CLINICAL AND LABORATORY STANDARDS INSTITUTE®

5th Edition

VET01S

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

This document includes updated tables for the Clinical and Laboratory Standards Institute veterinary antimicrobial susceptibility testing standard VET01.

A CLSI supplement for global application.

VET01S-Ed5
October 2020
Replaces VET08-Ed4

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

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Abstract

The data in the tables are valid only if the methodologies in CLSI document VET01[1] are followed. This standard contains information about disk and dilution susceptibility test procedures for aerobic and facultatively anaerobic bacteria. Clinicians need information from the microbiology laboratory for treating and/or confirming treatment decisions for their patients with bacterial infections and depend heavily on this information for treating their seriously ill patients. The clinical importance of antimicrobial susceptibility test results demands that these tests be performed under optimal conditions and that laboratories have the capability to interpret results based on the most current breakpoints and interpretive categories for antimicrobial agents used in veterinary medicine.

The tables presented in VET01S represent the most current information for drug selection, interpretation, and quality control using the procedures standardized in VET01.[1] Users should replace previously published tables with these new tables. Changes in the tables since the previous edition appear in boldface type.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*. 5th ed. CLSI supplement VET01S (ISBN 978-1-68440-092-8 [Print]; ISBN 978-1-68440-093-5 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2020.

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Suggested Citation

CLSI. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals.* 5th ed. CLSI supplement VET01S. Clinical and Laboratory Standards Institute; 2020.

Previous Editions:
May 2004, July 2013, June 2015, June 2018

VET01-Ed5-S-Ed5
ISBN 978-1-68440-092-8 (Print)
ISBN 978-1-68440-093-5 (Electronic)
ISSN 1558-6502 (Print)
ISSN 2162-2914 (Electronic) Volume 40, Number 13

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Overview of Changes

This supplement replaces the previous edition of the supplement, VET08, 4th ed., published in 2018. This list includes the major changes in this document. Other minor or editorial changes were made to the general formatting and to some of the table footnotes and comments. Changes to the tables since the previous edition appear in boldface type. The following are additions or changes unless otherwise noted as a "***deletion***."

- **General:**
  - Changed document code from VET08 to VET01S to associate it with the methods standard, CLSI document VET01[1] (cross-references to VET01S in VET01[1] will be updated in the next edition of VET01[1])
  - Updated nomenclature:
    - Genera formerly included in the family *Enterobacteriaceae* reorganized to an order (Enterobacterales) containing seven families: *Budviciaceae, Enterobacteriaceae, Erwiniaceae, Hafniaceae, Morganellaceae, Pectobacteriaceae, Yersiniaceae*[2]
    - *Eggerthella lenta* (formerly *Eubacterium lentum*)
  - Harmonized language and common information on methods and QC with CLSI documents M02,[3] the *M02 Disk Diffusion Reading Guide*,[4] M07,[5] and M100[6]
  - Revised Table 1, including:
    - Separating columns for dogs and cats
    - Expanding to five test/report groups (A to E) (ie, added new Group B to test/report groups, which resulted in relettering of former Groups B, C, and D to Groups C, D, and E, respectively; test/report groups in VET01[1] will be updated accordingly in the next edition of VET01[1])
    - Listing antimicrobial agents in alphabetical order within each test/report group
    - Revising footnotes
    - Cross-referencing new Appendix D
  - Revised Tables 2A through 2J:
    - Reorganized breakpoints within Tables 2A through 2J to be organized (with the exception of Table 2C-1):
      - First, by animal species (dogs, cats, horses, cattle, swine, and poultry)
      - Second, by alphabetical order of antimicrobial agent within each animal species (moved antimicrobial agent class or subclass to be a column heading rather than a row heading)
    - Added a general comment at the beginning of each table explaining the new organization, as applicable
  - Added general comment in each of Tables 2A through 2J indicating the location of dosage regimens used to establish the veterinary-specific breakpoints, listed in the new Appendix D, with the additional recommendation that laboratories share these dosage regimens with veterinarians, infectious diseases practitioners, clinical pharmacologists, and antimicrobial stewardship teams, if available

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, epidemiological cutoff values, QC parameters, and how the data are presented for evaluation are described in CLSI document VET02.[9]

The subcommittee's goal is to establish veterinary-specific breakpoints to decrease reliance on human breakpoints. However, human breakpoints are still listed in VET01S Table 2 series, identified with gray-shaded text, allowing comparison of veterinary-specific and human breakpoints. Human 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 all animal species.

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of 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 document are found in the meeting summary minutes of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing at www.clsi.org.

Instructions for Use of Tables

These instructions apply to:

- **Table 1:** suggested groupings of antimicrobial agents that could be considered for routine testing and reporting by microbiology laboratories. Placement of antimicrobial agents in Table 1 is either based on approval by relevant regulatory organizations or on use consistent with good clinical practice.

