CLINICAL AND LABORATORY STANDARDS INSTITUTE®

4th Edition

VET08

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.

VET08, 4th ed.
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Replaces VET01S, 3rd ed.

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 test procedures for aerobic and facultatively anaerobic bacteria.

Clinicians depend heavily on information from the microbiology laboratory 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 provide results for the newest antimicrobial agents.

The tables presented in VET08 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 editions appear in boldface type.

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Overview of Changes

This supplement replaces the previous edition of the supplement, VET01S, 3rd ed., published in 2015. 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 VET01S to VET08 to differentiate it from the methods standard, CLSI document VET01[1]
  - Harmonized language and common information on methods and QC with CLSI documents M02[2] and the *M02 Disk Diffusion Reading Guide,*[3] M07,[4] and M100[5]
  - Revised nomenclature:
    - *Clostridium difficile* to *Clostridioides* (formerly *Clostridium*) *difficile*
    - *Enterobacter aerogenes* to *Klebsiella* (formerly *Enterobacter*) *aerogenes*
    - β-lactam/β-lactamase inhibitor combinations to β-lactam combination agents
    - Folate pathway inhibitor to folate pathway antagonist
    - Methicillin-resistant *Staphylococcus aureus* (MRSA) salt agar to oxacillin salt agar
    - To align with the International Organization for Standardization, changed the name of the inoculum preparation method in all appropriate tables from growth method to broth culture method and changed direct colony suspension to colony suspension
- **Moved to CLSI document VET06[6]:**
  - Testing conditions for *Campylobacter* spp. and *Listeria* spp. (formerly in Table 7)
  - *Campylobacter* QC (formerly in Table 5B)
- **Summary of CLSI Processes for Establishing Breakpoints and Quality Control Ranges (p. xxi):**
  - Added new section
- **CLSI Reference Methods vs Commercial Methods and CLSI vs Regulatory Authority (p. xxii):**
  - Added new section
- **CLSI Veterinary-Specific Breakpoint Additions/Revisions Since 2015 (p. xxiii):**
  - Added new table of breakpoint additions and revisions since 2015, organized in order of appearance in the tables by organism group (2A, 2B, 2C, etc.) and animal species, and in alphabetical order by antimicrobial agent within the animal species (see bullets for Tables 2A through 2J for specific new breakpoints)
- **Subcommittee on Veterinary Antimicrobial Susceptibility Testing Mission Statement and Responsibilities (p. xxv):**
  - Added new section

Instructions for Use of Tables

These instructions apply to:

- **Table 1:** suggested groupings of antimicrobial agents that should 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 should be considered for routine testing and reporting by veterinary microbiology laboratories, as specified in Table 1 (test/report groups A, B, C, D)
  - 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. 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. 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, *Enterobacteriaceae* 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[7] and M23[8] 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, and D in Table 1 include recommendations for appropriate reporting. Antimicrobial agents listed in groups A, B, and C in Table 1 are the agents that have been approved by regulatory agencies or authorities for diseases in the indicated host animal. Only group A 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.

1. **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 medical breakpoints, because these compounds have demonstrated an acceptable level of correlation between *in vitro* susceptibility test results and clinical outcome.

2. **Group B** includes antimicrobial agents that use human medical 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.

3. **Group C** includes antimicrobial agents that are regulatory agency–approved for use in the specific animal species. Although QC data are available for these agents, they do not have veterinary- or human-specific CLSI-approved breakpoints and interpretive categories. These agents may be approved for use in other animal species and have veterinary-specific breakpoints in those animals. However, reporting interpretive categories determined by breakpoints set for a particular animal species is not recommended for application to other animal species because there are differences in dosages and pharmacokinetics between animals and people and between animal species. Thus, these agents should be reported selectively before extra-label use agents (group D) but after agents in group B.

4. **Group D** includes agents that are not approved but may be used in an extra-label manner per the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) guidelines[9] in the United States and per similar regulations in other countries for the listed animal. These supplemental agents may be selectively tested and selectively reported. Group D agents may be included in testing for monitoring antimicrobial resistance patterns or for surveillance programs (eg, oxacillin, vancomycin, carbapenems).

See VET01,[1] Subchapter 2.3 for additional information on routine reporting.

D. Selective Reporting

Each laboratory should decide which antimicrobial agents in Table 1 to report routinely (group A) and which might be reported only selectively. Results for antimicrobial agents tested but not reported routinely should be available on request, or they may be reported for selected specimen types.

