

# VET01

## Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals

This standard covers the current recommended methods for disk diffusion susceptibility testing and the reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution for veterinary use.

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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: +1.610.688.0100  
F: +1.610.688.0700  
[www.clsi.org](http://www.clsi.org)  
[standard@clsi.org](mailto:standard@clsi.org)

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## Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals

Michael T. Sweeney, MS  
Dubraska V. Diaz-Campos, DVM, PhD  
Robert Bowden, BS  
Thomas R. Fritsche, MD, PhD, FCAP, FIDSA  
Joshua Hayes, PhD  
Cory Langston, DVM, PhD  
Brian V. Lubbers, DVM, PhD, DACVCP

Tomás Martin-Jimenez, DVM, PhD, DACVCP,  
DECVPT  
Claire Miller, DVM, PhD, DACVM  
Christine Pallotta, MS, BS  
Mark G. Papich, DVM, MS  
Anne Parkinson, BS  
Stefan Schwarz, DVM  
Maria M. Traczewski, BS, MT(ASCP)

### Abstract

Antimicrobial susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy if its susceptibility cannot be reliably predicted from knowledge of the organism's identity. Susceptibility tests are most often indicated when the causative organism is thought to belong to a species capable of exhibiting resistance to commonly used antimicrobial agents.

Various laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many veterinary microbiology laboratories, an agar disk diffusion method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. Clinical and Laboratory Standards Institute standard VET01—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals* describes disk diffusion, as well as standard broth dilution (macrodilution and microdilution) and agar dilution, and it includes a series of procedures to standardize the way the tests are performed. The performance, applications, and limitations of the current CLSI-recommended methods are also described. The supplemental information (VET08<sup>1</sup> tables) used with this standard represents the most current information for antimicrobial agent selection, interpretation, and quality control using the procedures standardized in VET01.

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## Committee Membership

### Consensus Council

**Dennis J. Ernst, MT(ASCP),  
NCPT(NCCT)  
Chairholder  
Center for Phlebotomy Education  
USA**

**Mary Lou Gantzer, PhD, FACB  
Vice-Chairholder  
USA**

J. Rex Astles, PhD, FACB, DABCC  
Centers for Disease Control and  
Prevention  
USA

Lucia M. Berte, MA, MT(ASCP)SBB,  
DLM, CQA(ASQ)CMQ/OE  
Laboratories Made Better!  
USA

Karen W. Dyer, MT(ASCP), DLM  
Centers for Medicare & Medicaid  
Services  
USA

Thomas R. Fritsche, MD, PhD, FCAP,  
FIDSA  
Marshfield Clinic  
USA

Loralie J. Langman, PhD, DABCC,  
FACB, F-ABFT  
Mayo Clinic  
USA

Ross J. Molinaro, PhD,  
MLS(ASCP)CM, DABCC, FACB  
Siemens Healthcare Diagnostics, Inc.  
USA

James R. Petisce, PhD  
BD Diagnostic Systems  
USA

Andrew Quintenz  
Bio-Rad Laboratories, Inc.  
USA

Robert Rej, PhD  
New York State Department of Health –  
Wadsworth Center  
USA

Zivana Tezak, PhD  
FDA Center for Devices and  
Radiological Health  
USA

### Document Development Committee on Veterinary AST Methods Standard

**Michael T. Sweeney, MS  
Zoetis  
Chairholder  
USA**

**Dubrasca V. Diaz-Campos,  
DVM, PhD  
Vice-Chairholder  
College of Veterinary Medicine,  
The Ohio State University  
USA**

**Maria M. Traczewski, BS, MT(ASCP)  
Committee Secretary  
The Clinical Microbiology Institute  
USA**

Robert Bowden, BS  
University of Florida Veterinary  
Diagnostic Laboratories  
USA

