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

CLINICAL AND LABORATORY STANDARDS INSTITUTE ™

(Formerly NCCLS)
Providing NCCLS standards and guidelines,
ISO/TC 212 standards, and ISO/TC 76 standards

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

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

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Abstract

This document offers guidance for developing data on antimicrobial susceptibility testing of aerobic and anaerobic bacteria; data developed according to this guideline will be used in establishing interpretive and quality control criteria for NCCLS antimicrobial susceptibility testing standards. Human pharmacokinetics, *in vitro* drug characteristics, distributions of microorganisms, and correlation of test results with clinical outcome statistics are addressed from the perspective of interpretation of test results. In addition, the 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 together toward the best educated conclusions.

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Foreword

NCCLS is responsible for the development of standard reference methods for *in vitro* tests for measuring the susceptibility of bacteria to antimicrobial agents. In this regard, the NCCLS Subcommittee on Antimicrobial Susceptibility Testing is responsible for developing and updating the following susceptibility testing standards:

M2   *Performance Standards for Antimicrobial Disk Susceptibility Tests;*

M7   *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically;* and

M11   *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.*

M23-A2 provides guidance on the types of data that are useful to and/or required by the subcommittee when determining interpretive criteria and quality control limits for inclusion in the above noted documents. It also provides guidance both for new antimicrobial agents that are about to be made available for clinical use, as well as for marketed antimicrobial agents that require subsequent reassessment.

All sections of the guideline preceded by an asterisk (*) describe information required for review by the subcommittee. All other sections describe recommended information.

The previous edition of this document (M23-A) proved to be a needed and well-received starting point in defining the types of information necessary to assist the subcommittee in determining interpretive criteria and quality control limits. This edition builds upon M23-A, providing more complete guidance in numerous areas (i.e., pharmacokinetics/pharmacodynamics and clinical correlations). In addition, sections have been added to address the following topics: acceptability of both U.S. and non-U.S. data; use of data derived from previously accepted reference methods; and resolution of differences between NCCLS and regulatory agencies. The entire section on quality control limits has been revised following a thorough review of varying approaches to determine such limits. Wording has been added to minimize the possibility of creating unfair advantages or disadvantages for one antimicrobial relative to another. For example, this document states "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." This edition also clearly defines circumstances when a reassessment of interpretive criteria or QC parameters can be considered. When such reassessments are made, the guidelines outlined in this document are to be followed, and the data upon which the original decision was made are to be considered.

The working group devoted a great deal of effort to evaluating acceptable discrepancy rates when comparing susceptibility results obtained using dilution and disk diffusion methods in order to establish appropriate interpretive breakpoints. The acceptable discrepancy rates presented in this document are guidelines that take into account the distribution of the organisms tested. This should allow for a more precise evaluation of the agreement between the two standard methodologies. Over the coming years, the subcommittee will examine how well these guidelines work in practice.

The M23 guideline is a key foundation document for NCCLS's widely used "family" of susceptibility testing documents. It is intended to offer direction and guidance for developing data on antimicrobial susceptibility testing of aerobic and anaerobic bacteria; data developed according to M23 are used by the Subcommittee on Antimicrobial Susceptibility Testing as the basis for establishing interpretive and quality control criteria for NCCLS's antimicrobial susceptibility testing standards. The intent is to ensure that a "level playing field" is maintained, independent of manufacturer, healthcare professional, or government agency, in data presentation to the subcommittee and in subcommittee determinations based on those data.

Foreword (Continued)

In addition to the members of the working group, I wish to express my personal gratitude for the work contributed by the following persons in making this document what it is: Patricia Charache, Bruce Craig, Mary Jane Ferraro, William Gregory, Judith Johnston, James Jorgensen, Harriette Nadler, William Novick, James Poupard, and Raymond Testa.

