



CLINICAL AND  
LABORATORY  
STANDARDS  
INSTITUTE

6th Edition

# CLSI VET01™

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

Sample

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.

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

Brian V. Lubbers, DVM, PhD, DACVCP  
Dubraska V. Diaz-Campos, DVM, PhD  
Stefan Schwarz, DVM  
Michael T. Sweeney, MS  
Claire R. Burbick, DVM, PhD, DACVM  
Merran Govendir, PhD, BVSc, FHERDSA, MANZCVSc  
Beth Harris, PhD, MS  
Nicole M. Holliday, BA

Joshua Hayes, PhD  
Sara D. Lawhon, DVM, PhD, DACVM  
Xian-Zhi Li, PhD  
Ron A. Miller, PhD, MS  
Ian Morrissey, BSc, MBA, PhD, FRSM  
K. Marcia Murphy, DVM, DACVD  
Mark G. Papich, DVM, MS  
Shabbir Simjee, PhD, MSc

## 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<sup>1</sup> tables) used with this standard represents the most current information for antimicrobial agent selection, interpretation, and QC using the procedures standardized in CLSI VET01.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*. 6th ed. CLSI standard VET01 (ISBN 978-1-68440-202-1 [Print]; ISBN 978-1-68440-203-8 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2024.

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](http://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:**

**P:** +1.610.688.0100 **F:** +1.610.688.0700 **E:** [customerservice@clsi.org](mailto:customerservice@clsi.org) **W:** [www.clsi.org](http://www.clsi.org)

Copyright ©2024 Clinical and Laboratory Standards Institute. Except as stated below, any reproduction of content from a CLSI copyrighted standard, guideline, or other product or material requires express written consent from CLSI. All rights reserved. Interested parties may send permission requests to [permissions@clsi.org](mailto: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](mailto:permissions@clsi.org).

To read CLSI's full Copyright Policy, please visit our website at <https://clsi.org/terms-of-use/>.

## Suggested Citation

CLSI. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*. 6th ed. CLSI standard VET01. Clinical and Laboratory Standards Institute; 2024.

### Previous Editions:

August 1997, June 1999, May 2002, February 2008, July 2013, June 2018

CLSI VET01-Ed6

ISBN 978-1-68440-202-1 (Print)

ISBN 978-1-68440-203-8 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 44, Number 1

# Contents

Abstract	i
Committee Membership	iii
Foreword	ix
<b>Chapter 1: Introduction</b>	<b>1</b>
1.1 Scope	2
1.2 Background	3
1.3 Standard Precautions	5
1.4 Terminology	6
<b>Chapter 2: Indications for Performing Antimicrobial Susceptibility Tests</b>	<b>13</b>
2.1 Antimicrobial Agents for Routine Testing	15
2.2 Antimicrobial Agent Classes	16
2.3 Guidelines for Routine Reporting	20
2.4 Guidelines for Selective Reporting	21
<b>Chapter 3: Overview of Antimicrobial Susceptibility Testing Processes</b>	<b>23</b>
<b>Chapter 4: Disk Diffusion Antimicrobial Susceptibility Testing Process</b>	<b>25</b>
4.1 Reagents and Materials for Disk Diffusion Tests	28
4.2 Organism Growth for Inoculum and Testing Strains That Fail to Grow Satisfactorily	30
4.3 Preparing Inoculum for Disk Diffusion Tests	30
4.4 Inoculating the Test Plates	31
4.5 Applying Disks and Incubating Inoculated Agar Plates	32
4.6 Special Considerations for Fastidious Organisms	33
4.7 Reading Plates	35
4.8 Recording, Interpreting, and Reporting Results	36
4.9 Disk Diffusion Zone Diameter Equivalent Minimal Inhibitory Concentration Breakpoints	37
4.10 Disk Diffusion Method Limitations	38

