VET06
Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals

This document provides guidance for antimicrobial agent disk and dilution susceptibility testing, criteria for quality control testing, and breakpoints for fastidious and infrequently tested bacteria for veterinary use.

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Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals

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Abstract

Susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy. 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.

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 VET06—Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals describes the standard disk diffusion method, as well as standard broth dilution (macrodilution and microdilution) and agar dilution techniques for infrequently isolated or fastidious bacteria from animals. It also includes 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 test conditions, QC recommendations, agents to consider for primary testing, and breakpoints. In an increasing number of compounds, for which veterinary-specific breakpoints are not available, human breakpoints are used. As more veterinary-specific information becomes available, these changes will be incorporated into future revisions of this document.


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Foreword

In finalizing CLSI documents VET01\(^1\) and VET01S,\(^2\) the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) recognized that veterinary diagnostic laboratories often need to test many organisms for which there are no standardized test methods or breakpoints. Based on feedback from the user community, the subcommittee formed a working group to develop a document that is similar in scope to CLSI document M45.\(^3\) This new document provides veterinary diagnostic laboratories with recommendations for testing these veterinary pathogens, such as *Moraxella* and rapidly growing *Mycobacterium*, *Corynebacterium*, or *Brachyspira*. Continued user input will be critical to identifying organisms for which methods have been reported in the literature and that should be considered for inclusion in future editions of VET06.

This document was developed for the purpose of providing guidance to veterinary diagnostic, clinical, or public health microbiology laboratories regarding the performance of standardized susceptibility testing of infrequently isolated or fastidious bacteria. Potential agents of bioterrorism were included, because they are fastidious or infrequently encountered in most microbiology laboratories. Some organisms included are aerobic gram-negative bacilli that are not members of the family *Enterobacteriaceae* but may be tested by the standard CLSI broth microdilution or disk diffusion methods in the same manner as the much more commonly isolated *Enterobacteriaceae*. Some aerobic gram-positive cocci and bacilli that are periodically encountered by clinical laboratories may likewise be tested reliably by the standard CLSI minimal inhibitory concentration (MIC) or disk diffusion test methods in a manner analogous to *Staphylococcus* or *Enterococcus* spp. In addition, genera of fastidious gram-positive and gram-negative bacteria can be tested in the same manner as the streptococci, using blood-supplemented Mueller-Hinton media. For the purpose of this document, the term “fastidious” is used to describe bacteria that need media supplemented with blood or blood components and that possibly need an atmosphere other than ambient air (eg, with 5% CO\(_2\)) for acceptable growth. Because the standard CLSI media, reagents, and procedures can be used to test the organisms included in this document, the QC procedures, strains, and acceptable zone diameter and MIC limits that have been established through previous studies can be used for tests with the less common organisms that are included in this document. The working group used a thorough search of the published literature in conjunction with the members’ clinical experience to apply or adapt breakpoints from other organisms that could best be applied for interpreting tests of the less common organisms. Users of VET06 should be aware that the very extensive microbiological, clinical, and pharmacodynamic databases normally used for setting breakpoints by CLSI did not exist for the collection of infrequently isolated or fastidious veterinary organisms described. To facilitate further development of VET06, the working group requests laboratories that test these organisms to forward comments and suggestions for improvement with regards to the methods included herein (see specific request to laboratories below).

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 for the development of judicious use guidelines for veterinary antimicrobial agents. In particular, the Subcommittee on VAST has been concerned about mismatched methods and breakpoints 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 VET05\(^4\) was developed.

It is important for users of VET06 to recognize that commercial susceptibility testing devices are not covered in this document. The methods described herein are generic reference procedures that can be used for routine susceptibility testing by clinical laboratories, or that can be used by clinical laboratories to evaluate commercial devices for possible routine use. Results generated by reference methods, such as those contained in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial systems as part of the approval process. Clearance by a regulatory authority indicates that the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to
results generated using the reference methods for the organisms and antimicrobial agents described in the manufacturer’s approved package insert. Some laboratories could find that a commercial dilution, antibiotic gradient, colorimetric, turbidimetric, fluorometric, or other method is suitable for selective or routine use.

**Request for Data on Fastidious Pathogens for Inclusion in Future Editions of VET06**

The working group for VET06 would like to add the pathogens listed below to the next edition:

- *Bordetella avium*
- *Eikenella* spp.
- *Haemophilus parasuis*
- *Mycoplasma* spp.
- *Nicoletella semolina*

The working group is including the above list with hopes that laboratories with experience testing these organisms will send their methods and data to the VAST VET06 working group. Any information available can be submitted to CLSI directly at standard@clsi.org. In addition, any laboratories that would like to include other pathogens on the list for inclusion in future editions of VET06 may send their suggestions to CLSI.

**NOTE:** The content of this document is supported by the CLSI consensus process, and does not necessarily reflect the views of any single individual or organization.

**Key Words**

Agar dilution, antimicrobial agent, antimicrobial susceptibility, broth dilution, disk diffusion, microdilution, minimal inhibitory concentration
Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals

Chapter 1: Introduction

This chapter includes:

- Document’s scope and applicable exclusions
- Background information pertinent to the document’s content
- Standard precautions information
- “Note on Terminology” that highlights particular use and/or variation in use of terms and/or definitions
- Terms and definitions used in the document
- Abbreviations and acronyms used in the document

1.1 Scope

CLSI documents M02, M07, VET01, and M100 describe standardized methods and breakpoints for antimicrobial susceptibility testing (AST) of common aerobic bacteria, including some fastidious organisms. However, there are a number of less frequently encountered or fastidious veterinary bacteria that are not covered in those CLSI documents. Some are organisms that may cause serious infections in companion and livestock animals. This document provides recommendations to microbiology laboratories for how and when to determine the susceptibility of these diverse organisms. VET06 also includes some fastidious or unusual organisms potentially associated with bioterrorism.