Agents in groups A, B, and C may be reported routinely or selectively, as outlined in VET01,[1] Subchapter 2.4. However, some group A, B, and C agents are not approved by regulatory agencies or authorities in some countries, and others may be illegal or prohibited in some countries. For example, in the United States, AMDUCA prohibits the use of fluoroquinolones and glycopeptides

Table 1. Antimicrobial Agents That Could Be Considered for Routine Testing by Veterinary Microbiology Laboratories

Some drugs listed in Table 1 may not be approved in all countries and some animal-drug combinations may be considered prohibited or illegal uses in certain jurisdictions. The laboratory client is obligated to consult regulatory agencies in the reporting country to determine if these agents can be legally administered to the species listed for these uses (see **NOTE 5**).

Group A — Veterinary-Specific Breakpoints Primary Test and Report	Swine	Cattle[a]	Bovine Mastitis[b]	Poultry[c]	Horses	Dogs and Cats
	Ceftiofur[d]	Spectinomycin	Ceftiofur[d]	Enrofloxacin[d]	Amikacin Gentamicin[m]	Amikacin (dogs only) Gentamicin (dogs only)[m]
		Ceftiofur[d]				
		Gamithromycin Tildipirosin Tilmicosin Tulathromycin	Pirlimycin		Cefazolin[m] Ceftiofur	Amoxicillin-clavulanate (dogs only) **Piperacillin-tazobactam (dogs only)**
	Tildipirosin Tilmicosin Tulathromycin		Penicillin-novobiocin			
		Ampicillin[f] Penicillin G[m]				**Cefovecin** Cefpodoxime (dogs only) **Cephalexin (dogs only)**[m] Cephalothin (dogs only)[m] Cefazolin (dogs only)[m]
	Ampicillin[f,m] Penicillin G[m]				Ampicillin[f,m] Penicillin G[m]	Clindamycin (dogs only)
		Florfenicol				**Ampicillin (cats only)[f]** Ampicillin (dogs only)[f,m]
	Florfenicol	Danofloxacin[d] Enrofloxacin[d]			**Enrofloxacin[m]**	Difloxacin (dogs only) Enrofloxacin Marbofloxacin Orbifloxacin Pradofloxacin
	Tiamulin					
	Enrofloxacin[d]				**Doxycycline[m]** **Minocycline[m]**	Doxycycline (dogs only) **Minocycline (dogs only)** Tetracycline (dogs only)[i]
	Tetracycline[i]	Tetracycline[i]				

Table 1. (Continued)

	Swine	Cattle[a]	Bovine Mastitis[b]	Poultry[c]	Horses	Dogs and Cats
Group B — CLSI-Approved Human Breakpoints Primary Test, Selectively Report	Gentamicin	Sulfonamides	Cefoperazone[d] Cephalothin[g]	Spectinomycin	Sulfonamides Trimethoprim-sulfamethoxazole[j]	Amikacin (cats only) Gentamicin (cats only)
				Gentamicin	Erythromycin	Cephalothin (cats only) **Cephalexin (cats only)** Cefazolin (cats only)
	Clindamycin[e]	Erythromycin	Erythromycin	Sulfonamides Trimethoprim-sulfamethoxazole[j]		Sulfonamides Trimethoprim-sulfamethoxazole[j]
						Clindamycin (cats only)
						Erythromycin
	Sulfonamides		Ampicillin[f] Oxacillin[h] Penicillin	Erythromycin	Chloramphenicol[k]	Oxacillin[h] Penicillin
	Erythromycin			Penicillin (turkeys only)	Tetracycline[i]	Chloramphenicol[k]
						Doxycycline (cats only) Tetracycline (cats only)[i]
			Tetracycline[i]	Tetracycline[i]		
Group C — No Veterinary Species–Specific or Human-Specific Breakpoints Primary Test, Selectively Report	**Swine**	**Cattle[a]**	**Bovine Mastitis[b]**	**Poultry[c]**	**Horses**	**Dogs and Cats**
	Apramycin Spectinomycin	Cefquinome[d]	Kanamycin-cephalexin[d]	Spectinomycin	Cefquinome	Spectinomycin
				Ceftiofur (chickens only)[d]		Ceftiofur (dogs only)
	Cefquinome[d]	Tylosin	Cefquinome[d]			
				Clindamycin[e]		
	Tylosin					

Table 2A. Zone Diameter and MIC Breakpoints for *Enterobacteriaceae*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables **4A** and **5A** for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922
Pseudomonas aeruginosa ATCC® 27853 (for carbapenems)
E. coli ATCC® 35218 **(for modified instructions for QC of β-lactam combination agents, refer to CLSI document M100[1] Table 5A-2)**

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

Refer to Tables 7A and 7B for additional testing, reporting, and QC for *Enterobacteriaceae.*

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see VET01,[2] **Subchapter 4.5**). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter. **When testing *Enterobacteriaceae* against trimethoprim and the sulfonamides by broth microdilution, read the end point at the concentration in which there is ≥80% reduction in growth as compared with the control (see VET01,[2] Figure 6).**

(2) The dosage regimens shown in the comment column below are those needed to achieve plasma drug exposures (in animals with normal renal functions) on which breakpoints were based. When implementing new breakpoints, it is strongly recommended that laboratories share this information with veterinarians, infectious diseases practitioners, clinical pharmacologists, and antimicrobial stewardship teams, if available.

