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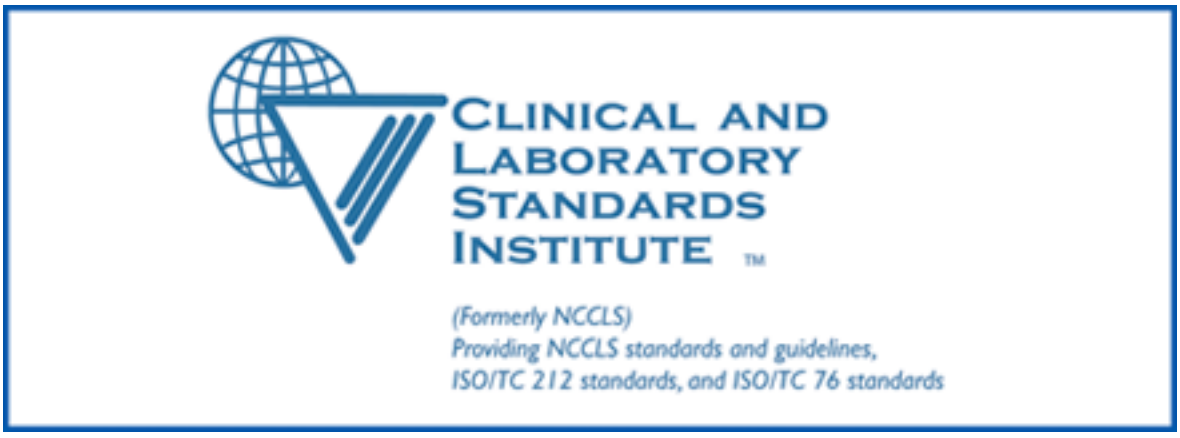
Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Second Edition



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

A guideline for global application developed through the NCCLS consensus process.

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- the revision of documents in response to comments by users
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Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Second Edition

Abstract

NCCLS document M37-A2—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline —Second Edition* offers guidance for developing agar disk diffusion zones of inhibition, dilution MIC breakpoints, and quality control limits for antimicrobial susceptibility testing of aerobic and anaerobic bacteria isolated from animals. It is intended to be used in establishing interpretive and quality control criteria for NCCLS 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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Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Second Edition

Volume 22 Number 7

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Contents

Contents (Continued)

Foreword

NCCLS document M37-A2 is intended to offer guidance for sponsors (corporate or individual) that want to list interpretive criteria and quality control information in NCCLS document M31—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals* (Table 1, Group A) for a new and/or approved, veterinary antimicrobial agent. Data developed according to M37 are used by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing as the basis for establishing interpretive and quality control criteria for inclusion in the M31 standard. As the word "guideline" implies, this is not a step-by-step detailed protocol to be applied to all new agents. It is intended to be a statement of philosophy for the types of data useful for and/or required for making better judgments on interpretive standards. The degree by which the guideline is followed remains the combined responsibility of the pharmaceutical company or other sponsor submitting a new agent and the Subcommittee on Veterinary Antimicrobial Susceptibility Testing. *All sections of the guideline preceded by an asterisk (*) describe information required for review by the subcommittee. All other sections describe recommended information that the subcommittee believes will be useful in developing interpretive guidelines for the specific drug.* The intent is to ensure that a "level playing field" is maintained, independent of manufacturer, veterinary healthcare professional, or government agency, in data presentation to the subcommittee and in subcommittee determinations based on those data. Since the *in vitro* testing of some antimicrobial agents may present unique problems, the minimal criteria outlined in this document might need to be expanded as problems become apparent during the process of data collection.

The first edition of M37 was adapted from NCCLS document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*[3] that outlines the data necessary to receive approval of interpretive and quality control criteria for antimicrobial agents from the NCCLS Subcommittee on Antimicrobial Susceptibility Testing and their subsequent listing in M100 supplemental tables. With the release and implementation of M37-A, *in vitro* tests for measuring the susceptibility of bacterial pathogens to veterinary antimicrobial agents are being carefully standardized. Additionally, interpretive criteria are being reviewed by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing for inclusion in NCCLS document M31—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals.*

Comments on M37-A have been addressed in this edition. M37-A2 has also been revised to include updated sections excerpted from M23-A2 and includes new sections based on lessons learned from implementation of M37-A. Specifically, M37-A2 now contains specific section updates on quality control testing "tiers" and extensive explanation of scattergram evaluation. As noted in M31-A2, the subcommittee will now review data packages for enteric disease applications of antimicrobial agents per the M37-A2 guidelines. In recognition of the many generic antimicrobial agents used in veterinary medicine that have been listed in M31 and whose interpretive criteria, based on human clinical data, have been imported into M31 from the M100 documents (Table 1, Group B), a new process to establish veterinary specific interpretive criteria is described. This document outlines the information that will be needed in order to facilitate the decision-making process. In the future, M37 will need to be modified to cover aquaculture pathogens, antimicrobial agents not currently marketed in the U.S., topical antimicrobial applications, and other new areas.

In closing, I would like to recognize the efforts of the Subcommittee on Veterinary Antimicrobial Susceptibility Testing. 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 NCCLS process and the advancement of the veterinary and microbiology professions. In particular, I thank Jeff Watts for his pioneering leadership that was instrumental in developing M37 and, now, in leading the revision process. I thank Bob Walker, Clyde Thornsberry, Ron Jones, and David White for their contributions.

Finally, I would like to express my sincere appreciation to the NCCLS Executive Offices' staff for their ongoing support with the countless revisions, meetings, phone calls, and e-mails that were necessary to produce this document.

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Subcommittee on Veterinary
Antimicrobial Susceptibility Testing

Key Words

Animal, antimicrobial agents, 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—Second Edition

1 General Considerations and Time Sequence

1.1 Subcommittee Requirements

In order for data to be considered by the Subcommittee on Veterinary Antimicrobial Susceptibility Testing to establish appropriate criteria for disk or minimum inhibitory concentration (MIC) breakpoints and quality control limits, they should conform to procedures presented in this document.

All sections of this document preceded by an asterisk (*) describe the minimum information recommended for review by the subcommittee (See Section 2.) All other sections describe information that may be helpful in supporting *in vitro* susceptibility test development.