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> *Control Parameters*

Key Words

Antimicrobial agents; standard dilution methods for bacteria that grow aerobically; standard disk diffusion test; standard reference method for anaerobes; susceptibility testing

NCCLS Subcommittee on Antimicrobial Susceptibility Testing Mission Statement

The NCCLS Subcommittee on Antimicrobial Susceptibility Testing is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, healthcare providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the NCCLS voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting.

The mission of the NCCLS Subcommittee on Antimicrobial Susceptibility Testing is to:

- Develop standard reference methods for antimicrobial susceptibility tests

- Provide quality control parameters for standard test methods

- Establish interpretive criteria for the results of standard antimicrobial susceptibility tests

- Provide suggestions for testing and reporting strategies that are clinically relevant and cost-effective

- Continually refine standards and optimize the detection of emerging resistance mechanisms through the development of new or revised methods, interpretive criteria, and quality control parameters

- Educate users through multimedia communication of standards and guidelines

- Foster a dialogue with users of these methods and those who apply them.

The ultimate purpose of the subcommittee's mission is to provide useful information to enable laboratories to assist the clinician in the selection of appropriate antimicrobial therapy for patient care.

The standards and guidelines are meant to be comprehensive and to include all antimicrobial agents for which the data meet established NCCLS guidelines. The values that guide this mission are quality, accuracy, fairness, timeliness, teamwork, consensus, and trust.

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

1   Introduction and Scope

1.1   Subcommittee Requirements

Guidelines for interpretive breakpoints and quality control (QC) limits are established by the Subcommittee on Antimicrobial Susceptibility Testing after the review of extensive data. This guideline describes the data needed for such determinations.

All sections of the guideline preceded by an asterisk (*) describe the minimum information required for review by the subcommittee. All other sections describe information that may be helpful in supporting the establishment of interpretive criteria and QC limits.

Guidelines presented in this document apply only to NCCLS documents. The guidelines do not apply to topical antibiotics.

Any reassessment (Section 1.7) of existing drugs should also follow the guidelines presented in this document whenever possible.

1.2   Definitions[a]

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 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.

Data, *n* - Individual facts, statistics, or items of information, or a body of facts or information; **NOTE:** The term "datum" is sometimes used to describe an individual fact, observation, or number.

Microorganism, *n* - Any organism that is too small to be viewed by the unaided eye, such as bacteria, viruses, molds, yeast, protozoa, and some fungi and algae.

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.

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[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.

1.3   Time Sequence for Presentation

To ensure a successful evaluation of a new drug or new data, each sponsor (company or individual) should review the guidelines for time sequence and for presentation (Section 1.4).

- As early as possible, the pharmaceutical sponsor will notify the subcommittee of the compound abbreviation (up to three letters) to be utilized by diagnostic companies for all commercial products used for susceptibility testing. This abbreviation will be added to Glossary 2 of NCCLS document M100--*Performance Standards for Antimicrobial Susceptibility Testing*. For those compounds not listed elsewhere in M100, a footnote will indicate that the compound has been included in Glossary 2 only with the intention of defining an antibiotic abbreviation.

- The data on quality control parameters for disk diffusion and dilution tests may be evaluated by the subcommittee any time they become available.

- The information on zone size, minimal inhibitory concentration (MIC) relationships, pharmacokinetics/pharmacodynamics may be made available to the subcommittee early in the development of a drug (Phase I or II). This can assist in the selection of "provisional" breakpoints to be used by clinical investigators (Phases II and III). "Provisional" breakpoints will not be published in NCCLS documents.

- Just before, or as soon as feasible after the new drug application (NDA) is submitted, all requested data defined in these guidelines (including previously presented data) should be formally presented to the subcommittee for selection of interpretive criteria and QC ranges.

- A drug's placement in Table 1 of NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests* and M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* requires an FDA-approved clinical indication for the specific organism(s) under which the drug is to be listed. Alternatively, placement in Table 1 without an FDA-approved indication may be made if a public health need requires such an action. FDA-approved clinical indications are not required for a drug's placement in any other tables.

- The subcommittee may reassess the need for altering interpretive criteria or QC parameters at any time (Section 1.7). Additional supportive data may be submitted to the subcommittee from any source whenever a change appears to be necessary.

