



VET01-A4

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

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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

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

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Jeffrey L. Watts, PhD, RM(NRCM)
Mark G. Papich, DVM, MS
Donald J. Bade, BS
Steven D. Brown, PhD, ABMM

Virginia R. Fajt, DVM, PhD, DACVCP
Thomas R. Fritsche, MD, PhD
Henry S. Heine, PhD
Robert P. Hunter, MS, PhD

Stefan Schwarz, DVM
Peter Silley, PhD
Ching Ching Wu, DVM, 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 in which the etiological 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 VET01-A4—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Fourth 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's supplement, VET01-S2, presents the most current information for drug selection, interpretation, and QC. 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.

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SAMPLE

Foreword

This version of VET01 represents the continued efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) to produce a globally useful, clinically relevant document for the standardized *in vitro* susceptibility testing of veterinary pathogens. Due to potential international differences in illegal or prohibited uses, some jurisdiction-specific restrictions are described in accompanying footnotes of Table 1 and in Table 2A comments. The subcommittee has worked diligently to improve the fourth edition of VET01 by incorporating relevant updates derived from CLSI documents M02¹ and M07,² developing new recommendations for emerging resistant veterinary pathogens, and restructuring the VET01-S2 tables to provide easier access to veterinary-specific interpretive criteria. The subcommittee expresses its appreciation to the users of VET01 for their continued support and application of the standard in their daily work routine, and encourages the user community to provide feedback so that VET01 can be updated frequently to maintain its clinical relevance.

There are significant changes in this version of VET01. For example, veterinary-specific interpretive criteria for categorizing methicillin-susceptible and methicillin-resistant strains of *Staphylococcus pseudintermedius* have been added. These recommendations are significant because they indicate that the strains of methicillin-resistant *S. pseudintermedius* (MRSP) are clearly different from methicillin-resistant *Staphylococcus aureus* (MRSA) and require veterinary-specific interpretive criteria. It should also be noted that as the scientific literature was reporting that the interpretive criteria for MRSA was not relevant to MRSP, the subcommittee was already engaged in revising the MRSP interpretive criteria and has published its new recommendations in the relevant literature.

The table revisions found in the supplement for VET01 (VET01-S2) reflect a milestone in the development of this document and are the second significant change in the document. When the Subcommittee on VAST first met in 1993, it was decided that human-derived breakpoints would remain in Table 2 as gray shaded text. It was hoped that over time, the human-derived breakpoints would be replaced with veterinary-specific breakpoints. The revisions to the tables, particularly VET01-S2 Table 2, result from the development of a sufficient number of animal species-specific interpretive criteria to warrant placing these into one table (Table 2A), while human-derived interpretive criteria are now placed in a second table (Table 2B). This emphasizes the subcommittee's opinion that veterinary-specific interpretive criteria should be used first, and that the human-derived interpretive criteria should not be considered equivalent. As the number of veterinary-specific interpretive criteria continues to increase, it is likely that human-derived interpretive criteria will no longer be included in future versions of VET01. While this version of VET01 makes reference to the tables in VET01-S2, it is possible that a new version of the supplement will publish before the guideline is revised in its entirety. As such, users should ensure that the current versions of the tables are used as new editions of the supplement are published, and should replace the previously published tables with the new tables. For ease of use, changes in the tables since the previous edition appear in boldface type.

The subcommittee also realizes that there remain many organisms for which there are no standardized test methods or interpretive criteria that veterinary diagnostic laboratories often need to test. Based on feedback from the user community, the subcommittee is investigating the development of a document that is similar in scope to CLSI document M45.³ This document would provide veterinary diagnostic laboratories with guidelines for testing the atypical or unusual veterinary pathogens, such as *Mycoplasma* or *Brachyspira*. User input will be critical to identifying those organisms for which methods have been reported in the literature and should be considered for inclusion in this forthcoming document.

The use of test methods and reporting of susceptibility test data have become critically important in understanding resistance development in veterinary (target and zoonotic) pathogens and the development of judicious use guidelines for veterinary antimicrobial agents. In particular, the Subcommittee on VAST is concerned about the mismatching of methods and interpretive criteria that have been reported in the literature. Moreover, using epidemiological or microbiological cutoffs and reporting these data as

equivalent to clinical breakpoints is also of concern to the subcommittee. In an effort to provide guidance on the development, implementation, and reporting of antimicrobial susceptibility data, CLSI document VET05⁴ was developed.

I would like to recognize the tremendous efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing in producing this document. In doing so, I would like to thank the heads of the various Working Groups for their commitment to the CLSI process, in particular, Virginia Fajt (Education), Mark Papich (Generics), Tom Shryock (International Harmonization), Ching Ching Wu (Mycoplasma), and Gary Zurenko (Editorial). I also wish to thank the representatives from the US Food and Drug Administration Center for Veterinary Medicine for their contributions. In particular, I would like to thank Melanie Berson, Marilyn Martinez, Patrick McDermott, and Ron Miller. Finally, I want to thank Luca Guardabassi, Dik Mevius, Stefan Schwarz, and Peter Silley for bringing the European Union perspective to the Subcommittee on VAST.

Jeffrey L. Watts, PhD, RM(NRCM)

Subcommittee on Veterinary Antimicrobial Susceptibility Testing

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.