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 breakpoints. The breakpoints 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.

This document does not cover commercial susceptibility testing devices.

1.2 Background

In order to have a positive effect on clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobial agents safely, and minimize the selection of resistant pathogens, laboratories should use a standardized, well-defined method for performing AST. A critical component of a veterinary AST (VAST) method is 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
exposure to the agent with label or normal dosage schedules and/or falls in the range in which specific microbial resistance mechanisms are likely. The purpose of the test method is not to mimic in vivo conditions but to establish a method that provides reproducible results. Therefore, to ensure that accurate, reproducible results are generated when performing AST on veterinary pathogens, laboratories should adhere to a standard, well-defined method that includes the appropriate QC information. VET06 provides AST methods that give accurate, reproducible, clinically relevant results for fastidious and infrequently tested veterinary pathogens. Judicious antimicrobial agent use in the veterinary setting is directly related to the breakpoints associated with AST in that a given set of breakpoints only applies to that specific antimicrobial agent and disease combination. Breakpoints in VET06 apply only if the laboratory has conducted susceptibility testing according to the specific methods found in the cited peer-reviewed journals or other documents that follow CLSI methods (see CLSI documents VET01, M45, M02, and M07).

Organisms that previously lacked defined methods for susceptibility testing and breakpoints include various Clostridium, Bacillus, and Trueperella species. Additionally, more detailed guidance for test performance and interpretation was needed, especially for breakpoints for fastidious and infrequently tested bacteria of animal origin. The lack of test methods or breakpoints made it difficult to assess the frequency of acquired resistance in these less frequently isolated or fastidious organisms and discouraged the testing of individual animal isolates by diagnostic laboratories. A variety of laboratory techniques exist that 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 and also describes current methodology applicable to therapeutic uses of antimicrobial agents used in veterinary medicine for diseases of animals. 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 enables 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.

Because infections due to organisms discussed in VET06 occur less frequently than many of the organisms presently covered in CLSI documents M02, M07, and VET01, the goal of this document is to recommend test conditions and breakpoints based on a careful review of published microbiological data (distributions of MICs), limited animal model studies, existing clinical literature regarding therapy for these organisms, and, in a few instances, a review of existing pharmacokinetic data on the drugs of interest. In some cases, limited in vitro studies were performed.

An increasing number of antimicrobial agents have established veterinary-specific breakpoints. In most cases in which veterinary-specific breakpoints are not established, human breakpoints have been used when appropriate (see CLSI documents M02, M07, M11, and M100). The veterinary-specific breakpoints have been established following CLSI document VET02 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 any associated supplements. Aquatic animal–specific breakpoints can be found in CLSI documents VET03, VET04, and VET03/VET04-S2.

This edition of VET06 includes several potential bacterial agents of bioterrorism that could be initially encountered by diagnostic microbiology laboratories and that should be forwarded to appropriate reference or public health laboratories for identification, confirmation, and possible susceptibility testing. The procedures included in this document are intended for use by those diagnostic, reference, or public health laboratories. It is hoped that VET06 will assist microbiology laboratories in determining an approach for testing these unusual organisms that is relevant to their individual practice settings.

The subcommittee will continue to consider new developments in test methodologies and procedures, as well as revisions to breakpoints for therapeutic agents. With respect to antimicrobial agents used for food
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system (QMS) approach in the development of standards and guidelines, which facilitates project management; defines a document structure using a template; and provides a process to identify needed documents. The QMS 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:

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VET06 does not cover any of the QSEs. For a description of the documents listed in the grid, please refer to the Related CLSI Reference Materials section.

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Related CLSI Reference Materials*

**M02**  
*Performance Standards for Antimicrobial Disk Susceptibility Tests. 12th ed., 2015.* This standard 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.

**M07**  

**M11**  
*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 8th ed., 2012.* This standard provides reference methods for the determination of minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.

**M23**  
*Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters. 4th ed., 2016.* This guideline discusses the necessary and recommended data for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents.

**M24**  
*Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes. 2nd ed., 2011.* This standard provides protocols and related quality control parameters and interpretive criteria for the susceptibility testing of mycobacteria, *Nocardia* spp., and other aerobic actinomycetes.

**M29**  
*Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.* 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.

**M45**  
*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed., 2015.* This guideline informs clinical, public health, and research laboratories on susceptibility testing of infrequently isolated or fastidious bacteria that are not included in CLSI documents M02, M07, or M100. Antimicrobial agent selection, test interpretation, and quality control are addressed.

**M100**  

**VET01**  
*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 4th ed., 2013.* 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.

**VET01S**  
*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 3rd ed., 2015.* This document provides updated tables for the CLSI antimicrobial susceptibility testing standard VET01.

**VET02**  
*Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents. 3rd ed., 2008.* This document addresses the required and recommended data needed for selection of appropriate interpretive standards and quality control guidance for new veterinary antimicrobial agents.

**VET03**  
*Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 1st ed., 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.

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* 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  
Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 2nd ed., 2014. This document provides the most up-to-date techniques for the determination of minimal inhibitory concentrations of aquatic bacteria by broth micro- and macrodilution, and criteria for data interpretation and quality control testing.

VET03/VET04S  
Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 2nd ed., 2014. This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing guidelines VET03-A and VET04-A2.

VET05  
Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin. 1st ed., 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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