1.4   Presentation

Individuals or sponsors who wish to present data to the subcommittee must have a hard copy of their presentation (including supportive data and recommended actions) included in the agenda book for the subcommittee meeting. Submissions should be sent to the NCCLS Executive Offices. Agenda priority will be given for the final formal presentation of new drugs to be included in NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests,* M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, and M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*.

All data packages requesting an 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.

Materials and methods used in studies presented to NCCLS should be documented in the report. Materials used in these studies should be qualified for susceptibility testing or identified as "investigational." The identities of study materials and participants are generally masked in the data presentation to prevent bias in analyzing the results. Discussion of aberrant or unusual results should focus on the investigation of the cause. Identity of participants or material sources may be unmasked as needed in the investigation.

Variations from the NCCLS standard **reference** methods should also be identified in the study report. Data from these studies may be useful to detect trends and potential issues.

1.5   Acceptability of Data

Data generated from within or from outside the U.S. must meet the same high standards.

For microbiologic data, NCCLS reference methods are to be used and so documented, including proper quality control tests. When establishing QC criteria, NCCLS reference methods must be used. For other purposes broth microdilution panels (e.g., dried panels) may be prepared by a commercial supplier that produces panels cleared by the FDA for testing similar drugs against the organisms to be tested. These panels and procedures should closely follow NCCLS reference methods, including the final organism inoculum density, media, total volume included in each panel well, length (16 to 24 hours) and conditions of incubation, and the method of visual interpretation of MIC endpoints. Any variations from the NCCLS reference broth dilution method must be presented. The reasons for any variations and the potential impact on susceptibility results must be explained. When using commercially prepared panels, proper quality control tests are to be performed and documented – a minimum of 20 replicates performed over at least three separate days. Comparability of the commercial broth microdilution panels must be demonstrated by parallel testing of the new compound and a control drug (if available) of the same class, using the NCCLS reference broth microdilution method and the commercially prepared panels. At least 100 isolates are to be tested. When interpretive criteria are being developed for groups of organisms that have separate interpretive criteria (as per the groupings in Tables 2 of M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically,* e.g., Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus pneumoniae*, *Haemophilus* spp., and *Enterococcus* spp.) 100 isolates of each group are to be tested and presented individually. The isolates tested should belong to clinically relevant species, and should include susceptible and resistant (if recognized) clinical strains. In addition, the test population should include isolates with known resistance mechanisms that are relevant to the type of antimicrobial being studied. It would be preferable to include a subset of isolates with MICs around the proposed breakpoint. When assessing the comparability of MICs determined using commercially prepared nonreference panels with the NCCLS reference method, an analysis that examines the correlation, variability, and bias of the nonreference panel must be presented. Comparability to the NCCLS reference broth microdilution method may not be necessary if the commercially prepared panels were equivalent to those cleared by the FDA for the specific drug and species under evaluation, and have been demonstrated to be comparable to the NCCLS reference broth microdilution method as delineated above.

1.6   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 reference method.

1.7   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 or when there becomes a public health need requiring action with limited clinical information. The following represents situations under which a reassessment can 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 actions and/or data from the FDA or other regulatory authorities, CDC, College of American Pathologists, or other sources suggest the need for reassessment;

- when changes in NCCLS-approved reference methods are proposed and adapted and such changes may have an impact on interpretive criteria and/or QC parameters; and

- 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 manufacturer submits a request to the FDA for revisions in interpretive breakpoints or QC limits, they are encouraged to submit such data for consideration by the subcommittee   (see Section 7).

If the need for reassessment is brought 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.

When a change is made with limited information or when the guidelines outlined in this document are not followed due to a pressing public health need, this should be so noted within the document.

2   Data for Determining Susceptibility Test Breakpoints

2.1   *In Vitro* Drug Characteristics

*Data on the stability of appropriate concentrations of the drug at incubation and storage temperatures specified for NCCLS dilution methods (M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically* and M11—*Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*) must be provided.