The guidance in this document applies to therapeutic antibiotics (control or treatment claim) that are intended for the treatment or control of infectious disease processes in animals. Applications of antibiotics for uses such as growth promotion and prophylaxis are not included in this document. (See the discussion in NCCLS document M31—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals* regarding this issue). However, the testing methodology described for development of quality control standards may be applicable for those antibiotics which would be tested for epidemiological survey purposes.

The subcommittee believes that there are a substantial number of antibiotics used to treat a variety of enteric infections in animals; thus, a concerted attempt to include them within M31 should be made to guide practitioners in the proper selection of agents. The types of information that would be necessary to provide a "complete" pharmacokinetic package for sponsor presentation according to the guidance presented in this document includes the following:

- concentration of antibiotic at various sites in the gut;
- binding of the antibiotic to cells, fecal solids, or intracellular uptake;
- absorption and recycling;
- metabolism;
- excretion;
- pH effects on bioactivity;
- effects of feed components;
- atmosphere;
- effect of antibiotic on other gut microflora which subsequently alters the target pathogen's niche;
- pathogen localization along the gut;
- intracellular vs. extracellular pathogens; and
- the effects of dosing intervals.

This list is not exhaustive, but is intended to outline the types of information that could be used to explain the *in vivo* activity of the antibiotic. As such, it is viewed as unrealistic to expect a sponsor to provide all of this information, some of which would have to be generated in model systems, which have not been proven to be consistently correlated to the *in vivo* outcome. Sponsors seeking interpretive criteria approval are encouraged to provide as much of this information as is available when making a

presentation to the subcommittee. However, since there is no established "formula" for comparing MICs to gut pharmacokinetic data, the value of the data should be informational only.

Thus, recognizing the need to incorporate enteric infections and treatment into the M31 document, the subcommittee will evaluate a sponsor's presentation for the clinical efficacy data and the *in vitro* MIC/zone data as described in this document, with the pharmacokinetic data "package" presented as supportive evidence. The interpretive criteria and breakpoints would be included in the M31 tables and footnoted to indicate that the antibiotic is used to treat enteric infections.

Drugs that were approved prior to the implementation of this guideline and that are to be re-evaluated should also follow the procedures presented in this document.

Since not all antimicrobial agents have veterinary specific interpretive criteria (See the most current version of NCCLS document M31—*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*, Table 1, Group A), the subcommittee has imported M100 breakpoints and zone diameters and designated them by gray shaded listing in Table 2, Group B of M31. Since these interpretive criteria have been developed for human treatment applications, there is uncertainty as to how they apply to specific animal species and disease treatments. In order to facilitate moving M100—*Performance Standards for Antimicrobial Susceptibility Testing,* interpretive criteria to veterinary-specific approved status, the Working Group on Generics will serve a gatekeeper function to ensure that presentations to the full subcommittee will conform to M37 requirements as much as possible. This will allow for a consistent approach to address those situations where veterinary specific data are not readily available in the public domain or where sponsors (i.e. manufacturers) are not able or willing to provide data on their products.

1.2 Time Sequence for Presentation

To ensure a successful evaluation of a new drug or new data, each sponsor (corporate or individual) should review the procedures presented here and in the following section.

(1) The data on quality control parameters for disk diffusion and/or dilution tests may be evaluated by the subcommittee any time this information becomes available.

(2) The data on zone of inhibition diameter size, MICs, scattergrams, and pharmaco-kinetics/pharmacodynamics may be presented to the subcommittee early in the drug development process. This would permit selection of investigational breakpoints and zone interpretive criteria that would be available to clinical investigators. These recommendations can be listed in M31, Table 1, Group B, as "Investigational Use Only."

(3) Prior to, or after the submission of the New Animal Drug Application (NADA) to the FDA Center for Veterinary Medicine (CVM), a formal presentation of the data requested in this document (M37) should be made. This will enable the subcommittee to establish approved entries for inclusion in M31, in particular the listing in Table 1, Group A and Table 2 (non-gray shaded).

(4) At any time after FDA approval of a drug, the subcommittee may reassess the need to alter interpretive criteria or quality control parameters. Additional supportive data may be submitted to the subcommittee from any source whenever a change appears to be necessary. (See Section 1.6.)

1.3 Presentation

Sponsors or others, including the Generic Working Group, wishing to present data to the subcommittee should have a hard copy of their presentations (including supportive data and recommended actions)

included in the agenda book for the subcommittee meeting. Agenda priority will be given for the final formal presentation of new drugs to be included in M31.

All data packages requesting action by the subcommittee will have a table of contents and a cover page summary containing background information that is relevant to the request. (See Appendix A.) In addition to tabular data, graphic presentations of data should also be included when appropriate to facilitate review. Tentative breakpoints may be granted by the subcommittee to allow for additional data to be generated by a sponsor. Tentative breakpoints are published only in the subcommittee minutes and expire after one year.

1.4 Acceptability of Data

Data generated from within or from outside the U.S. must meet the same standards. For microbiologic data, NCCLS reference methods, including proper quality control tests, are to be used and documented. See Table 3 in NCCLS document M31. If NCCLS methods are not used, sufficient data must be made available to demonstrate the comparability of such methods to NCCLS reference methods. The design and evaluation of clinical studies should conform to the most recent guidelines from the CVM or other official regulatory authority in the country/region of origin. Data from the U.S. and from different countries or regions should be presented separately, but may be presented combined if comparability of such data can be established. Differences between data from the U.S. and outside the U.S. should be noted, e.g., distribution of microorganisms, resistance mechanisms, dosages, etc.

1.5 Use of Data Derived from Previously Accepted Reference Methods

If NCCLS reference methods change, or if new reference methods are created, data from previously accepted methods will be acceptable for consideration if the relationship between the methods is known or can be demonstrated. Studies initiated after NCCLS publication of modified or new reference methods should use the modified or new method. The subcommittee will allow a grace period for acceptability of data generated during the transition period between methods; however, it is preferred that data be generated using the same method (new or old) for the entire study.

1.6 Reassessment of Interpretive Criteria or QC Parameters

Reassessment of interpretive criteria or QC parameters may become necessary as new information becomes available. Reassessment should only be considered when there is adequate information for making a decision. The following represents situations under which a reassessment should be considered.

