M31-A3
Vol. 28 No. 8
Replaces M31-A2
Vol. 22 No. 6

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition

This document provides the currently recommended techniques for antimicrobial agent disk and dilution susceptibility testing, criteria for quality control testing, and interpretive criteria for veterinary use.

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

CLINICAL AND LABORATORY STANDARDS INSTITUTE®
(Formerly NCCLS)

M31-A3
ISBN 1-56238-659-X
Volume 28 Number 8 ISSN 0273-3099

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition

Jeffrey L. Watts, PhD, RM(AAM)
Thomas R. Shryock, PhD
Michael Apley, DVM, PhD
Donald J. Bade
Steven D. Brown, PhD
Jeffrey T. Gray, PhD
Henry Heine, PhD
Rob P. Hunter, MS, PhD
Dik J. Mevius, DVM, PhD
Mark G. Papich, DVM, MS
Peter Silley, PhD
Gary E. Zurenko, MS

Abstract

If the susceptibility of a bacterial pathogen to antimicrobial agents cannot be predicted based on the identity of the organism alone, *in vitro* antimicrobial susceptibility testing of the organism isolated from the disease processes in animals is indicated. Susceptibility testing is particularly necessary in those situations where the etiologic agent belongs to a bacterial species for which resistance to commonly used antimicrobial agents has been documented, or could arise.

A variety of laboratory techniques can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. Clinical and Laboratory Standards Institute document M31-A3—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition* describes the standard agar disk diffusion method, as well as standard broth dilution (macrodilution and microdilution) and agar dilution techniques. It also includes a series of procedures designed to standardize test performance. The performance, applications, and limitations of the current CLSI-recommended methods are described.

The tabular information in this document presents the most current information for drug selection, interpretation, and quality control. In an increasing number of compounds where veterinary-specific interpretive criteria are not available, human interpretive criteria are used. As more veterinary-specific information becomes available, these changes will be incorporated into future revisions of this document.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition*. CLSI document M31-A3 (ISBN 1-56238-659-X). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2008.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI/NCCLS documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If your organization is not a member and would like to become one, and to request a copy of the catalog, contact us at: Telephone: 610.688.0100; Fax: 610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org

Contents

Contents (Continued)

Contents (Continued)

Contents (Continued)

Foreword

This revision of the M31 standard represents a continuation of the collective efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) to produce a globally useful consensus document for standardized *in vitro* susceptibility testing of veterinary pathogens. The subcommittee has worked diligently to improve M31-A2—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second Edition* primarily by incorporating relevant updates derived from CLSI documents M2 and M7,[1,2] as well as referencing other relevant CLSI documents. The subcommittee recognizes the ongoing CLSI efforts that are necessary to maintain CLSI documents M2 and M7[1,2] as "state of the art," and expresses its appreciation to the Subcommittee on Antimicrobial Susceptibility Testing for its contributions. In addition, it is also appropriate to acknowledge the users of M31-A2 for their continued support and application of the standard in their daily work routine. The user community is encouraged to demonstrate responsible application of the CLSI methods when publishing work in peer-reviewed journals.

Global events and perceptions regarding the use of antimicrobial agents in animals have placed even more importance on the essential role of antimicrobial susceptibility testing of bacteria isolated from animals. For example, Judicious Use Guidelines (consensus directions for the appropriate use of antimicrobial agents in animals) have been developed worldwide by veterinary societies, food animal production organizations, government agencies, and international groups for both food and companion animals that emphasize the critical need for obtaining susceptibility data using standardized test methods and interpretive criteria. M31-A3 was updated to help meet these needs. Additionally, with an increased emphasis on national "resistance surveillance" programs, the use of a standardized antimicrobial susceptibility testing methodology and quality control (QC) provides a means to harmonize testing across national boundaries to facilitate data comparisons. As a first step toward worldwide applications of susceptibility testing using CLSI methods, a separate section lists QC strain entries in various international repositories. A workflow sheet for QC testing and a troubleshooting checklist are now available that should aid laboratories to track their performance for increased proficiency. Development of specific test methods and QC values for several antimicrobial agents effective against *Campylobacter* have also been developed, with the expectation that they will contribute to improved surveillance efforts.

Several additional antimicrobial agents have been reviewed since the previous edition of the document and have received veterinary-specific interpretive criteria approval from the subcommittee. M31-A3 features these new antimicrobial agents in Table 1, Group A (Veterinary-Specific Interpretive Criteria, Primary Test and Report). Further revisions were made to designate CLSI Approved Human Interpretive Criteria, Primary Test, Selectively Report (Group B); No Veterinary Species Specific- or Human-Specific Interpretive Criteria, Primary Test, Selectively Report (Group C); and Supplemental "AMDUCA-use" (US Animal Medicinal Drug Use Clarification Act) products, Selectively Test, Selectively Report (Group D). Additionally, refinements to definitions for "susceptible" and "resistant" designations are now included. Using the principles for breakpoint establishment, several older, generic antimicrobial agents have had veterinary-specific breakpoints set. The user should be aware that, while M31 is primarily derived from CLSI documents M2 and M7,[1,2] which are "human" documents, this version incorporates more veterinary-specific information than before, although there is still ample opportunity for expansion. This standard should not be considered a static document because it will change as additional methods for testing veterinary pathogens become available, and in response to changes in antimicrobial agent usage in veterinary medicine. The subcommittee is committed to making every effort to incorporate the latest information into future versions of this standard.