*Data on the preparation of stock solutions, including diluent and solvent information, must be presented for inclusion in Table 4 of NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*.

*Because different methods could be used to determine MIC endpoints, data showing the relationship or comparability of these endpoints for a relevant subset of representative organisms for NCCLS standard (i.e., broth and agar) methods and media should be presented.

2.2   Distribution of Microorganisms

*Dilution and disk diffusion tests should be done on at least 500 isolates according to NCCLS methods and should contain examples of clinically relevant isolates appropriate both for the class of compound being evaluated and for the anticipated clinical use of the compound. They should also include isolates showing important resistance mechanisms. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative staphylococci should be included in the evaluation of antistaphylococcal agents.

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

2.3   Pharmacokinetics/Pharmacodynamics

*An analysis that examines the relation between pharmacokinetic/pharmacodynamic parameters and efficacy should be included. This analysis might include, but not be limited to, the following: time that serum or plasma levels exceed the MIC; peak serum or plasma level: MIC ratio; and area-under-the-serum drug concentration (AUC): MIC ratio. Data for this analysis might be derived from experimental models of infection, comparison of MICs with serum levels in humans following proposed dosage regimens, or even results from clinical studies. If such data are not available, data on the postantibiotic effect and impact of increasing drug concentrations on bacterial killing may be helpful. If an experimental model of infection is used, it should be established to be predictive of human outcome, and appropriate drug and untreated controls should be included.

*The pharmacokinetic/pharmacodynamic parameters of interest should be calculated for aerobic and fastidious organisms for which breakpoints are sought. The serum concentrations resulting from proposed dosage regimens in humans should be used. Similar calculations for related drugs of the same class should be presented.

In order to assist the subcommittee in making a timely decision, the following points should be considered when putting together the pharmacokinetics/pharmacodynamics data package:

Methods for measurement of drug concentrations in serum and body fluids should be provided. If bioassay and other analytical methods (e.g., HPLC) for drug-level measurement are available, the relative performance of the assays and comparisons between bioassay and other methods should be provided.

*Actual plots of serum or plasma levels in humans over time following the expected method of administration should be provided. If available, data from target patient populations should be presented. The number of subjects or patients, characteristics, and the intra- and interindividual variability of measurements should be included.

*The pharmacokinetic parameters useful for simulation, calculation of pharmacokinetic/ pharmaco-dynamic relationships, and comparisons with existing agents of a class should be provided. The parameters should include AUC, bioavailability, volume of distribution, clearance, and elimination half-life. The number of subjects in any study should be governed by good statistical approaches.

*Data concerning the effects of protein binding on MIC and pharmacokinetic/pharmacodynamic parameters should be included.

*Data on the metabolism and excretion of the drug in humans 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.

*Concentrations in cerebrospinal fluid must be presented if the drug will be used for treatment of meningitis.

Tissue, body fluid, and intracellular concentrations of the drug may be presented.

Where data are available, it is helpful to present data on differences in AUC, expected peak and trough serum concentrations, and pharmacokinetic/pharmacodynamic parameters (e.g., AUC: proposed MIC breakpoint ratio) with anticipated dosage regimens. While data in normal human subjects are generally available, data in special target patient populations which might include children, the elderly, and populations where special dosage adjustments will be made (e.g., renal or hepatic impairment) are desirable.

2.4   *Correlation of Test Results with Clinical Outcomes Statistics

During the clinical evaluation of antimicrobial agents, *in vitro* susceptibility (dilution or disk diffusion) test results should be correlated with therapeutic outcomes. 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 patients. Dilution and disk diffusion results are to be obtained from NCCLS standard reference methods or from systems approved by the U.S. Food and Drug Administration for such use. If any results are obtained from methods or systems which do not fit into the aforementioned categories, then data must be presented to demonstrate the comparability of such methods to NCCLS reference methods (see Section 1.5).

