Sample management
				M02 M07 M27 M38	X M02 M07 M27 M38	X M02 M07 M27 M38	X M02 M07 M27 M38	M27 M38

Adapted from CLSI document HS01—*A Quality Management System Model for Health Care.*

Related CLSI Reference Materials*

M02-A10 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition (2009).** This document contains the current Clinical and Laboratory Standards Institute–recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.

M06-A2 **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2006).** This document provides procedures for evaluating production lots of dehydrated Mueller-Hinton agar, and for developing and applying reference media.

M07-A8 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition (2009).** This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M23-A3 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition (2008).** This document addresses the required and recommended data needed for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents.

M27-A3 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition (2008).** This standard addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

M29-A3 **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005).** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

M38-A2 **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition (2008).** This document addresses the selection of antifungal agents; preparation of antifungal stock solutions and dilutions for testing implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive and cutaneous fungal infections.

* CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

NOTES

NOTES

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Canadian Society for Medical Laboratory Science
COLA
College of American Pathologists
College of Medical Laboratory Technologists of Ontario
College of Physicians and Surgeons of Saskatchewan
Elkin Simson Consulting Services
ESCMID
Family Health International
Hong Kong Accreditation Service Innovation and Technology Commission
International Federation of Biomedical Laboratory Science
International Federation of Clinical Chemistry
Italian Society of Clinical Biochemistry and Clinical Molecular Biology
JCCLS
The Joint Commission
The Korean Society for Laboratory Medicine
National Society for Histotechnology, Inc.
Ontario Medical Association Quality Management Program-Laboratory Service
RCPA Quality Assurance Programs PTY Limited
SIMeL
Sociedad Espanola de Bioquimica Clinica y Patologia Molecular
Sociedade Brasileira de Analises Clinicas
Sociedade Brasileira de Patologia Clinica
Turkish Society of Microbiology
USP
World Health Organization

