Evaluation of Lots of Dehydrated Mueller-Hinton Broth for Antimicrobial Susceptibility Testing; Proposed Guideline

PLEASE

This proposed document is published for wide and thorough review as the first step in the NCCLS consensus-review process. Please send your comments on scope, approach, and technical and editorial content to the Executive Offices.

Comment period ends
12 March 2002

The subcommittee responsible for this document will assess all comments received by the end of the comment period. Based on this assessment, a new version of the document will be issued. Readers are encouraged to send their comments to the NCCLS Executive Offices, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898; Fax: (610) 688.0700, or to the following e-mail address: standard@nccls.org

COMMENT

This document provides protocols for evaluating the performance characteristics of production lots of dehydrated Mueller-Hinton broth (dMHB).

A guideline for global application developed through the NCCLS consensus process.

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Evaluation of Lots of Dehydrated Mueller-Hinton Broth for Antimicrobial Susceptibility Testing; Proposed Guideline

Abstract

This document describes methods for evaluation of production lots of Mueller-Hinton broth by manufacturers of the dehydrated product. Performance characteristics of production lots and broth prepared with the dehydrated product are determined by testing defined organism/antimicrobial agent combinations. The results of testing must conform to defined quality control limit ranges for each combination of antimicrobial agent and ATCC quality control strain. Each production lot should be tested at least against these combinations of antimicrobial agents and quality control strains. Lots falling outside these limits should be retested, and ion contents should be checked to ensure that they are within suggested ranges. Concentration ranges or limits of calcium (20 to 25 mg/L), magnesium (10 to 12.5 mg/L) and zinc (< 3mg/L) are provided. It is expected that media that contain these ranges or limits of cation concentrations will provide results within the defined quality control ranges.

This document will be updated when primary and secondary "standard" lots of Mueller-Hinton broth powder have been selected, so that manufacturers can check production lots against these standard lots of Mueller-Hinton broth media. The studies on which this proposed document is based were designed to test selected class-representative antimicrobial agents against specific quality control strains. As new antimicrobial agents are discovered for which Mueller-Hinton broth dilution quality control ranges are defined by NCCLS and FDA, the primary and secondary "standard" Mueller-Hinton broth media lots should be checked against those agents also. That information will permit the NCCLS Subcommittee on Culture Media to keep future revisions to this document up to date.

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Contents

Foreword

This proposed guideline is the result of the effort of the NCCLS Subcommittee on Culture Media to establish a standard protocol by which manufacturers of dehydrated Mueller-Hinton broth (dMHB) may determine its performance characteristics. This guideline is limited to preparation of production lots of dMHB for use according to NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. The objectives of the project were:

- to evaluate possible variations in performance among currently manufactured lots of dMHB that are prepared for use in fresh or frozen reference MIC microdilution trays;
- to select performance criteria that will guide manufacturers of dMHB to produce lots of media that give accurate and reproducible results for broth microdilution antimicrobial susceptibility tests; and
- to characterize and quantitate medium components (especially cations and anions) that may influence the activity of an antimicrobial agent when tested *in vitro*.

The subcommittee attempted to define the characteristics of acceptable lots of dMHB. Nine laboratories participated in the investigations. Two lots of dMHB that had been released for sale were obtained from four different manufacturers. The identities of medium lots were blinded and coded, and the source of the media was known neither to the investigators nor to the manufacturer of the frozen broth microdilution trays. The control organisms were supplied from a single lot through the auspices of the American Type Culture Collection. Technologists in the participating laboratories were provided with specific instructions on how to thaw, inoculate, incubate, and read the broth microdilution trays so that each laboratory was performing the same methodology.

Cation and anion content was determined on samples from each lot of dMHB by inductively coupled plasma (ICP) analysis in laboratories at the Alberta Research Council. Subsequently these same samples were analyzed for major cations and anions by two different methods (ICP and flame atomic absorption spectroscopy) at BD Diagnostic Systems and Dade Behring Microscan to check for comparability of results among different testing laboratories. Results of ion concentrations in the eight lots of dMHB were found to vary by less than 5% among the three testing laboratories.

