Guidance for Industry and for FDA Reviewers

Guidance on Review Criteria for Assessment of Antimicrobial Susceptibility Devices

Draft Guidance – Not for Implementation
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U.S. Department of Health and Human Services
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Microbiology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

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Preface

Public Comment:

For 90 days following the date of publication in the Federal Register of the notice announcing the availability of this guidance, comments and suggestions regarding this document should be submitted to the Docket No. assigned to that notice, Dockets Management Branch, Division of Management Systems and Policy, Office of Human Resources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852.

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Guidance[1] on Review Criteria for Assessment of Antimicrobial Susceptibility Devices

1. INTRODUCTION

1.1. Definition

The generic type device is intended for use in clinical laboratories as an *in vitro* test for determining resistance of bacteria from isolated colonies to antimicrobial agents.

1.2. Purpose

This draft guidance document describes a means by which fully automated short-term incubation cycle antimicrobial susceptibility devices may comply with the requirement of special controls for class II devices, if that device is reclassified. Designation of this guidance document as a special control means that manufacturers attempting to establish that their device is substantially equivalent to a predicate device must demonstrate that the proposed device complies with either the specific recommendations of this guidance or some alternative control that provides equivalent assurances of safety and effectiveness. Fully automated short-term incubation cycle antimicrobial susceptibility devices remain subject to premarket approval unless and until reclassified by FDA.

The purpose of this document is to ensure well-standardized, reliable, and reproducible tests for determining the *in vitro* susceptibility of infectious bacteria. Clinically, an *in vitro* antimicrobial susceptibility test is useful for therapeutic guidance whenever the susceptibility of a bacterial pathogen may be unpredictable or appears appropriate. Additionally, susceptibility testing in a relevant format may also be indicated in studies of the epidemiology of resistance and in studies of new antimicrobial agents. There is no intent to include the evaluation of anti-mycobacterial, anti-viral, or anti-fungal agents in this document.

This document is an adjunct to the CFR and other FDA guidance documents for the preparation and review of 510(k) submissions. It does not supersede those

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[1] This document is intended to provide guidance. It represents the Agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

publications, but provides additional clarification on what should be provided before FDA clears a device for marketing. The primary reference for the information contained in a premarket notification (510(k)) for a medical device is found in 21 CFR 807.87. Antimicrobial susceptibility testing devices are regulated under CFR 866.1640 and 866.1620. Labeling for *in vitro* devices is addressed in 21 CFR 809.10.

Substantial equivalence to a legally marketed device is established with respect to, but not limited to, intended use, design, energy used/delivered, materials, performance, safety, effectiveness, labeling, and other applicable characteristics. A determination that the device is substantially equivalent to a legally marketed predicate device is based on the performance of the device in comparison to the National Committee for Clinical Laboratory Standards (NCCLS) referenced method.[1,2,3,4]

The intent of this document is for premarket recommendations. Each manufacturer is responsible for complying with the 21 CFR 820 Quality System Regulation for Class II or Class III devices, which includes Design Controls and Corrective and Preventive Action.

2.   BACKGROUND

Laboratory procedures used for determining resistance or susceptibility of bacteria to different antimicrobial agents have developed over the past five decades to reach the level of sophistication known today. Historically, there have been two general principles applied to susceptibility testing, i.e. dilution and diffusion procedures. There are other manual methods based on modifications and refinements of older techniques such as gradient diffusion. Because susceptibility testing yields results that are antimicrobial agent/organism/methodology dependent, broad-based voluntary consensus agreements on methodology and interpretive categories supported by some degree of regulation were implemented. The NCCLS is the major organization in the United States to establish voluntary standards or guidelines for standardizing and maintaining performance of laboratory tests including susceptibility tests. A system has been established for continual assessment and upgrading of the recommendations and additional test criteria for new antimicrobial agents and older agents particularly when resistance emerges. A separate subcommittee was established in 1986 to outline the specific information that is needed for developing *in vitro* susceptibility testing criteria and is now used within the pharmaceutical industry.[4]

The NCCLS standard reference methods are based on 16-24 hours of incubation for aerobic bacteria and 48 hours for anaerobes. Because earlier results may provide clinical advantages, a number of manufacturers have developed automated procedures designed to

generate results more rapidly, generally by the use of shortened incubation times (<16 hours). The results of reference overnight (16-24 hours of incubation) tests should also be accepted as standards for evaluating methods with a shortened incubation because: (i) all accepted reference and standard tests use 16 to 24 hour incubation, (ii) the knowledge and experience on laboratory-clinical correlation has been based on 16 to 24 hour incubation tests, (iii) where discrepancies have occurred, they have most often involved failure of shortened incubation procedures to detect bacterial resistance. Failure of *in vitro* tests to detect *in vivo* bacterial resistance has been shown to be clinically significant.

A susceptibility result may suggest that an uncomplicated bacterial infection can be effectively treated if *in vitro* tests indicate that the bacterial isolate is susceptible to the antimicrobial agent selected. The inability of a new device to determine a susceptible result for an organism that is susceptible to the antimicrobial agent being tested is considered a major error (see Section 4 for definitions). In this case, if the *in vitro* result shows resistance, the antimicrobial agent may not be made available for treatment when in fact it could be an effective choice. Conversely, the inability to detect resistance is assessed by the "very major error rate" and therapy with that antimicrobial agent may lead to treatment failure, particularly for serious infections or altered host conditions. *In vitro* susceptibility tests are of greater clinical value if they are accurately able to detect resistance, whether the mechanism of resistance is intrinsic, genetically acquired or selected during therapy.

Resistance to antimicrobial agents can generally be classified into four basic mechanisms: (i) production of antimicrobial-inactivating enzymes; (ii) substitution of antimicrobial-insensitive targets; (iii) alteration in the target site; and (iv) decreased drug entry. The time needed for expression of resistance varies with different antimicrobial/organism combinations and the respective resistance mechanism involved. The delay of expression of resistance can range from one to many hours. Studies comparing results of shorter incubation test results with conventional 16 to 24 hour incubation methods have documented the difficulties of detecting delayed resistance expression. Manufacturers of devices with shortened incubation periods have adopted a variety of strategies to bring these results as close to conformity as possible with results of the reference methods as recommended by the NCCLS. Examples of these strategies include: the use of higher concentrations of bacteria in the inoculum, adjusting media to optimize resistance detection, and computer assisted reading determinations and adjustment of results for some antimicrobial/organism combinations. At present, however, there is no NCCLS reference standard utilizing < 16-hour incubation.