## Contents (Continued)

<b>Chapter 5: Broth Dilution Antimicrobial Susceptibility Testing Process</b> .....	<b>39</b>
5.1 Reagents and Materials for Broth Dilution Tests .....	42
5.2 Organism Growth for Inoculum and Testing Strains That Fail to Grow Satisfactorily .....	46
5.3 Preparing Inoculum for Dilution Tests.....	46
5.4 Inoculum Preparation and Inoculation.....	47
5.5 Inoculum Suspension Colony Counts .....	49
5.6 Incubation.....	49
5.7 Special Considerations for Fastidious Organisms.....	50
5.8 Determining Broth Macro- or Microdilution End Points .....	52
5.9 Recording, Interpreting, and Reporting Results.....	55
5.10 Dilution Test Method Limitations .....	56
<b>Chapter 6: Agar Dilution Antimicrobial Susceptibility Testing Process</b> .....	<b>57</b>
6.1 Reagents and Materials for Agar Dilution Tests .....	60
6.2 Organism Growth for Inoculum and Testing Strains That Fail to Grow Satisfactorily .....	64
6.3 Preparing Inoculum for Dilution Tests.....	64
6.4 Inoculating Agar Dilution Plates .....	66
6.5 Incubating Agar Dilution Plates.....	66
6.6 Special Considerations for Fastidious Organisms.....	67
6.7 Determining Agar Dilution End Points.....	70
6.8 Recording, Interpreting, and Reporting Results.....	70
6.9 Dilution Test Method Limitations.....	72
<b>Chapter 7: Tests to Detect Resistance</b> .....	<b>73</b>
7.1 Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Resistance to Antimicrobial Agents .....	74
7.2 Detecting and/or Confirming Resistance in Staphylococci.....	75
7.3 Detecting Resistance in Enterococci.....	81
7.4 Detecting Resistance in Streptococci.....	82
7.5 Detecting $\beta$ -Lactam Resistance in Gram-Negative Bacilli.....	83

## Contents (Continued)

<b>Chapter 8: Quality Control and Quality Assurance</b> .....	<b>87</b>
8.1 Quality Control Purpose .....	88
8.2 Quality Control Responsibilities .....	88
8.3 Selecting Strains for Quality Control .....	89
8.4 Maintaining and Testing Quality Control Strains .....	91
8.5 Batch or Lot Quality Control .....	93
8.6 Acceptable Quality Control Ranges .....	94
8.7 Quality Control Testing Frequency (also refer to Appendix G and CLSI VET01S Tables 4D and 5F) .....	94
8.8 Out-of-Range Results With Quality Control Strains and Corrective Action .....	96
8.9 Reporting Patient Results When Out-of-Range Quality Control Results Are Observed .....	99
8.10 Confirming Results When Testing Patient Isolates .....	99
8.11 Reporting Minimal Inhibitory Concentration Results .....	100
8.12 End-Point Interpretation Control .....	100
<b>Chapter 9: Additional Antimicrobial Susceptibility and Resistance Reporting</b> .....	<b>101</b>
9.1 Cumulative Antimicrobial Susceptibility Test Data Summary Reports .....	102
9.2 Veterinary Antimicrobial Resistance Monitoring Surveillance Programs .....	102
<b>Chapter 10: Conclusion</b> .....	<b>105</b>
<b>Chapter 11: Supplemental Information</b> .....	<b>107</b>
<b>References</b> .....	108
<b>Appendix A.</b> Preparation of Media, Supplements, Reagents .....	114
<b>Appendix B.</b> Conditions for Disk Diffusion Antimicrobial Susceptibility Tests .....	123
<b>Appendix C.</b> Conditions for Broth and Agar Dilution Antimicrobial Susceptibility Tests .....	128
<b>Appendix D.</b> Screening Test Methods to Detect Resistance .....	134
<b>Appendix E.</b> Quality Control Strain Maintenance .....	137
<b>Appendix F.</b> Antimicrobial Susceptibility Testing Quality Control Form .....	139
<b>Appendix G.</b> Quality Control Protocol Flow Charts .....	140
<b>The Quality Management System Approach</b> .....	144

## Foreword

CLSI VET01S,<sup>1</sup> 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-notice/>
  - Added antimicrobial susceptibility testing (AST) methods and QC information for *Campylobacter* spp.
- **Subchapter 1.1, Scope:**
  - Added reference to CLSI VET03<sup>2</sup> 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<sup>3</sup> and CLSI VET04<sup>4</sup>
- **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

