

5th Edition

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.

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

Clinical and Laboratory Standards Institute

Setting the standard for quality in medical laboratory testing around the world.

The Clinical and Laboratory Standards Institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Consensus Process

Consensus—the substantial agreement by materially affected, competent, and interested parties—is core to the development of all CLSI documents. It does not always connote unanimous agreement but does mean that the participants in the development of a consensus document have considered and resolved all relevant objections and accept the resulting agreement.

Commenting on Documents

CLSI documents undergo periodic evaluation and modification to keep pace with advances in technologies, procedures, methods, and protocols affecting the laboratory or health care.

CLSI's consensus process depends on experts who volunteer to serve as contributing authors and/or as participants in the reviewing and commenting process. At the end of each comment period, the committee that developed the document is obligated to review all comments, respond in writing to all substantive comments, and revise the draft document as appropriate.

Comments on published CLSI documents are equally essential and may be submitted by anyone, at any time, on any document. All comments are managed according to the consensus process by a committee of experts.

Appeal Process

When it is believed that an objection has not been adequately considered and responded to, the process for appeal, documented in the CLSI *Standards Development Policies and Processes*, is followed.

All comments and responses submitted on draft and published documents are retained on file at CLSI and are available upon request.

Get Involved—Volunteer!

Do you use CLSI documents in your workplace? Do you see room for improvement? Would you like to get involved in the revision process? Or maybe you see a need to develop a new document for an emerging technology? CLSI wants to hear from you. We are always looking for volunteers. By donating your time and talents to improve the standards that affect your own work, you will play an active role in improving public health across the globe.

For additional information on committee participation or to submit comments, contact CLSI.

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 standard@clsi.org

VET01, 5th ed. June 2018 Replaces VET01-A4

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 (VET08¹ tables) used with this standard represents the most current information for antimicrobial agent selection, interpretation, and quality control using the procedures standardized in VET01.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*. 5th ed. CLSI standard VET01 (ISBN 978-1-68440-008-9 [Print]; ISBN 978-1-68440-009-6 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

The Clinical and Laboratory Standards Institute consensus process, which is the mechanism for moving a document through two or more levels of review by the health care community, is an ongoing process. Users should expect revised editions of any given document. Because rapid changes in technology may affect the procedures, methods, and protocols in a standard or guideline, users should replace outdated editions with the current editions of CLSI documents. Current editions are listed in the CLSI catalog and posted on our website at www.clsi.org. If you or your organization is not a member and would like to become one, or to request a copy of the catalog, contact us at: Telephone: +1.610.688.0100; Fax: +1.610.688.0700; E-Mail: customerservice@clsi.org; Website: www.clsi.org.



Copyright [©]2018 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, derivative product, or other material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to permissions@clsi.org.

CLSI hereby grants permission to each individual member or purchaser to make a single reproduction of this publication for use in its laboratory procedures manual at a single site. To request permission to use this publication in any other manner, e-mail permissions@clsi.org.

Suggested Citation^a

CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 5th ed. CLSI standard VET01. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Previous Editions:

August 1997, June 1999, May 2002, February 2008, July 2013

ISBN 978-1-68440-008-9 (Print) ISBN 978-1-68440-009-6 (Electronic) ISSN 1558-6502 (Print) ISSN 2162-2914 (Electronic)

Volume 38, Number 13

^a 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-notices/) for more information.

Committee Membership

Consensus Council

Dennis J. Ernst, MT(ASCP), NCPT(NCCT) Chairholder Center for Phlebotomy Education	Karen W. Dyer, MT(ASCP), DLM Centers for Medicare & Medicaid Services USA	James R. Petisce, PhD BD Diagnostic Systems USA
USA		Andrew Quintenz
	Thomas R. Fritsche, MD, PhD, FCAP,	Bio-Rad Laboratories, Inc.
Mary Lou Gantzer, PhD, FACB Vice-Chairholder	FIDSA Marshfield Clinic	USA
USA	USA	Robert Rej, PhD
J. Rex Astles, PhD, FACB, DABCC Centers for Disease Control and Prevention	Loralie J. Langman, PhD, DABCC, FACB, F-ABFT Mayo Clinic	New York State Department of Health – Wadsworth Center USA
USA	USA	Zivana Tezak, PhD FDA Center for Devices and
Lucia M. Berte, MA, MT(ASCP)SBB, DLM, CQA(ASQ)CMQ/OE Laboratories Made Better! USA	Ross J. Molinaro, PhD, MLS(ASCP)CM, DABCC, FACB Siemens Healthcare Diagnostics, Inc. USA	Radiological Health USA