Joshua Hayes, PhD  
FDA Center for Veterinary Medicine  
USA

Cory Langston, DVM, PhD  
Mississippi State University  
USA

Claire Miller, DVM, PhD, DACVM  
Washington State University  
USA

Christine Pallotta, MS, BS  
Thermo Fisher Scientific  
USA

Anne Parkinson, BS  
Ohio Animal Disease Diagnostic  
Laboratory  
USA

Stefan Schwarz, DVM  
Freie Universität Berlin  
Germany

## Subcommittee on Veterinary Antimicrobial Susceptibility Testing

<b>Mark G. Papich, DVM, MS</b> <b>Chairholder</b> College of Veterinary Medicine, North Carolina State University USA	Mark Fielder, PhD School of Life Science, Kingston University London United Kingdom	Thomas R. Shryock, PhD Antimicrobial Consultants, LLC USA
<b>Brian V. Lubbers, DVM, PhD,</b> <b>DACVCP</b> <b>Vice-Chairholder</b> Kansas State Veterinary Diagnostic Laboratory USA	Cynthia C. Knapp, MS, BS, MT(ASCP) Thermo Fisher Scientific USA	Virginia Sinnott-Stutzman, DVM, DACVECC Angell Animal Medical Center (MSPCA) USA
<b>Stefan Schwarz, DVM</b> <b>Committee Secretary</b> Freie Universität Berlin Germany	Cory Langston, DVM, PhD Mississippi State University USA	Maria M. Traczewski, BS, MT(ASCP) The Clinical Microbiology Institute USA
Dubraska V. Diaz-Campos, DVM, PhD College of Veterinary Medicine, The Ohio State University USA	Xian-Zhi Li, PhD Health Canada Veterinary Drugs Directorate Canada	Darren Trott, BSc(Hon), BVMS(Hon), PhD School of Animal and Veterinary Sciences, The University of Adelaide Australia
<b>Staff</b>		
Clinical and Laboratory Standards Institute USA	Megan L. Tertel, MA, ELS <i>Editorial Manager</i>	Kristy L. Leirer, MS <i>Editor</i>
Lori T. Moon, MS, MT(ASCP) <i>Project Manager</i>	Catherine E.M. Jenkins <i>Editor</i>	Laura Martin <i>Editor</i>

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CLSI, the Consensus Council, the Document Development Committee on Veterinary AST Methods Standard, and the Subcommittee on Veterinary Antimicrobial Susceptibility Testing gratefully acknowledge the Expert Panel on Microbiology for serving as technical advisors and subject matter experts during the development of this standard.

## Expert Panel on Microbiology

<b>Richard B. Thomson, Jr., PhD,</b> <b>D(ABMM), FAAM</b> <b>Chairholder</b> Evanston Hospital, NorthShore University HealthSystem USA	Carey-Ann Burnham, PhD, D(ABMM) Washington University School of Medicine USA	David H. Pincus, MS, RM/SM(NRCM), SM(ASCP) bioMérieux, Inc. USA
<b>Mary Jane Ferraro, PhD, MPH</b> <b>Vice-Chairholder</b> Massachusetts General Hospital and Harvard Medical School USA	German Esparza, BSc Proasecal SAS Colombia	Audrey N. Schuetz, MD, MPH, D(ABMM) Mayo Clinic USA
Lynette Y. Berkeley, PhD, MT(ASCP) FDA Center for Drug Evaluation and Research USA	Mark G. Papich, DVM, MS College of Veterinary Medicine, North Carolina State University USA	Ribhi M. Shawar, PhD, D(ABMM) FDA Center for Devices and Radiological Health USA
	Jean B. Patel, PhD, D(ABMM) Centers for Disease Control and Prevention USA	Barbara L. Zimmer, PhD Beckman Coulter – West Sacramento USA

## Acknowledgment

CLSI, the Consensus Council, the Document Development Committee on Veterinary AST Methods Standard, and the Subcommittee on Veterinary Antimicrobial Susceptibility Testing gratefully acknowledge the following volunteers for their important contributions to the development of this standard:

