CLINICAL AND LABORATORY STANDARDS INSTITUTE®

6th Edition

CLSI 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 for 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.

CLSI VET01-Ed6
January 2024
Replaces CLSI VET01-Ed5

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. Clinical and Laboratory Standards Institute 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 (CLSI VET01S[1] tables) used with this standard represents the most current information for antimicrobial agent selection, interpretation, and QC using the procedures standardized in CLSI VET01.

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Foreword

CLSI VET01S,[1] a volume of tables, is updated along with this standard to ensure users are aware of the latest recommendations related to the methods described in CLSI VET01. Many editorial and procedural changes in this edition of CLSI VET01 resulted from decisions made at CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing meetings held since 2018. The most important changes in CLSI VET01 are summarized below.

Overview of Changes

This standard replaces CLSI VET01-Ed5, published in 2018 (re-released in 2019). Several changes were made in this edition, including:

- **General**:
  - Nomenclature updates:
    - Replaced *Enterobacteriaceae* with Enterobacterales
    - Replaced *Staphylococcus sciuri* with *Mammaliicoccus sciuri*
    - Replaced *Staphylococcus vitulinus* with *Mammaliicoccus vitulinus*
    - Deleted "coagulase-negative staphylococci" (CoNS) throughout the document and replaced with "staphylococci other than *Staphylococcus aureus*" (SOSA) where appropriate
  - Replaced veterinary fastidious medium with Mueller-Hinton fastidious broth medium with yeast extract throughout the document. For more information, see Revisions for CLSI VET01-Ed5 (August 2019) on the CLSI website: https://clsi.org/standards-development/document-correction-notices/
  - Added antimicrobial susceptibility testing (AST) methods and QC information for *Campylobacter* spp.
- **Subchapter 1.1, Scope:**
  - Added reference to CLSI VET03[2] on standardized AST of bacterial pathogens from aquatic animals
- **Subchapter 1.2, Background:**
  - Moved paragraph previously in **Subchapter 1.2.2, Standard Dilution (Macrodilution, Microdilution, Agar Dilution),** that also applies to disk diffusion and describes methods, media, and pathogens included in this standard vs pathogens included in CLSI VET06[3] and CLSI VET04[4]
- **Subchapter 1.2.4, Antimicrobial Agents for Treatment and Control:**
  - Clarified the differences between treatment and control
- **Subchapter 1.2.6, Antimicrobial Agents for Production Use:**
  - Clarified explanation that the use of antimicrobial agents for production purposes is outside the scope of the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing's work and not considered judicious use
- **Subchapter 1.4.1, Definitions:**
  - Added definition for **control//metaphylaxis** per the American Veterinary Medical Association (AVMA)
  - Added separate definitions for **interpretive category (for breakpoints)** and **interpretive category (for epidemiological cutoff values)**
  - Updated definitions for **prevention//prophylaxis** and **treatment** per the AVMA

Chapter 1

Introduction

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

1 Introduction

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 M100,[5] M02,[7] and M07.[8] Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI M11.[9] Guidelines for standardized antimicrobial susceptibility testing (AST) of bacterial pathogens from aquatic animals are available in CLSI VET03.[2] Guidelines for AST of infrequently isolated or fastidious bacteria that are not included in CLSI M100,[5] M02,[7] M07,[8] or M11[9] are available in CLSI VET06[3] and CLSI M45.[10] The AST methods provided in this standard can be used in laboratories around the world, including but not limited to:

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

This standard and its supplement (CLSI VET01S[1]) 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. CLSI VET01 presents AST methods that provide accurate, reproducible, clinically relevant results for veterinary pathogens. Veterinary-specific breakpoints were established following guidelines presented in CLSI VET02,[6] with particular attention given to product label indications. Recommendations[11] 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 published[12] 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 CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) as recommended in CLSI VET02.[6]

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, pathogen, anatomical site or disease, and host species combination. Breakpoints and interpretive categories presented in the CLSI VET01S[1] informational supplement apply only if the laboratory has conducted AST according to the specific methods described in CLSI VET01.