The CLSI Subcommittee on VAST believes the document also serves as an educational resource. The subcommittee believes diagnostic laboratory personnel, veterinarians, students, and allied professionals will benefit from this document. Thus, a glossary of antimicrobial agents and resistance mechanisms, and a listing of antimicrobial resistance tests have been assembled for the convenience of investigators interested in this area.

The subcommittee anticipates that, as a result of the efforts of several new working groups, additional fastidious pathogens will be included in future editions, such as the intestinal spirochetes, *Haemophilus parasuis*, and mycoplasmas. Additionally, the inclusion of antimicrobial agents not currently marketed in the United States is welcomed for future editions, with the goal of making this document as globally useful as possible. A new working group within the subcommittee has been formed to address this need. As mentioned before, the inclusion of international entries in culture collections of QC strains is viewed as just the first step in this process, so leading laboratories might begin to use CLSI methods and compare them with their own national methods. Perhaps the most important future revision will be shifting antimicrobial agents from Table 1, Group C or B, to A. Because approvals of new antimicrobial agents for veterinary medicine are foreseen to be a rare event, the subcommittee will begin to shift its focus to the Generics Working Group to develop data in conformance with the M37 guideline to continue to advance generic drugs into Group A. I encourage and welcome input regarding ways to improve the document.

In closing, I recognize the tremendous efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing in producing this revised document. I would like to particularly acknowledge the individual members of the Editorial Working Group. Their willingness to sacrifice significant amounts of their personal time for the editing process and to address controversial topics demonstrates a real commitment to the CLSI process and the advancement of the veterinary and microbiology professions. I thank Jo Abraham, Jeff Gray, Janine Matlak, Maria Traczewski, Ching Ching Wu, and Steve Yan. Of particular note are Bob Walker, Pat McDermott, and Tom Fritsche, for their work in developing QC values for susceptibility testing of *Campylobacter*.

Thomas R. Shryock, PhD
Subcommittee on Veterinary
Antimicrobial Susceptibility Testing

Mission Statement

To develop and promote performance standards and interpretive criteria for *in vitro* antimicrobial susceptibility testing of bacteria isolated from animals.

Key Words

Agar diffusion, agar dilution, antimicrobial agent, antimicrobial susceptibility, broth dilution, susceptibility testing, veterinary

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition

1 Scope

This document provides veterinary diagnostic laboratories with currently recommended antimicrobial agent disk and dilution susceptibility test methods for bacteria isolated from animals; criteria for quality control (QC) testing; and interpretive criteria. The interpretive criteria are intended only to support therapeutic label claims for animal antimicrobial agent use and do not apply to label claims for disease prevention or performance enhancement. Additionally, the document provides a brief overview of the various antimicrobial classes and mechanisms of resistance to them, including specific tests for antimicrobial resistance.

In order to have a positive impact on clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobials safely, and minimize selection of resistant pathogens, laboratories must use a standardized, well-defined method for performing antimicrobial susceptibility testing (AST). A critical component of an AST method is the relationship between the test outcome and clinical outcome following treatment of the animal. In other words, an isolate yielding a susceptible AST result would be expected to respond clinically to that agent at the appropriate dosages while an AST result of resistant would imply that the treatment would fail. The purpose of the test method is not to mimic *in vivo* conditions; rather, it is to establish a method that provides reproducible results. Therefore, to ensure the generation of accurate, reproducible results when performing ASTs on veterinary pathogens, laboratories must adhere to a standard, well-defined method that includes the appropriate QC information. The M31-A3 document is predicated on providing AST methods that give accurate, reproducible, clinically relevant results for veterinary pathogens. It is important to consider that the judicious use of antimicrobials in the veterinary setting is directly related to the interpretive criteria associated with AST in that a given set of interpretive criteria only applies to that specific antimicrobial and disease combination. It is also important to note that the interpretive criteria in M31-A3 apply only if the laboratory has conducted susceptibility testing according to the specific methods found in the documents.

