CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

4th Edition

VET02

Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine

This guideline discusses the necessary and recommended data for selecting appropriate quality control ranges, breakpoints, and interpretive categories for antimicrobial agents for veterinary use.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine

Marilyn N. Martinez, PhD
Mark G. Papich, DVM, MS
Robert P. Hunter, MS, PhD
Xian-Zhi Li, PhD
Markus Rose, DVM
Peter Silley, PhD
John D. Turnidge, MD, BS, FRACP, FASM, FRCPA
Jeffrey L. Watts, PhD, RM(NRCM), M(ASCP)

Elke Abbeloos, MSc
Donald J. Bade, BS
Joshua Hayes, PhD
John Dustin Loy, DVM, PhD, DACVM
Carolin Ludwig, DVM
Hilde Moyaert, DVM, PhD
Stefan Schwarz, DVM
Michael T. Sweeney, MS

Abstract

Clinical and Laboratory Standards Institute guideline VET02—*Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine* provides recommendations for developing QC ranges, agar disk diffusion zones of inhibition breakpoints, and dilution minimal inhibitory concentration breakpoints for antimicrobial susceptibility tests for aerobic bacteria isolated from animals and performed by CLSI antimicrobial susceptibility testing standards. It describes the data used by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing to establish these QC ranges, breakpoints, and interpretive categories for antimicrobial agents intended for veterinary use. Host-specific pharmacokinetics, *in vitro* drug characteristics, distributions of microorganisms, and correlation of test results with outcome statistics are described for interpretation of test results. As antimicrobial agents are used in practice, additional experience accrued may be used to reassess QC ranges, breakpoints, or interpretive categories. Users of this guideline should understand that susceptibility test results cannot predict clinical outcomes with absolute certainty. Susceptibility test results should be used along with experienced clinical judgment and laboratory support to reach conclusions resulting in the best possible outcome for the patient.

Clinical and Laboratory Standards Institute (CLSI). *Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine.* 4th ed. CLSI guideline VET02 (ISBN 978-1-68440-102-4 [Print]; ISBN 978-1-68440-103-1 [Electronic]). Clinical and Laboratory Standards Institute, USA, 2021.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Suggested Citation

Contents

Contents (Continued)

Foreword

CLSI develops standardized reference methods that measure the *in vitro* susceptibility of bacteria isolated from animals to antimicrobial agents. Developed by a team of scientists with expertise in microbiology, clinical pharmacology, and veterinary medicine, this guideline provides information on the development of QC ranges, breakpoints, and interpretive categories for new or approved veterinary antimicrobial agents. In this regard, the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) is responsible for developing and updating the following antimicrobial susceptibility testing (AST) documents:

- VET01—P*erformance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*[1]

- VET01S—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals*[2]

- VET03—*Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals*[3]

- VET04—*Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals*[4]

- VET05—*Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin*[5]

- VET06—*Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals*[6]

- VET09—*Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings*[7]

VET02 is a foundation document that supports these AST standards, guidelines, reports, and supplements. The purpose of VET02 is to provide guidance on the data submitted by sponsors of antimicrobial agents for veterinary use and on the procedures followed by the Subcommittee on VAST to establish or revise AST interpretive categories and QC ranges for inclusion in CLSI documents. Once approved by the Subcommittee on VAST, the approved QC ranges and breakpoints are incorporated into the supplemental tables (CLSI document VET01S[2]) presented with the standard (CLSI document VET01[1]), with details important for veterinarians using the data included in CLSI document VET09[7] or in the appropriate document in the CLSI veterinary library.

This guideline is not intended to serve as a mandatory, step-by-step, detailed protocol to be applied to all antimicrobial agents. This guideline recognizes that submissions may be made by a wide variety of organizations or individuals and that it is important to ensure that the same processes are followed regardless of the data source (eg, manufacturer, veterinary health care professional, government agency). Nevertheless, it recognizes that the extent of the data that can be provided to support new or revised QC ranges, breakpoints, and interpretive categories may be highly variable because of factors that include but are not limited to the antimicrobial agent's age and whether the sponsor has access to raw data or only published data.

Sponsors are encouraged to consult the Subcommittee on VAST chairholder at any time to ensure the completeness of their proposed presentation. As problems become apparent or unique situations arise during the data collection process or during subcommittee discussions, the minimal criteria outlined in this guideline may need to be expanded. Additionally, it is expected that sponsors are aware of the value of presenting as much information as possible to achieve approval of QC ranges or to support the determination of breakpoints and interpretive categories. Sponsors are encouraged to begin data collection as early as possible during their product development.