Sample



# 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,<sup>5</sup> M02,<sup>7</sup> and M07.<sup>8</sup> Standards for testing the *in vitro* antimicrobial susceptibility of bacteria that grow anaerobically are found in CLSI M11.<sup>9</sup> Guidelines for standardized antimicrobial susceptibility testing (AST) of bacterial pathogens from aquatic animals are available in CLSI VET03.<sup>2</sup> Guidelines for AST of infrequently isolated or fastidious bacteria that are not included in CLSI M100,<sup>5</sup> M02,<sup>7</sup> M07,<sup>8</sup> or M11<sup>9</sup> are available in CLSI VET06<sup>3</sup> and CLSI M45.<sup>10</sup> 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<sup>1</sup>) 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,<sup>6</sup> with particular attention given to product label indications. Recommendations<sup>11</sup> 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<sup>12</sup> 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.<sup>6</sup>

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<sup>1</sup> 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.<sup>1</sup> Guidelines for AST of other fastidious or infrequently isolated bacteria, including anaerobes, are found in CLSI VET06.<sup>3</sup> Aquatic animal-specific breakpoints, epidemiological cutoff values (ECVs), and interpretive categories can be found in CLSI VET04.<sup>4</sup>

In cases in which veterinary-specific breakpoints are not established, human breakpoints and interpretive categories have been used when appropriate (see CLSI M02,<sup>7</sup> M07,<sup>8</sup> M11,<sup>9</sup> M24,<sup>13</sup> M45,<sup>10</sup> and M100<sup>5</sup>). CLSI VET09<sup>14</sup> 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.

