This document addresses the required and recommended data needed for selection of appropriate interpretive standards and quality control guidance for new veterinary antimicrobial agents.

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

CLSI document VET02-A3—Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition offers guidance for developing agar disk diffusion zones of inhibition, dilution MIC breakpoints, and quality control limits for antimicrobial susceptibility testing of aerobic bacteria isolated from animals. It is intended to be used in establishing interpretive and quality control criteria for CLSI antimicrobial susceptibility testing standards 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 addressed from the perspective of interpretation of test results. In addition, this document addresses clinical confirmation of interpretive criteria and quality control limits. For clinical confirmation, the “ideal” data set may not be obtained during development of a new drug. Users of this guideline should understand the limitations and work toward the best-educated conclusions.


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Foreword

CLSI document VET02-A3 is intended to offer guidance for sponsors (corporate or individual) that want to list interpretive criteria and quality control information in CLSI document M31 (Table 1, Group A) for a new and/or approved veterinary antimicrobial agent. CLSI welcomes presentations for antimicrobial agents originating from any country, not just the United States. Data developed according to VET02, using relevant testing methods in other CLSI documents, are used by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing (VAST) as the basis for establishing interpretive and quality control criteria for inclusion in the CLSI standard M31. As the word “guideline” implies, this is not a mandatory step-by-step detailed protocol to apply to all new agents. Rather, it is intended as a statement of philosophy for the types of data useful for and/or required for making better judgments on interpretive criteria. The extent to which the guideline is followed remains the combined responsibility of the sponsor submitting a new agent and the Subcommittee on VAST. The sponsor is encouraged to consult the chairholder at any time to ensure the completeness of the presentation. The intent is to ensure that a “level playing field” is maintained, independent of manufacturer, veterinary health care professional, or government agency, in data presented to the subcommittee and in subcommittee determinations based on those data. Since the in vitro testing of some antimicrobial agents may present unique unanticipated situations, the minimal criteria outlined in this document might need to be expanded as problems become apparent during the data collection process.

This edition of VET02, originally adapted from CLSI/NCCLS document M23, has been modified to address more veterinary-specific issues, including a new paradigm to establish primary interpretive criteria. However, it retains basic guidelines on topics such as testing methodology, and quality control criteria that are consistent with those used for human-use antimicrobial agents in the CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST). It is important to note that VET02-A3 is not an alternative guideline to CLSI/NCCLS document M23 for those sponsors that seek to establish interpretive criteria for human use antimicrobial agents. Users of the document are referred to the Statement of Policy of the Antimicrobial Susceptibility Testing (AST) Standing Subcommittee of CLSI 20 February 2007, which does not apply the VAST. With the concurrent update of CLSI document M31, in vitro tests for measuring the susceptibility of bacterial pathogens to veterinary antimicrobial agents are now available. Also, in CLSI documents M42 and M49, testing methods for pathogens of aquatic species are now in place, and it is anticipated that VET02-A3 will be used to generate interpretive criteria for those pathogens.

VET02-A3 includes new sections based on lessons learned from implementation of M37-A2. Specifically, VET02-A3 contains a new appendix that provides more rationale for the process of establishing breakpoints and interpretive criteria. As noted in CLSI document M31, the subcommittee will now review data packages for treatments such as skin and soft tissue infections or enteric disease applications of antimicrobial agents per the VET02-A3 guidelines. In recognition of the many generic antimicrobial agents used in veterinary medicine (that have been listed in CLSI document M31 and whose interpretive criteria, based on human clinical data, imported into CLSI document M31 from CLSI document M100’s Table 1, Group B), a new process to establish veterinary-specific interpretive criteria for them has been implemented. This document outlines the information needed to facilitate the decision-making process. Unlike the previous version, there are no mandatory requirements because it is expected that drug sponsors are now aware of the value of presenting as much information as possible to the subcommittee to achieve approval of quality control ranges or interpretive criteria for their products. To facilitate data presentation to CLSI VAST, sponsors are encouraged to begin data collection as early as possible in the clinical development phase.

In closing, I would like to recognize the outstanding efforts of the Subcommittee on VAST that made this revision possible. I would like to particularly acknowledge the individual members of the Editorial Working Group. Their willingness to sacrifice significant amounts of their personal time for the editing process and to address controversial topics demonstrates a real commitment to the CLSI process and the
advancement of the veterinary and microbiology professions. In particular, I thank Marilyn Martinez for her leadership on drafting the appendix that outlines the process of establishing breakpoints, and Jo Abraham, Melanie Berson, Bob Walker, Jeff Watts, and Steve Yan for their contributions. I would like to express my sincere appreciation to the CLSI staff for their ongoing support with the countless revisions, meetings, phone calls, and e-mails necessary to produce this document.