- When less susceptible and/or resistant strains develop to an antimicrobial agent whose breakpoints were determined when only susceptible strains were available.
- When organisms with new mechanisms of resistance are not reliably detected using current breakpoints.
- When new dosages or formulations of an antimicrobial agent and/or new clinical usage require(s) a change.
- When new clinical and/or pharmacologic data suggest a need for reassessment.
- When NCCLS approved reference methods change and such changes will have an impact on interpretive criteria and/or QC parameters.

When other *in vitro* testing data suggest the need for reassessment.

When a reassessment is made, the guidelines presented in this document are to be followed to the extent possible. The data upon which the original decision was made should also be considered in any reassessment.

If the need for reassessment is brought to NCCLS from a source other than the manufacturer of the product, then the manufacturer must be notified that a reassessment is being considered. This notice must allow reasonable time for the manufacturer to prepare a packet of relevant data for incorporation into the meeting agenda book, if the manufacturer so desires.

When a reassessment is considered that could potentially impact and/or apply to other similar products, then all products so affected should be considered at the same time. In such instances, NCCLS will formally notify all subcommittee members and advisors, and all manufacturers whose drugs could be impacted by such a reassessment. This notice must allow reasonable time for the preparation of relevant data for incorporation into the meeting agenda book.

1.7 Scope

This document offers guidance to a sponsor to develop quality control limits and interpretive criteria for antimicrobial susceptibility testing, performed by disk diffusion and dilution testing with bacteria isolated from animals, for subcommittee review and, upon approval, inclusion in the NCCLS document M31-*Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. It has been adapted from the M23 for application with antimicrobial agents used to treat bacteria isolated from animals.

1.8 Definitions[a]

Breakpoint/Interpretive criteria, *n* - MIC or zone diameter value used to indicate **susceptible, intermediate, and resistant.** (See these terms defined below.)

For example, for antimicrobial X with interpretive criteria (µg/mL) of:

	MIC (µg/mL)	Zone Diameter (mm)
Susceptible	≤ 4	≥ 20
Intermediate	8-16	15-19
Resistant	≥32	≤14

"Susceptible breakpoint" is 4 µg/mL or 20 mm
"Resistant breakpoint" is 32 µg/mL or 14 mm

Antimicrobial Susceptibility Test Interpretive Category, *n* - **1)** A classification based on an *in vitro* response of an organism to an antimicrobial agent at levels of that agent corresponding to blood or tissue levels attainable with usually prescribed doses of that agent; **2) Susceptible Antimicrobial Susceptibility Test Interpretive Category,** *n* - A category that implies that an infection due to the isolate may be appropriately treated with the dosage of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated; **3) Intermediate Antimicrobial Susceptibility Test Interpretive Category,** *n* - A category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high 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,** *n* - Resistant isolates are not inhibited by the usually

[a] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

achievable concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., beta-lactamases), and clinical efficacy has not been reliable in treatment studies.

Minimal inhibitory concentration, MIC, *n* - The lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.

2 Data for Determining Susceptibility Test Breakpoints

2.1 Target Animal Pharmacokinetics and Pharmacodynamics

Data on pharmacokinetics/pharmacodynamics (PK/PD) should be developed that are relevant to the method of drug administration and animal species used in the clinical efficacy trials (e.g. route, dose, time, etc.) so that their relationship to efficacy can be evaluated. This evaluation might include, but is not limited to the following: time that serum or plasma concentration exceeds the MIC; peak serum or plasma concentration; MIC ratio; and area-under-the-curve (AUC) serum drug concentration:MIC ratio. The actual drug concentration and active metabolite concentration (if relevant) at the infection site(s) should be demonstrated using appropriate analytical methodology. Data should be generated from healthy representatives of the target species that consider age, weight, gender, and other physical factors relevant to the intended treatment population. Where appropriate, data using diseased animals could also be developed. The number of subjects used in any study should be governed by the appropriate statistical approaches. Moreover, all such studies should be in accordance with the animal use guidelines of the sponsor's institution. The PK/PD rationale for establishing the dose or dose range is desired. Factors that should be discussed include withdrawal time, animal safety, efficacy, and resistance selection.

(1)* Traditional pharmacokinetic information (e.g., AUC, $T_{½}$,C_{max}) is of primary interest and may be studied for either single administration or multiple administration of antimicrobial agents. Depending on the antimicrobial agent, tissue and body fluid accumulation as well as cellular concentrations may be determined as appropriate. (For intramammary infusion products, milk residue depletion data, which is acquired by tracking milk levels during lactation, may be sufficient).

(2)* Data should be presented for drug stability *in vivo*, including metabolism and excretion.

(3)* Data concerning protein binding should be presented for target animal serum or other body fluids (e.g., milk) as appropriate.

(4)* In general, a validated high performance liquid chromatography (HPLC) or other acceptable method such as microbiological assay should be used to determine *in vivo* concentration levels.

(5)* Pharmacodynamic parameters (e.g., postantibiotic effect, concentration-dependent killing or time-kill kinetics) as appropriate for the drug class should be presented.

(6)* Data on the metabolism and excretion of the drug in target animals should be presented. If metabolized, the microbiological activity of the metabolites should be provided. If a drug is to be used for urinary tract infections, data showing the kinetics of the drug in urine should be provided. The effect of pH and cations on antimicrobial activity in urine should also be included.

2.2 *In-Vitro* Drug Characteristics

Data on the solubility and stability of appropriate concentrations of the drug at incubation and storage temperatures specified for NCCLS dilution methods (M31) should be provided.

Data on the preparation of stock solutions, including diluent and solvent information, must be presented for inclusion in Table 8 of NCCLS document M31.

2.3 *Correlation of Test Results with Outcome Statistics

During clinical evaluation of antimicrobial agents, *in vitro* susceptibility (dilution or disk diffusion) test results should be correlated with therapeutic outcome. For assignment of interpretive criteria for both MICs and zone sizes, therapeutic outcome results based on both methods should be presented. This does not mean that both tests must be performed on isolates from all target animals.