Thomas R. Fritsche, MD, PhD, FCAP, FIDSA  
Marshfield Clinic  
USA

Tomás Martin-Jimenez, DVM, PhD, DACVCP, DECVPT  
College of Veterinary Medicine, University of Tennessee  
USA

Brian V. Lubbers, DVM, PhD, DACVCP  
Kansas State Veterinary Diagnostic Laboratory  
USA

Mark G. Papich, DVM, MS  
College of Veterinary Medicine,  
North Carolina State University  
USA



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## Foreword

In this revision of VET01, several sections were added or revised, as outlined in the Overview of Changes. One of the main updates is the reformatting of the standard to follow a laboratory's path of workflow, defined as the sequential processes of preexamination, examination, and postexamination. An overview of the antimicrobial susceptibility testing process is provided in the beginning of the standard in the new Figure 1 (see Chapter 3) and at the beginning of each method chapter (Chapters 4 through 6), with various testing methods shown in easy-to-follow step-action tables throughout the standard. Other improvements have been made in VET01 by incorporating relevant updates derived from CLSI documents M02<sup>2</sup> and M07<sup>3</sup> and by adding new antimicrobial agents or testing standards for veterinary pathogens.

The most current edition of CLSI document VET08<sup>1</sup> (formerly VET01S), a volume of tables published every 2 to 3 years, is made available with this standard to ensure users are aware of the latest Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) performance standards related to both methods and the information presented in the tables. Previously published tables should be replaced with the current editions for interpreting breakpoints. Because of potential international differences that restrict use of certain antimicrobial agents, some jurisdiction-specific restrictions are described in VET08<sup>1</sup> Table 1 footnotes and in VET08<sup>1</sup> Table 2A comments.

Significant changes in the revision of the VET08<sup>1</sup> tables since 2013 include veterinary-specific breakpoints for categorizing methicillin-susceptible and methicillin-resistant strains of *Staphylococcus pseudintermedius*, which are different from *Staphylococcus aureus* breakpoints. Newly approved antimicrobial agents, such as the fluoroquinolone pradofloxacin, the macrolides gamithromycin and tildipirosin, and the cephalosporin cefovecin have been added to VET08<sup>1</sup> using data presented by the sponsors. For testing of first-generation cephalosporins in dogs, cephalothin has been replaced with cephalexin, which is more predictive of susceptibility and is also used more commonly in dogs. These and other specific changes to the VET08<sup>1</sup> tables are summarized at the beginning of VET08.<sup>1</sup>

Other important additions to the VET08<sup>1</sup> tables are breakpoints for antimicrobial agents that did not previously have a veterinary-specific breakpoint. These are often human antimicrobial agents that are not approved in all countries for animals but may be used legally in some countries by veterinarians in their generic forms. The new additions include doxycycline (for dogs and horses), minocycline (for dogs), amikacin (for dogs and horses), cephalexin (for dogs), cefazolin (for dogs and horses), ampicillin/amoxicillin (for dogs, pigs, and horses), amoxicillin-clavulanate (for dogs and cats), and piperacillin-tazobactam (for dogs), among others. The veterinary diagnostic and related laboratory community is encouraged to provide feedback so that VET01 and its supplement VET08<sup>1</sup> can be kept up to date, maintaining clinical relevance.

Many other editorial and procedural changes in this edition of VET01 were made since 2013 following meetings of the Document Development Committee on Veterinary AST Methods Standard and the Subcommittee on VAST. The most important changes in this standard are summarized below.

## Overview of Changes

This standard replaces the previous edition of the approved standard, VET01-A4, published in 2013. Several changes were made in this edition, including:

- **General:**

- To harmonize with the International Organization for Standardization, the terms for the methods for inoculum preparation have been changed. “Growth method” has been changed to “broth culture method,” and “direct colony suspension method” has been changed to “colony suspension method” throughout the standard.