Comparison to the reference method is used to establish equivalency for all commercial devices for determining *in vitro* susceptibility results. There are many variables when performing an *in vitro* susceptibility test, all of which should be in control before results can be compared to the reference method results. This would include, but is not limited

to, all manufacturer recommended inoculation preparation methods and interpretation of results (i.e., turbidity standard inoculation preparation method/manual reading, turbidity standard inoculation preparation method/automated readings, direct colony suspension method/manual readings, etc.) that the device labeling recommends. See Attachment 1 for recommendations.

3. **TYPES OF DEVICES AND PREDICATE DEVICES**

   3.1. **Types**

   The types of commercial devices FDA has reviewed are based on the following methods/technologies:

   3.1.1. Disk Diffusion - These are paper disks containing defined contents of antimicrobial agents used in disk diffusion susceptibility tests to determine a qualitative susceptibility category for bacteria after 16 to 24 hour incubation. The test procedure is based on the method described by Bauer et al. and is commonly called the Bauer - Kirby method.[6] Refer to the NCCLS approve standard: M2—Performance Standards for Antimicrobial Disk Susceptibility Testing[1] for specific details of the test methodology.

   3.1.2. Minimal Inhibitory Concentration (MIC) Systems - These systems utilize either broth or agar and may be marketed in varying concentrations of antimicrobial agents, in the form of four or more serial, two-fold dilutions of an antimicrobial in a frozen, lyophilized, or dehydrated microdilution format for broth microdilution tests, in agar plates for agar dilution procedures, or as a predefined gradient of antimicrobial agents on a plastic strip for testing on agar plates. They include a minimum of two dilutions below the breakpoint in order to assess developing resistance for epidemiology using trending and tracking patterns. These devices use the traditional non-automated 16-24 hour incubation period (overnight incubation) and provide quantitative MIC results.

   3.1.3. Dilution Breakpoint System - These systems are manufactured in the same format as the full MIC system; however only 1-3 concentrations of each antimicrobial agent are included. These concentrations are based on the FDA/NCCLS interpretive categorical MIC breakpoint for each antimicrobial agent. Like the disk diffusion test, the dilution breakpoint system yields qualitative (category) susceptibility results, i.e., susceptible (S), intermediate (I), or resistant (R).

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3.1.4. Automated systems and non-traditional systems - With the advent of new technology, computers, sophisticated optical scanning devices, and available computer software, new techniques for deriving susceptibility results have been developed. This category includes any system which generates an *in vitro* result using automated technology and/or based on non-traditional methods, e.g., devices using shortened incubation periods (< 16 hours), disk elution techniques, algorithmically derived growth rate comparisons, and the detection of microbial growth by fluorogenic compounds and redox markers. These systems may generate MIC results or susceptibility category results.

3.1.5. Genotypic methods - Determination of the presence or absence of resistant genes. Although not expected to generate MIC results, a comparison is made to a phenotypic result.

3.2. Predicate Devices

The following is not all-inclusive, but is meant to provide some examples of predicate devices that may be appropriate for susceptibility test systems that require a premarket notification:

3.2.1. Antimicrobial disks for the Disk Diffusion Method. These are 510(k) submissions; however performance data for these devices are limited to a labeling review by the Division of Clinical Laboratory Devices (DCLD) as stated under CFR 809.10. The scientific evaluation for these disks is performed by the Center for Drug Evaluation Research (CDER) which regulates antimicrobial agents under 21 CFR Sections 430, 431 and 460, therefore the In Vitro Diagnostic (IVD) manufacturer should only provide, for review, the labeling with the FDA approved interpretive criteria and Quality Control recommendations. The information on the performance should be maintained at the manufacturing facility (Attachment 1).

Predicate device:
- Becton Dickinson – BBL disks

3.2.2. Microdilution MIC or Breakpoint (16-24 hour incubation). These systems may be manual, semi-automated, or fully automated and may use methods for endpoint detection, which rely on fluorometric, spectrophotometric, or colorimetric detection of endpoints.

Predicate devices:
- Dade MicroScan Inc. – MicroScan

- MicroMedia Systems
- Pasco Laboratories
- Trek Diagnostics Systems Inc. - Sensititre

3.2.3. Any instrumented device that is not based on a traditional 16 - 24 hour incubation diffusion or dilution method.

Predicate devices:
- bioMérieux Vitek Inc. - Vitek Systems
- Dade MicroScan Inc. - Rapid Fluorogenic Panels

3.2.4. Other nontraditional formats, which use 16 to 24 hour incubation, but employ dilution schemes and formats other than broth or agar dilution (predefined antibiotic gradients).

Predicate device:
- AB Biodisk – Etest®

4.   PERFORMANCE CRITERIA

A comparative study should provide data on the ability of the system and each antimicrobial agent included in the test format to determine susceptibility test results when compared to results obtained by an NCCLS reference method. The reference method that is recommended for comparative testing is a MIC (broth or agar) method.[2,3] Refer to the most recent appropriate NCCLS standard for specific technical details on the type and procedure of the reference method. For best results there should be no deviation from the NCCLS reference method. A descriptive protocol for the comparative study should be in place at each testing site for both the reference method and test method. The protocols should include the exact procedure to follow for both methods including the media recommended, methods of inoculation, incubation conditions, etc. Comparative data are also recommended for all methods of inoculation (growth method, direct colony suspension or any other variations to be recommended in the procedural instructions of the package insert), incubation conditions, or reading (visual vs. automated). This is especially helpful for certain organism/antimicrobial agent combinations that are affected by variance in inoculum, and have growth patterns that may be interpreted differently when read manually or automatically (See Attachment 1). If the package insert recommends a different method of inoculation or additional dilutions of the inoculum suspension for certain groups of organisms (e.g., *Proteus sp.*), these should also be evaluated.

For suggested data presentations see the appropriate Tables 1-4. The following explanations are offered to assure uniformity in data submission.

4.1. **Essential Agreement**

Essential agreement (EA) analysis is applied to devices with a full (minimum of 4) two-fold dilution MIC format or devices using antimicrobial gradients covering a range of concentrations that include at least one dilution above and below the interpretive breakpoint values. EA occurs when the device result agrees exactly with or within ± one two-fold dilution of the reference result. Suggested EA data presentation for all strains tested can be found in Table 2 and Table 2A.

4.2. **Category Agreement**

Category agreement (CA) is assessed for all devices based on the interpretation only. CA occurs when the device and the NCCLS reference interpretive result agree (susceptible, intermediate, and resistant). The FDA interpretive criteria should be used. Suggested CA data presentation for all strains tested can be found in Table 2 and Table 2A.

4.3. **Error Rate Determination**

Determine minor, major, and very major error rates for all organisms tested using the following criteria:
- Minor error - reference result is R or S and device result is I; reference result is I and device result is R or S.
- Major error - reference result is S and device result is R.
- Very major error (VME)- reference result is R and device result is S.