The clinical cure and bacteriological eradication rates should be correlated with appropriate *in vitro* test results to confirm the validity of the proposed interpretive criteria. There may be occasions when a clear breakpoint can be determined from the clinical data; however, due to the inherent difficulties and variabilities encountered in clinical studies this will frequently not be the case. In these situations, the clinical data will serve more to support other types of data (microbiologic and pharma-cokinetic/pharmacodynamic) in determining interpretive criteria.

In the course of the development of an antimicrobial agent, the manufacturer will conduct clinical studies that will yield a large volume of data. The manufacturer should provide the subcommittee, in a summarized fashion, all relevant data needed to make breakpoint determinations. If such data have already been submitted, or will be submitted in the near future to the FDA for review, the manufacturer should note if the data being presented are in any way different from that submitted, or to be submitted, to the FDA. If the data are different, such differences should be so noted, as well as the reasons for such differences. Optimally, the design and evaluation of clinical studies should conform to the most recent guidelines from the FDA or the Infectious Diseases Society of America. In order to assist the subcommittee in making a timely decision, the following points should be considered when putting together the data package:

- A clear description of the clinical protocol(s) used should be given. This should include:

    — description of the population studied;
    — study-specific inclusion/exclusion criteria;

— dosage and duration of study and comparative drug therapies;
— times of initial, on-therapy, and follow-up microbiologic and clinical assessments;
— visits and test of cure;
— evaluability criteria; and
— definitions of "clinical" and "bacteriological" response.

- When the category of "improved" is used as a clinical outcome, this should be clearly defined.

- If adjunctive therapy is permitted, this should be described.

- If patients are permitted to switch from one study antimicrobial to another (i.e., parenteral to oral switch), this should be so stated and criteria for such a change should be clearly defined.

- If surgical procedures are part of the routine care of an infection type, details concerning such procedures within the study should be discussed.

All clinical data relevant to breakpoint analysis, including an analysis of evaluable cases AND an intent to treat analysis of microbiologically documented cases, should be presented regardless of pending or approved FDA indications. In addition, summary results for the comparative arms should be presented by individual study to assist the subcommittee in evaluation of the data. To allow optimal evaluation, clinical data must be presented separately for sites and types of infection in accordance with FDA categories, e.g., urinary tract infection (complicated and uncomplicated), pneumonia (community acquired and nosocomial), etc. In addition, subsets of patients with bacteremia should be presented.

The data analysis should be based on responses at the "test of cure" AND at the subsequent long-term follow-up visits. All "failures" should be carried forward to subsequent evaluations.

The data presented must be relevant for the anticipated use of the antimicrobial agent in clinical practice. In addition, the organisms presented should be relevant for the site of infection being studied.

All clinical and/or bacteriological failures should be presented separately according to the infecting bacteria, MIC and/or zone of inhibition, and site of infection.

In vitro data should be presented as actual MIC or zone diameter of inhibition, not simply susceptible or resistant. Quality control data should be generated and recorded for all clinical isolate susceptibility determinations (MIC and zone of inhibition).

For bacterial species that are relevant for the anticipated use of the drug, data should be presented by individual zone diameter of inhibition and/or MIC.

Infections due to single or predominant pathogens should be presented separately from true polymicrobial infections.

Data should be presented for both clinical (cure, improved, failure) and bacterial responses. When the category of "improved" is used, clinical response data should be broken down into "cure," "improved," and "failure" as separate categories. Bacteriological response data should be presented with and without "presumed" outcomes included. In addition, data should be presented for each MIC and for all zone diameters relevant to those MICs.

Information about species that are resistant or that have MIC or zones of inhibition near the breakpoints is of particular interest and should be presented. These data should be presented for both evaluable and intent-to-treat populations.

3   Disk Diffusion Test Methods (M2)

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.

3.2   Evaluation of Disk Diffusion Susceptibility Tests

3.2.1   *Selection of Isolates

3.2.1.1   *Sample Size

In general, studies should be performed with at least 500 isolates representing all species that are likely to be tested by the disk diffusion procedure; the majority of which should belong to clinically relevant species.