- Formatting has been changed throughout the standard:
  - The information and techniques needed for performing each type of methodology are divided into three separate chapters:
    - Chapter 4, Disk Diffusion Antimicrobial Susceptibility Testing Process
    - Chapter 5, Broth Dilution Antimicrobial Susceptibility Testing Process
    - Chapter 6, Agar Dilution Antimicrobial Susceptibility Testing Process
  - Information and special techniques needed for detecting resistance are in a new, separate chapter (Chapter 7, Screening Tests to Detect Resistance), with new step-action tables included in Appendix D.
- Easy-to-follow step-action tables are introduced, consistent with CLSI’s goal to make standards and guidelines more user friendly. Most of these tables reflect reformatted text that appeared in the previous edition of VET01. Any changes to the testing recommendations are summarized here in the Overview of Changes.
  - The new step-action tables for disk diffusion tests include:
    - Subchapter 4.1.2.1, Storing and Handling Antimicrobial Disks
    - Subchapter 4.3.2, Colony Suspension Method for Inoculum Preparation
    - Subchapter 4.3.3, Broth Culture Method for Inoculum Preparation
    - Subchapter 4.4, Inoculating the Test Plates
    - Subchapter 4.5, Applying Disks and Incubating Inoculated Agar Plates
  - The new step-action tables for broth dilution tests include:
    - Subchapter 5.1.3, Preparing and Storing Diluted Antimicrobial Agents (for both broth macrodilution [tube] method and broth microdilution method)
    - Subchapter 5.3.2, Colony Suspension Method for Inoculum Preparation
    - Subchapter 5.3.3, Broth Culture Method for Inoculum Preparation
    - Subchapter 5.4, Inoculum Preparation and Inoculation (for both broth macrodilution [tube] method and broth microdilution method)
    - Subchapter 5.6, Incubation (for both broth macrodilution [tube] method and broth microdilution method)
    - Subchapter 5.8, Determining Broth Macro- or Microdilution End Points
  - The new step-action tables for agar dilution tests include:
    - Subchapter 6.1.4, Preparing Agar Dilution Plates
    - Subchapter 6.3.2, Colony Suspension Method for Inoculum Preparation
    - Subchapter 6.3.3, Broth Culture Method for Inoculum Preparation
    - Subchapter 6.4, Inoculating Agar Plates
    - Subchapter 6.5, Incubating Agar Dilution Plates
    - Subchapter 6.7, Determining Agar Dilution End Points
- **Subchapter 1.4.1, Definitions:**
  - Clarified definitions for breakpoint, interpretive category, susceptible, intermediate, resistant, nonsusceptible, and quality control
  - Added definitions for test method and test system

- **Subchapter 2.2.3, Folate Pathway Antagonists:**
  - Revised nomenclature from “folate pathway inhibitor” to “folate pathway antagonist”
- **Subchapter 2.3, Guidelines for Routine Reporting:**
  - Provided additional information on the location of test and report group designations in VET08<sup>1</sup>
- **Subchapter 2.4, Guidelines for Selective Reporting:**
  - Provided additional information on the reasons for selective reporting, with subchapters containing examples and warnings about potentially misleading results
- **Chapter 3, Overview of Antimicrobial Susceptibility Testing Processes:**
  - Added flow chart (Figure 1) that provides an overview of antimicrobial susceptibility testing processes
- **Chapter 4, Disk Diffusion Antimicrobial Susceptibility Testing Process:**
  - Added flow chart (Figure 2) that provides an overview of the disk diffusion susceptibility testing process
- **Subchapters 4.6, 5.7, and 6.6, Special Considerations for Fastidious Organisms:**
  - Added tables that summarize special testing conditions (eg, media, incubation time, and temperature) for fastidious organisms in each method chapter
- **Subchapter 4.7, Reading Plates:**
  - Added reference to the *M02 Disk Diffusion Reading Guide*<sup>4</sup>
  - Noted that the penicillin zone edge test can be useful for determining  $\beta$ -lactamase production in *Staphylococcus aureus* strains with penicillin zones  $\geq 29$  mm
- **Subchapters 4.8, 5.9, and 6.8, Recording, Interpreting, and Reporting Results:**
  - Added subchapters on recording results, determining interpretive categories, and reporting results, with consideration of warnings and intrinsic resistance
- **Subchapters 4.8.1, 5.9.1, and 6.8.1, Recording Results and Determining Interpretive Categories:**
  - Added explanation of nonsusceptible to disk diffusion and minimal inhibitory concentration (MIC) interpretive categories
  - Added explanation of and suggestion to record results in individual data fields for quantitative (zone measurement values) and qualitative test interpretation or interpretive category (ie, whether the isolate is classified as resistant, intermediate, or susceptible)
- **Subchapters 4.8.2, 5.9.2, and 6.8.2, Reporting Results:**
  - Added considerations needed before reporting results:
    - Warnings against the use of specific antimicrobial agents regardless of *in vitro* results
    - Intrinsic resistance
    - Additional species-specific and screening tests to detect resistance
    - Evaluation of QC results
- **Subchapters 4.8.3, 5.9.3, and 6.8.3, Warnings and Intrinsic Resistance:**
  - Added warnings about misleading results
  - Added reference to new intrinsic resistance table (Appendix B in VET08<sup>1</sup>)