A "perfect" database for this evaluation will not be generated in the usual NADA-type clinical trials. However, both the sponsor and the subcommittee should attempt to evaluate the available data with the awareness that certain potential shortcomings are possible. To this end, the following points should be considered:

(1)* Data on clinical efficacy of the antimicrobial agent in the target species should include:

- A protocol describing the study, which includes animal selection, treatments, clinical evaluations, bacteriological culture and susceptibility test results, and interpretation of treatment effects (i.e., cure, improved, failure, etc.). Multiple studies may be presented.

- Where possible, information on individual isolate susceptibility test results (MIC and/or zone size) should be provided and related to the therapeutic outcome in an individual animal. Alternatively, MIC and zone information from a collection of isolates (described in Section 3) relevant to the approved use of the antimicrobial agent should be related to the therapeutic outcome in a group of diseased animals with the same etiologic agent(s).

(2)* The efficacy data should be compared to the PK/PD data (based on the same dose, route, treatment regimen, etc.). MIC data will be related to the pharmacokinetic data to establish investigational or approved breakpoints.

(3) For those situations where investigational interpretive criteria are to be established; efficacy data of sufficient quantity and quality may not be available. For generic agents, a CVM approval listing will be accepted as sufficient evidence of efficacy and appropriate dose, with the recognition that isolates from clinical trials will not be available.

2.4 Development of Interpretive Criteria for Compounds With a Flexible Label

A flexible label interpretive category is allowed for those agents meeting the criteria below. This category is reserved for drugs with specific label information for organisms with higher MIC values that cannot be effectively treated using dosages for organisms falling into the susceptible or intermediate categories. Sponsors seeking organism or dose-based interpretive criteria for compounds with an FDA-approved flexible label must provide data supporting the following categories:

(1) Demonstrated clinical efficacy against the target pathogens at the higher dose level, as well as data showing a lack of clinical efficacy at the “normal” dose and MIC.

(2) The flexible label category will be granted for organisms with higher MICs than the most susceptible organism and for which efficacy was demonstrated at a higher dose. The effective dose for this organism must be clearly defined on the label.

(3) A copy of the FDA approved or submitted insert must be available in the submitted packet for review by the subcommittee.

(4) Actual pharmacokinetic data demonstrating that achieved drug levels meet or exceed MICs of the target pathogen at specific, label-indicated, dose regimen that differs from the "base" dose. A graph of the pharmacokinetics extrapolating the dose range alone is insufficient for supporting the flexible label category.

(5) For those situations where a drug is concentrated (such as the urinary tract), substantial clinical data must be presented to justify the need for a flexible label category rather than an intermediate category.

2.5 Development of Interpretive Criteria for Generic or Older Compounds

The development of interpretive criteria for generic or older compounds is problematic due to limited sponsor support for generation of new data. In particular, efficacy and pharmacokinetic data may be dated and of limited value. Sponsors seeking interpretive criteria for generic or older compounds should consider the following:

- Interpretive criteria for new indications for compounds in these categories must provide pharmacokinetic/pharmacodynamic data as outlined in Section 3.
- Pharmacokinetic and efficacy data must be presented, but the subcommittee may allow some latitude based on the age of the data; and
- The sponsor is strongly encouraged to contact the chairholder for guidance prior to the presentation to the full subcommittee.

3 Development of Interpretive Criteria

3.1 *Selection of Isolates

Dilution and disk diffusion tests should be performed according to NCCLS methods using a distribution of organisms similar to those commonly isolated from disease situations relevant to the drug's use, and from multiple geographical locations. In addition, this distribution should contain **examples of clinically important isolates relevant to the class** of compound being evaluated and should include isolates showing important resistance mechanisms. This includes not only resistant veterinary pathogens but also other pathogens (e.g., methicillin-resistant *Staphylococcus aureus* (MRSA) should be included in evaluations of antistaphylococcal agents).

3.1.1 Multiple Genera Sample Size

In general, studies should be performed with at least 300 (preferably up to 600) isolates representing all species that are likely to be treated by the drug. A few isolates which represent species that are outside of the study drug's expected spectrum of activity should be included, but the majority should belong to clinically relevant species. Ideally, for those organisms for which the antimicrobial product is intended, it is desirable to have separate scattergrams presented for each species (100 isolates minimum) in addition to the total combined scattergram.

3.1.2 Single Species or Genus Sample Size

(1)* Comparisons of broth microdilution and agar dilution MICs should be done on 100 or more clinical isolates (on scale) with a distribution similar to that described in Section 3.1.

(2) If commercially prepared broth microdilution panels are used to determine MICs and there is appropriate documentation as to quality assurance of the plate and the performance of the test, the use of broth microdilution panels alone may be acceptable.

When interpretive criteria are being developed for test procedures that are designed for a single genus or species (e.g., *Haemophilus somnus* or *Actinobacillus pleuropneumoniae*), fewer isolates can be used. However, those isolates should exhibit clinically relevant susceptibility or resistance mechanisms important to the antimicrobial agent being studied. In such cases, 100 will usually suffice.

3.1.3 Characteristics of Species and MIC Distributions Used in Regression Analysis

For regression analysis, all clinically relevant species should be represented, but efforts should be made to provide a reasonably even distribution of MICs over the range of concentrations tested, particularly in the range near the proposed susceptibility threshold. Such an even distribution of MICs may not be possible for some drugs; however, in these cases, error rate-bounding may be a preferred statistic and regression statistics should not be calculated.

3.1.4 Culture Collections and Error Rate-Bounding

For error rate bounding, the nature of the culture collection studied is critically important. When reporting the results of such studies, the type of culture collection used must be specified. Three types of culture collections are listed below. At least one of these must be evaluated. Ideally, data generated by culture collections (a) and (b) should be available for review by the subcommittee.

(a) A carefully selected challenge set of microorganisms may be gathered to include isolates with all known resistance mechanisms that may be relevant to the type of antimicrobial agent that is being evaluated. A similar number of susceptible isolates with no known resistance mechanisms should be included in such a challenge set of isolates, and all relevant species should be represented.

(b) A large collection of isolates (over 500) should be gathered from several geographically separate institutions to represent consecutively isolated strains that are normally subjected to susceptibility tests. Except for antimicrobials being developed for limited indications, no more than 20 to 30% of these isolates will be of the same species. In this type of collection, the more common species will predominate, and resistant isolates will be included as they are being encountered in the institutions contributing isolates.