When interpretive criteria are being developed for those organisms or groups of organisms that have separate interpretive criteria (e.g., *Streptococcus pneumoniae, Haemophilus* spp*., Enterococcus* spp.), data and scattergrams are to be presented separately for each of these. Fewer isolates can be used; however, those isolates should represent susceptible and resistant (if recognized) clinical strains that are relevant to the antimicrobial agent being studied. A total of 100 isolates of each will usually suffice.

3.2.1.2 *Single Species or Genus Sample Size

Fewer isolates can be used; however, those isolates should represent susceptible and resistant (if recognized) clinical strains that are relevant to the antimicrobial agent being studied. A total of 100 isolates of each will usually suffice.

3.2.1.3 *Regression Analysis

For regression analysis, all clinically relevant species should be represented, but efforts must 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 and, in that case, error rate bounding may be a preferred statistic and regression statistics should not be calculated.

3.2.1.4 *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) may 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.

3.2.2   *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).

3.2.3   *Regression Line Determination

Statistical analysis of these data may involve the calculation of a regression line correlating MICs and zone diameters of inhibition. Calculations must exclude undefined measurements (such as no zone of inhibition or off-scale MICs). To evaluate the linear portion of the 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.

3.2.4   *Error Rate-Bounded Method

3.2.4.1 *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. 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 (that is, 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 3.2.4.2).

3.2.4.2 *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 two-fold concentration above the intermediate MIC (I+1) and one two-fold 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 along with other factors (see Section 3.2.5) 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 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 Acceptable Discrepancy Rates (With Intermediate Ranges) (see Note)

		Discrepancy Rates		
1-dilution Intermediate Range	**2-dilution Intermediate Range**	**Very Major**	**Major**	**Minor**
$\geq$ I+2	$\geq$ I$_{High}$+2	< 2%	NA	< 5%
I+1 to I-1	I$_{High}$+1 to I$_{Low}$−1	<10%	<10%	< 40%
$\leq$ I-2	$\leq$ I$_{Low}$-2	NA	<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 no intermediate ranges for both disk diffusion and dilution testing, minor discrepancies are not a consideration.

Table 2. Guideline Acceptable Discrepancy Rates (see Note)

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

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

3.2.4.3 *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.

3.2.4.4 *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.

3.2.5   *Final Selection of Breakpoints

The final selection of breakpoints must include evaluation of pharmacokinetics, regression line analysis, overall discrepancy rates, and clinical verification of breakpoints by clinical and bacteriological response rates.

3.2.6   *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.

4   Dilution Test Methods for Aerobic Bacteria (M7)

The MICs may be determined by approved methods to establish parity of results.

*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 3.2.1.

*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.

5   Anaerobe Susceptibility Tests

*If the spectrum of the new drug includes anaerobic bacteria, intermethod comparisons may be made of results obtained with one or more of the alternative methods to the agar dilution reference method (see the most current edition of NCCLS document M11—*Methods for Antimicrobial Susceptibility Testing of*

Anaerobic Bacteria). These studies should contain a reasonable number (300 or more) of clinically relevant isolates. Results should be reported for broth microdilution versus agar dilution.

6   Quality Control Limits

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 documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests* and M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*.

6.2.1   Disk Diffusion Tests (M2)

*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. The evaluation should involve two lots of disks from two different manufacturers, if possible.

Three lots of Mueller-Hinton agar (MHA) from different manufacturers (see the most current edition of NCCLS document M6—*Protocols for Evaluating Dehydrated Mueller-Hinton Agar)* should be used. 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., M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests,* M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 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. 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, [3] 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 (M7) and Anaerobic Bacteria (M11)

*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 laboratories from 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 drugs' 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 µg/mL	1	12	10
1.00 µg/mL	0	0	0
Range (µg/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 the most current editions of 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 should be used to provide on-scale endpoints for all determinations.

The results from all laboratories must be presented. 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 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.

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 1.7).