To date, an increasing number of antimicrobial agents have established veterinary-specific interpretive criteria. In most cases where veterinary-specific interpretive criteria are not established, human interpretive criteria are used when appropriate (see CLSI documents M2, M7, and M11[1-3]). The veterinary-specific interpretive criteria have been established following M37, with particular attention given to product label indications and directions as approved by regulatory authorities. As more veterinary-specific information becomes available, changes in the listing of the agents will be incorporated into future revisions of this document and associated supplements. AST of bacteria from aquaculture environments has been advanced with the publication of two CLSI documents: M42 and M49.[4,5]

2 Introduction

A variety of laboratory techniques can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. These include disk diffusion as well as broth and agar dilution techniques. This document includes a series of recommendations to help standardize the way these tests are performed. The performance, applications, and limitations of the currently recommended methods are described. Recommendations by the International Collaborative Study (ICS), as well as regulations established by the US Food and Drug Administration and other regulatory agencies, have been reviewed and the appropriate sections have been incorporated into this standard.[6-8] This document describes current methodology applicable to therapeutic uses of antimicrobial agents used in veterinary medicine for diseases of animals, as described in Section 5. In recognition of the need for a global standard for AST for

©*Clinical and Laboratory Standards Institute. All rights reserved.*

bacteria isolated from animals, the Office International des Epizooties (OIE) published test method guidelines in its Terrestrial Code that are consistent with those contained in this document (www.oie.int). The need for globally harmonized test methods is essential if interlaboratory minimal inhibitory concentration (MIC) or zone size data are to be compared in journals, Web postings, resistance monitoring program reports, etc. The application of a single methodology also allows drug sponsors in countries other than the United States to prepare data packages for presentation to the Veterinary AST subcommittee by means of CLSI document M37.[9]

The subcommittee believes the development of new, or modified, *in vitro* testing procedures to determine interpretive criteria to guide therapeutic uses of antimicrobial drugs in veterinary practice is not realistic for two reasons:

First, there is no apparent variable that can be easily modified in the current procedure to reflect a key factor that will correlate to *in vivo* efficacy. For example, any alteration in the inoculum (which might be reflective of an initial low infectious dose early in an infectious process) would need to be validated with efficacy studies in animals. Furthermore, redesigning the current methodology would require the development of new QC guidelines; possibly disks with lowered antimicrobial content or extended dilutions on MIC dilution panels.

Second, even if such a procedure were developed, how realistic is it to expect a laboratory to use it and explain the outcome to a veterinarian or other client? As explained in the current edition of CLSI document M37,[9] interpretive criteria are based, in part, on the directions listed on the drug product label, so any methods that were developed to support extra-label usage would place undue responsibility for antibiotic decision-making on the laboratorian.

The subcommittee will continue to consider new developments in test methodologies and procedures, as well as revisions to interpretive criteria for therapeutic agents. With respect to antimicrobial agents used to promote or enhance food animal growth, the beneficial effects of antimicrobial agents can neither be entirely ascribed to effects on the metabolic activities of microbial gut flora or suppression of "subclinical disease," nor correlated to physiological or immunological effects on the animal, so susceptibility testing is of no value to predict an *in vivo* response. New *in vitro* techniques for prediction of clinical outcome will be considered only when a better understanding of the mode of action of antimicrobial agents in this situation becomes available.

3 Definitions

The selection of the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with the pharmacy (where available) and veterinarians. The lists in Table 1 comprise agents of proven clinical efficacy for treatment of infections in that animal group, and which show acceptable *in vitro* test performance. Agents listed are currently approved for treatment or control of disease. Agents used for other purposes (such as prevention or growth promotion) are not listed, because the correlation of AST for these uses cannot be established. Tests on selected agents can also be useful for epidemiological or research purposes.

The subcommittee believes it is both appropriate and necessary to provide the rationale used to limit the scope of this document to therapeutic and control uses of antimicrobial agents in veterinary medicine. For the most part, these limitations reflect antimicrobial uses in livestock and poultry used for food production, and do not necessarily apply to equine or small-animal medicine. Currently, antimicrobial agents are approved by regulatory authorities for certain indications; treatment (ie, therapy), control, or prevention of disease; or growth promotion. Inclusion of antimicrobial agents approved for use in countries other than the United States is anticipated. The subcommittee has used the following definitions of antimicrobial uses, which are consistent with current regulatory interpretations in its deliberations:

 ©*Clinical and Laboratory Standards Institute. All rights reserved.*

Related CLSI Reference Materials*

M2-A9 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition (2006).** This standard contains recommended techniques, interpretive criteria, and quality control parameters for disk susceptibility testing.

M6-A2 **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard—Second Edition (2005).** This standard contains procedures for evaluating production lots of Mueller-Hinton agar, and for the development and application of reference media.

M7-A7 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition (2006).** This newly revised standard provides updated reference methods for the determination of minimal inhibitory concentrations (MICs) for aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M11-A7 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007).** This document provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

M23-A2 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001).** This document addresses the required and recommended data needed for the selection of appropriate interpretative standards and quality control guidelines for new antimicrobial agents.

M37-A3 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition (2008).** This document addresses the recommended data needed for selection of appropriate interpretive standards and quality control guidelines for new veterinary antimicrobial agents.

M39-A2 **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Second Edition (2005).** This document describes methods for the recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of epidemiologically significant microorganisms.

M100-S18 **Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement (2008).** This document provides updated tables for the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards M2-A9 and M7-A7.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

©*Clinical and Laboratory Standards Institute. All rights reserved.*