The following variables were analyzed for each of the antimicrobial/organism combinations tested:

- performance of each lot compared to quality control criteria in NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically;*
- precision of the MIC observations; and
- interlaboratory variations.

The variability of cation and anion content in the lots of dMHB between and among manufacturers make it difficult to establish a "golden pound" of dMHB. The results of these laboratory investigations indicate that manufacturers should ensure that their production lots of dMHB conform to the quality control performance criteria identified in NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Contents of ions that are known or have been determined to affect *in vitro* activity of antimicrobial agents should be adjusted so that performance of the medium is appropriate. The content of critically important ions should be specifically identified on a certificate of analysis that is available to the user of the dMHB.

Invitation for Participation in the Consensus Process

An important aspect of the development of this and all NCCLS documents should be emphasized, and that is the consensus process. Within the context and operation of NCCLS, the term “consensus” means more than agreement. In the context of document development, “consensus” is a process by which NCCLS, its members, and interested parties (1) have the opportunity to review and to comment on any NCCLS publication; and (2) are assured that their comments will be given serious, competent consideration. Any NCCLS document will evolve as will technology affecting laboratory or healthcare procedures, methods, and protocols; and therefore, is expected to undergo cycles of evaluation and modification.

The NCCLS Area Committee on Microbiology has attempted to engage the broadest possible worldwide representation in committee deliberations. Consequently, it is reasonable to expect that issues remain unresolved at the time of publication at the proposed level. The review and comment process is the mechanism for resolving such issues.

The NCCLS voluntary consensus process is dependent upon the expertise of worldwide reviewers whose comments add value to the effort. At the end of a 90-day comment period, each subcommittee is obligated to review all comments and to respond in writing to all which are substantive. Where appropriate, modifications will be made to the document, and all comments along with the subcommittee's responses will be included as an appendix to the document when it is published at the next consensus level.

Key Words

Antimicrobial agent, broth microdilution, dehydrated Mueller-Hinton broth, minimal inhibitory concentration (MIC), susceptibility.

Evaluation of Lots of Dehydrated Mueller-Hinton Broth for Antimicrobial Susceptibility Testing; Proposed Guideline

1 Introduction

NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* recommends the use of Mueller-Hinton broth for routine susceptibility testing for the following reasons:

- Historically there is good batch-to-batch reproducibility of QC results;
- The base medium, like Mueller-Hinton agar, is low in known inhibitors of sulfonamides, trimethoprim, and tetracycline;
- Mueller-Hinton broth supports the growth of most nonfastidious pathogens; and
- A large body of data is available using this medium to establish breakpoints and reproducible QC limits.

Earlier data from multicenter studies with Mueller-Hinton agar and other culture media showed that many variables affect agar diffusion susceptibility results. It appears that the culture medium itself is a critical component of that variability. The development of new antimicrobial agents and the more extensive use of Mueller-Hinton broth in laboratory studies from multicenter clinical trials indicate that more objective evaluation of possible variability of Mueller-Hinton broth is required.

This document provides protocols for evaluating the performance characteristics of production lots of dehydrated Mueller-Hinton broth (dMHB). The document only addresses lots of dMHB used to prepare fresh or frozen microdilution trays used in the broth dilution susceptibility method as described in the most recent version of NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. It does not evaluate any variability that might occur as a result of use in dried or lyophilized media that are manufactured in microdilution MIC trays.

2 Scope

This proposed guideline is the result of the effort of the NCCLS Subcommittee on Culture Media to establish a standard protocol by which manufacturers of dehydrated Mueller-Hinton broth (dMHB) may determine its performance characteristics. This guideline is limited to preparation of production lots of dMHB for use according to NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*.

This document only addresses lots dMHB used to prepare fresh or frozen microdilution trays in the broth dilution susceptibility method as described in the most recent edition of NCCLS documents M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. It does not evaluate any variability that might occur as a result of use in dried or lyophilized media that are manufactured in microdilution MIC trays.

3 Definitions

Dehydrated Mueller-Hinton broth, *n* – A dry-form complex bacteriological medium produced by several commercial manufacturers which is used to prepare broth dilution antimicrobial susceptibility media for testing routine nonfastidious aerobic and facultative anaerobic bacterial organisms.