Finally, I would like to thank CLSI and the many participants in the CLSI consensus process for allowing me the privilege of serving as the VAST Chairholder.

Thomas R. Shryock, PhD, Past Chairholder
Subcommittee on Veterinary Antimicrobial Susceptibility Testing

Key Words
Animal, antimicrobial agents, breakpoints, interpretive criteria, pharmacokinetics/pharmacodynamics, PK-PD, standard dilution methods for bacteria that grow aerobically, standard disk diffusion test, susceptibility testing, veterinary

Mission Statement
To develop and promote performance standards and interpretive criteria for in vitro antimicrobial susceptibility testing of bacteria isolated from animals
Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition

1 Scope

This document offers guidance for the development of quality control (QC) limits and interpretive criteria for antimicrobial susceptibility testing (AST), performed by disk diffusion and dilution testing with bacteria isolated from animals, for subcommittee review and, upon approval, inclusion in CLSI document M31.

The guidance in this document applies to therapeutic antimicrobial agents intended for the treatment or control of systemic or organ-specific infectious disease processes in domestic animals (terrestrial or aquatic). Antimicrobial agents used for growth promotion or prophylaxis (disease prevention) are not included in this document. (See the discussion in CLSI document M31 for more details regarding this issue.) However, the testing methodology described for the development of QC standards may be applicable for those antimicrobial agents that are tested for epidemiological survey or other purposes for which a validated test is required. The subcommittee recognizes that antimicrobial agents are used to treat a variety of enteric infections in animals; thus, a concerted attempt to include them within CLSI document M31 should be made to guide practitioners in the proper selection of agents. NOTE: The guidelines do not apply to directly applied topical antimicrobials such as lotions, cream, ointments, or eye drops.

Since not all antimicrobial agents have veterinary-specific breakpoints or interpretive criteria, the subcommittee has imported breakpoints and zone diameters from CLSI document M100 (ie, human treatments) into Table 1, Group B of CLSI document M31, and designated them by gray shaded listing. Since these breakpoints and interpretive criteria have been developed for human treatment applications, there is uncertainty as to how they apply to specific animal species and disease treatments. To facilitate moving CLSI document M100 interpretive criteria to veterinary-specific approved status, the Working Group on Generics will provide a gatekeeper function to ensure that presentations to the full subcommittee conform as much as possible to VET02 requirements. This will allow for a consistent approach to address those situations where veterinary-specific data are not readily available within the public domain or where sponsors (ie, manufacturers) are not able or willing to provide data on their products.

Additionally, should there be a need to reevaluate previously established breakpoints or interpretive criteria, a process is outlined in Section 3.6.

2 Definitions

Susceptibility Testing

agar dilution susceptibility test – an in vitro antimicrobial susceptibility test method conducted using serial concentrations of an antimicrobial agent incorporated into an agar growth medium in separate petri dishes that are inoculated with a bacterial suspension to determine the minimal inhibitory concentration (MIC).

agar disk diffusion susceptibility test – an in vitro antimicrobial susceptibility test conducted using disks impregnated with a specified single concentration of an antimicrobial agent applied to the surface of an agar medium that has been inoculated with the test organism. The diameter of the zone of growth inhibition that results from the diffusion of an antimicrobial agent from the disks is measured with calipers or ruler and recorded in millimeters.
**broth dilution susceptibility test** – an *in vitro* antimicrobial susceptibility test method conducted using serial concentrations of an antimicrobial agent incorporated in liquid nutrient media that are inoculated with a bacterial suspension to determine the MIC of the antimicrobial agent. When this procedure is carried out in test tubes, it is referred to as broth macrodilution; when performed in microdilution plates, it is called broth microdilution.

**minimal inhibitory concentration (MIC)** – the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

**Breakpoints/Interpretive Criteria**

**interpretive criteria/breakpoint** – MIC or zone diameter values used to indicate susceptible, intermediate, and resistant as defined below.