- **Tables 2A through 2J:** tables for each organism group that contain:
  - Recommended testing conditions
  - Routine QC recommendations (also see Chapter 8 in VET01[1])
  - General comments for testing the organism group and specific comments for testing particular agent/organism combinations
  - Suggested agents that could be considered for routine testing and reporting by veterinary microbiology laboratories, as specified in Table 1 (test/report groups A, B, C, D, E)
  - Zone diameter and minimal inhibitory concentration (MIC) breakpoints

- **Tables 3 through 5:** tables for acceptable QC organisms, sources, and acceptable result ranges

- **Table 6:** table of solvents and diluents for preparing stock solutions of antimicrobial agents

- **Tables 7A through 7G:** tables describing tests to detect particular resistance types in specific organisms or organism groups (also see Chapter 7 in VET01[1])

I. Selecting Antimicrobial Agents for Testing and Reporting

A. Appropriate Agents for Routine Testing

Selecting the most appropriate antimicrobial agents to test and report is a decision best made by each laboratory in consultation with veterinarians, infectious diseases practitioners, clinical pharmacologists, and antimicrobial stewardship teams, if available. The recommendations for each organism group include antimicrobial agents that show acceptable *in vitro* test performance. Considerations in the assignment of antimicrobial agents to specific test/report groups include clinical efficacy, prevalence of resistance, minimizing emergence of resistance, cost, regulatory agency–approved clinical indications for use, and current consensus recommendations for first-choice and alternative agents. Tests of selected agents may be useful for infection control and/or monitoring purposes.

B. Equivalent Agents

Antimicrobial agents listed together in a single box are agents for which interpretive categories (susceptible, intermediate, or resistant) and clinical efficacy are similar. Within each box, an “or” between agents indicates agents for which cross-resistance

and cross-susceptibility are nearly complete. Results from one agent connected by an "or" can be used to predict results for the other agent. For example, **Enterobacterales** susceptible to ampicillin can be considered susceptible to amoxicillin. The results obtained from testing ampicillin could be reported along with a comment that the isolate is also susceptible to amoxicillin. For drugs connected with an "or," combined major and very major errors are fewer than 3%, and minor errors are fewer than 10%, based on a large population of bacteria tested (see CLSI documents VET02[9] and M23[11] for description of error types). "Or" is also used for comparable agents when tested against organisms for which "susceptible-only" breakpoints are provided (eg, ampicillin or amoxicillin with *Streptococcus canis*). When no "or" connects agents within a box, testing of one agent cannot be used to predict results for another, owing either to discrepancies or insufficient data.

C. Test/Report Groups

The antimicrobial agents listed in groups A, B, C, D, **and E** in Table 1 include recommendations for appropriate reporting. Antimicrobial agents listed in groups A, C, **and D** in Table 1 are the agents that have been approved by regulatory agencies or authorities for **treatment of** diseases in the indicated host animal. Only group A **and B** designations are restated in the Table 2 series that lists the breakpoints and interpretive categories for species-specific breakpoints in each organism group. To avoid misinterpretation, routine reports to veterinarians should include antimicrobial agents appropriate for therapeutic use.

- **Group A** includes antimicrobial agents with veterinary-specific breakpoints and interpretive categories that are considered appropriate for inclusion in a routine, primary testing panel for food and companion animals, as well as for routine reporting of results for the specified organism groups. The recommended hierarchy for reporting is to first report group A agents over those using human breakpoints, because these compounds have demonstrated an acceptable level of correlation between *in vitro* susceptibility test results and clinical outcome.

- **Group B includes antimicrobial agents with veterinary-specific breakpoints and interpretive categories that are considered appropriate for testing and selective reporting. Antimicrobial agents listed in Group B are considered by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing to be "drugs of last resort," eg, so as not to select for resistance in veterinary patients. Testing may be conducted routinely as part of an antimicrobial resistance surveillance program; however, reporting should be done cautiously and with guidance from an antimicrobial stewardship program[12] and/or by generating an antibiogram.**

- **Group C** includes antimicrobial agents that use human breakpoints and interpretive categories and are next in the hierarchy to report. These agents may perform adequately, but outcome for many veterinary applications has not been demonstrated. The veterinary laboratory may use its discretion to decide whether to selectively report the results from testing these agents.