- **Chapter 5, Broth Dilution Antimicrobial Susceptibility Testing Process:**
  - Added flow chart (Figure 3) that provides an overview of the broth dilution susceptibility testing process
- **Subchapter 5.1.2.2, Broth Media for Testing Fastidious Organisms:**
  - Added Mueller-Hinton fastidious broth medium with yeast extract (MHF-Y)<sup>b</sup>
- **Subchapter 5.7, Special Considerations for Fastidious Organisms:**
  - Included MHF-Y as an acceptable medium for broth dilution testing of *A. pleuropneumoniae* and *H. somni*<sup>b</sup>
- **Subchapter 5.8, Determining Broth Macro- or Microdilution End Points:**
  - Added new figures (Figures 4 through 7) to illustrate growth control wells, trailing end points, partial inhibition, and skipped well examples of MIC reporting
- **Chapter 6, Agar Dilution Antimicrobial Susceptibility Testing Process:**
  - Added flow chart (Figure 8) that provides an overview of the agar dilution susceptibility testing process
- **Subchapter 7.2.2, Methicillin/Oxacillin Resistance:**
  - Expanded explanation of mechanisms and generic determinants of oxacillin resistance in staphylococci, which includes *mecC* in *S. aureus*
- **Subchapter 7.2.2.1, Methods for Detecting Oxacillin Resistance:**
  - Expanded the discussion of oxacillin resistance and added a table that summarizes the tests available to detect oxacillin resistance in staphylococci
  - Clarified time of incubation for testing of cefoxitin against *Staphylococcus* spp.: 24 hours for coagulase-negative staphylococci and 16 to 18 hours for *S. aureus*
- **Subchapter 7.2.2.2, Reporting Oxacillin for Staphylococci:**
  - Clarified several reporting recommendations to include application of oxacillin results to other penicillinase-stable penicillins and reporting results for *mecA*-negative *S. aureus* and/or penicillin-binding protein 2a-negative *S. aureus* with oxacillin MICs  $\geq 4$   $\mu\text{g/mL}$
- **Subchapter 7.2.3.2, Reporting Vancomycin for Staphylococci:**
  - Emphasized the need to confirm and communicate results to appropriate authorities when *S. aureus* and coagulase-negative staphylococci with vancomycin MICs of  $\geq 8$   $\mu\text{g/mL}$  and  $\geq 32$   $\mu\text{g/mL}$ , respectively, are encountered
- **Subchapter 7.3.3, High-Level Aminoglycoside Resistance:**
  - Noted that high-level resistance to both gentamicin and streptomycin implies resistance to all aminoglycosides
- **Subchapter 7.4, Detecting  $\beta$ -Lactam Resistance in Gram-Negative Bacilli:**
  - Expanded previous section on detection of extended-spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae* to include enzyme classifications and characteristics (Table 6)

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<sup>b</sup> VET01, 5th ed. was re-released in November 2019 to include a new broth medium approved for antimicrobial susceptibility testing of the veterinary fastidious pathogens *Actinobacillus pleuropneumoniae* and *Histophilus somni*. Please see the full memo on the CLSI website (<https://clsi.org/standards-development/document-correction-notice/>) for more information.