Suggested data presentation for error rates can be found in Table 2 and Table 2A.

5. **REFERENCE METHOD**

Microbroth or agar dilution (MIC) methods – Prepare all reference panels/plates according to the most recent appropriate NCCLS standards. For best results there should be no variance from the recommended method, inoculation preparation, incubation, or reading as recommended in the appropriate standard. Special care should be taken in the preparation of these panels since the reference result will be used in the final analysis. The reference microbroth plates or agar plates should contain two-fold dilutions of the antimicrobial agent for which FDA clearance is sought. The selection of dilutions should include the FDA/NCCLS interpretive breakpoint concentrations with one two-fold dilution above and several below the breakpoint concentrations to provide a range for evaluating the results. For example, if interpretative criteria are: S as $\leq 1\mu g/mL$, I as $2\mu g/mL$, and R as $\geq 4\mu g/mL$, then a typical panel would include serial two-fold dilutions

between 0.25 µg/mL and 8 µg/mL. Including dilutions more than one dilution above the resistant concentration provides little evaluable data but dilutions below the susceptible category may be evaluable for trending of specific organisms. The selection of a range of concentrations will allow for more evaluable test results but will not limit the final product to include all concentrations tested in the clinical trial. The final product concentrations should be stated in the submission.

6.   **ORGANISMS SELECTED**

The organisms selected for the comparative study should be representative of those for which the antimicrobial agent has clinical indications and are within its spectrum of activity as shown in the Microbiology and Indication and Usage Sections of the approved pharmaceutical antimicrobial agent package insert[7] and/or the NCCLS most recent standard M100[4], Table 1 "Suggested Groupings of U.S. FDA-Approved Antimicrobial Agents That Should be Considered for Routine Testing and Reporting of Nonfastidious Organisms by Clinical Microbiology Laboratories". This would include those organisms for which clinical efficacy and *in vitro* activity have been demonstrated. A 50% susceptible, 50% resistant distribution within species is considered an ideal situation although this seldom occurs in the clinical setting. The lack of resistant strains should be addressed in the labeling (see Section 13 for labeling considerations). Organisms with known mechanisms of resistance should be included in the comparison study. Inclusion of organisms for which there is no approved indication for use should be avoided. Repeat isolates from the same patient should not be used. There are situations where the spectrum of activity of the antimicrobial agent has not been demonstrated in bacteriological and/or clinical failures in clinical studies. In this instance the antimicrobial approval process results in only a susceptible breakpoint and any result other than susceptible should be referred to a reference laboratory for further analysis.

If the antimicrobial agent approved labeling includes fastidious organisms (e.g., streptococci, haemophilus etc.), and there is an NCCLS approved standard methodology, with FDA interpretive criteria; the recommendations for evaluation are similar but the numbers necessary for review should be statistically relevant (see Attachment 1). The recommendation for testing rare isolates for which an antimicrobial agent may be approved for use should be discouraged since sufficient data for the appropriate organism is usually difficult to acquire in a clinical setting. The routine testing of these is usually not necessary and is best left for testing by reference laboratories. Refer to the recommendations in the NCCLS Approved Standard[4], Table 1 for relevant testing and reporting. Comments such as the following are included in this document: "Susceptibility testing of penicillins and other-β-lactams approved by the FDA for treatment of Group A and Group B streptococci is not necessary for clinical purposes and need not be done routinely, since as with vancomycin, resistant strains have not been recognized."

Source of organisms should be comprised of the following:

6.1. **Clinical Isolates**
Fresh and recent clinical isolates – testing should include organisms isolated from clinical specimens at the test site during the study, that have never been frozen and have been on agar for less than 7 days. Organisms for which the antimicrobial agent being tested has been shown to have no activity could be tested in the random clinical setting of the comparative study, but should not be specifically selected for testing. The numbers recommended are included in Attachment 1.

6.2. **Challenge**
The selection of these isolates should be based on organisms with clinical utility and within the spectrum of activity of the antimicrobial agent as shown in the Microbiology and Indications for Usage Sections of the approved labeling with particular attention to the organisms listed in the Indications for Usage Section. This should not include organism groups for which the antimicrobial agent being tested has been shown to have no activity.

6.2.1. Stock – any organism isolated from a clinical specimen at the test site and stored for > 7 days. These are usually saved due to some unusual susceptibility pattern and/or mechanism of resistance and may be used to enhance the resistant organisms from the clinical evaluation but should not comprise more than 50% of any group of organisms or the total number tested. Each site will have its own selection for testing on the reference method and the new device.

6.2.2. Reference strains – The selection of such isolates should favor resistant strains and include organisms for which the antimicrobial agent's MIC is close to the intermediate breakpoint. (If interpretive criteria are S ≤ 4, I= 8, R >8, organisms with known results in all dilutions in the range of 0.25 μg/mL to 32 μg/mL could be included). A source for these would be the CDC or a reference laboratory that collects and characterizes strains based on their resistance patterns or particular uniqueness. The IVD manufacturer may add to this set a selection of organisms that were not used in the developmental stages of the antimicrobial agent algorithm for susceptibility testing, but should be clinically indicated organisms for *in vitro* testing as stated in the FDA approved pharmaceutical antimicrobial package insert. If the organisms have been characterized phenotypically using the NCCLS reference method, this should be used as the "expected result". If the "expected result" is not known the isolates should have multiple MIC testing performed using the reference method only, prior to

entering into this study to determine the mode or "expected result". This testing can be performed internally or at an outside site using the NCCLS recommended methods. Only the reference method results should be used to determine the mode, the new device should not be performed at this time. These strains are meant to challenge the device to reliably detect intermediate and resistant strains. Since all challenge isolates will have known expected values (reproducibly obtained using the reference method), the testing site need only generate results using the device under evaluation. The set should be coded, the results masked and sent to one site for performance on the test device only. For suggested data presentations see Table 2.

7. QUALITY CONTROL

For methods and recommended quality control organisms for testing, refer to the appropriate NCCLS approved standard[1,2,3] or the most recently NCCLS approved M100 supplement. The FDA approved pharmaceutical antimicrobial agent labeling will provide the expected quality control range for each organism. On-scale dilutions for the recommended NCCLS quality control organism range should include 1 two-fold dilution below the lowest dilution in the range and 1 two-fold dilution above the highest dilution in the range. For example, if the expected range is 1 - 4 ug/mL, the reference panel should include 0.5 - 8 ug/mL. For rare instances where the quality control organisms are significantly above or below the interpretive dilutions, on scale results may not be possible and additional manufacturer selected organisms should be recommended. If additional NCCLS recommended quality control organisms would be expected to give on-scale results for the final product format, they should also be tested each day of the comparative studies.