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 1.5)

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.

7   Resolving Differences Between NCCLS and Regulatory Authorities

MIC and zone diameter breakpoints, and quality control limits are established by the Subcommittee on Antimicrobial Susceptibility Testing after review of extensive data. Regulatory agencies independently establish interpretative criteria and quality control limits based on evaluation of extensive data submitted by pharmaceutical manufacturers. This independent process can at times produce interpretations that are discrepant. Every effort should be made to minimize or resolve such discrepancies. When the subcommittee makes a change that will result in a discrepancy, the manufacturer of the product will be encouraged to submit the data upon which the change is made to the appropriate regulatory authority, and/or request additional analysis or reassessment by the subcommittee.

References

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

[2] 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.

[3] Gavan TL, 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.

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) If a request is being made for the addition of the drug to NCCLS document M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, provide solvents and diluents needed for stock solution preparation (for Table 4).

(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"

MIC Range	Number	Number of Discrepancies (Discrepancy Rate)		
		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 9.0 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

M23-T3: *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters; Tentative Guideline – Third Edition*

Foreword

1. Page xiii- "In addition, sections have been added to address the following topics: acceptability of both U.S. and non-U.S. data;..."

    It is important to qualify the quality of materials used in non-U.S. data because although the procedure may be regarded to be equivalent to NCCLS standards, the quality or performance of the reagent may be different. The use of non-U.S. commercial MIC microdilution panels or Mueller Hinton agar (non-U.S. sources) or local antibiotic discs may involve reagents which have not fulfilled the FDA 510(k) regulatory process. Thus, the quality of these reagents would not be "substantially equivalent" to those clinically approved for use in the U.S. However, if reagents fulfill at least FDA standards, and are used per NCCLS procedures, the non-U.S.-generated data would be fully acceptable.

-   **These concerns are addressed in appropriate sections of the document.**

Section 1.4 (now Section 1.5)

2. "Data generated from within or from outside of the U.S. must meet the same high standards. For microbiologic data, NCCLS reference methods are to be used and so documented, including proper quality control tests. If NCCLS methods are not used, then sufficient data must be available to demonstrate the comparability of such methods to NCCLS reference methods."

    Qualify what is meant by "sufficient data."

    The FDA 510(k) Review Criteria for Assessment of Antimicrobial Susceptibility Devices, if used, can demonstrate substantial equivalence, which means in essence, non-U.S. materials and methods, especially those which are non-NCCLS, require this extensive process of validation. Again, this suggests that reagents and methods used should preferably conform to FDA standards, regardless of where they are geographically conducted.

-   **Wording has been added to Section 1.5 to further clarify this.**

Section 2.1 (now Section 4)

3. "Because different methods could be used to describe MIC endpoints, data showing the relationship or comparability of these endpoints for a relevant subset of representative organisms for NCCLS standard (i.e., broth and agar) methods and media should be presented."

    This requires more specific definition of the criteria for comparability judgement. Does this mean 200 or less challenge strains run in parallel with NCCLS broth and agar dilution to demonstrate, for example, MIC/MIC equivalence?

- **This is addressed in Section 4, which states "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 3.2.1."**

Section 3.2.1.2 (now Section 3.2.1.1)

4. Even for rapidly growing Gram negatives, nonfermentors such as *Pseudomonas, Stenotrophomonas* and *Acinetobacter* are known to be slower growing (longer critical times) than their enterobacteriaceae counterparts. Even they should be viewed separately, especially in the context of zones (disc) versus MIC regression analysis.

- **This section includes *Pseudomonas, Stenotrophomonas,* and *Acinetobacter,* since these are "organisms or groups of organisms that have separate interpretive criteria."**

Section 3.2.2

5. Quality control can vary significantly with regard to the source of discs and standards used for manufacture. More stringent specifications should be provided before a disc source could be considered acceptable for use.