- Divided into subchapters with details on extended-spectrum  $\beta$ -lactamases, AmpC  $\beta$ -lactamases, and carbapenemases
- **Subchapter 7.4.1, Extended-Spectrum  $\beta$ -Lactamases:**
  - Updated discussion of extended-spectrum  $\beta$ -lactamases
  - Updated nomenclature for *Enterobacter aerogenes* to *Klebsiella* (formerly *Enterobacter aerogenes*<sup>5</sup>)
- **Subchapter 7.4.2, AmpC Enzymes:**
  - Added discussion of AmpC  $\beta$ -lactamases in gram-negative bacilli
- **Subchapter 7.4.3, Carbapenemases (Carbapenem-Resistant Gram-Negative Bacilli):**
  - Added discussion of carbapenemases in gram-negative bacilli
  - Added reference to the CarbaNP colorimetric microtube assay to detect carbapenemase activity
  - Added examples of  $\beta$ -lactamases with carbapenemase activity (Table 7)
- **Subchapter 7.5.2, Inducible Lincosamide Resistance in *S. pneumoniae* and  $\beta$ -Hemolytic *Streptococcus* spp.:**
  - Noted that infections due to streptococci with inducible lincosamide resistance may fail to respond to lincosamide therapy
- **Subchapter 8.3, Selecting Strains for Quality Control:**
  - Expanded description of routine and supplemental QC strains
- **Subchapter 8.4.2, Subculturing Frozen or Freeze-Dried Quality Control Strains:**
  - Introduced the terms “F1,” “F2,” and “F3” to indicate “frozen” or “freeze-dried” subcultures of QC strains and provided enhanced recommendations for handling QC strains
- **Subchapter 8.7.2, Performance Criteria for Reducing Quality Control Frequency to Weekly:**
  - Introduced the 15-replicate (3-  $\times$  5-day) QC plan as an alternative to the 20- or 30-day QC plan
- **Appendixes:**
  - Reorganized to reflect the order in which they are mentioned in the main text:
    - **Appendix A. Preparation of Media, Supplements, and Reagents** (new)
    - **Appendix B. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests** (new)
    - **Appendix C. Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests** (new)
    - **Appendix D. Screening Test Methods to Detect Resistance** (new)
    - **Appendix E. Quality Control Strain Maintenance** (new)
    - **Appendix F. Antimicrobial Susceptibility Testing Quality Control Form**
    - **Appendix G. Quality Control Protocol Flow Charts** (formerly Appendixes B and C)
  - Deleted **Disk Diffusion Quality Control Troubleshooting Guide** (formerly Appendix D1; currently Table 4C, Disk Diffusion Reference Guide to QC Frequency, in VET08<sup>1</sup>)
  - Deleted **Minimal Inhibitory Concentration Quality Control Troubleshooting Guide** (formerly Appendix D2; currently Table 5C, MIC QC Ranges for Anaerobes [Agar Dilution Method], in VET08<sup>1</sup>)