7.1. Reference Method Quality Control
The purpose of performing daily quality control on the reference panel is to ensure that the reference method is in control for each day of comparative testing. Daily quality control testing should adhere to the NCCLS reference standard method and include; (i) all recommended quality control strains for that antimicrobial agent, (ii) manufacturer non-NCCLS recommended quality control strains, (iii) inoculum colony counts, and (iv) purity of the organism.

7.2. Test Device Quality Control

Quality control should be performed daily on the test device and include (i) all recommended quality control strains for that antimicrobial agent, (ii) manufacturer non- NCCLS recommended quality control strains, (iii) inoculum colony counts, and (iv) purity of the organism. These should be performed with any procedural modifications such as the use of a growth enhancers, additional methods of inoculation (growth method, direct colony suspension) and reading differences (manual vs. automated).

Quality Control Recommendations:

7.2.1. Selection of organisms – Test all recommended quality control strains daily on the test device to ensure that the user will be able to achieve the FDA/NCCLS recommended results in the ranges for that organism. The IVD manufacturer may select additional isolates for quality control if the NCCLS recommended strains do not fall within the range of the test device.

7.2.2. Inoculum density check – The purpose of the inoculum density check is to ensure that the final test concentration of an organism will result in the concentration recommended in the reference procedure (broth dilution of approximately 5×10^5 CFU/mL) and the test procedure. Some antimicrobial agents are affected by variance in the final inoculum and performance may be compromised. This quality control procedure should be performed as recommended in the NCCLS M7 Approved Standard on all methods of inoculum preparation that are to be recommended for the test procedure. Ideally this would include all quality control isolates daily, isolates for precision testing, and 10% of fresh isolates. This should provide information on all organisms for which the antimicrobial agent has approved indications. It is especially important to perform the colony counts directly from the inoculated panel, in the case of a broth dilution test, to ensure the time period from the initial inoculum adjustment and the final time of inoculation has not adversely affected the inoculum density. In the case of a non-broth device, a colony count determination should be performed just prior to conducting the test.

7.2.3. Purity check – The purpose of the purity check is of particular importance in broth susceptibility testing to recognize mixed cultures that may go undetected when performing broth dilutions. As recommended in the NCCLS M7, these should be performed after inoculation of the final test panel. Purity check plates should be performed on all isolates tested from each test device.

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7.2.4. Recommended number of test results – There should be a minimum of 20 test results per site with only one test result performed daily.

8. BIAS/PRECISION TESTING

Bias is defined as the "deviation of results from truth" which in devices is usually a systematic (non-random) tendency of any factor associated with the design, conduct, analysis, or interpretation of results of a clinical laboratory study to make an estimate of the device's performance different from its true value. Precision/reproducibility is the certainty with which a measurement or estimate is made due to random errors.

Reproducibility should be performed on 25 selected organisms. These isolates should have multiple test results only on the reference method prior to entering into this study to determine the mode or "expected result". The selection should include organisms for which the antimicrobial agent is intended for testing with known results in the interpretive range with an additional concentration allowed on each end or the range (range of 1 -32 μg/mL when the interpretive criteria are: S $\leq$ 4, I = 8, R >8). These may be selected from the challenge isolates. Isolates should be coded, the results masked and sent to three sites for testing: one time at each site on the test system only. Results should be sent back to the manufacturer for uncoding and recording on the data sheet (Table 3) for evaluation and submission to the FDA.

The testing of more than one antimicrobial agent during a clinical trial may result in the testing of isolates which are not included in the Microbiology and Indications for Usage Sections of the FDA approved labeling for that antimicrobial agent. These should also be included in the data in the format recommended in Table 3 but should not be considered in the number of appropriate isolates recommended for testing of each antimicrobial agent. This will provide results for more than the minimum number of isolates but may not provide much additional data if they are off-scale. Nevertheless, all isolates tested on each antimicrobial agent should be presented in table format.

The same reproducibility/precision testing should be performed for all recommended methods of inoculum preparation and/or reading variations recommended in the package labeling (Attachment 1).

9. CLINICAL TESTING

Performance from the clinical studies should be representative of the finished product, as intended for use in the clinical laboratory. It is not uncommon for IVD manufacturers to include several antimicrobial agents on one device or even multiple devices at the time of clinical testing. This is acceptable if it does not interfere with the routine use of the test

(multiple devices should not be inoculated from the same initial inoculum broth if the time interval from initial inoculum preparation and device inoculation will be compromised) or impede the number of appropriate test results for the evaluation of each antimicrobial agent. If multiple antimicrobial agents are included on a test device, all isolate test results should be presented for each antimicrobial agent. The review process concentrates on the organisms for which the antibiotic has approved indications for use. The additional testing results may also be evaluated.

9.1.   **Reference Method**

As described in Section 5, the reference method should be performed on all clinical isolates, and quality control isolates daily. It is not necessary to perform the reference method on isolates with known expected results e.g., challenge, precision isolates.

9.2.   **Test Methods**

9.2.1.   Broth or agar dilution format

Regardless of the final marketed format of the MIC device, the comparative test panel should match the reference panel full dilution format. The testing of a full range of dilutions, when cleared by the FDA, will allow the manufacturer to choose selected dilutions in their final product format. The testing of a full range of dilutions will also provide more test results in an evaluable range. In order to market a MIC device the antimicrobial agent concentrations selected should include at least 4 two-fold dilutions that include the interpretive criteria range. It is important to include one concentration above the resistant interpretative value for determining essential agreement evaluations.

9.2.2.   Nontraditional devices

For devices with or without computer/instrument assisted result interpretation, (e.g., Vitek, Etest) the final device format should be tested and compared to a broth or agar dilution reference method with full two-fold dilutions as previously outlined.

9.3.   **Test Sites**

The following information should be submitted:

9.3.1.   Name and address of test site.

9.3.2. Financial interests and arrangements of clinical investigators (Federal Register/Vol. 63, No. 21/Monday, February 2, 1998).

9.3.3. Name and telephone number of principal investigator.

9.3.4. Testing protocol to contain the type of quality control recommended, procedures for the reference and test method, including the procedures for the method (s) of inoculation, media recommended, conditions of incubation, recommendations for the selection of organisms, etc.

9.4. Testing

9.4.1. Clinical Isolates – Fresh and/or recent clinical isolates as described in Section 6.1, to be set up on both the reference and test device. For minimal numbers see Attachment 1.

9.4.2. Stock Strains – as described in Section 6.2.1, to be tested in both the reference and test method.

9.4.3. Challenges Strains – as described in Section 6.2.2, challenge strains are tested at only one site on the new device only. It is not necessary to test using the reference method.