The methods described in this standard are intended primarily for testing commonly isolated aerobic or facultative bacteria that grow well after overnight incubation on unsupplemented Mueller-Hinton agar (MHA) or in Mueller-Hinton broth (MHB). Alternative media and other testing conditions for *Actinobacillus pleuropneumoniae, Campylobacter* spp., *Histophilus somni, Mannheimia haemolytica, Pasteurella multocida,* and *Streptococcus* spp. are described in Subchapters 4.6, 5.7, and 6.6 and in CLSI VET01S.[1] Guidelines for AST of other fastidious or infrequently isolated bacteria, including anaerobes, are found in CLSI VET06.[3] Aquatic animal–specific breakpoints, epidemiological cutoff values (ECVs), and interpretive categories can be found in CLSI VET04.[4]

In cases in which veterinary-specific breakpoints are not established, human breakpoints and interpretive categories have been used when appropriate (see CLSI M02,[7] M07,[8] M11,[9] M24,[13] M45,[10] and M100[5]). CLSI VET09[14] also provides in-depth information about the appropriate application of breakpoints from other species. **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 extralabel 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.

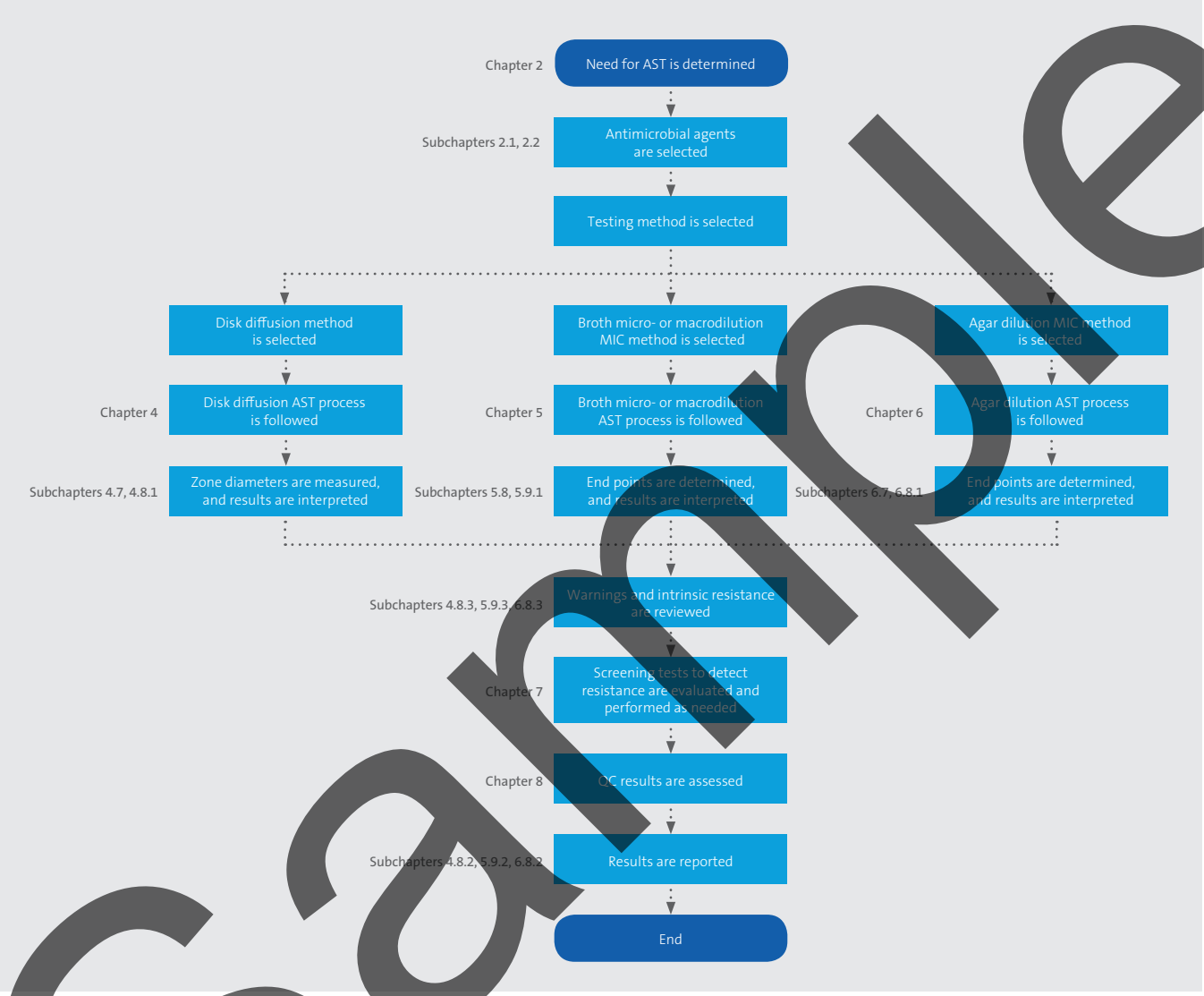
1.2.1 Disk Diffusion

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

3 Overview of Antimicrobial Susceptibility Testing Processes

Figure 1 provides an overview of AST processes for disk diffusion methods and MIC testing by broth dilution (macrodilution or microdilution) and agar dilution methods. Detailed information for each step is provided in each designated chapter and subchapter.

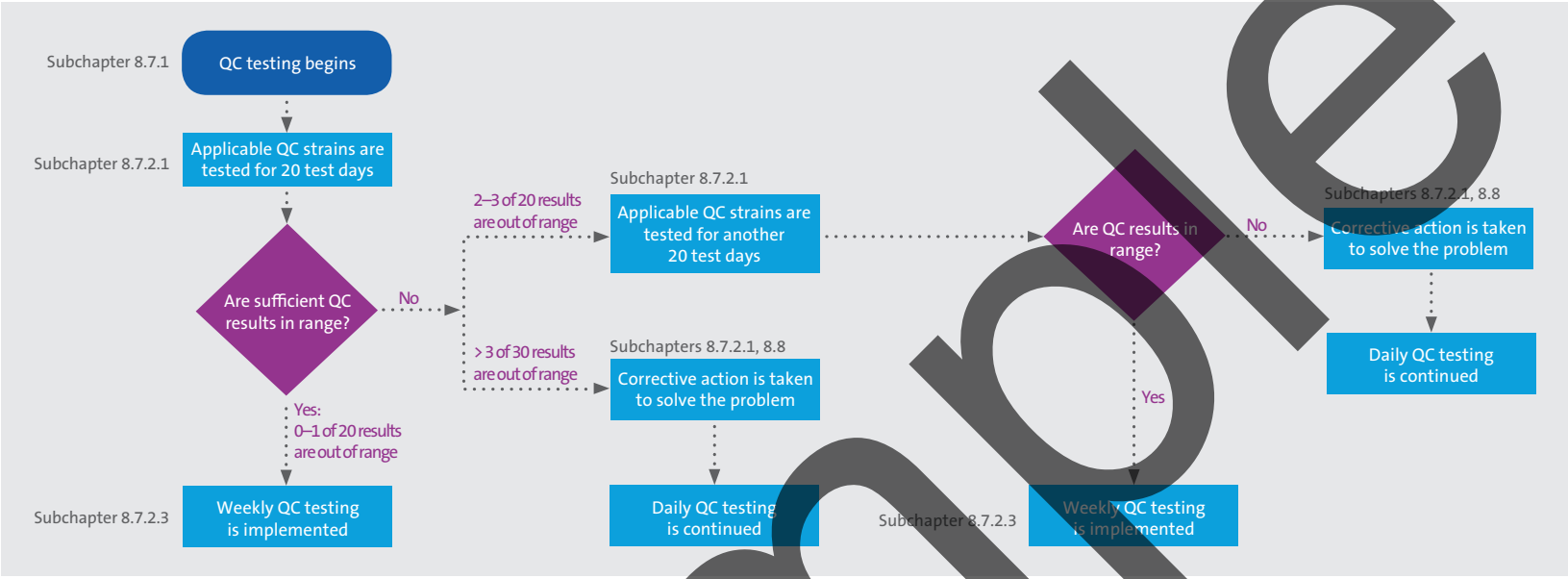


Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; QC, quality control.
[a] Three basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities).

Figure 1. Overview of AST Processes[a]

G1 Quality Control Protocol: Conversion From Daily to Weekly Testing (20- or 30-Day Plan)[a]

NOTE: All subchapter references are to subchapters in this standard.



Abbreviation: QC, quality control.

[a] Four basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities), diamond (includes a question with alternative "Yes" and "No" responses).

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