- **Wording has been added to address this concern. ("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].")**

Sections 3.2.3, 3.2.4, and 3.2.4.1

6. Currently the practice of using MIC on the y-axis, i.e., dependent variable and zone on the *x-axis* independent variable is statistically incorrect. The MIC, i.e., the known quantitative variable, is the independent variable. The unknown or dependent variable is the zone!

- **Although this comment is statistically correct, the current standard among microbiologists is to present such data in this manner. Since this is a document geared towards the microbiology community, their standard for presentation of such data will be followed.**

Section 3.2.4.2

7. The acceptable VME (1.5%) and ME (3%) error rates should be defined relative to the sample size and the denominator. The current stringent FDA requirements for AST devices should be discussed in this context and the acceptable rates for both types of calculations should be specified.

- **Sections 3.2.4.2 and 3.2.4.3 have been changed to help clarify acceptable discrepancy rates. The intent of these sections is to address discrepancies between NCCLS standard broth and disk methodologies. It is not the intent of this document to address discrepancies which may occur between NCCLS standard methodologies and AST devices.**

Section 4

8. "The MICs may be determined by approved methods to establish parity of results." Please define what is meant by "approved." Does it include devices cleared by FDA to be "substantially equivalent" to NCCLS?

- **Standard reference methods refer specifically to those methods described in NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests*; M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically;* and M11— *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. Standard reference methods for the purposes of this document do not include "devices cleared by FDA to be substantially equivalent to NCCLS."**

9. "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 3.2.1." It would be important to include certain species and resistance mechanisms where agar-broth methods would be expected to differ.

- **Wording has been added in Section 3.2.1.1 to address this concern.**

Section 5

10. "…one or more of the alternative methods to the agar dilution…" Does this imply FDA-cleared, NCCLS substantially equivalent devices?

- **Standard reference methods refer specifically to those methods described in NCCLS documents M2—*Performance Standards for Antimicrobial Disk Susceptibility Tests;* M7—*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically;* and M11— *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*. Standard reference methods for the purposes of this document do not include "devices cleared by FDA to be substantially equivalent to NCCLS."**

Section 6.1

11. The currently recommended NCCLS QC strains have serious limitations as useful candidates for QC, especially for newer drugs where they may be especially susceptible. The need to add more "intermediate" QC strains, i.e., those that give steep log dose-response curves, must be addressed.

- **Wording has been added to this section to discuss situations where current "NCCLS-recommended QC strains" appear to be inadequate.**

Section 7

12. "When the subcommittee makes a change that will result in a discrepancy, the manufacturer of the product will be encouraged to submit the data upon which the change is made to the appropriate regulatory authority..." Does this mean the manufacturer of the drug, or does it also include the device manufacturer?

- **This refers to the manufacturer of the antimicrobial.**

13. If NCCLS changes FDA-approved criteria, why should NCCLS criteria have precedence in a judicial process whereby the manufacturer is now encouraged by NCCLS to submit more data to FDA? Whose criteria would stand up in a court of law?

Currently for AST device manufacturers, if the NCCLS and FDA criteria differ, by law, the manufacturer must use FDA criteria in its labeling. This is equally confusing for the laboratory end-user and creates a dilemma all around.

It is important to resolve this issue in principle and define FDA's precedence relative to NCCLS, as clinical testing ultimately impacts patient care. Regulatory requirements and consensus recommendations are two completely different issues.

- **This section is not meant to imply that NCCLS breakpoints have "precedence" relative to the FDA. The drug manufacturer is "encouraged" to submit to the FDA the data presented to NCCLS. This will give the FDA an opportunity to review such data and determine if a product's label should be changed to agree with NCCLS.**

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).** The document discusses a protocol for the development of primary and secondary reference lots of disk diffusion susceptibility testing medium.

M7-A5   **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Fifth Edition (2000)**. The document discusses reference methods for the determination of minimal inhibitory concentration (MIC) of antimicrobial agents with bacteria that grow aerobically, as well as broth macrodilution, broth microdilution, and agar dilution methods.

M11-A4   **Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Fourth Edition (1997)**. 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.

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.

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[*] 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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