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VET02-A3

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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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

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Jeffrey L. Watts, PhD, RM(AAM)
Michael Apley, DVM, PhD
Donald J. Bade
Steven D. Brown, PhD
Jeffrey T. Gray, PhD
Henry Heine, PhD
Rob P. Hunter, MS, PhD
Marilyn N. Martinez, PhD

Dik J. Mevius, DVM, PhD
Mark G. Papich, DVM, MS
Thomas R. Shryock, PhD
Peter Silley, PhD
Robert D. Walker, PhD
Ching Ching Wu, DVM, PhD
Gary E. Zurenko, MS

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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Committee Membership

Area Committee on Microbiology

Mary Jane Ferraro, PhD, MPH
Chairholder
Massachusetts General Hospital
Boston, Massachusetts

Fred C. Tenover, PhD, ABMM
 Centers for Disease Control and
 Prevention
 Atlanta, Georgia

Michael A. Pfaller, MD
 University of Iowa College of
 Medicine
 Iowa City, Iowa

James H. Jorgensen, PhD
Vice-Chairholder
University of Texas Health
Science Center
San Antonio, Texas

John D. Turnidge, MD
 Women's and Children's Hospital
 North Adelaide, Australia

Robert P. Rennie, PhD
 University of Alberta Hospitals
 Edmonton, Alberta, Canada

Donald R. Callihan, PhD
 BD Diagnostic Systems
 Sparks, Maryland

Michael L. Wilson, MD
 Denver Health Medical Center
 Denver, Colorado

Jana M. Swenson, MMSc
 Centers for Disease Control and
 Prevention
 Atlanta, Georgia

Freddie Mae Poole
 FDA Center for Devices and
 Radiological Health
 Rockville, Maryland

Advisors

Ellen Jo Baron, PhD
 Stanford University Hospital &
 Medical School
 Stanford, California

Melvin P. Weinstein, MD
 Robert Wood Johnson Medical
 School
 New Brunswick, New Jersey

John H. Rex, MD, FACP
 AstraZeneca
 Cheshire, United Kingdom

Lynne S. Garcia, MS
 LSG and Associates
 Santa Monica, California

Matthew A. Wikler, MD, MBA,
 FIDSA
 Pacific Beach BioSciences
 San Diego, California

Daniel F. Sahm, PhD
 Eurofins Medinet
 Herndon, Virginia

Richard L. Hodinka, PhD
 Children's Hospital of Philadelphia
 Philadelphia, Pennsylvania

Gail L. Woods, MD
 University of Arkansas for
 Medical Sciences
 Little Rock, Arkansas

Subcommittee on Veterinary Antimicrobial Susceptibility Testing

Jeffrey L. Watts, PhD, RM(AAM)
Chairholder
Pfizer Animal Health
Richmond, Michigan

Dik J. Mevius, DVM, PhD
 Central Institute for Animal Disease
 Control-CIDC-Lelystad
 Lelystad, Netherlands

Diane M. Citron, M(ASCP)
 R.M. Alden Research Laboratory
 Santa Monica, California

Mike Apley, DVM, PhD
 Kansas State University
 Manhattan, Kansas

Mark G. Papich, DVM, MS
 North Carolina State University
 Raleigh, North Carolina

Ronald N. Jones, MD
 JMI Laboratories
 North Liberty, Iowa

Donald J. Bade
 Microbial Research, Inc.
 Fort Collins, Colorado

Peter Silley, PhD
 MB Consult Limited
 Lymington, Hampshire,
 United Kingdom

Cindy Lindeman
 Pfizer Animal Health
 Kalamazoo, Michigan

Steven D. Brown, PhD
 The Clinical Microbiology Institute
 Wilsonville, Oregon

Gary E. Zurenko, MS
 Micromyx, LLC
 Kalamazoo, Michigan

Jennifer Lorbach
 Trek Diagnostic Systems, Inc.
 Cleveland, Ohio

Jeffrey T. Gray, PhD
 Des Moines University
 Des Moines, Iowa

Advisors

Jo Abraham, DVM, MS
 Bayer Health Care LLC
 Shawnee Mission, Kansas

Carol W. Maddox, PhD
 University of Illinois
 Urbana, Illinois

Henry Heine, PhD
 USAMRIID
 Ft. Detrick, Maryland

Melanie R. Berson, DVM
 FDA Center for Veterinary
 Medicine
 Rockville, Maryland

Marilyn N. Martinez, PhD
 FDA Center for Veterinary
 Medicine
 Rockville, Maryland

Rob P. Hunter, MS, PhD
 Elanco Animal Health
 Greenfield, Indiana

Patrick McDermott, PhD
 FDA Center for Veterinary
 Medicine
 Laurel, Maryland

Advisors (Continued)

Stefan Schwarz, PhD
Institut Für Tierzucht (FAL)
Neustadt-Mariensee, Germany

Thomas R. Shryock, PhD
Elanco Animal Health
Greenfield, Indiana

Clyde Thornsberry, PhD
Eurofins Medinet
Franklin, Tennessee

John D. Turnidge, MD
Women's and Children's Hospital
North Adelaide, Australia

Robert D. Walker, PhD
Glade Park, Colorado

Ching Ching Wu, DVM, PhD
Purdue University School of
Veterinary Medicine
West Lafayette, Indiana

S. Steve Yan, PhD
FDA Center for Veterinary
Medicine
Rockville, Maryland

Staff

Clinical and Laboratory Standards
Institute
Wayne, Pennsylvania

Lois M. Schmidt, DA
*Vice President, Standards
Development and Marketing*

Tracy A. Dooley, BS, MLT(ASCP)
Staff Liaison

Melissa A. Lewis
Editor

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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 M31¹ (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 M31.¹ 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 *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 M23,² 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 VET02-A3 is not an alternative guideline to CLSI/NCCLS document M23² 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 *in vitro* tests for measuring the susceptibility of bacterial pathogens to veterinary antimicrobial agents are now available. Also, in CLSI documents M42³ and M49,⁴ 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 M31,¹ 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 VET02-A3 guidelines. In recognition of the many generic antimicrobial agents used in veterinary medicine (that have been listed in CLSI document M31¹ and whose interpretive criteria, based on human clinical data, imported into CLSI document M31¹ from CLSI document M100's⁵ 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 M31.¹

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 M31¹ 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 M31¹ 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 M100⁵ (ie, human treatments) into Table 1, Group B of CLSI document M31,¹ 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 M100⁵ 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.