- **Appendix A. Preparation of Media, Supplements, and Reagents:**
  - Added appendix with instructions for preparation of media and reagents used for all methodologies (agar media, supplements, broth media, reagents, and turbidity standard)
  - Added a new section (A3.5.2, Mueller-Hinton Fastidious Medium With Yeast Extract) with a step-action table that describes preparation of MHF-Y for broth microdilution testing of *A. pleuropneumoniae* and *H. somni*<sup>c</sup>
- **Appendix B. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests:**
  - Added tables with testing conditions for nonfastidious and fastidious organisms
- **Appendix C. Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests:**
  - Added tables with testing conditions for nonfastidious and fastidious organisms
  - Added MHF-Y for broth microdilution testing of *A. pleuropneumoniae* and *H. somni* to Table C2, Conditions for Dilution Antimicrobial Susceptibility Tests for Fastidious Organisms<sup>c</sup>
- **Appendix D. Screening Test Methods to Detect Resistance:**
  - Added appendix with methodology for screening tests to detect resistance described in Chapter 7
- **Appendix E. Quality Control Strain Maintenance:**
  - Revised schematic that depicts stages of subculture and testing of QC strains that originate from “frozen” or “freeze-dried” stock cultures
- **Appendix G. Quality Control Protocol Flow Charts:**
  - Revised and expanded flow charts to better convey the QC testing process (for either disk diffusion or dilution antimicrobial susceptibility tests), with options to convert from daily to weekly QC testing (20- or 30-day plan and 15-replicate [3- × 5-day] plan)
  - Added flow charts for corrective action for daily and weekly QC testing

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

### Key Words

Agar diffusion, agar dilution, antibiotic, antimicrobial agent, antimicrobial susceptibility testing, broth dilution, broth macrodilution, broth microdilution, disk diffusion, Kirby-Bauer, minimal inhibitory concentration, veterinary

Use of Supplement C<sup>TM</sup> in this standard is not an endorsement on the part of CLSI. With each use of the trade name, the words “or the equivalent” are added to indicate that this standard also applies to any equivalent products.

<sup>c</sup> VET01, 5th ed. was re-released in November 2019 to include a new broth medium approved for antimicrobial susceptibility testing of the veterinary fastidious pathogens *A. pleuropneumoniae* and *H. somni*. Please see the full memo on the CLSI website (<https://clsi.org/standards-development/document-correction-notices/>) for more information.

## Summary of CLSI Processes for Establishing Breakpoints and Quality Control Ranges

The Clinical and Laboratory Standards Institute (CLSI) is an international, voluntary, not-for-profit, interdisciplinary, standards-developing, and educational organization accredited by the American National Standards Institute that develops and promotes the use of consensus-developed standards and guidelines within the health care community. These consensus standards and guidelines are developed in an open and consensus-seeking forum to cover critical areas of diagnostic testing and patient health care. CLSI is open to anyone or any organization that has an interest in diagnostic testing and patient care. Information about CLSI is found at [www.clsi.org](http://www.clsi.org).

The CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing reviews data from a variety of sources and studies (eg, *in vitro*, pharmacokinetics-pharmacodynamics, and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and QC parameters. The details of the data necessary to establish breakpoints, QC parameters, and how the data are presented for evaluation are described in CLSI document VET02.<sup>6</sup>

The subcommittee's goal is to establish veterinary-specific breakpoints to decrease reliance on human medical breakpoints. However, human medical breakpoints are still listed in VET08<sup>1</sup> Table 2 series, identified with gray-shaded text, allowing comparison of veterinary-specific and human medical breakpoints. Human medical breakpoints are occasionally necessary to provide zones of inhibition for some categories and a breakpoint for laboratories to consider when there are no veterinary breakpoints available for some antimicrobial agents and organisms for that animal species.

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in decreased clinical efficacy and/or safety. In addition, microbiological methods and QC parameters may be refined to ensure more accurate and better performance of susceptibility test methods. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment.

Additional information, updates, and changes in this standard are found in the meeting summary minutes of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing at [www.clsi.org](http://www.clsi.org).

## CLSI Reference Methods vs Commercial Methods and CLSI vs Regulatory Authority Breakpoints

It is important for users of VET01 and the VET08<sup>1</sup> supplement to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of patient isolates. CLSI recognizes that commercial susceptibility testing devices are commonly used by veterinary diagnostic laboratories. Commercial testing devices used in veterinary medicine may not have demonstrated that test results from such systems are substantially equivalent to those generated using reference methods. For example, the US Food and Drug Administration does not have preapproval or regulatory clearance requirements for use of commercial testing devices for veterinary isolates. Manufacturers of commercial testing devices are expected to validate their methods against CLSI reference methods, but CLSI does not evaluate these data. Laboratories should follow the manufacturer's instructions for quality assurance and quality control testing. The laboratory is responsible for ensuring that the performance of commercial test systems has been validated against the reference method(s).