Inoculum, *n* – A substance or portion of a specimen, implanted in a culture medium.

Minimal inhibitory concentration (MIC), *n* – The lower concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

4 Manufacturers' Protocol for Testing Production Lots of Dehydrated Mueller-Hinton Broth

4.1 Preparing Control Cultures

4.1.1 Stock Cultures

Stock cultures are prepared from lyophilized cultures obtained from the American Type Culture Collection (ATCC®). Cultures should be reconstituted according to procedures obtained from the ATCC® and maintained in such a way that selection of genetic variants is minimized. The cultures required for the purposes of this protocol are:

Staphylococcus aureus	ATCC®29213
Staphylococcus aureus	ATCC®43300
Escherichia coli	ATCC®25922
Pseudomonas aeruginosa	ATCC®27853
Enterococcus faecalis	ATCC®29212
Streptococcus pneumoniae	ATCC®49619

4.1.2 Preparing the Initial Stock Cultures

Using a sterile loop or swab, inoculate two to three plates of soybean-casein digest agar (tryptic soy agar; [TSA]) containing 5% sheep blood with each control culture listed in Section 4.1.1. Streak each plate to obtain isolated colonies. Incubate the plates for 18 to 24 hours at 35 ± 1 °C in ambient air, except for *S. pneumoniae* which should be incubated in an atmosphere of 5 to 7% CO_2.

4.1.3 Preparing Frozen Stock Cultures

After incubation, check for purity and harvest the entire growth from each set of plates and suspend it in soybean-casein digest broth (tryptic soy broth [TSB]) containing 15% glycerol. To prepare the TSB, add the manufacturer's recommended amount of TSB medium to make one liter to 500 mL of deionized water and add 150 mL of glycerol. Adjust the volume to 1 L, mix well, and sterilize it at 121 °C for 15 minutes. The bacterial suspension may be adjusted to the turbidity of a 0.5 McFarland standard (about 1 to 2 x 10^8 CFU/mL), if a known viability is desired. Dispense at least 0.5 mL of the suspension into small, sterile vials. Store the vials at -60 °C, or a lower temperature. With this method, cultures should be viable for at least one year. Other methods of preparing stock cultures may be used if they provide adequate viability and stability. Periodically renew stock cultures from fresh lyophilized cultures obtained from ATCC®.

4.1.4 Preparing the Stock Culture Test Inoculum

The day before the inoculation of the plates (day 1), thaw a vial of each of the control cultures that will be needed. Inoculate each culture onto a plate of TSA with 5% sheep blood and incubate it for 18 to 24 hours (time is not critical for this step) at 35 °C in ambient air (5 to 7% CO_2 for *S. pneumoniae* ATCC® 49619). After incubation, check for purity. If satisfactory, growth from these plates is then used to prepare standardized inocula, as described in NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*.

4.2 Performing the Tests

4.2.1 Preparing the Trays

Testing of each antimicrobial - organism combination should be performed to conform to the QC ranges indicated below. The minimum and maximum concentration on each tray should bracket the quality control limit range by at least two doubling dilutions beyond each limit. The following organisms should be tested against these agents using twofold dilutions and must fall within the current quality control limit ranges published in the most current edition of NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* (and/or NCCLS document M100—*Performance Standards for Antimicrobial Susceptibility Testing*). The following values are included in the most current edition of NCCLS document M100—*Performance Standards for Antimicrobial Susceptibility Testing.*