(c) A randomly selected collection of stock cultures can be gathered to represent all relevant bacterial species without prior knowledge of the study drug's activity. With many broad-spectrum antimicrobial agents, few resistant isolates are likely to be included in this type of culture collection.

3.2 Dilution Tests for MIC Breakpoint Determinations

3.2.1 Performance of Dilution Tests

The MICs are to be determined by NCCLS approved methods using isolate populations described in Section 3.1.

When an antimicrobial is developed that is a combination product (e.g., beta-lactam/beta-lactamase inhibitor, trimethoprim-sulfa, etc.), then data supporting the selected ratio(s) of the various components to be used in dilution test methods must be presented.[4]

3.3 Disk Diffusion Susceptibility Tests

3.3.1 Disk Content Studies

In most cases, the content of the antimicrobial disk will be the same as that for other established antimicrobials that are structurally related. This generalization does not apply if the new antimicrobial represents a new class of antimicrobial agents, or exhibits different physicochemical characteristics, or if the MIC breakpoints or human pharmacokinetics are substantially different from those of related antimicrobials.

If necessary, preliminary studies may be carried out to determine the antimicrobial content in the disk that should be evaluated more thoroughly. The ideal disk content is one that provides zone diameters greater than 15 mm and less than 45 mm with most susceptible strains but only small zone diameters of inhibition (or no detectable zone diameters of inhibition) with resistant strains. However, susceptible breakpoint(s) should, ideally, be between 15 and 25 mm.

*When an antimicrobial disk is developed for a drug that is a combination product (e.g., beta-lactam/beta-lactamase inhibitor, trimethoprim-sulfa, etc.) then a justification for the selected ratio of the components of the disk must be presented.

3.3.2 Reagent Disks

All studies should be conducted with reagent disks, regardless of source (commercial or other), that meet the requirements stipulated in the U.S. Code of Federal Regulations (CFR).

3.3.3 Performance of Disk Diffusion Tests

The MICs are to be determined by NCCLS approved methods using isolate populations described in Section 3.1.

3.4 Evaluation of Dilution MIC and Disk Diffusion Data

3.4.1 *Regression Line Determination

Statistical analysis of these data may involve the calculation of a linear regression line that correlates MICs and zone diameters of inhibition. Calculations should exclude undefined measurements such as no zone (i.e., 6 mm) of inhibition or off-scale MICs (i.e., < or ≥ values). To avoid problems with a parabolic regression curve, the regression statistics may be recalculated using only isolates with MICs two-to-three dilutions above and below the proposed MIC breakpoint. In either case, all data should be presented as scattergrams, including the endpoints that were excluded when calculating the regression line.

3.4.2 *Error Rate-Bounded Method

3.4.2.1 *Interpretive Criteria and Discrepancy Rates

The error rate-bounding method of Metzler and DeHaan[1] may be used to select zone-size interpretive criteria and to calculate interpretive discrepancy rates. The Metzler and DeHaan method usually needs to be modified[2] because two MIC breakpoints are normally described to define an "intermediate" category. An easy procedural approach for this method is available.[3] Data should be displayed as a scattergram with zone diameters on the x-axis and MICs on the y-axis, and with horizontal and vertical lines showing the proposed interpretive breakpoints. In practice, the proposed zone-size breakpoints are simply adjusted until the number of false-susceptible disk diffusion test results (very major discrepancies) and false-resistant disk tests (major discrepancies) are held to a minimum. Minor discrepancies (i.e., when one of

the test results is intermediate and the other is susceptible or resistant) should also be considered in these determinations. When a large proportion of strains is close to the proposed or approved breakpoint for an antimicrobial agent, a high percentage of minor discrepancies may be expected. (See Section 3.1.4).

3.4.2.2 *Acceptable Discrepancy Rates for Challenge Sets of Organisms

Because of the inherent +1 dilution variation in MIC endpoints, discrepancy rates will be directly proportional to the percentage of isolates with antibiotic MICs in the range of one twofold concentration above the intermediate MIC (I+1) and one twofold concentration below the intermediate MIC (I-1). Thus, when an entire population is used as the denominator for calculating discrepancy rates, the rate will be determined largely by the population of MICs in the I+1 to I-1 range. For example, when 90% of the isolates have highly susceptible drug MICs (as is common with newer antibiotics), the discrepancy rate will be considerably less than that of a population in which 40% of the MICs fall in the I+1 to I-1 range. If the total I+1 to I-1 subpopulation is used as the denominator for calculating the discrepancies in this range, the discrepancy rates should be more comparable.

Of greater concern are discrepancies that occur with MICs two or more twofold concentrations above (>I+2) or below (<I-2) the intermediate MIC. These should be uncommon, and when they do occur, both the MIC and disk diffusion test should be repeated and the repeated values used in the scattergram. A notation of all such repeated tests should be made in the report. Based in part on the data from the scattergrams of drugs for which interpretive criteria have been approved by NCCLS, the table below is provided as a guideline for acceptable discrepancy rates using specific MIC subpopulations as the denominator. These guidelines will be weighed in assessing the appropriateness of proposed interpretive criteria.

When there is a two-dilution intermediate range, the process for determining discrepancy rates remains the same with one modification. The MIC range of I+1 to I-1 will include both intermediate MICs plus one dilution above the higher intermediate MIC and one dilution below the lower intermediate MIC. As an example, if the intermediate range is between 2 and 4 mcg/mL, the MIC range of I+1 to I-1 will include 8, 4, 2 and 1 mcg/mL. (See Table 1.)

Table 1. Guideline for Acceptable Discrepancy Rates (With Intermediate Ranges) (see Note)

		Discrepancy Rates		
1-dilution Intermediate Range	**2-dilution Intermediate Range**	**Very Major**	**Major**	**Minor**
≥ I+2	≥ I_{High}+2	< 2%	N/A	< 5%
I+1 to I-1	I_{High}+1 to I_{Low} -1	<10%	<10%	< 40%
≤ I-2	≤ I_{Low}-2	N/A	<40%	< 5%

NOTE: I_{High} and I_{Low} are the higher and lower MICs in a two-dilution intermediate range. See example in Appendix B.