Currently, there are no regulations that apply to veterinary laboratories regarding susceptibility testing. Veterinary-specific breakpoints are not set by regulatory agencies but have been developed and approved solely by the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing. The guidelines used by CLSI to evaluate data and determine breakpoints are outlined in CLSI document VET02.<sup>6</sup>

CLSI proactively evaluates the need for changing breakpoints. Following a decision by CLSI to change an existing breakpoint, a delay of one or more years may be needed if a breakpoint and interpretive category change is to be implemented by a device manufacturer. Each laboratory should check with the manufacturer of its commercial susceptibility testing device for additional information on the breakpoints and interpretive categories used in its system's software. In addition, newly approved or revised breakpoints may be implemented by veterinary diagnostic laboratories. If approved by CLSI, new or revised breakpoints will be published in VET08.<sup>1</sup>

## **Subcommittee on Veterinary Antimicrobial Susceptibility Testing Mission Statement and Responsibilities**

### **Mission Statement:**

Develop and promote performance standards, breakpoints, and interpretive categories for *in vitro* antimicrobial susceptibility testing of bacteria isolated from animals.

### **Responsibilities:**

The Subcommittee on Veterinary Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, health care providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting. Responsibilities of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing include:

- Developing standard reference methods for antimicrobial susceptibility tests
- Providing quality control parameters for standard test methods
- Establishing breakpoints and interpretive categories for the results of standard antimicrobial susceptibility tests performed on veterinary pathogens
- Providing suggestions for testing and reporting strategies that are clinically relevant and cost-effective
- Continually refining standards through development of new or revised methods, breakpoints, interpretive categories, and quality control parameters
- Educating users through multimedia communication of standards and guidelines
- Fostering a dialogue with users of these methods and those who apply them

The ultimate purpose of the subcommittee's mission is to provide useful information to enable veterinary diagnostic laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care. The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established CLSI guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.



# Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals

## Chapter 1: Introduction

This chapter includes:

- Standard's scope and applicable exclusions
- Background information pertinent to the standard's content
- Standard precautions information
- Terms and definitions used in the standard
- Abbreviations and acronyms used in the standard

### 1.1 Scope

This standard describes reference agar disk diffusion techniques, as well as standard broth (macrodilution and microdilution) and agar dilution methods used to determine *in vitro* antimicrobial susceptibility of bacteria that grow aerobically. It includes:

- Agar plate preparation
- Broth and agar dilution test preparation
- Testing conditions, including inoculum preparation and standardization, incubation time, and incubation temperature
- Results interpretation and reporting considerations
- QC procedures
- Disk diffusion and dilution test method limitations

To assist the veterinary laboratory, suggestions are provided for selecting antimicrobial agents for routine testing and reporting. Additionally, a brief overview of the various antimicrobial classes, bacterial mechanisms of antimicrobial resistance (AMR), and specific tests for detecting AMR are included.

For additional resources, standards for testing the *in vitro* antimicrobial susceptibility of bacteria isolated from humans that grow aerobically using disk or dilution methods are found in CLSI documents M100,<sup>7</sup> M02,<sup>2</sup> and M07,<sup>3</sup> respectively. Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI document M11.<sup>8</sup> Guidelines for standardized antimicrobial susceptibility testing (AST) of infrequently isolated or fastidious bacteria that are not included in CLSI documents M100,<sup>7</sup> M02,<sup>2</sup> M07,<sup>3</sup> or M11<sup>8</sup> are available in CLSI documents VET06<sup>9</sup> and M45.<sup>10</sup> The AST methods provided in this standard can be used in laboratories around the world, including but not limited to:

- Veterinary diagnostic laboratories
- Public health laboratories
- Research laboratories
- Food laboratories
- Environmental laboratories