Chapter ❶

Introduction

This chapter includes:

- Guideline's scope and applicable exclusions

- Background information pertinent to the guideline's content

- Terminology information, including:

  - Terms and definitions used in the guideline

  - Abbreviations and acronyms used in the guideline

Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine

① Introduction

1.1 Scope

This guideline provides direction for determining QC ranges, breakpoints, and interpretive categories for veterinary antimicrobial agents that have a direct action on microorganisms. This guideline applies to therapeutic antimicrobial agents intended to treat or control systemic or organ-specific infectious diseases processes in nonhuman, animal species (terrestrial or aquatic). The intended audience includes sponsors (eg, antimicrobial agent manufacturers) planning to submit data to establish or revise antimicrobial susceptibility testing (AST) QC ranges, breakpoints, and interpretive categories for inclusion in CLSI AST documents. Guidance presented in VET02 applies only to CLSI procedures and documents. The methods described in this guideline do not apply to:

- Antimicrobial agents used for growth promotion or prophylaxis (disease prevention)
  - See CLSI document VET01[1] for details.
- Antimicrobial agents formulated for direct administration to skin or mucous membranes (eg, topical antimicrobial agents such as lotions, creams, ointments, or eye drops) or for inhalation

1.2 Background

AST QC ranges, breakpoints, and interpretive categories are established by the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) after comprehensive review of all available relevant data. This guideline describes the procedures to be followed by the Subcommittee on VAST and by sponsors intending to submit data to facilitate timely review and decision-making processes. Data requirements that support amending existing or setting new QC ranges, breakpoints, interpretive categories are described.

The Generic Drug Working Group (GWG) of the Subcommittee on VAST, which develops breakpoints for generic or unsponsored drugs, is responsible for establishing veterinary-specific breakpoints for antimicrobial agents with only human breakpoints established or for veterinary agents with no previously established breakpoints (eg, older drugs). The GWG provides the information needed by the Subcommittee on VAST to approve breakpoints and move these drugs into CLSI document VET01S[2] Table 1, Group A or B. When these drugs are evaluated, the GWG ensures that presentations to the Subcommittee on VAST follow recommendations made in this guideline, providing a consistent approach for breakpoint determinations when a veterinary drug sponsor (ie, manufacturer) is not able or willing to provide data on an antimicrobial agent. The GWG is also responsible for establishing QC ranges for these antimicrobial agents and obtaining approval from the Subcommittee on VAST if QC ranges are not available in CLSI document M100.[8]

❸ Quality Control

A critical component of any antimicrobial susceptibility test method and corresponding dataset is method validation. In turn, method validation is contingent on the selection of appropriate QC strains. Each QC strain should be obtained from a recognized source (eg, American Type Culture Collection [ATCC®]) or an institution that has demonstrated it can reliably store and correctly use the organisms. To date, a full set of QC strains that have adequate or optimal zone sizes or end points for all commonly used antimicrobial agents is not available. However, several strains have been tested repeatedly over the years and have proven genetically stable. When it is necessary to consider the use of new QC strains, their acceptability must first be evaluated in a Tier 1 study to establish initial feasibility. Considerations for a Tier 1 study to evaluate a new QC strain include:

- Reproducibility of test results

- Performance for the objective(s) of using the QC strain

- Stability of the strain and relevant resistance mechanisms through:

  - Multiple subculture passages

  - Multiple freeze-thaw cycles

- Effect of culture age on test results

- Any known problems with lyophilization of similar strains

Subsequently, candidate strains are qualified in a Tier 2 study to obtain formal CLSI approval for use. To qualify a new QC strain and establish the expected range for the antimicrobial agent, Tier 2 study guidance should be followed as described in the following subchapters. If QC expected ranges already exist for the antimicrobial agent, sponsors should include at least one current QC strain in the information submitted. The intention of the Subcommittee on VAST is to ensure that the development of QC ranges described in this guideline is consistent with the process published in CLSI document M23.[9] However, owing to revision cycles, CLSI document M23[9] may present slightly different methods for the development of QC limits than those found in this guideline. At present, methods detailed in this guideline or in CLSI document M23[9] may be used by sponsors to complete a work in progress that was initiated under any of the above guidelines. However, the Subcommittee on VAST encourages sponsors to follow the most current guidelines.