When there is no intermediate range, i.e., when there is only a susceptible and resistant breakpoint, the process for determining discrepancy rates is done in a similar manner. (See Table 2.) If there are intermediate ranges for both disk diffusion and dilution testing, minor discrepancies are not a consideration.

Table 2. Guideline for Acceptable Discrepancy Rates (see Note)

MIC Range	Discrepancy Rates		
No Intermediate Range	**Very Major**	**Major**	**Minor**
≥ R+1	< 2%	N/A	< 5%
R+S	<10%	<10%	<40%
≤ S-1	N/A	< 2%	< 5%

NOTE: R is the resistant breakpoint MIC; S is the susceptible breakpoint MIC.

3.4.2.3 *Acceptable Discrepancy Rates for Unselected Clinical Isolates

Ideally, when evaluating a large collection of unselected clinical isolates, very major discrepancy rates should be less than 1.5%, and major discrepancies should occur with less than 3% when calculated based on all isolates.

3.4.2.4 *Comprehensive Tabulation

A separate tabulation should be provided showing the total number of isolates for each species tested and the number of minor, major, or very major discrepancies that were recorded for each species.

3.4.2.5 *Cross-Resistance and Cross-Susceptibility Studies

Cross-resistance and cross-susceptibility studies should be conducted by dilution and disk diffusion tests using available drugs in the same class. These studies should be done with 300 or more representative clinical isolates. When possible, representative isolates of uncommonly encountered organisms should also be included. Tables showing results with different species should be presented.

3.5 *Final Selection of Proposed Breakpoints

The final selection of proposed breakpoints should be based upon subcommittee evaluation of pharmacokinetic and pharmacodynamic parameters, regression line analysis, overall error rates, and clinical verification of breakpoints by clinical and bacteriological response rates.

3.6 *MIC/Zone Diameter Distribution

The MIC/zone diameter distribution used for the *in vitro* test development should be compared with those obtained from a large survey of recent clinical isolates.

4 Comparison of Dilution Test Methods for Aerobic Bacteria

The MICs are to be determined by approved standardized testing methods to establish parity of results. Susceptibility test comparative studies are described below:

(1)*Comparisons of broth microdilution and agar dilution MICs should be done on 100 or more clinical isolates (on scale) with a distribution similar to that described in Section 3.1.

(2) If commercially prepared broth microdilution panels are used to determine MICs and there is appropriate documentation as to quality assurance of the plate and the performance of the test, the use of broth microdilution panels alone may be acceptable.

5 Tests for Anaerobic Bacteria

(1) If the spectrum of the new drug includes anaerobic bacteria, susceptibility testing will be consistent with NCCLS document M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*.

(2) These studies should contain a reasonable number (100 or more) of clinically relevant isolates obtained from different sources.

(3) All other parameters should conform to the M37 guideline.

6 Quality Control Limits

The M23-A2 document has established slight modifications in the process for development of quality control limits. At the present, the use of methods detailed in M37-A, or M37-A2 or those in M23-A2 may be used to allow sponsors to complete work in progress that may have been initiated under any one of the above guidelines. However, the subcommittee encourages sponsors to follow the most recent guidelines.

6.1 *Preliminary QC Testing (Tier 1 Preliminary QC Study)

During the drug development process, testing of NCCLS-recommended QC strains should be performed to establish preliminary QC limits and to determine the impact of procedural variations on test performance. Testing should be performed using all appropriate NCCLS reference methods to establish equivalency of methods (e.g., agar dilution and broth microdilution). Testing may be done at one laboratory.

If this preliminary testing is not done, 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 (e.g., ATCC).

6.2 *Requirements for Establishing Acceptable QC Ranges (Tier 2 QC Study)

A Tier 2 QC study is designed to provide adequate data to establish expected ranges for quality control. These studies evaluate reproducibility of the method within a lab, between labs, and between reagent lots. All testing will be performed using NCCLS reference methods. Expected ranges established with Tier 2 QC studies are published in NCCLS document M31—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline—Second Edition.*

6.2.1 Disk Diffusion Tests

*To monitor the performances of *in vitro* disk diffusion susceptibility tests, it is necessary to know the limits of acceptable variability in zone sizes using appropriate QC strains. These strains should be standard strains from a recognized source (e.g., the American Type Culture Collection [ATCC]). All NCCLS-recommended QC organisms appropriate for the drug should be evaluated.

To establish QC limits for disk diffusion tests, acceptable results from the laboratories of at least seven separate and distinct institutions should be analyzed. If possible, include at least one veterinary laboratory. The evaluation should involve two lots of disks from two different manufacturers, if possible.

Three lots of Mueller-Hinton agar (MHA) from different manufacturers should be used. (See the most current edition of NCCLS document M6—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar.)* Each laboratory should use each MHA lot. Each lot should meet the M6 performance requirements.

At least seven laboratories should test each QC strain on each MHA lot and each disk lot for ten days. This results in 70 data points for each individual MHA and disk lot and 420 total data points. The same principles should be used when other media are required (e.g., fastidious organisms). NCCLS methods must be followed as appropriate for each organism (i.e., M31 and M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*).

A control drug of similar class as the study drug should also be tested (one lot of media 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. The results for the control drug must be within the expected control limits each day of testing. If this is not the case, an investigation as to the cause of the problem should be conducted, and the day's testing should be repeated.

The results from all laboratories must be presented in a blinded format. Results for both the study drug and the control drug should be presented as a distribution of zone diameters of inhibition by each QC strain for each laboratory and MHA lot. Statistical methods (e.g., Gavan et al[4] which may include mean, standard deviation, range of zone sizes, and median of ranges) should also be used. Ideally, at least 95% of values should be included in the proposed range.

6.2.2 Dilution Tests for Aerobic and Fastidious Organisms and Anaerobic Bacteria

*****For purposes of susceptibility testing, fastidious organisms are defined as those that will not grow satisfactorily in (or on) unsupplemented Mueller-Hinton (MH) medium within 24 hours.

To monitor the performance of *in vitro* dilution tests, it is necessary to establish the limits of acceptable variability in MICs using appropriate QC strains. These strains should be standard strains taken from a recognized source (e.g., ATCC). All NCCLS-recommended QC strains appropriate for the study drug should be evaluated.