9.4.4. Quality Control

9.4.4.1. Selection of quality control isolates as in 7.2.1 - All selected quality control strains should be tested each comparative test day on the reference method and the test method. If more than one quality control strain with "on-scale" results is used and, if on any given day during the comparative testing, one strain has results that are outside of the expected range in the reference method, the quality control strain should be repeated. If the repeat testing is within the expected quality control range, the device data from the previous test day is acceptable and can be included in the comparative summary tables. However, if the repeat testing result is still outside of the expected range, the data from the previous day's testing is invalid and should be repeated. If multiple quality control strains have results that are outside of the expected results in the reference method on any test day, data from that test day should not be included in the submission. Strain testing should be repeated in both reference

and test devices until quality control results in the reference panel are in control. A minimum of 20 results (once daily) should be obtained for each quality control strain at each site.

9.4.4.2. Inoculum density check as described in 7.2.2 should be performed on quality control isolates daily (both reference and test method), on all reproducibility strains and on 10% of fresh isolates. Quality control strains and reproducibility strains should include the performance of colony counts for all variations of the inoculum preparation for the test method.

9.4.4.3. Purity checks as described in 7.2.3 should be performed on all reference and test devices.

9.4.5. Bias/Precision Testing – The testing should be performed as described in Section 8 on a minimum of 25 isolates on the test method only. Colony count testing on each isolate should be performed.

10. **REPEAT TESTING**

Repeat testing is an option for the determination of a systematic error. The FDA realizes that the reference method may have occasional errors in the clinical evaluation of the fresh isolates but the evaluation of the challenge and reproducibility results will be compared to an expected value and repeat testing would not be necessary.

11. **PRESENTATION OF RESULTS**

11.1. **Clinical-fresh and stock**

Results from comparative testing should be presented as outlined in Table 2 for full MIC devices and for nontraditional (non-MIC) devices including breakpoint options. Using this format, data should be submitted in separate tables for each individual test site. Summary data should be presented as in Table 2A for all sites combined.

A list of organisms tested should be presented in chart format by site, designating the numbers that are stock and fresh.

11.2. **Challenge strains**

Results from challenge strains may be presented as in Table 2 and Table 2A with the comparison to the expected value.

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11.3. **Quality Control**

Quality control strains should be presented as in Table 1 and have a minimal of 20 per site.

11.4. **Bias/Precision**

Bias/precision should be presented as in Table 3 for all methods of inoculation, methods of reading and any other factors that may affect bias or precision. An overall summary should be included.

12. **EVALUATION OF DATA**

12.1. **Fresh and Stock**

Calculate the EA and CA as described in Table 2 and Table 2A. All results are to be included in Table 2, but only those listed in the Microbiology and Indications for Usage Sections of the FDA pharmaceutical approved labeling are to be included in Table 2A. These tables are used to identify the evaluable test results based on the interpretative criteria of the antimicrobial agent and the concentrations tested on both the reference panel and the test panel. Tables 5 and 6 provide guidance as to the recommended maximum error rate and the minimum acceptable EA rate.

Particular attention will be paid to the organisms with clinical utility and within the spectrum of activity of the antimicrobial agent as shown in the Microbiology and Indications for Usage Sections of the FDA pharmaceutical approved labeling. If the essential and category agreements for the organisms that are listed in the FDA approved antimicrobial agent labeling are unacceptable, additional testing may be necessary prior to clearance or a limitation statement may be appropriate until further data can be collected. The use of Limitation Statements are not recommended if the errors occur with organisms included in the Indications for Usage Section of the FDA approved antimicrobial agent labeling; further testing may be necessary. A limitation statement is not necessary for organisms (genus or species) for which the antimicrobial agent has no clinical utility and/or is inactive against, and has not been approved for use by the FDA (i.e., cefdinir with Enterococcus and Pseudomonas).

The overall performance of the device in the clinical testing will also be evaluated for the number of relevant resistant isolates tested. The challenge and stock may be of particular importance. In the event that less than a statistical relevant number

of resistant isolates (Table 5) were tested with a similar mechanism of resistance for the antimicrobial agent, a statement would be included in the labeling (Section 13). If and when resistance develops, additional testing is needed before the limitation statement can be removed from the labeling. If, at the time of testing, there is only an interpretive category of susceptible provided by the FDA/NCCLS, a limitation statement as stated in section 13.5 is appropriate as well as a recommendation in the labeling to submit all isolates with a non- susceptible category to a reference laboratory for confirmation.

Refer to Tables 5 and 6 for agreement as a function of the number of strains tested. The following would be considered acceptable performance for the clinical data for all organisms with an approved indication for use:

12.1.1. Percent essential and/or category agreement > 90 %.

12.1.2. A major error (ME) rate based on the number of susceptible strains tested of < 3%.

12.1.3. A very major error (VME) rate based on the number of resistant strains tested. The numbers recommended are included in Table 5 with proposed statistical criteria for acceptance that include an upper 95% confidence limit for the true VME rate of $\leq$ 7.5% and the lower 95% confidence limit for the true VME $\leq$ 1.5%.

12.1.4. Growth failure rates in the system exceeding 10% for any genus or species tested should be listed. Any specific group that had a no growth rate >10% would be contraindicated since the results, if obtained, might be unreliable.

12.2. Challenge data

Using the suggested chart format (Table 2), the data can be used to calculate % EA, and/or CA by organism group and overall for the challenge data alone. The challenge data should also be evaluated with the fresh and stock isolates and presented in summary format (See Table 2A). The use of the challenge data results will allow for the evaluation of organisms that have been selected to have results closer to the breakpoint interpretation and provide an assurance that an adequate number of resistant isolates is available for evaluation. This should also enhance the data around the critical interpretive range.

12.3. Quality Control Expectations

Any day the reference method has unacceptable performance the clinical testing data for that day should not be used. The test method results for the recommended quality control isolates should be within the expected range 95% of

the time. In the rare event that the device expected result does not agree with the NCCLS recommended ranges for that antimicrobial agent, additional data following NCCLS M23[5] "Development of *In vitro* Susceptibility Testing Criteria and Quality Control Parameters" recommendations, should be submitted to demonstrate the bias/precision of the newly requested range plus supportive data that all parameters of the test method are in control. The data should include all Quality Control parameters, e.g., colony counts. With appropriate data, a statement would be included in the product insert.