(3) **Zone diameter** and MIC breakpoints for antimicrobial agents with gray shading are human data taken from CLSI document M100.[1,*] eterinary-specific breakpoints for indicated organisms isolated from designated animal species (with defined disease) are also provided in this table. The user should apply the gray-shaded breakpoints based on human data only if the animal species/antimicrobial agent combinations are not listed in this table. The laboratory should inform the clinician of the species from which the breakpoints were derived (eg, dog, cat, human).

(4) **Unless otherwise listed in the comments, the dose used for evaluation of each breakpoint is the approved dose by regulatory authorities in the country in which the antimicrobial agent is approved.**

NOTE: Information in boldface type is new or modified since the previous edition.

[*] VET08 was developed according to the 28th edition of CLSI document M100,[1] published in January 2018. M100[1] is updated annually; users should refer to the most current edition when using human breakpoints.

Table 2B. Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: **Broth culture** method or colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air
Disk diffusion: 16–18 hours
Dilution methods: 16–20 hours

Routine QC Recommendations (see Tables **4A** and **5A** for acceptable QC ranges)

Escherichia coli ATCC®[a] 25922
P. aeruginosa ATCC® 27853
E. coli ATCC® 35218 **(for modified instructions for QC of β-lactam combination agents, refer to CLSI document M100[1] Table 5A-2)**

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see VET01,[2] Subchapter 4.5). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) Zone diameter and MIC breakpoints for antimicrobial agents with gray shading are human data taken from CLSI document M100.[1,*] Veterinary-specific breakpoints for indicated organisms isolated from designated animal species (with defined disease) are also provided in this table. The user should apply the gray-shaded breakpoints based on human data only if the animal species/antimicrobial agent combinations are not listed in this table. The laboratory should inform the clinician of the species from which the breakpoints were derived (eg, dog, cat, human).

(3) *P. aeruginosa* may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.

(4) Unless otherwise listed in the comments, the dose used for evaluation of each breakpoint is the approved dose by regulatory authorities in the country in which the antimicrobial agent is approved.

NOTE: Information in boldface type is new or modified since the previous edition.

[*] VET08 was developed according to the 28th edition of CLSI document M100,[1] published in January 2018. M100[1] is updated annually; users should refer to the most current edition when using human breakpoints.

Appendix A. Suggestions for Confirming Resistant, Intermediate, or Nonsusceptible Antimicrobial Susceptibility Test Results and Organism Identification

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I[b]	**Category II**	**Category III**
		Uncommon and of veterinary importance, not reported or only rarely reported to date	**Uncommon in most institutions**	**May be common, but is generally considered of epidemiological concern**
		Action Steps:		
		• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection control in the facility to determine if special reporting procedures or additional action are needed. • Check with local rules and regulations to determine which isolates should be reported.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection control in the facility to determine if special reporting procedures or additional action are needed. • Check with local rules and regulations to determine which isolates should be reported.	• Confirm ID and susceptibility if uncommon in the institution.[a] • Check with infection control in the facility to determine if special reporting procedures or additional action are needed.
Any *Enterobacteriaceae*	Carbapenem – I or R[c]		X	
	Colistin[d] – NWT		X	
	Amikacin, gentamicin, and tobramycin – R			X
Escherichia coli *Klebsiella* spp. *Enterobacter* spp. *Proteus mirabilis*	Extended-spectrum cephalosporin[e] – I or R			X
Escherichia coli	Ampicillin – R (urine, dogs) Amoxicillin-clavulanate – R (urine, dogs)			X
Salmonella and *Shigella* spp.	Extended-spectrum cephlosporin[e] – I or R Fluoroquinolone – I or R		X	
Acinetobacter baumannii	Colistin – R		X	
	Carbapenem – I or R			X
Actinobacillus pleuropneumoniae	Macrolide – NS or R Ceftiofur – I or R	X		
Pseudomonas aeruginosa	Carbapenem – I or R			X
Stenotrophomonas maltophilia	Trimethoprim-sulfamethoxazole – I or R		X	