To establish QC limits for dilution tests, results from at least seven separate and distinct institutions should be analyzed. Three lots of Mueller-Hinton broth, each from a different manufacturer, should be used. For anaerobic bacteria and other special organisms, three lots of agar or broth (according to the applicable standards) should be used from different manufacturers, if possible. Each laboratory should use each lot of media. 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 which can be "accurately" prepared (i.e., dilutions lower then 0.03 mcg/mL should be avoided) and no more than five dilutions below the drug's susceptibility breakpoint. A three-dilution range is preferred; however a four-dilution range may sometimes be needed (see examples below).

	Example # 1	Example # 2	Example # 3
0.03 µg/mL	0	0	0
0.06 µg/mL	5	15	0
0.12 µg/mL	100	73	30
0.25 µg/mL	104	110	170
0.50 ug/mL	1	12	10
1.00 ug/mL	0	0	0
Range (ug/mL)	0.06-0.50	0.06-0.50	0.12-0.50

- Example #1 illustrates results where the MIC is distributed evenly across two dilutions.

- Example #2 shows a mode of 0.25. However, there are a large number of results at 0.12 (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, and 95% of the results are within one dilution of the mode.

Each of the seven laboratories should test ten replicates of each QC strain on each media lot. Each replicate should use individually prepared inoculum suspensions. The study should be conducted over a minimum of three days with a maximum of four replicates per day. This results in 70 data points for each individual media lot and 210 total data points. The same principles should be used when other media are required (e.g., fastidious or anaerobic organisms [see NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests,* M6—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar*, and M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*]). When using agar dilution, ten replicates of each QC strain should be tested for a minimum of two days. Each replicate should use individually prepared inoculum suspensions. All ten replicates of each strain can be inoculated onto the same set of agar dilution plates. This will result in 140 data points for each individual media lot and 420 total data points for each QC strain.

A control drug of similar class as the drug under development should also be tested (one lot of media 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. The results for the control drug must be within the expected control limits each day of testing. If this is not the case, an investigation as to the cause of the problem should be conducted, and the day's testing should be repeated.

For each study drug and control drug, a twofold dilution schedule, with 1 µg/mL as the central point should be used to provide on-scale endpoints for all determinations.

The results from all laboratories must be presented in a blinded format. The results for both the study drug and control drug should be presented as MIC distributions for each lot of media, for each laboratory, and for all data points combined.

Additionally, the results of the testing to establish the equivalency of methods (e.g., agar dilution vs. broth microdilution) should be presented. In the absence of equivalency data, the accepted QC limits will be noted to apply only to the method used to obtain the data. Inoculum concentration and pH effects should be evaluated.

6.3 Confirmation and Reassessment of Quality Control Ranges (Tier 3 QC Monitoring)

Quality control ranges should be monitored as additional data are collected. Additional QC data may be obtained as various groups gain testing experience with the drug (e.g., clinical trial of the drug, research studies, development of commercial diagnostic tests, and routine clinical laboratory use). QC data from

the drug clinical trial should be presented to NCCLS to confirm the appropriateness of the expected range established during the Tier 2 QC study or to determine the need to reassess the expected range. Other individuals may also present QC data to NCCLS and request a reassessment of the expected range. Occasionally, QC ranges may need to be revised to adequately monitor performance of *in vitro* susceptibility tests (see Section 1.6).

Tier 2 QC data used to establish the current expected range should be reviewed to reassess QC ranges when possible. If the Tier 2 QC data are not available, Tier 2 QC study requirements should be fulfilled by collecting data with a new study or compiling retrospective data. (See Section 1.4.)

To reassess a QC range, a new Tier 2 study should be conducted or additional QC data, using NCCLS reference methods, may be collected to supplement the original Tier 2 study. Supplemental QC data may be acceptable if the sample size and study design is sufficient and adequate controls are used (e.g., control drug and/or second QC strain). Supplemental QC studies should focus on the source of variability to better assess current performance (e.g., lab-to-lab, lot-to-lot, within-lab variability).

Data from the two studies (original and new) should be analyzed separately. If the two data sets are similar or show a slight shift (e.g., one twofold dilution), the new data may also be combined with the original Tier 2 data as part of the analysis. A comparison of the standard deviation and geometric mean may also be useful. If the new data is significantly different from the original data (e.g., greater than one dilution shift), further investigation may be required to identify the cause of the difference or assess the impact of the allowable tolerances of the standard method. The process of revising the QC range should be similar to the initial selection process. The QC range may be left unchanged, changed, or enlarged (e.g., four-dilution range) as appropriate.

References

[1] Bradford PA, Sanders CC. Use of a predictor panel for development of a new disk for diffusion tests with cefoperazone-sulbactam. *Antimicrob Agents Chemother.* 1992;36:394-400.

[2] Metzler DM, DeHaan RM. Susceptibility tests of anaerobic bacteria: Statistical and clinical considerations. *J Infect Dis*. 1974;130:588-594.

[3] Gavan TL, Jones RN, Barry AL, et al. Quality control limits for ampicillin, carbenicillin, mezlocillin, and piperacillin disk diffusion susceptibility tests: A collaborative study. *J Clin Microbiol*. 1981; 14:67-72.

[4] Brunden MN, Zurenko GE, Kapik B. Modification of the error-bounded classification scheme for use with two MIC breakpoints. *Diagn Microbiol Inf Dis*. 1992;15:135-140.

Appendix A. Suggested Information to Be Contained on Package Cover Page (see Note)

(a) Specific request being made by the company (e.g., inclusion in Table 1, breakpoints, QC data, etc.).
(b) FDA status (package insert attached if approved) and status in other countries.
(c) FDA-approved and/or proposed indications for use (include organisms).
(d) Clinical conditions for which the drug is targeted.
(e) Dosage of drug to be used for indications/data being evaluated.
(f) Provide solvents and diluents needed for stock solution preparation (for Table 8).
(g) Antimicrobial agent abbreviation to be used by diagnostic manufacturers.

NOTE: Only information relevant for the request being made need be included.

