

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.

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

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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.

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Suggested Citation

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

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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 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

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:

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

This standard and its supplement (VET08¹) are not intended to guide the use of antimicrobial agents that are used for production or disease prevention purposes.

1.2 Background

To positively affect clinical outcomes, help maintain antimicrobial effectiveness, assist clinicians in using antimicrobial agents safely, and minimize the selection of resistant pathogens, laboratories must use a standardized, well-defined method for performing AST. VET01 presents AST methods that provide accurate, reproducible, clinically relevant results for veterinary pathogens. Veterinary-specific breakpoints were established following guidelines presented in CLSI document VET02,⁶ with particular attention given to product label indications. Recommendations¹¹ have been reviewed, with the appropriate sections incorporated into this standard. In recognition of the need for a global standard for AST of bacteria isolated from animals, test method guidelines have been published¹² that are consistent with those contained in this standard. The need for globally harmonized test methods is essential if interlaboratory minimal inhibitory concentrations (MICs) or zone-size data are to be compared in journals, Web postings, AMR monitoring program reports, etc. The application of a single methodology also allows drug sponsors in countries other than the United States to prepare data packages for presentation to the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) as recommended in CLSI document VET02.⁶

Breakpoints and interpretive categories presented in the VET08¹ informational supplement apply only if the laboratory has conducted AST according to the specific methods described in VET01. Aquatic animal-specific breakpoints can be found in CLSI documents VET03¹³ and VET04¹⁴ and their supplement, CLSI document VET03/VET04.¹⁵ Judicious use of antimicrobial agents in the veterinary setting is directly related to the breakpoints associated with AST in that a given set of breakpoints and interpretive categories applies only to that specific antimicrobial, disease, and host species combination. In cases in which veterinary-specific breakpoints are not established, human medical breakpoints and interpretive categories have been used when appropriate (see CLSI documents M02,² M07,³ M11,⁸ M45,¹⁰ and M100⁷). For antimicrobial agents not approved for use in indicated food animal species, the laboratory client or veterinarian assumes all responsibility for efficacy, safety, and residue avoidance with the extra-label use of these agents.

Other AST methods provide results essentially equivalent to the CLSI methods described herein. Also, commercial systems based primarily or in part on some of these methods may provide results essentially equivalent to the CLSI methods described. CLSI does not approve or endorse commercial products or devices.

1.2.1 Disk Diffusion

Various laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. In many veterinary and medical laboratories, agar disk diffusion is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens. This standard describes the performance, applications, and limitations of the standardized disk diffusion test method.

Disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the zone's size are not acceptable for determining antimicrobial susceptibility. Reliable results can be obtained only with disk diffusion tests that use standardized methodology and zone diameter measurements correlated with MICs with strains known to be susceptible or resistant to various antimicrobial agents. The methods described herein must be followed explicitly to obtain reproducible results. The standardized method currently recommended by the CLSI Subcommittee on VAST is based on the original and most thoroughly described disk diffusion method¹⁶ for which breakpoint and interpretive categories have been developed and supported by laboratory and clinical data.

This standard describes methods, QC, breakpoints, and interpretive categories currently recommended for disk diffusion susceptibility tests. For most agents, these criteria are developed by first comparing zone

Chapter 2: Indications for Performing Antimicrobial Susceptibility Tests

This chapter includes:

- Indications for when AST is necessary
- Selecting appropriate antimicrobial agents for routine testing
- Descriptions of the various antimicrobial agent classes
- Guidelines for routine reporting
- Guidelines for and examples of selective reporting

AST 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 or if there is a reason to believe that the initial antimicrobial agent of choice would not be acceptable because of adverse effects or other factors. Antimicrobial 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. Resistance mechanisms include:

- Production of drug-inactivating enzymes
- Alteration of drug targets
- Altered drug uptake or efflux

Some organisms have predictable susceptibility to antimicrobials agents, and empiric therapy for these organisms is widely accepted. Antimicrobial susceptibility tests are seldom necessary when the infection is due to a microorganism recognized as susceptible to a highly effective antimicrobial agent (eg, the continued susceptibility of *Streptococcus equi* subsp. *zooepidemicus* to penicillin). Susceptibility test methods, QC ranges, and/or breakpoints and interpretive categories have not been established for all pathogens of animals, so standardized AST may not be available.

AST should be performed on isolates from cultures of clinical specimens (or tissues) from animals suspected of having an infectious disease. Isolated colonies of each organism type that may be pathogenic should be selected from primary, nonselective agar plates and tested individually for susceptibility. A statistical model for assessing sample size for bacterial colony selection has been proposed.²² Culture and identification procedures should be performed in conjunction with or precede *in vitro* susceptibility tests. Susceptibility tests are also important in studies of epidemiology or AMR monitoring, as well as in studies of new antimicrobial agents.

The following “short-cut” methods of AST are discouraged, because they provide misleading outcomes and could result in making a poor treatment decision:

- Mixtures of different types of microorganisms should not be tested on the same susceptibility test plate.
- The practice of conducting susceptibility tests directly with clinical material (eg, normally sterile body fluids and urine) should be avoided, except:
 - When using a commercially available, approved, validated method.
 - In clinical emergencies when a direct Gram stain suggests a single pathogen (results should be reported as preliminary, and AST must be repeated using the standard method).
- When the nature of the infection is not clear and the specimen contains mixed organisms or normal flora, in which the organisms probably bear little relationship to the infectious process.

Chapter 4: Disk Diffusion Antimicrobial Susceptibility Testing Process

This chapter includes:

- An overview of the disk diffusion AST process
- Suggested media for disk diffusion testing, including supplements for fastidious organisms
- Instructions for proper storage of antimicrobial disks
- Description of the methods for inoculum preparation and standardization, plate inoculation, application of disks, and incubation
- Testing considerations for fastidious organisms, including recommended media and incubation conditions
- Instructions for reading disk diffusion plates, measuring zone diameters, and interpreting the results
- Considerations for reporting results
- Limitations of disk diffusion methods, development of resistance, and testing of repeat isolates

In many veterinary diagnostic laboratories, the agar disk diffusion method is used for testing common, rapidly growing bacterial pathogens. If results are to be reliable, the technical details of such procedures must be carefully standardized and controlled. Agar disk diffusion methods based solely on the presence or absence of a zone of inhibition or on achievement of an arbitrary size, regardless of the diffusion characteristics of the agent and the disk content, are not acceptable.

The standardized method currently recommended by the CLSI Subcommittee on VAST for agar disk diffusion testing was adapted from the method in CLSI document M02,² which is based on the original and most thoroughly described disk diffusion method.¹⁶ This method is the most thoroughly described disk diffusion method for which breakpoints and interpretive categories have been developed and supported by laboratory and clinical data. Figure 2 provides an overview of the AST process for disk diffusion susceptibility testing. Detailed information for each step is provided in each designated subchapter.