Document Development Committee on Veterinary AST Methods Standard

Michael T. Sweeney, MS Zoetis Chairholder USA

Dubraska 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

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

Acknowledgment for the Expert Panel on Microbiology

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

David H. Pincus, MS, RM/SM(NRCM),		
SM(ASCP)		
bioMérieux, Inc.		
USA		
Audrey N. Schuetz, MD, MPH,		
MM)		
Clinic		
Ribhi M. Shawar, PhD, D(ABMM) FDA Center for Devices and		
a L. Zimmer, PhD		
an Coulter – West Sacramento		

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

Brian V. Lubbers, DVM, PhD, DACVCP Kansas State Veterinary Diagnostic Laboratory USA Tomás Martin-Jimenez, DVM, PhD, DACVCP, DECVPT College of Veterinary Medicine, University of Tennessee USA

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

Contents

Abstracti			
Committee M	Membership	iii	
Foreword		ix	
Chapter 1:	Introduction	1	
1.1	Scope	1	
1.2	Background	2	
1.3	Standard Precautions		
1.4	Terminology		
Chapter 2:	Indications for Performing Antimicrobial Susceptibility Tests	9	
2.1	Selecting Antimicrobial Agents for Routine Testing	10	
2.2	Antimicrobial Agent Classes		
2.3	Guidelines for Routine Reporting	14	
2.4	Guidelines for Selective Reporting		
Chapter 3:	Overview of Antimicrobial Susceptibility Testing Processes		
Chapter 4:	Disk Diffusion Antimicrobial Susceptibility Testing Process		
4.1	Reagents and Materials for Disk Diffusion Tests		
4.1	•		
4.2	Organism Growth for Inoculum and Testing Strains That Fail to Grow	22	
4.3	Satisfactorily		
4.5	Preparing Inoculum for Disk Diffusion Tests		
4.4	Inoculating the Test Plates		
4.5	Applying Disks and Incubating Inoculated Agar Plates		
4.0	Special Considerations for Fastidious Organisms Reading Plates		
4.7	Recording, Interpreting, and Reporting Results		
4.8	Disk Diffusion Zone Diameter Equivalent Minimal Inhibitory Concentration	29	
т.)	Breakpoints	30	
4.10	▲		
Chapter 5:	Broth Dilution Antimicrobial Susceptibility Testing Process	33	
5.1	Reagents and Materials for Broth Dilution Tests		
5.2	Organism Growth for Inoculum and Testing Strains That Fail to Grow		
5.2	Satisfactorily	40	
5.3	Preparing Inoculum for Dilution Tests		
5.4	Inoculum Preparation and Inoculation		
5.5	Inoculum Suspension Colony Counts		
5.6	Incubation		
5.7	Special Considerations for Fastidious Organisms		
5.8	Determining Broth Macro- or Microdilution End Points		
5.9	Recording, Interpreting, and Reporting Results		
5.10			
Chapter 6:	Agar Dilution Antimicrobial Susceptibility Testing Process		
6.1	Reagents and Materials for Agar Dilution Tests		
6.2	Organism Growth for Inoculum and Testing Strains That Fail to Grow		
0.2	Satisfactorily	60	
6.3	Preparing Inoculum for Dilution Tests		
6.4	Inoculating Agar Plates		
		-	

Contents (Continued)