Appendix B. Drug "X" MIC vs. Zone Diameter (495 challenge organisms)

		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	>35
I + 3	**≥ 16**	13		1	2	1	3	2		1																						
I + 2	**8.0**	1		1	3	1	2		1	1	2				1																	
I + 1	**4.0**						1	4	2	3	1						1															
I	**2.0**								1	2	1	5	2	1	4		1			1					1							
I – 1	**1.0**											3	3	5	7	3	3	3	1	2	1	3		1								
I – 2	**0.5**			1									2	3	1	2	3	5	8	13	4	4	1		2	1		1	1			
I – 3	**0.25**														1	1	2	3	5	6	11	13	9	6	5	3	1	4	1			
I – 4	**0.12**															1			1	6	13	9	6	7	6	1	3	1				
I – 5	**0.06**														1				1	4	5	6	5	4	3	8	10	5	7	3		1
I – 6	**≤ 0.03**																			3	7	17	20	20	9	15	12	10	10	1	1	6

Table B1. Discrepancy Rates for Drug "X"

		Number of Discrepancies (Discrepancy Rate)		
MIC Range	Number	Very Major (%)	Major (%)	Minor (%)
≥ I + 2	36	1 (2.8)[†]	NA	2 (5.6)
I + 1 to I – 1	66	1 (1.5)	0	18 (27.3)
≤ I – 2	393	NA	1 (0.3)	2 (0.5)
Total	495	2 (0.4)	1 (0.2)	22 (4.4)

[†]This figure exceeds the proposed limit. However, since only 36 MICs fell in the ≥ I + 2 range (and at least 50 would be required for one discrepancy to equal 2%), this should be considered acceptable, particularly since the overall discrepancy rates are so low. Furthermore, it appears this discrepant result was not repeated, and it very likely would not be discrepant on repeat testing.

NCCLS consensus procedures include an appeals process that is described in detail in Section 9 of the Administrative Procedures. For further information contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Subcommittee Responses

M37-A: *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters for Veterinary Antimicrobial Agents; Approved Guideline*

1. Page 1, Section 1.1: Why speak only about enteric infections in animals and gut pharmacokinetic data? And the other types of infection and pharmacokinetic data?

- **NCCLS document M37 specifically states that it applies to therapeutic antimicrobial agents used to treat systemic diseases in animals. At the time of the development of M37, there were no interpretive criteria available for drugs used to treat enteric infections in animals. This section is designed to encourage sponsors to develop interpretive criteria for agents with enteric disease indications.**

2. Page 4, Section 2.1: It would be interesting to add a point about resistance selection in integrating data of drug resistance developed *in vitro* and/or *in vivo*, and also data concerning the mechanism of resistance when these mechanisms are partially known.

- **Data requirements specifically related to resistance and resistance mechanisms are indicated in Section 3. While there is no specific requirement for resistance selection, this is a key characteristic that would be determined early in the drug development process and this information should be available from the sponsor.**

3. Page 8, Section 3.4.2: Why calculate the discrepancy rates? What is the formula? What is exactly its use? Is it useful for determination of MICs only by microdilution antimicrobial susceptibility tests (and not by antimicrobial disk susceptibility tests)?

- **The discrepancy rate method allows calculation of the number of interpretive errors that occur when comparing MIC and disk interpretive categories. In particular, it allows one to determine both the overall error rates as well as the error rates that occur with specific subpopulations. The method of calculating discrepancy rates is detailed in this section.**

4. Page 9-10, Section 3.4.2.5: What does it mean "Clinical verification of breakpoints by clinical and bacteriological response rates"? What are exactly the studies to do this verification?

- **The studies required for clinical verification of breakpoints are described in Sections 2.3 and 2.4.**

5. Pages 16-17, Appendix B, Table B1: It would be interesting to explain the calculation of the discrepancy rates with the formula in this example. In particular, I don't understand the results of the number of discrepancies for the MIC Range I+1 and I-1.

- **In the cited example, the total number of isolates yielding MIC values ranging from one dilution higher than the selected intermediate category to those yielding one dilution lower is 66. One of the isolates was resistant when tested by the MIC test but categorized as susceptible by the disk test (isolate with MIC of 4.0 µg/ml, zone of 21 mm). This type of error is a very major error**

and is 1.5% of this total population (1/66 = 1.5%). The same type of calculation is done for the minor errors. By calculating error rates, interpretive categories for the disk test can be selected that minimize the number of errors.

6. General Comment: This publication is not very easy to understand at the first reading. The abstract is not sufficient to understand really the objectives of the publication. In addition, there are many general considerations about requirements but not lots of details or examples which could improve the understanding.

- **As stated in the Foreword, "M37 is intended to offer guidance for sponsors that wish to list interpretive criteria and quality control information in NCCLS document M31." Also, the Foreword states that this document is intended to be a guideline and not a step-by-step protocol due to the different characteristics and indications that antimicrobial agents are developed to treat. While the subcommittee appreciates this viewpoint, practical considerations prevent development of a specific protocol that would be adequate for all current and future antimicrobial agents.**

Related NCCLS Publications*

M2-A7 **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Seventh Edition (2000).** This document provides current recommended techniques for disk susceptibility testing, new frequency criteria for quality control testing, and updated tables for interpretive zone diameters.

M6-A **Protocols for Evaluating Dehydrated Mueller-Hinton Agar; Approved Standard (1996).** This standard contains procedures for evaluating production lots of Mueller-Hinton agar, and for the development and application of reference media.

M7-A5 **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard —Fifth Edition (2000).** This newly revised standard provides updated reference methods for the determination of minimal inhibitory concentrations (MICs) for aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution. This document contains MIC interpretive criteria and quality control parameters tables updated for 2000.

M11-A5 **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Fifth Edition (2001)**. This document provides reference methods for the determination of minimal inhibitory concentrations (MICs) of anaerobic bacteria by broth macrodilution, broth microdilution and agar dilution. Interpretive and quality control tables are included.

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-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections—Second Edition; Approved Guideline (2002).** This document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

M31-A2 **Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second Edition (2002).** This document provides current recommended techniques for antimicrobial agent disk and dilution susceptibility testing criteria for quality control testing; and interpretive criteria for veterinary use.

M100-S12 **Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement (2002).** This document provides updated tables for the NCCLS antimicrobial susceptibility testing standards M2-A7 and M7-A5.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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