Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Third Edition

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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Advancing Quality in Health Care Testing

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Abstract

CLSI document M37-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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Contents

Abstract .................................................................................................................................................... i

Committee Membership .................................................................................................................................................... iii

Foreword .............................................................................................................................................. vii

1 Scope .......................................................................................................................................... 1

2 Definitions ........................................................................................................................................... 1

3 General Considerations and Time Sequence ............................................................................. 4

3.1 Subcommittee Requirements ........................................................................................ 4

3.2 Time Sequence for Presentation ................................................................................... 4

3.3 Presentation Format ...................................................................................................... 5

3.4 Acceptability of Data .................................................................................................... 5

3.5 Use of Data Derived From Previously Accepted Reference Methods .......................... 5

3.6 Reassessment of Breakpoints/Interpretive Criteria and QC Parameters ....................... 5

3.7 Development of Interpretive Criteria for Generic or Older Compounds ...................... 6

4 Data Generation and Establishment of QC Limits ..................................................................... 7

4.1 Preliminary QC Testing (Tier 1 Preliminary QC Study) .............................................. 7

4.2 Requirements for Establishing Acceptable QC Ranges (Tier 2 QC Study) .................. 9

4.3 Confirmation and Reassessment of QC Ranges (Tier 3 QC Monitoring) .................. 11

5 Data to Provide to Establish Breakpoints and Interpretive Criteria ......................................... 12

5.1 Clinical Effectiveness Studies..................................................................................... 12

5.2 Isolate Collections and Susceptibility Test Data Presentation .................................... 13

5.3 Cross-Resistance Studies ............................................................................................ 14

5.4 Comparison of Dilution Test Methods for Aerobic Bacteria ...................................... 15

5.5 Use of Commercially Prepared Microdilution Panels ................................................ 15

5.6 Pharmacology ............................................................................................................. 15

6 Process of Establishing Breakpoints and Interpretive Criteria ................................................ 17

6.1 Decision Tree for Setting Breakpoints ........................................................................ 17

6.2 Evaluation of Dilution MIC and Disk Diffusion Data ................................................ 20

6.3 Final Determination of Breakpoints and Interpretive Criteria .................................... 21

References ............................................................................................................................................. 22

Appendix A. Information to Appear on Presentation Coverage Page .................................................. 23

Appendix B. Drug “X” MIC vs Zone Diameter (495 challenge organisms) ............................... 24

Appendix C. The Use of PK and PD Relationships in the Setting of Susceptibility Breakpoints for Veterinary Antimicrobial Agents .......................................................................................................... 25

The Quality Management System Approach ........................................................................................ 42

Related CLSI Reference Materials .............................................................................................. ......... 43
Foreword

CLSI document M37-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\(^1\) (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 M37, 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.\(^1\) 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 \textit{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 M37, originally adapted from CLSI/NCCLS document M23,\(^2\) 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 M37-A3 is not an alternative guideline to CLSI/NCCLS document M23\(^2\) 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,\(^1\) \textit{in vitro} tests for measuring the susceptibility of bacterial pathogens to veterinary antimicrobial agents are now available. Also, in CLSI documents M42\(^3\) and M49,\(^4\) testing methods for pathogens of aquatic species are now in place, and it is anticipated that M37-A3 will be used to generate interpretive criteria for those pathogens.

M37-A3 includes new sections based on lessons learned from implementation of M37-A2. Specifically, M37-A3 contains a new appendix that provides more rationale for the process of establishing breakpoints and interpretive criteria. As noted in CLSI document M31,\(^1\) 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 M37-A3 guidelines. In recognition of the many generic antimicrobial agents used in veterinary medicine (that have been listed in CLSI document M31\(^1\) and whose interpretive criteria, based on human clinical data, imported into CLSI document M31\(^1\) from CLSI document M100’s\(^5\) 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
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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 (i.e., 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 M37 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 (i.e., 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.