Quality Control Strain	Antimicrobial Agent	Quality Control Range (µg/mL)
Pseudomonas aeruginosa ATCC®27853	Tobramycin	0.25 – 1.0
	Imipenem	1.0 – 4.0
	Ciprofloxacin	0.25 – 1.0
Escherichia coli ATCC®25922	Ampicillin	2.0 – 8.0
	Cefotaxime	0.03 – 0.12
	Tetracycline	0.5. – 2.0
	Aztreonam	0.06 – 0.25
Staphylococcus aureus ATCC®29213	Clindamycin	0.06 – 0.25
	Azithromycin	0.5 – 2.0
	Oxacillin	0.12 – 0.5
	Erythromycin	0.25 – 1.0
Enterococcus faecalis ATCC®29212	Trimethoprim-sulfamethoxazole	≤ 0.5/9.5
	Vancomycin	1.0 – 4.0
Staphylococcus aureus ATCC®43300	Oxacillin	1.0 – 4.0
Streptococcus pneumoniae ATCC®49619	Penicillin	0.25 – 1.0
	Trimethoprim-sulfamethoxazole	0.12/2.4 – 1/19

4.2.2 Preparing Stock Antimicrobial Solutions

Stock solutions of antimicrobial agents should be prepared according to the methodology identified in NCCLS document M100—*Performance Standards for Antimicrobial Susceptibility Testing* (M7, Table 4). Final concentrations of antimicrobial agent should be prepared in the rehydrated Mueller-Hinton broth, adjusting cations if necessary. Dilutions may be prepared by making serial two/fold dilutions of the stock or by using the procedure described in M100—*Performance Standards for Antimicrobial Susceptibility Testing* (M7, Table 6).

- The final concentrations in each well of the trays should be contained in a total volume of 100 µL, and each tray should include a growth and negative control well.

- The addition of the final inoculum from a microdilution inoculator will not appreciably alter the final concentrations of antimicrobial agents if the volume of inoculum delivered is ≤10 µL.

4.2.3 Preparing the Test Inoculum

A single inoculum for each quality control strain should be prepared using the direct colony suspension method as described in the most current version of NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*.

- Using sterile saline, adjust the turbidity of the suspension either visually or with a nephelometer or spectrophotometer to equal a 0.5 McFarland suspension.

- Within 15 minutes of preparation, dilute the adjusted inoculum with sterile saline so that, after inoculation, each tube or well contains approximately 5 x 10^5 CFU/mL. The dilution required to obtain this final inoculum varies according to the method of delivery of the inoculum to the individual wells or tubes and must be calculated for each situation. For microdilution tests the exact inoculum volume delivered to the wells must be known to make this calculation. For example, when the volume of medium in the well is 0.1 mL and the inoculum volume is 0.01 mL, then the 0.5 McFarland suspension (~1 x 10^8 CFU/mL) should be diluted 1:20 to yield 5 x 10^6 CFU/mL. When 0.01 mL of this dilution is inoculated into the broth, the final test concentration of bacteria will be approximately 5 x 10^5 CFU/mL (or 5 x 10^4 CFU/well).

4.2.4 Performing Colony Counts

Perform colony counts on the inoculated plate to verify that the final inoculum is approximately 5 x 10^5 CFU/mL as per NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. (Section 7.3.1[4]).

- Immediately after inoculating, transfer 0.01 mL from the growth control well into a tube containing 10 mL of sterile 0.9% saline.

- Transfer a 0.1-mL sample onto the surface of a suitable agar medium, and spread the liquid over the entire plate surface.

- After incubation the presence of 50 colonies indicates an inoculum density of 5 x 10^5 CFU/mL.

4.2.5 Incubating the Microdilution Trays

Trays should be incubated in ambient air at 35 °C:

- Incubate *S. pneumoniae* ATCC® 49619 and *S. aureus* ATCC® 43300 (for oxacillin) for 24 hours.

- Incubate all other strains for 16 to 18 hours.

4.2.6 Reading the Microdilution Trays

After incubation, trays should be removed from the incubator and read within one hour. The endpoint (MIC) is defined as the lowest concentration that completely inhibits the growth of the organism. For trimethoprim-sulfamethoxazole (TMP-SMX), the endpoint (MIC) is defined as 80% or greater reduction of growth compared to the control well (no antimicrobial agent).

4.3 Interpreting the Results

The MIC for each antimicrobial-organism concentration should be within the quality control limits identified in the M7 section of NCCLS document M100—*Performance Standards for Antimicrobial Susceptibility Testing* for that combination. If the susceptibility test results are within MIC quality control limits for each antimicrobial agent-organism combination, the dMHB lot is acceptable. If any organism-antimicrobial agent susceptibility result falls outside the established quality control MIC limits, corrective action must be taken. That antimicrobial agent-organism test should be repeated, so that the test results fall within the quality control limits at least 95% of the time (i.e., 19 of 20 times).