For example, for antimicrobial X with interpretive criteria of:

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>Zone Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Intermediate</td>
<td>8-16</td>
</tr>
<tr>
<td>Resistant</td>
<td>≥ 32</td>
</tr>
</tbody>
</table>

“Susceptible breakpoint” is 4 µg/mL or less; or 20 mm or more.
“Resistant breakpoint” is 32 µg/mL or greater; or 14 mm or less.

**antimicrobial susceptibility test interpretive category** – 1) classification of projected clinical outcome of patient treatment based on the causative microorganism’s *in vitro* response to an antimicrobial agent relative to the exposure to that agent, which is attainable using the labeled dose regimen for the target animal species for that type of infection and infecting organism; 2) **susceptible antimicrobial susceptibility test interpretive category** – a category that implies that an infection due to the isolate may be successfully treated with the normal dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated; 3) **intermediate antimicrobial susceptibility test interpretive category** – a category that implies that an infection due to the isolate may be successfully treated in body sites where the drugs are physiologically concentrated or when a higher approved dosage of drug can be used; also indicates a “buffer zone” that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations; 4) **resistant antimicrobial susceptibility test interpretive category** – resistant isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage regimens and/or fall in the range where specific microbial resistance mechanisms are likely (eg, β-lactamases), and clinical efficacy has not been reliable in treatment studies.

**CO<sub>CL</sub>** – the value selected by inspecting clinical/microbiological outcome vs MIC from prospective clinical studies will be called the “clinical” cutoff” and will be abbreviated CO<sub>CL</sub>. This estimate is to be used in the establishment of interpretive criteria by the subcommittee, as described in Appendix C. It is not intended to be reported to clinical laboratories and will not be published within CLSI documents.

**CO<sub>PD</sub>** – the “breakpoint” that can be calculated using PK/PD parameters and Monte Carlo simulation will be called the “pharmacodynamic cutoff” and will be abbreviated CO<sub>PD</sub>. It is established solely on the basis of the relationship between physiologic drug concentrations (eg, blood, or possibly urine or milk) and a microbial susceptibility parameter, generally MIC values. This estimate is to be used in the establishment of interpretive criteria by the subcommittee, as described in Appendix C. It is not intended to be reported to clinical laboratories and will not be published within CLSI documents.
The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. The quality management system approach applies a core set of “quality system essentials” (QSEs), basic to any organization, to all operations in any health care service’s path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager’s guide. The QSEs are:

- **Documents & Records**
- **Organization**
- **Personnel**
- **Equipment**
- **Purchasing & Inventory**
- **Information Management**
- **Process Improvement**
- **Occurrence Management**
- **Assessments—External and Internal**
- **Process Control**
- **Information Management**
- **Facilities & Safety**

VET02-A3 addresses the QSEs indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory’s services, namely quality laboratory information.

VET02-A3 addresses the clinical laboratory path of workflow steps indicated by an “X.” For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

<table>
<thead>
<tr>
<th>Preexamination</th>
<th>Examination</th>
<th>Postexamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination ordering</td>
<td>Sample collection</td>
<td>Sample transport</td>
</tr>
<tr>
<td>M2</td>
<td>M7</td>
<td>M11</td>
</tr>
</tbody>
</table>

Adapted from CLSI/NCCLS document HS1—*A Quality Management System Model for Health Care*. ©Clinical and Laboratory Standards Institute. All rights reserved.
Related CLSI Reference Materials


M11-A7  Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition (2007). This standard provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by agar dilution and broth microdilution.

M23-A2  Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Second Edition (2001). This document addresses the required and recommended data needed for the selection of appropriate interpretative standards and quality control guidelines for new antimicrobial agents.

M29-A3  Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Third Edition (2005). Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

M31-A3  Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition (2008). This document provides the currently recommended techniques for antimicrobial agent disk and dilution susceptibility testing, criteria for quality control testing, and interpretive criteria for veterinary use.

M42-A  Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (2006). This document provides the most up-to-date techniques for disk diffusion susceptibility testing of aquatic species isolates, and criteria for quality control testing.

M49-A  Methods for Broth Dilution Susceptibility testing of Bacteria Isolated From Aquatic Animals; Approved Guideline (2006). This document provides the most up-to-date techniques for the determination of minimal inhibitory concentrations (MICs) of aquatic bacteria by broth micro- and macrodilution, and criteria for quality control testing.

M100-S18  Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement (2008). This document provides updated tables for the antimicrobial susceptibility testing standards M2-A9 and M7-A7.

* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.
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