

# 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<sup>1</sup> and VET01S,<sup>2</sup> 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.<sup>3</sup> 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<sub>2</sub>) 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<sup>4</sup> 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

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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](mailto: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,<sup>5</sup> M07,<sup>6</sup> VET01,<sup>1</sup> and M100<sup>7</sup> 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