4.4 Label Statement

If all performance criteria for all organism-antimicrobial combinations are within acceptable limits, the manufacturer may indicate for that production lot that the dMHB (lot number) conforms to the performance criteria laid out in NCCLS document M32 for antimicrobial susceptibility testing. For standard production lots of dMHB, the broth prepared from the dehydrated product should contain no greater than 25 mg/L of calcium, 12.5 mg/L of magnesium, and must have less than 3 mg/L of zinc ions. A certificate of analysis should be available to the user of dMHB that specifies the concentrations of these ions in the lot of dehydrated medium and indicates if additional calcium and/or magnesium is required to achieve the concentrations specified in NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*

The label or package insert statement should state:

"This lot of dehydrated Mueller-Hinton broth has been tested according to, and meets quality control criteria as stated in NCCLS document M32."

5 Determining the Ion Content for Production Lots of Dehydrated Mueller-Hinton Broth

Ion content for major cations and anions known or suspected as having an effect on antimicrobial-organism interactions should be tested by inductively coupled plasma analysis (ICP) or flame atomic absorption spectroscopy (FAAS) using a recognized standard method that has been validated. A brief summary description of these methods with sensitivity and detection limit data is given in the appendix. Available data indicates that interactions between ions and the following antimicrobial agents are known or may cause changes in broth dilution antimicrobial susceptibility test results.

5.1 Aminoglycosides

Suggested levels of calcium (20 to 25 µg/mL) and magnesium (10 to 12.5 µg/mL) are based on studies [1,2] comparing Mueller-Hinton agar and broth dilution for clinical strains of *P. aeruginosa*.

5.2 Carbapenems

The concentration of zinc should be below 3 µg/mL to avoid false resistance interpretations.[3] This is known to be true for imipenem and may apply to other carbapenems.

5.3 Quinolones

Data from studies of quinolone activity in human urine suggest that decreased quinolone activity may occur when magnesium concentrations are in the range of 8 to 10 mM (> 100 to 150 µg/mL) in Mueller-

Hinton medium.[4] Other data indicate that 35 to 60 µg/mL of magnesium cause a reduction in zone diameters and an increase in MICs for several species of bacteria.[5, 6]

5.4 Tetracycline

Mueller-Hinton broth supplemented to contain 50 µg/mL of calcium and 25 µg/mL of magnesium has been shown to increase MICs of *E. coli*, *P. aeruginosa,* and other species of *Pseudomonas* by 2- to 32-fold.[7]

5.5 Ion Effects on Other Antimicrobial Agents (Not Tested in This Document)

Although ion effects on other antimicrobial agents not included in this document are known to affect susceptibility results, they should be considered for Mueller-Hinton broth dilution susceptibility tests. (These include daptomycin,[8-10] polymyxin,[7] and mecillinam.[11]) Manufacturers and others should refer to these references and to NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* for appropriate instructions on preparation and testing.

5.6 Cation Concentrations

If test results of production lots of dMHB do not conform to the quality control ranges identified in the M7 section of M100—*Performance Standards for Antimicrobial Susceptibility Testing* and the ion concentrations of calcium, magnesium, and zinc are within those identified in Section 4.4 of this document and in NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, concentrations of other ions or medium components should be checked to determine if they affect the results.

6 Testing New Antimicrobial Agents with Production Lots of Dehydrated Mueller-Hinton Broth

When *in vitro* data on new antimicrobial agents are being developed, dMHB production lots meeting the criteria in this document should be used to develop *in vitro* quality control parameters for these new agents. Testing of production lots of dMHB with new antimicrobial agents should follow the procedures outlined in this document. Ion content should be examined to determine if the new agent may be affected by specific cations or anions or concentrations of ions in the medium that differ from the ranges suggested in this guidance document. Other medium components that may affect *in vitro* quality control susceptibility tests that come to light during these investigations should be identified. Adjustments should be made as necessary to achieve stable, reproducible test results, and this information should be widely circulated to others involved in antimicrobial susceptibility testing and quality control including the NCCLS Subcommittees on Culture Media and Antimicrobial Susceptibility Testing.