12.4. Bias/Precision

If the results of any bias/precision study from all test sites for any antimicrobial agent show less than 95% (+/- 1 dilution) as compared to the expected result, the device cannot be recommended for a substantial equivalence decision. If there is a bias/precision problem with an additional methodology e.g., inoculum preparation, automated reading, a limitation similar to that recommended in Section 13 would be included in the labeling. Results should not be reported. This type of limitation could apply if additional recommendations of the procedure (method of inoculum, reading method etc.) were considered unacceptable while another was acceptable.

13. LABELING CONSIDERATIONS

Labeling should conform to 21 CFR 809.10. The product insert should be considered a living document with the possibility of the addition of newer antimicrobial agents. Charts should be utilized when possible for ease of adding antimicrobial agents, limitations and performance characteristics. The user should always be kept in mind when considering presentation and organization of the ongoing additional information.

13.1. Intended Use Statement

The Intended Use statement should clearly state:

13.1.1. If the assay is quantitative (MIC) or qualitative (breakpoints or disks).
13.1.2. If the assay is specific for certain organisms or contraindicated for certain organisms.
13.1.3. If the assay is to be used only with a special instrument.

> A typical intended use statement should read "ABC's system is intended for use for the *in vitro* quantitative or qualitative determination of antimicrobial susceptibility of rapidly growing aerobic non-fastidious Gram positive and Gram negative organisms utilizing the ABC automated system."

13.2. Format

Antimicrobials agents should be presented with concentration ranges and any abbreviations used. With multiple antimicrobial agents, a list of each final antimicrobial agent concentration to be included in the finished device should be included. This could be included under reagents in the labeling or on each package container if different for different devices.

State the interpretive criteria for each antimicrobial agent when testing a MIC or breakpoint format (S, I, R). The FDA/NCCLS interpretive criteria used in the evaluation should be clearly stated. The use of commercial systems provide results for all types of organisms that may be appropriate for some, but not all, of the antimicrobial agents provided on a test panel/system. For this reason, the interpretive criteria section should carry a statement similar to the following: [There are antimicrobial agents included in this panel/device/section that are not proven to be effective for treating infections for all organisms that may be tested. For interpreting and reporting results of antimicrobial agents that have shown to be active against organism groups both *in vitro* and in clinical infections refer to the individual pharmaceutical antimicrobial agent labeling. Alternately, refer to the most recent NCCLS M100 Performance Standard, Table 1 "Suggested groupings of U.S. FDA approved antimicrobial agents that should be considered for routine testing and reporting by clinical laboratories" and Table 2 "MIC Interpretive Standards"].

13.3. Performance Characteristics

Performance should be described in a paragraph stating the reference method used, number of sites, etc. The percent EA and/or CA with the NCCLS reference method for each antimicrobial agent from comparative testing should be stated in chart format. Results of bias/precision studies should also be included in either a chart format or a summary paragraph describing the studies and a statement that all results were acceptable at $\geq 95\%$.

13.4. Quality Control

The manufacturer should specify all recommended Quality Control strains whether NCCLS or other, and the expected results when tested with each antimicrobial agent.

13.5. Limitations of the Test

All limitations are included in the labeling. If the device has software driven interpretations, these same limitations should be incorporated. The following are some examples of limitation statements:

13.5.1. Recommend the use of an alternative method for testing prior to reporting of any results (if software driven, results should be blocked from reporting) when the spectrum of activity for any antimicrobial agent includes organisms with unacceptable i) very major error (VME) and/or ii) major error (ME) rate. Depending on the type of error and/or the group of organism affected, this may include additional testing prior to clearance.

13.5.2. In the event that sufficient resistance strains with an approved indication for use for the antimicrobial agent were not tested, a statement should be included in the labeling that states: "The ability of the ABC system to detect resistance to ("Antimicrobial") among the Enterobacteriaceae (or other organisms) is unknown because resistant strains were not available at the time of comparative testing".

13.5.3. If the results of any bias/precision study from all test sites for any antimicrobial agent shows less than 95% (+/- 1 dilution) as compared to the expected result, a limitation similar to the following should be included in the labeling: "The results of testing of ("antimicrobial") showed less than 95% agreement (+/- 1 dilution) to the expected result." Results should not be reported. This would apply if any recommended alternate methods of the procedure (method of inoculum, reading method, etc.) were unacceptable while another was acceptable. A particular antibiotic may not be cleared if the overall reproducibility is <95%.

13.5.4. Any specific organism group that had a no growth rate >10% should be recommended to use an alternative method for testing prior to reporting of any results (if software driven, results should be blocked from reporting) since the results if obtained might be unreliable.

14. REMOVAL OF LIMITATIONS

Additional testing should be performed to support the removal of any limitation included in the labeling as a result of unacceptable performance during the original clinical studies or post-market evaluations. These testing procedures are described below and detailed in

Attachment 2. The testing results should be submitted as a new 510(k) with a reference to the 510(k) number of the submission that recommended the Limitation Statement. If changes have been made to the device to alter the overall performance, the testing should include all organisms previously tested.

14.1. **Performance**

If essential agreement and/or category agreement were unacceptable, a comparative clinical laboratory study should be performed after final device modifications to address the problem to verify that performance is now acceptable. This testing should utilize reference and test devices similar to those from the original comparative study. The organism mix should concentrate on those groups/species that originally provided the unacceptable results but also include all groups that might be affected by the changes. The testing data should be reported in the formats outlined in the Tables. All quality control organisms should be ·tested each day of the comparative testing.

14.2. **Insufficient Resistant Strains**

A comparative clinical laboratory study should be performed to verify the detection of resistance in organisms with approved indications for use. This testing should utilize reference and test devices similar to those from the original comparative study. A special challenge set containing the resistant isolates and some susceptible strains may be substituted for fresh isolates. The testing data should be reported in the formats outlined in the Tables. All quality control organisms should be tested each day of the comparative testing.

14.3. **Bias/Precision**

If the bias/precision was <95%, a bias/precision study should be performed to verify that the test method is now acceptable. This study should involve the problematic organism(s) or procedural variation (alternate methods of inoculation, alternate reading procedures, etc.) which originally showed unacceptable results. Twenty to twenty-five strains should be tested at three test sites. The strains selected should include organisms for which the antimicrobial agent is intended for testing with known results in the interpretive range, with an additional concentration allowed on each end or the range. Any problematic organisms as determined in the original bias/precision study should be included. This testing should utilize test devices identical to those from the original comparative study. The new testing data should be reported in the formats outlined in Tables 3. All quality control strains should be included in the study. The poor bias/precision results of an alternate method of inoculation/reading may indicate additional

concerns with this particular procedure and additional challenge data may be performed. If the inoculum were determined to be a concern, evaluation of colony count data should be performed.