Disclaimer

Note that the trade name Supplement CTM is included in Section 12.2. It is Clinical and Laboratory Standard Institute's policy to avoid using a trade name unless the product identified is the only one available, or it serves solely as an illustrative example of the procedure, practice, or material described. In this case, Supplement CTM is the only available product at the time of this document's development, and the subcommittee and consensus committee believe the trade name is an important descriptive adjunct to the document. In such cases, it is acceptable to use the product's trade name, as long as the words "or the equivalent" are added to the references. It should be understood that information on this product in this standard also applies to any equivalent products. Please include in your comments any information that relates to this aspect of VET01.

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—Fourth 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 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 (AMR).

In order to have a positive impact on clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobials safely, and minimize the selection of resistant pathogens, laboratories must use a standardized, well-defined method for performing antimicrobial susceptibility testing (AST). A critical component of a veterinary AST (VAST) method is the ability to enable a clinician to choose the appropriate antimicrobial agent for which there is likelihood of achieving a favorable clinical outcome and minimize an unfavorable clinical response. In other words, a susceptible result implies that the infection may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, whereas a VAST result of resistant implies that the isolate is not inhibited by the usually achievable concentration of the agent with label or normal dosage schedules and/or falls in the range where specific microbial resistance mechanisms are likely. 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 AST on veterinary pathogens, laboratories must adhere to a standard, well-defined method that includes the appropriate QC information. VET01 is predicated on providing AST methods that give accurate, reproducible, clinically relevant results for veterinary pathogens. 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. Interpretive criteria in VET01 apply only if the laboratory has conducted susceptibility testing according to the specific methods found in the documents.

An increasing number of antimicrobial agents have established veterinary-specific interpretive criteria. In most cases in which veterinary-specific interpretive criteria are not established, human interpretive criteria have been used when appropriate (see CLSI documents M02,¹ M07,² M11,⁵ and M100^{6a}). The veterinary-specific interpretive criteria have been established following CLSI document VET02,⁷ with particular attention given to product label indications and directions as approved by regulatory authorities. For those antimicrobial agents not approved for use in indicated food animal species, the laboratory client or veterinarian assumes all responsibility for efficacy, safety, and residue avoidance with the extra-label use of these agents. 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. Aquatic animal-specific interpretive criteria can be found in CLSI documents VET03,⁸ VET04,⁹ and their supplement, VET03/VET04.¹⁰

^a VET01-A4 and VET01-S2 were developed according to CLSI document M100-S22, published in 2012. M100 is subject to yearly updates; please refer to the most current edition when using human interpretive criteria.

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 (FDA) and other regulatory agencies, have been reviewed and the appropriate sections have been incorporated into this standard.¹¹⁻¹³ This document describes current methodology applicable to therapeutic uses of antimicrobial agents used in veterinary medicine for diseases of animals, as described in Section 6. In recognition of the need for a global standard for AST for bacteria isolated from animals, the Office International des Epizooties published test method guidelines in its Terrestrial Manual¹⁴ that are consistent with those contained in this document. The need for globally harmonized test methods is essential if interlaboratory minimal inhibitory concentrations (MICs) 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 Subcommittee on VAST as recommended in CLSI document VET02.⁷

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 the two reasons cited below.

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, which could possibly include 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 CLSI document VET02,⁷ 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 for food animal production uses, the beneficial effects of antimicrobial agents cannot be entirely ascribed to effects on the metabolic activities of microbial gut flora or the 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 the clinical outcome will be considered only when a better understanding of the mode of action of antimicrobial agents in this situation becomes available.

3 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 known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. The Centers for Disease Control and Prevention address this topic in published

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, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. 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:

- Organization
- Customer Focus
- Facilities and Safety
- Personnel
- Purchasing and Inventory
- Equipment
- Process Management
- Documents and Records
- Information Management
- Nonconforming Event Management
- Assessments
- Continual Improvement

VET01-A4 addresses the QSE indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section, beginning on page 72.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X M02 M06 M07 M11 M23 M39 M45 VET02 VET03 VET03/VET04 VET04 VET05	M07				

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. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

VET01-A4 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
				X M02 M07	X M02 M07 M11 VET03 VET03/VET04 VET04 M100	X M02 M07 M11 VET03 VET03/VET04 VET04 M100	X M02 M07 M11 VET03 VET03/VET04 VET04 M100	

Related CLSI Reference Materials*

- M02-A11** **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition (2012).** 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-A9** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition (2012).** This document addresses reference methods for the determination of minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.
- M11-A8** **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Eighth Edition (2012).** This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.
- 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.
- 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.
- M39-A3** **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline—Third Edition (2009).** This document describes methods for recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.
- M45-A2** **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition (2010).** This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI documents M02 or M07. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.
- M100-S23** **Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement (2013).** This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A11, M07-A9, and M11-A8.
- VET02-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 required and recommended data needed for selection of appropriate interpretive standards and quality control guidelines for new veterinary antimicrobial agents.
- VET03-A** **Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (2006).** This document provides the most up-to-date techniques for disk diffusion susceptibility testing of aquatic species isolates, and criteria for quality control testing.
- VET03/
VET04-S1** **Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals; First Informational Supplement (2010).** This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing guidelines VET03-A and VET04-A.

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

Related CLSI Reference Materials (Continued)

- VET04-A** **Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (2006).** This document provides the most up-to-date techniques for the determination of minimal inhibitory concentrations (MICs) of aquatic bacteria by broth micro- and macrodilution, and criteria for quality control testing.
- VET05-R** **Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin; A Report (2011).** This report offers guidance on areas in which harmonization can be achieved in veterinary antimicrobial surveillance programs with the intent of facilitating comparison of data among surveillance programs.

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