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Appendix. Methods for Ion Analysis of Dehydrated Mueller-Hinton Broth

Flame Atomic Absorption Spectroscopy (FAAS). The instrument consists of an atomizer, burner, light source, wavelength selector, and detector. The sample is first atomized, and then burned. The light source is a hollow cathode lamp (HCL). The light from the HCL is passed through the flame, through a wavelength selector, and to the detector where the amount of light is measured. When a sample is introduced into the flame, analyte atoms will absorb the quantized light from the HCL and reach an excited state. When the atoms de-excite most of the re-emitted quantized light is radiated away from the monochromator and the detector, so the amount of light passing from the HCL through the flame is reduced. The reduction in light intensity, or absorbance, is proportional to the number of atoms of the element of interest in the flame and subsequently to the concentration of the sample introduced into the flame.

Inductively Coupled Plasma Spectroscopy (ICP). The instrument consists of an atomizer, plasma source, wavelength selector, and detector. The sample is mixed with argon gas, which is then ionized and forced to move in a circular path by a radio frequency generator. The resistance of the ions to the applied radio frequency results in extremely hot temperatures, up to 10,000 ^{o}K. The energy that results from heating and subsequent cooling is in the form of electromagnetic radiation, or light. The light from the plasma is passed through a wavelength selector and to the detector where the amount of light is measured. The increase in light intensity, or absorbance, is proportional to the number of atoms of the element of interest in the plasma and subsequently to the concentration of the sample introduced into the plasma.

Sensitivity, Detection Limit, and Accuracy. The sensitivity and detection limit of FAAS and ICP are affected by variables such as flame/plasma temperature, spectral bandwidth, and detector sensitivity. In the absence of sample matrix effects, sensitivity and detection limit are generally close to each other. For flame atomic absorption spectroscopy, sensitivities from about 3×10^{-4} to 20 µg/mL are observed. For inductively coupled plasma spectroscopy, detection limits from about 3×10^{-6} to 1×10^{-3} µg/mL are observed. The table lists some detection limits and sensitivities for FAAS and ICP. The accuracy of FAAS and ICP are generally less than 5%.

Table. Sensitivity and Detection Limits for FAAS and ICP. (Reprinted with permission from Morrison GH. *Critical Reviews in Analytical Chemistry.* 1969;(14):28A. ©CRC Press, Boca Raton, Florida.)

Element	FAAS Sensitivity (µg/mL)	ICP Detection Limit (µg/mL)
Calcium (Ca)	0.02	0.000001
Magnesium (Mg)	0.003	0.000003
Iron (Fe)	0.06	0.0005
Copper (Cu)	0.04	0.0004
Sodium (Na)	0.003	0.0002
Zinc (Zn)	0.009	0.0001

Related NCCLS Publications*

M2-A7 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Seventh Edition (2000).** American National Standard.* This standard contains updated recommended techniques, interpretive criteria, and quality control parameters for disk susceptibility testing.

M6-A **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard (1996).** This document provides procedures for evaluating production lots of Mueller-Hinton agar, and for the development and application of reference media.

M7-A5 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fifth Edition (2000).** American National Standard.* This standard provides updated reference methods for the determination of minimal inhibitory concentrations (MICs) for aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M22-A2 **Quality Assurance for Commercially Prepared Microbiological Culture Media—Second Edition; Approved Standard (1996).** This document provides quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections—Second Edition; Approved Guideline (2001).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

M31-A **Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard (1999).** Reference methods for the determination of minimum inhibitory concentration (MIC) and agar disk diffusion susceptibility tests with bacteria isolated from animals, broth macrodilution, broth microdilution, agar dilution, and agar disk diffusion methods.

M100-S12 **Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement (2002).** This document provides updated tables for the NCCLS antimicrobial susceptibility testing standards M2-A7 and M7-A5.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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