14.4. Quality Control

When quality control ranges did not match NCCLS acceptable ranges, a minimum of 20 replicates per site of each quality control strain should be tested on 3 lots of the test devices to verify that a quality control range now matches the acceptable NCCLS quality control range. This testing should be done at three sites over a minimum of three test days and each test device should be setup from a different inoculum suspension. The results of this quality control study should be reported in the format outlined in Table 1. Colony counts should be performed once on each test day using the NCCLS recommendations for sampling from the inoculated test device. If the recommendation is for additional methods of inoculation and/or reading, testing should be performed on all variables.

15. ATTACHMENTS AND TABLES

LITERATURE CITED

1.   National Committee for Laboratory Standards Approved Standard. M2. (most recent approved supplement) Performance Standards for Antimicrobial Disk Susceptibility Tests. NCCLS, Wayne, PA.

2.   National Committee for Clinical Laboratory Standards. Approved Standard. M7. (most recent approved supplement) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. NCCLS, Wayne, PA.

3.   National Committee for Clinical Laboratory Standards. Approved Standard. M11. (most recent approved supplement) Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. NCCLS, Wayne, PA.

4.   National Committee for Clinical Laboratory Standards. Approved Standard. M100. (most recent approved supplement) Performance Standards for Antimicrobial Susceptibility Testing. NCCLS, Wayne, PA.

5.   National Committee for Clinical Laboratory Standards. Approved Standard. M23. (most recent approved supplement) Development of *In vitro* Susceptibility Testing Criteria and Quality Control Parameters. NCCLS, Wayne, PA.

6.   Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. Antibiotic Susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966; 45: 493 - 496.

7.   Physicians Desk Reference. 47th ed. Oradell, NJ: Medical Economics Company, Inc.

ATTACHMENT 1: Recommendations for Antimicrobial Susceptibility Devices[a]

		MIC/BP Formats	Fastidious[b]	Additional methods of Inoculation/Reading[c]	Impregnated Disks[d]
Number of Sites (including 1 in-house)		3	3	3	1
Organisms	Fresh[e] Clinical/stock [f]	100/site	75/site	0	0
	CDC Challenge[g]	75/one site	50/one site	75/one site	0
Bias/Precision		25/site	25/site	25/site	0
Interpretive Breakpoint		FDA/NCCLS	FDA/NCCLS	FDA/NCCLS	FDA
Stability (3 lots)		Real time (on file)	Real time (on file)	Real time (on file)	Real time (on file)
QC Reference and Test Device Results	NCCLS Strains	20 results/site	20 results/site	20 results/site	100 results on file
	(Other Mfg. Recommended)	Optional	Optional	Optional	
	On-scale	At least 1	At least 1	At least 1	NCCLS organisms
	Inoculum density check[h]	QC, precision, fresh	QC, precision, fresh	QC, precision	
NCCLS Reference Method		MIC	MIC	MIC	Disk

a   See Tables 2, 5, and 6 for statistical numbers and evaluable results.

b   For Fastidious organisms such as Streptococcus, Haemophilus, anaerobes, etc. that have an NCCLS approved standard methodology, FDA/NCCLS interpretive criteria and Quality Control recommendations, refer to NCCLS approved standard M100[4] Table 1.   The routine testing of rare isolates such as Listeria is not recommended.

c   Minimal data to establish performance should be presented for each variations of the method of inoculation (growth method, direct colony suspension etc.), reading of results (manual vs. automated), or any other variance.

d   Labeling review performed only with data on file.

e   Fresh clinical isolates - an organism isolated from a clinical specimen and which has been on an agar plate for less than 7 days and never frozen.

f   Stock organisms - any organism from a clinical specimen which has been isolated greater than 7 days prior to testing or which has been stored in a frozen state. May not include organism for which the antimicrobial agent is not intended. Selection should be supplemental based on the listing in the FDA approved package insert and should not comprise more than 50% of the clinical isolates.

g   Challenge - CDC or reference laboratory source with known results to be tested on the test system. Organisms that are intended for the testing with the antimicrobial agent as stated in the pharmaceutical approved package labeling (microbiology section) should be selected for testing on the test device.

h   Inoculum density check should be performed daily on the QC isolates, on precision isolates, and 10% fresh isolates.

ATTACHMENT 2: Recommendations for the Removal of Limitations from Antimicrobial Susceptibility Devices[a]

Items		Performance	Insufficient Resistant Strains	QC Not match NCCLS	Bias/precision
Number of Sites		3[b]	1	3	3
Organisms	Fresh or Recent Clinical	100/site	0	0	NA
	Stock/challenge	75/site	as needed	0	
Reproducibility/Precision		NA	NA	NA	25/site
Quality Control		Daily	Daily	20 x 3 lots	Daily

a For Statistical evaluable numbers see Tables 2, 5 and 6
b one may be in-house

Note: If changes have been made to the device to alter the overall performance the testing should include all organisms previously tested, refer to Attachment 1.

TABLE 1: Example of Reporting Format for Quality Control Data

Antimicrobial agent: _____

QC Organism	Expected Result (NCCLS or Mfg)		Reference Panel Frequency			Test Device Frequency		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
E. coli ATCC 25922	.25 – 1.0 µg/mL	<.25				1	1	
		.25	14	18	14	4	14	14
		.5	6	2	6	4	5	6
		1.0				11		
		>1.0						
E. cloacae Ref 1611	2 – 8 µg/ml	<2						
		2				12	14	4
		4	14	15	12	2	6	5
		8	6	5	8	8		11
		>8						
Pseudomonas aeruginosa ATCC 27853	0.5-2 µg/mL	<.25			2			2
		.5	10	10	18		2	
		1	5	8	2	20	18	18
		2	5	2				4
		4						
Enterococcus faecalis ATCC 29212	4 – 16 µg/mL	2						
		4	18	2	18	20	12	6
		8	2	18	2		8	14
		16						

Performed daily with a minimum of 20 per site.

List all reference and test results including out of range results that required repeat testing.