6.5	Incubating Agar Dilution Plates	
6.6	Special Considerations for Fastidious Organisms	
6.7	Determining Agar Dilution End Points	
6.8	Recording, Interpreting, and Reporting Results	
6.9	Dilution Test Method Limitations	
Chapter 7:	Screening Tests to Detect Resistance	67
7.1	Screening Tests	
7.2	Detecting Resistance in Staphylococci	
7.3	Detecting Resistance in Enterococci	
7.4 7.5	Detecting β-Lactam Resistance in Gram-Negative Bacilli	
7.5	Detecting Resistance in Streptococci	
	Detecting Penicillin Resistance and β -Lactamase in <i>Haemophilus</i> spp	
Chapter 8:	Quality Control and Quality Assurance	
8.1	Quality Control Purpose	
8.2	Quality Control Responsibilities	
8.3	Selecting Strains for Quality Control.	
8.4	Maintaining and Testing Quality Control Strains	
8.5	Batch or Lot Quality Control	
8.6 8.7	Acceptable Quality Control Ranges Quality Control Testing Frequency	
8.8	Out-of-Range Results With Quality Control Strains and Corrective Action.	
8.9	Reporting Patient Results When Out-of-Range Quality Control Results Are	
0.0	Observed	
8.10	Confirming Results When Testing Patient Isolates	90
8.11	Reporting Minimal Inhibitory Concentration Results	
8.12	End-Point Interpretation Control	91
Chapter 9:	Additional Antimicrobial Susceptibility and Resistance Reporting	
9.1	Cumulative Antimicrobial Susceptibility Test Data Summary Reports	
9.2	Veterinary Antimicrobial Resistance Monitoring Surveillance Programs	
Chapter 10:	Conclusion	
Chapter 11:	Supplemental Information	96
Refe	rences	97
Appe	endix A. Preparation of Media, Supplements, and Reagents	100
Appe	endix B. Conditions for Disk Diffusion Antimicrobial Susceptibility Tests	111
Appe	endix C. Conditions for Broth and Agar Dilution Antimicrobial Susceptibility	Tests 115
	endix D. Screening Test Methods to Detect Resistance	
	endix E. Quality Control Strain Maintenance	
	endix F. Antimicrobial Susceptibility Testing Quality Control Form	
	endix G. Quality Control Protocol Flow Charts	
	Quality Management System Approach	
	ted CLSI Reference Materials	
Rela	ובע כבסו ועדובובוונב ועומובוומוצ	

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 M02² and M07³ and by adding new antimicrobial agents or testing standards for veterinary pathogens.

The most current edition of CLSI document VET08¹ (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 VET08¹ Table 1 footnotes and in VET08¹ Table 2A comments.

Significant changes in the revision of the VET08¹ 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 VET08¹ 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 VET08¹ tables are summarized at the beginning of VET08.¹

Other important additions to the VET08¹ 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 VET08¹ 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 VET 08^1
- 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 Guide⁴
- Noted that the penicillin zone edge test can be useful for determining β -lactamase production in *Staphylococcus aureus* strains with penicillin zones $\geq 29 \text{ mm}$

• 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:
 - o 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 VET08¹)

- 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)^b
- 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*^b
- 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 penicillinbinding protein 2a–negative *S. aureus* with oxacillin MICs ≥4 µg/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 $\geq 8 \ \mu g/mL$ and $\geq 32 \ \mu g/mL$, 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 β-Lactam Resistance in Gram-Negative Bacilli:
 - Expanded previous section on detection of extended-spectrum β-lactamase-producing *Enterobacteriaceae* to include enzyme classifications and characteristics (Table 6)

^b 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-notices/) for more information.

- Divided into subchapters with details on extended-spectrum β -lactamases, AmpC β -lactamases, and carbapenemases
- Subchapter 7.4.1, Extended-Spectrum β-Lactamases:
 - Updated discussion of extended-spectrum β-lactamases
 - Updated nomenclature for *Enterobacter aerogenes* to *Klebsiella* (formerly *Enterobacter*) *aerogenes*⁵
- Subchapter 7.4.2, AmpC Enzymes:
 - Added discussion of AmpC β-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 β -lactamases with carbapenemase activity (Table 7)
- Subchapter 7.5.2, Inducible Lincosamide Resistance in *S. pneumoniae* and β-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- × 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 VET08¹)
 - Deleted Minimal Inhibitory Concentration Quality Control Troubleshooting Guide (formerly Appendix D2; currently Table 5C, MIC QC Ranges for Anaerobes [Agar Dilution Method], in VET08¹)

• 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 stepaction table that describes preparation of MHF-Y for broth microdilution testing of *A. pleuropneumoniae* and *H. somni*^c
- 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^c
- 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 microdilution, disk diffusion, Kirby-Bauer, minimal inhibitory concentration, veterinary

Use of Supplement C^{TM} 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.

^c 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.

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 VET02.⁶

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 VET08¹ 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.

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

It is important for users of VET01 and the VET08¹ 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 VET02.⁶

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 VET08.¹

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 M100,⁷ M02,² and M07,³ respectively. Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI document M11.⁸ Guidelines for standardized antimicrobial susceptibility testing (AST) of infrequently isolated or fastidious bacteria that are not included in CLSI documents M100,⁷ M02,² M07,³ or M11⁸ are available in CLSI documents VET06⁹ and M45.¹⁰ The AST methods provided in this standard can be used in laboratories around the world, including but not limited to:

1

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

[©]Clinical and Laboratory Standards Institute. All rights reserved.