3.1 Preliminary Quality Control Testing (Tier 1 Quality Control Study)

3.1.1 Selecting Quality Control Strains and Reference Methods for Preliminary Quality Control Studies

During the drug development process, CLSI-recommended QC strains should be tested to establish preliminary QC limits and to determine the effect of procedural variations on test performance. Testing should be performed using all appropriate CLSI reference methods to establish the equivalence of methods (eg, agar dilution and broth microdilution). This testing may be conducted at one laboratory. If this preliminary testing is not performed, future QC development testing should include all testing methods for which a QC limit is desired. If currently used QC strains are inadequate, the sponsor should suggest alternative strains. These alternative strains should be standard strains taken from or deposited to a recognized source (eg, ATCC®).

The sponsor should provide data on the solubility and stability of appropriate drug concentrations at incubation and storage temperatures specified for CLSI dilution methods (see CLSI document VET01[1]). Data that describe the interactions between the test agent and medium pH, cation levels, and CO_2 incubation atmospheres may be needed for certain antimicrobial agent classes. Data on the preparation of stock solutions, including diluents and solvent information, must be presented for inclusion in CLSI document VET01S[2] Table 6.

broth (according to the applicable standards) should be used, from different manufacturers if possible. Each of the seven laboratories should test 10 replicates of each QC strain on each lot of medium. Ideally, at least 95% of the values should be included in the proposed range and will include mode ± 1 log. Whenever possible, the low end of the QC range should include dilutions that can be accurately prepared (ie, dilutions lower than 0.03 µg/mL should be avoided, if possible). A three-dilution range is preferred. However, a four-dilution range may sometimes be needed (see examples in Table 2). Ideally, the QC range should be no more than five dilutions below the dilution that ultimately serves as the drug's susceptibility breakpoint.

Table 2. MIC Distributions for Establishing QC Expected Ranges

MIC, µg/mL	Example 1	Example 2	Example 3
0.03	0	0	0
0.06	5	15	0
0.12	100	73	30
0.25	104	110	170
0.50	1	12	10
1.00	0	0	0
Proposed QC range	0.06—0.50	0.06—0.50	0.12—0.50

Abbreviations: MIC, minimal inhibitory concentration; QC, quality control.

In Table 2:

- Example 1 illustrates results in which the MIC is distributed evenly across two dilutions.

- Example 2 shows a mode of 0.25 µg/mL. However, there are a large number of results at 0.12 µg/mL, which represents 66% of the frequency of the mode. In addition, use of a three-dilution range would not include at least 95% of the results.

- Example 3 has a clear mode of 0.25 µg/mL, and at least 95% (in this example, 100%) of the results are within one dilution of the mode.

When broth or agar dilution is used, each of the seven laboratories should test 10 replicates of each QC strain on each medium lot for a minimum of three days with a maximum of four replicates per day. Each replicate should use individually prepared inoculum suspensions. This test procedure results in 70 data points for each individual medium lot and 210 total data points. The same principles should be used when other media are needed (eg, fastidious or anaerobic organisms). With agar dilution, all 10 replicates of each strain can be inoculated onto the same set of agar dilution plates.

A control (ie, comparator) drug from a class similar to that of the study drug should also be tested (one lot of medium is sufficient). If a similar class or compound is not available, a drug with a similar spectrum of activity should be used as a control. For each study drug and control drug, a twofold-dilution schedule should be used to provide on-scale end points for all determinations. The results for the control drug must be within the expected control limits on each day of testing. If this is not the case, an investigation into the cause of the problem should be conducted, and the day's testing should be repeated.

If the control drug included in a QC development study is from another sponsor and the data affect the QC ranges for the control drug, the Subcommittee on VAST chairholder should be notified in writing before presentation of the QC data. The comparator drug sponsor must agree to the recommended changes to the QC range being recommended for the control drug.

Related CLSI Reference Materials[a]

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.

M52 **Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems. 1st ed., 2015.** This guideline includes recommendations for verification of commercial US Food and Drug Administration–cleared microbial identification and antimicrobial susceptibility testing systems by clinical laboratory professionals to fulfill regulatory or quality assurance requirements for the use of these systems for diagnostic testing.

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.

VET01S **Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. 5th ed., 2020.** This document includes updated tables for the Clinical and Laboratory Standards Institute veterinary antimicrobial susceptibility testing standard VET01.

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.

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.

---

[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)

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.

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®