TABLE 2: **Example fo Reporting Format for Clinical and Challenge Data**

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	<0.25	0.5*	1*	2* S	4* R	8	>16
<0.25	6		1				
0.5*	10	100	21	2			
1*		10	8	1			
2* S		6	11	1			
4* R							
>8							31
Evaluable Results*	16	116*	41*	4*			31

Organism: Staphylococcus aureus

Evaluation		
Overall EA	199/208	95.70%
EA based on evaluable results*	152/161	94.40%
CA based on interpretation		100%

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	<0.25 S	0.5* R	1*	2*	4*	8	>16
<0.25 S	29						
0.5* R							
1*							
2*			1	1		1	
4*					1		
>8				7	12	12	52
Evaluable Results*	29		1*	8*	1*ª, 13	1*ª, 13	52

Organism: Staphylococcus epidermidis

Evaluation		
Overall EA	90/98	91.80%
EA based on evaluable results*	3/11	27.30%
CA based on interpretation		100%

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	<0.25 S	0.5* R	1*	2*	4*	8	>16
<0.25 S	25	1 VME					1 VME
0.5* R		8	3				
1*							1
2*							
4*							
>8	1 MAJ				2	3	26
Evaluable Results*	26	9*	3*		2*	3	27

Organism: other CNS

Evaluation		
Overall EA	68/70	97%
EA based on evaluable results*	12/12	100%
CA based on interpretation	67/70	96%

see footnotes on Table 2A

Draft – Not for Implementation

Table 2A.   Example of Reporting Format for Summary Data on Combined Sites

Summary data

Antimicrobic agent: Ciprofloxacin; S=< 1; I=2; R=> 4   **Organism:** all listed in Microbiology Section of approved antibiotic labeling

Test Results	Reference Result									Evaluation		
	<01.25	0.25*	0.5*	1* S	2* I	4* R	8	16		Overall EA		398/407 = 97.8%
<01.25	259											
0.25*		4	2							EA based on evaluable results*		85/88 = 96.6%
0.5*	6	4	2									
1* S			10	10	5	1				CA	minor [b]	35/407 = 8.6%
2* I			2	9	10	11					major [c]	0
4* R					8	10					very major [d]	1/76 = 1.3%
>8						1	7	46				
Evaluable results*	265	8*	16*	19*	23*	22*a, 23	7	46				

* denotes the evaluable results based on the concentrations tested in both the reference and the test panel.

[a] test results that fall outside the evaluable range are not included

[b] $\frac{\text{\# minor erros based on interpretation}}{\text{Total strains tested}} \times 100$

[c] $\frac{\text{\# major erros based on interpretation}}{\text{Total susceptible strains}} \times 100$

[d] $\frac{\text{\# very major erros based on interpretation}}{\text{Total resistant strains}} \times 100$

TABLE 4: Report Format for Inoculum Density

ORGANISM[a]	Number Tested	SOURCE	METHOD[b]	MEAN	MINUMUM	MAXIMUM
S. aureus ATCC #	20	QC ATCC	Reference	6×10^5	2×10^5	8×10^5
S. aureus ATCC #	20	QC ATCC	Direct inoculum	5×10^5	2×10^5	6×10^5
S. aureus ATCC #	20	QC ATCC	Growth inoculum	5×10^5	2×10^5	6×10^6
MRSA	13	Precision, clinical	Direct inoculum	7×10^5	4×10^5	8×10^6
MRSA	13	Precision, clinical	Growth inoculum	6×10^5	2×10^5	7×10^5
MSSE	3	Precision	Direct inoculum	8×10^5	5×10^5	12×10^5
MSSE	3	Precision	Growth inoculum	7×10^5	4×10^5	8×10^6
MRSE	19	Precision	Direct inoculum	6×10^5	2×10^5	7×10^5
MRSE	19	Precision	Growth inoculum	7×10^5	5×10^5	9×10^5
Enterococcus	4	Clinical	Direct inoculum			
Enterococcus	4	Clinical	Growth inoculum			
MSSA	15	Clinical	Direct inoculum			
MSSA	15	Clinical	Growth inoculum			
			Direct inoculum			
			Growth inoculum			

a   Data should be available upon request for by site evaluation, by organism, etc.
b   Inoculum density should be performed on all methods of inoculation.

TABLE 5: Maximum Number of VMEs as Function of the Number of Resistant Strains Tested

Number of Resistant Strains	Acceptable Number of Errors	Estimated Rate[a]	95% Confidence Interval[b] for True VME Rate
48	0	0.00	(0.00, 7.40)
50	0	0.00	(0.00, 7.11)
60	0	0.00	(0.00, 5.96)
70	0	0.00	(0.00, 5.13)
72	1	1.39	(0.04, 7.50)
80	1	1.25	(0.03, 6.77)
90	1	1.11	(0.03, 6.04)
94	2	2.13	(0.26, 7.48)
100	2	2.00	(0.24, 7.04)
110	2	1.82	(0.22, 6.41)
120	3	2.50	(0.52, 7.13)
130	3	2.31	(0.48, 6.60)
140	4	2.86	(0.78, 7.15)
150	4	2.67	(0.73, 6.69)
160	5	3.13	(1.00, 7.20)
170	5	2.94	(0.94, 6.78)
180	6	3.33	(1.21, 7.16)
190	7	3.68	(1.48, 7.48)
200	7	3.50	(1.40, 7.12)
250	8	3.20	(1.38, 6.24)
300	9	3.00	(1.37, 5.64)
400	11	2.75	(1.37, 4.88)

a   Est. Rate = estimated VME rate = number of errors divided by number of resistant strains.

b   Exact confidence intervals based on the binomial distribution.

TABLE 6: Essential Agreement as Function of the Number of Evaluable Strains Tested

Number of Evaluable[a] Strains	Acceptable Number of Disagreements	Estimated Essential Agreement (EA)[b]	95% Confidence Interval[c] for True EA
35	0	100.00 %	(90.00, 100.00)
54	1	98.15	(90.11, 99.95)
55	1	98.18	(90.28, 99.95)
60	1	98.33	(91.06, 99.96)
65	1	98.46	(91.72, 99.96)
70	2	97.14	(90.06, 99.65)
75	2	97.33	(90.70, 99.68)
80	2	97.50	(91.26, 99.70)
85	3	96.47	(90.03, 99.27)
90	3	96.67	(90.57, 99.31)
95	3	96.84	(91.05, 99.34)
100	4	96.00	(90.07, 98.90)
110	4	96.36	(90.95, 99.00)
120	5	95.83	(90.54, 98.63)
130	6	95.38	(90.22, 98.29)
140	6	95.71	(90.91, 98.41)
150	7	95.33	(90.62, 98.10)
160	8	95.00	(90.39, 97.82)
170	9	94.71	(90.19, 97.55)
180	10	94.44	(90.02, 97.30)
190	10	94.74	(90.53, 97.45)
200	11	94.50	(90.37, 97.22)

a   Evaluable strains are those that fall within the interpretive range plus and minus 2 dilutions (for a range of S = 4, I = 8, R = 16; evaluable results would be those that have a MIC result of 1, 2, 4, 8, 16, 32 or 64 μg/mL) if the device contains these dilutions. Any test or reference result that falls in the < or > category is considered not evaluable.

b   Estimated Essential Agreement = percent agreement = number of evaluable test results that are equal to or with in one dilution of the expected result divided by number of strains that are evaluable.

c   Exact confidence intervals based on the binomial distribution.