Table 1. (Continued)

Test/Report Group	Animal Species						
	Swine	Cattle[a]	Bovine Mastitis[b]	Poultry[c]	Horses	Dogs	Cats[d]
Group C – Human Breakpoints							Amikacin
			Ampicillin[e]				
					Azithromycin	**Azithromycin**	
							Cefazolin
							Cephalexin
					Chloramphenicol[h]	Chloramphenicol[h]	Chloramphenicol[h]
	Clindamycin[f]						Clindamycin[f]
						Colistin	
							Doxycycline
	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin
	Gentamicin			Gentamicin			Gentamicin
							Minocycline
						Nitrofurantoin	
	Oxacillin[i]	**Oxacillin[i]**	Oxacillin[i]	**Oxacillin[i]**	**Oxacillin[i]**	Oxacillin[i]	Oxacillin[i]
			Penicillin	Penicillin		Penicillin	Penicillin
					Rifampin	**Rifampin**	**Rifampin**
				Spectinomycin			
	Sulfonamides	Sulfonamides		Sulfonamides		Sulfonamides	Sulfonamides
			Tetracycline[g]	Tetracycline[g]	Tetracycline		Tetracycline
						Tobramycin	**Tobramycin**
				Trimethoprim-sulfamethoxazole[j]	Trimethoprim-sulfamethoxazole[j]	Trimethoprim-sulfamethoxazole[j]	Trimethoprim-sulfamethoxazole[j]

Table 2A. Enterobacterales (Continued)

Test/ Report Group	Body Site	Antimicrobial Agent	Antimicrobial Agent Class or Subclass	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
						S	I	R	S	I	R	
Dogs (Continued)												
A	UTI	Amoxicillin-clavulanate	ß-lactam combination agents	*E. coli*	**20/ 10 µg**	≥ 18	-	-	≤ 8/4	-	-	See comments **(5)** and **(6).**
A	SST	Ampicillin	Penicillinase-labile penicillins	*E. coli*	-	-	-	-	≤ 0.25	0.5	≥ 1.0	**(7)** Systemic breakpoints were derived from microbiological and PK/PD data. The dosage regimen used for PK/PD analysis of amoxicillin was **11** mg/kg every 12 hours orally. See comment **(6).**
A	UTI	Ampicillin	Penicillinase-labile penicillins	*E. coli*	-	-	-	-	≤ 8	-	-	**(8)** This breakpoint for UTIs was derived from published literature in which amoxicillin 11 mg/kg was administered orally to healthy dogs at 8-hour intervals and produced urine concentrations in dogs > 300 µg/mL. See comment **(6).**
A	SST	Cefazolin	Cephalosporin I	*E. coli*	-	-	-	-	≤ 2	4	≥ 8	**(9)** Cefazolin breakpoints were determined from an examination of MIC distribution data and PK/PD analysis of cefazolin. The dosage regimen used for PK/PD analysis of cefazolin was 25 mg/kg every 6 hours IV in horses and dogs.

Related CLSI Reference Materials[a]

EP23™ **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

M02 **Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed., 2018.** This standard covers the current recommended methods for disk susceptibility testing and criteria for quality control testing.

M02QG **Disk Diffusion Reading Guide. 1st ed., 2018.** The Disk Diffusion Reading Guide provides photographic examples of the proper method for reading disk diffusion susceptibility testing results.

M07 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed., 2018.** This standard covers reference methods for determining minimal inhibitory concentrations of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

M11 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 9th ed., 2018.** This standard provides reference methods for determining minimal inhibitory concentrations of anaerobic bacteria by agar dilution and broth microdilution.

M23 **Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters. 5th ed., 2018.** This guideline discusses the necessary and recommended data for selecting appropriate breakpoints and quality control ranges for antimicrobial agents.

M39 **Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data. 4th ed., 2014.** This document describes methods for recording and analysis of antimicrobial susceptibility test data, consisting of cumulative and ongoing summaries of susceptibility patterns of clinically significant microorganisms.

M45 **Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed., 2016.** 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 **Performance Standards for Antimicrobial Susceptibility Testing. 30th ed., 2020.** This document includes updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02, M07, and M11.

VET01 **Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 5th ed., 2018.** 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.

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 Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 2nd ed., 2020.** This guideline provides the most up-to-date techniques for the determination of minimal inhibitory concentrations and zones of inhibition of aquatic bacteria and criteria for data interpretation and quality control testing.

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

Related CLSI Reference Materials (Continued)

VET04 **Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals. 3rd ed., 2020.** This document includes updated tables for the Clinical and Laboratory Standards Institute veterinary antimicrobial susceptibility testing guideline VET03.

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.

VET06 **Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals. 1st ed., 2017.** 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.

VET09 **Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings. 1st ed., 2019.** This report provides veterinarians with the information needed to successfully acquire and interpret antimicrobial susceptibility test results. It promotes common understanding between the veterinarian and the veterinary microbiology laboratory by providing example culture and susceptibility reports and animal species-specific guidance on applying breakpoints to interpret susceptibility test results.

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PRINT ISBN 978-1-68440-092-8

ELECTRONIC ISBN 978-1-68440-093-5

VET01-Ed5-S-Ed5