Government Members

Armed Forces Institute of Pathology
BC Centre for Disease Control
CAREC
Centers for Disease Control and Prevention
Centers for Disease Control and Prevention, China
Centers for Disease Control and Prevention – Ethiopia
Centers for Disease Control and Prevention – Namibia
Centers for Disease Control and Prevention – Nigeria
Centers for Disease Control and Prevention – Tanzania
Centers for Disease Control and Prevention RETRO -CI CDC/PEPFAR
Centers for Medicare & Medicaid Services
Centers for Medicare & Medicaid Services/CLIA Program
Chinese Committee for Clinical Laboratory Standards
Danish Institute for Food and Veterinary Research
Department of Veterans Affairs
DFS/CLIA Certification
FDA Center for Biologics Evaluation and Research
FDA Center for Devices and Radiological Health
FDA Center for Veterinary Medicine
Health Authority of Abu Dhabi
Health Canada
Indiana State Department of Health
Institut Pasteur de Cote D' Ivoire
Institute of Tropical Medicine Dept. of Clinical Sciences
Instituto Nacional de Saude
Kern County Department of Health Services
Laboratoire National de La Sante Publique
MA Dept. of Public Health Laboratories
Malaria Research Training Center
Meuhedet Central Lab
Ministry of Health and Social Welfare – Tanzania
Namibia Institute of Pathology
National Cancer Institute
National Center of Infectious and Parasitic Diseases (Bulgaria)
National Health Laboratory Service (South Africa)
National HIV & Retrovirology Lab
National Institute of Standards and Technology
National Pathology Accreditation Advisory Council (Australia)
New York City Office of Medical Examiner
New York State Department of Health
NJ State Department of Health and Senior Services
Ontario Agency for Health –Protection and Promotion
Orange County Health Care Agency – Public Health Lab
Pennsylvania Dept. of Health
SAIC Frederick Inc – NCI-Frederick Cancer Research & Development Center
Saskatchewan Health-Provincial Laboratory
Scientific Institute of Public Health
State of Alabama
State of Wyoming Public Health Laboratory
University of Iowa, Hygienic Lab
US Naval Medical Research Unit #3
USAMC – AFRIMS
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Abbott Point of Care Inc.
Access Genetics
AdvaMed
Al Borg Laboratory
Ammirati Regulatory Consulting
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Anna Longwell, PC
Arpida Ltd.
Astellas Pharma
AstraZeneca Pharmaceuticals
Aviir, Inc.
Axis-Shield PoC AS
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BD Diagnostic Systems
BD Vacutainer Systems
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Bioanalyse, Ltd.
Bio-Development S.r.l.
Biohit Oyj.
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bioMérieux, Inc. (MO)
bioMérieux, Inc. (NC)
bioMérieux Spain
Bio-Rad Laboratories, Inc. – France
Bio-Rad Laboratories, Inc. – Irvine, CA
Bio-Rad Laboratories, Inc. – Singapore
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Diagnostica Stago
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Instrumentation Laboratory (NY)
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Olympus America Inc.
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Powers Consulting Services
PPD
Predictive Physiology and Medicine
ProteoGenix, Inc.
QML Pathology
QSE Consulting
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59th MDW/859th MDTS/MTL Wilford Hall Medical Center (TX)
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Chatham – Kent Health Alliance (Canada)
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Children's Hospital of Orange County (CA)
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Children's Hospitals and Clinics (MN)
Children's Medical Center (OH)
Children's Medical Center (TX)
Children's Memorial Hospital (IL)
The Children's Mercy Hospital (MO)
Childrens Hospital Los Angeles (CA)
Childrens Hospital of Wisconsin (WI)
Christiana Care Health Services (DE)
Christus St. John Hospital (TX)
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Clinton Memorial Hospital (OH)
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Commonwealth of Virginia (DCLS) (VA)
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Community Medical Center (NJ)
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Effingham Hospital (GA)
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Elmhurst Hospital Center (NY)
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Fox Chase Cancer Center (PA)
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Hôpital Sacré-Coeur de Montreal (Quebec, Canada)
Hopital Santa Cabrini Ospedale (Canada)
Hospital Albert Einstein (Brazil)
Hospital das Clinicas-FMUSP (Brazil)
Hospital de Sousa Martins (Guarda) (Portugal)
Hospital Dirino Espirito Santa (Portugal)
The Hospital for Sick Children (Canada)
Hôtel-Dieu de Lévis (Canada)
Hôtel Dieu Grace Hospital Library (Windsor, ON, Canada)
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Johns Hopkins University (MD)
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Laboratory Corporation of America (NJ)
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Mayo Clinic (MN)
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Meadows Regional Medical Center (GA)
Medecin Microbiologiste (Canada)
Medical Center Hospital (TX)
Medical Center of Louisiana at NO-Charity (LA)
Medical Center of McKinney (TX)
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Medical Univ. of South Carolina (SC)
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Memorial Hospital at Gulfport (MS)
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St. Joseph Mercy Hospital (MI)
St. Joseph's Medical Center (CA)
St. Joseph's Regional Medical Center (NJ)
St. Jude C[illegible] rch Hospital (TN)
St. Louis Univers[illegible]
St. Luke's Hospita[illegible]
St. Luke's Hospital [illegible]
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Sunnybrook Health Science Centre (ON, Canada)
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Sutter Health (CA)
Suter[illegible]ville M[illegible]
Swedish [illegible] C[illegible]
Sydney So[illegible] Pa[illegible]
[illegible]
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Tufts New England Medical Center (MA)
Tulane Medical Center Hospital & Clinic (LA)
Turku University Central Hospital (Finland)
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UCLA Medical Center Clinical Laboratories (CA)
UCSD Medical Center (CA)
UCSF Medical Center – China Basin (CA)
UMass Memorial Medical Center (MA)
UMC of Southern Nevada (NV)
UNC Hospitals (NC)
Union Clinical Laboratory (Taiwan)
United Christian Hospital (Hong Kong)
United Clinical Laboratories (IA)
United States Air Force School of Aerospace Medicine/PHE (TX)
Unity HealthCare (IA)
Universita Campus Bio-Medico (Italy)
Universitair Ziekenhuis Antwerpen (Belgium)
University College Hospital (Ireland)
University Hospital Center Sherbrooke (CHUS) (Canada)
University Medical Center at Princeton (NJ)
University of Alabama Hospital Lab (AL)
University of Chicago Hospitals Laboratories (IL)
University of Colorado Health Sciences Center (CO)
University of Colorado Hospital (CO)
University of Illinois Medical Center (IL)
University of Iowa Hospitals and Clinics (IA)
University of Kentucky Med. Ctr. (KY)
University of Missouri Hospital (MO)
University of MN Medical Center – Fairview (MN)
University of MS Medical Center (MS)
University of Pennsylvania Health System (PA)
University of So. Alabama Children's and Women's Hospital (AL)
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The University of Texas Medical Branch (TX)
University of the Ryukyus (Japan)
University of Virginia Medical Center (VA)
UPMC Bedford Memorial (PA)
U.S. Naval Hospital Guantanamo Bay
US Naval Hospital Naples (FPO)
USC University Hospital (CA)
UW Hospital (WI)
VA (Bay Pines) Medical Center (FL)
VA (Biloxi) Medical Center (MS)
VA (Chillicothe) Medical Center (OH)
VA (Cincinnati) Medical Center (OH)
VA (Dayton) Medical Center (OH)
VA (Decatur) Medical Center (GA)
VA (Hampton) Medical Center (VA)
VA (Indianapolis) Medical Center (IN)
VA (Miami) Medical Center (FL)
VA New Jersey Health Care System (NJ)
VA (San Diego) Medical Center (CA)
VA (Sheridan) Medical Center (WY)
Valley Health (VA)
Vancouver Coastal Health Regional Laboratory (BC, Canada)
Vancouver Island Health Authority (SI) (Canada)
Vanderbilt University Medical Center (TN)
Veterans Hospital (FL)
Via Christi Regional Medical Center (KS)
Virga Jessezieukenhuis (Belgium)
Virginia Beach General Hospital (VA)
Virginia Regional Medical Center, (MN)
Virtua – West Jersey Hospital (NJ)
WakeMed (NC)
Walter Reed Army Medical Center (DC)
Warren Hospital (NJ)
Washington Hospital Center (DC)
Waterbury Hospital (CT)
Waterford Regional Hospital (Ireland)
Wayne Memorial Hospital (NC)
Wellstar Health System (GA)
West China Second University Hospital, Sichuan University (China)
West Jefferson Medical Center (LA)
West Shore Medical Center (MI)
West Valley Medical Center Laboratory (ID)
Western Baptist Hospital (KY)
Western Healthcare Corporation (Canada)
Western Pennsylvania Hospital (PA)
Wheaton Franciscan and Midwest Clinical Laboratories (WI)
Wheeling Hospital (WV)
William Osler Health Centre (Canada)
Winchester Hospital (MA)
Winn Army Community Hospital (GA)
Wishard Health Sciences (IN)
Womack Army Medical Center (NC)
York Hospital (PA)
Zulekha Hospital Dubai (United Arab Emirates)

Method for antifungal disk diffusion susceptibility testing of yeasts; approved guideline

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