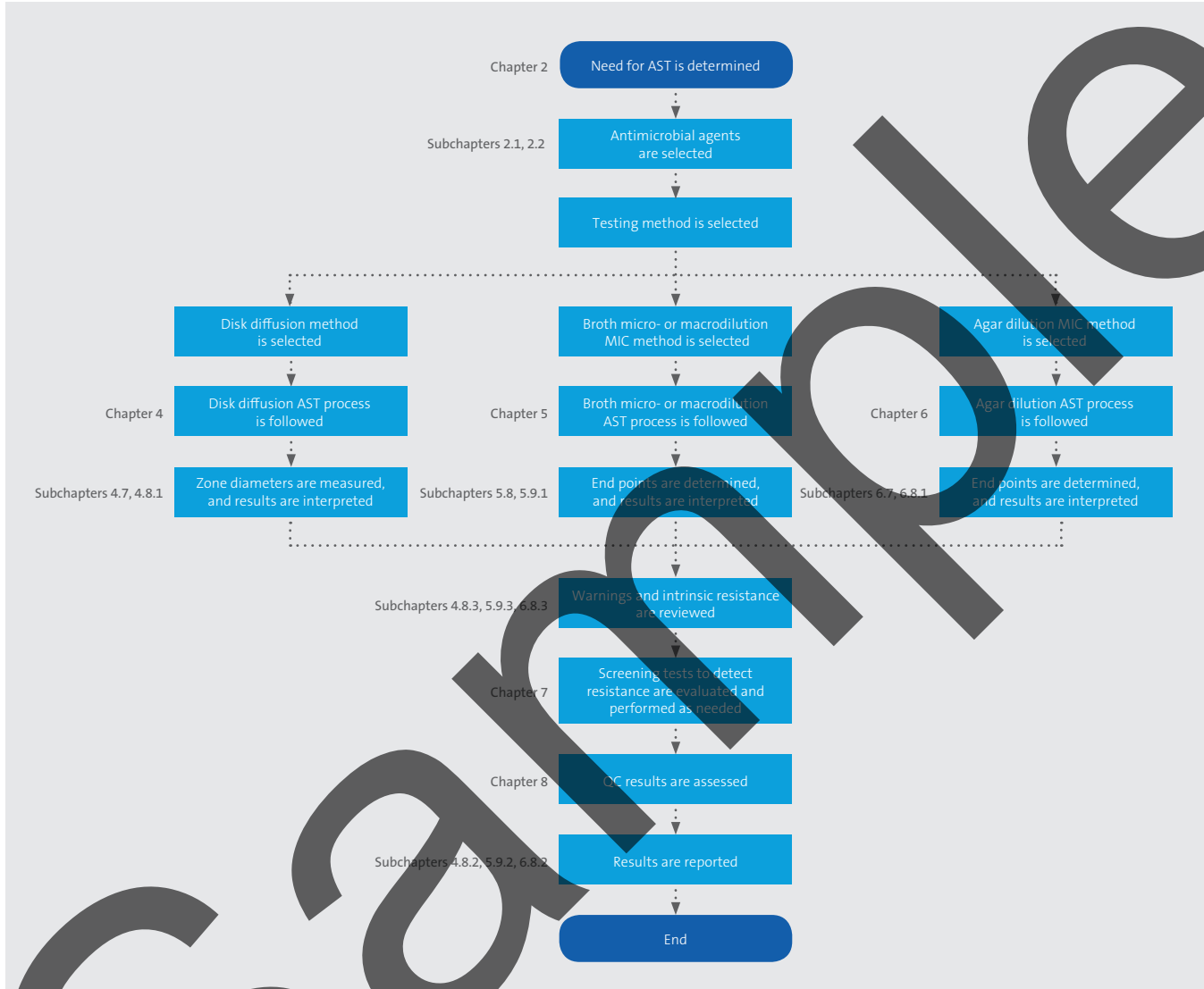
### 1.2.1 Disk Diffusion

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.



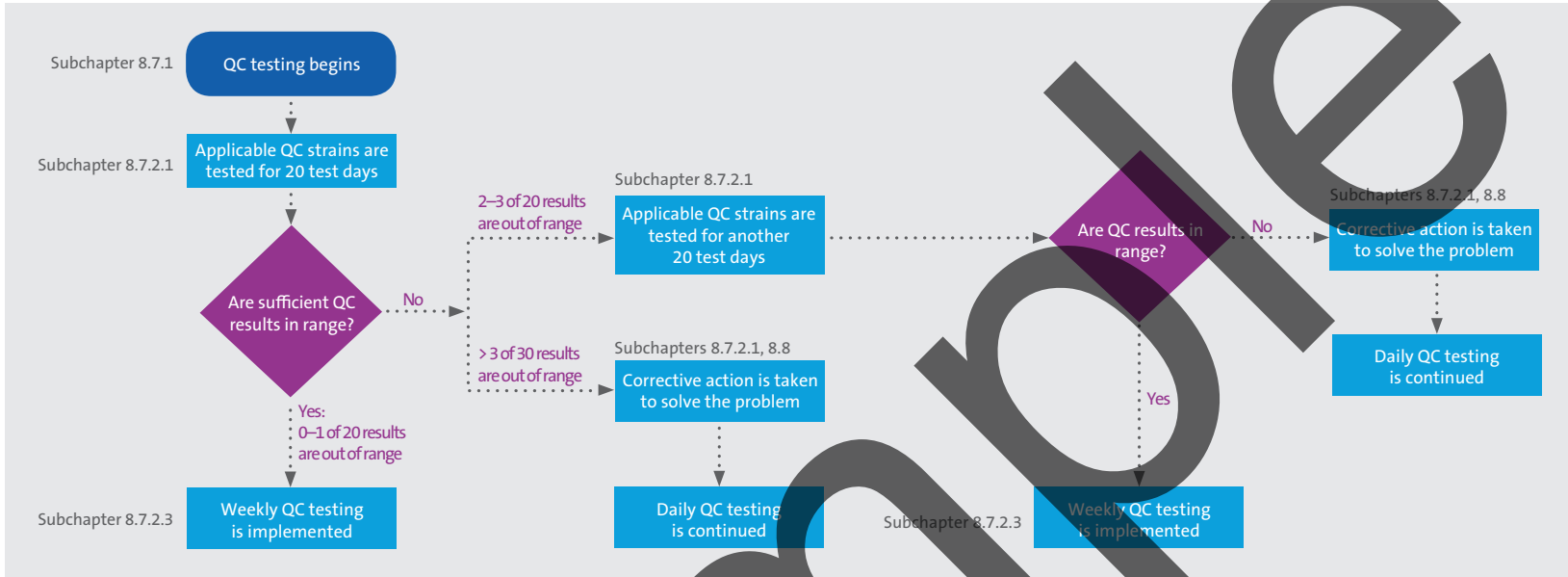
Abbreviations: AST, antimicrobial susceptibility testing; MIC, minimal inhibitory concentration; QC, quality control.

<sup>a</sup> 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<sup>a</sup>**

G1 Quality Control Protocol: Conversion From Daily to Weekly Testing (20- or 30-Day Plan)<sup>a</sup>

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



Abbreviation: QC, quality control.

<sup>a</sup> 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).

# Sample



CLINICAL AND  
LABORATORY  
STANDARDS  
INSTITUTE.

PRINT ISBN 978-1-68440-202-1

ELECTRONIC ISBN 978-1-68440-203-8

CLSI VET01-Ed6