Guidance for Industry and FDA

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Bacteriology Branch
Division of Microbiology Devices
Office of In Vitro Diagnostic Device (OIVD)
Evaluation and Safety

Preface

Public Comment:

Comments and suggestions may be submitted at any time for Agency consideration to 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. When submitting comments, please refer to Docket No. 00D-0109. Comments may not be acted upon by the Agency until the document is next revised or updated.

For questions regarding the use or interpretation of this guidance contact Freddie Poole at (301) 796-5457 or by email at freddie.poole@fda.hhs.gov.

Additional Copies:

Additional copies are available from the Internet at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm. You may also send an e-mail request to dsmica@fda.hhs.gov to receive an electronic copy of the guidance, or send a fax request to 240-276-3151 to receive a hard copy. Please use the document number (631) to identify the guidance you are requesting.

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Guidance for Industry and FDA

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems

I. Introduction

This guidance document was developed as a special control guidance to support the reclassification of the antimicrobial susceptibility test (AST) system, when the device is a system employing short-term incubation (less than 16 hours) from class III into class II (special controls). The device is intended to determine the *in vitro* susceptibility of bacterial pathogens from clinical specimens.

This guidance was originally issued March 8, 2000, in conjunction with a Federal Register notice announcing the reclassification of the automated short-term incubation cycle AST system. Following the effective date of that final reclassification rule any firm submitting a 510(k) premarket notification for an automated short-term incubation cycle AST system needs to address the issues covered in the special control guidance. However, the firm need only show that its device meets the recommendations of the guidance or in some other way provides equivalent assurances of safety and effectiveness.

This guidance updates the previous guidance and includes additional labeling considerations (see Section XI.J).

II. Background

FDA believes that special controls, when combined with the general controls, will be sufficient to provide reasonable assurance of the safety and effectiveness of automated short-term incubation cycle AST system. Thus, a manufacturer who intends to market a device of this generic type must (1) conform to the general controls of Section 513(a)(1)(B) of the Federal Food, Drug & Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with automated short-term incubation cycle AST system identified in this guidance under Section 513(a)(1)(B) of the Act and 21 CFR 866.1645(b) and, (3) obtain a substantial equivalence determination from FDA prior to marketing the device under Section 510(k) of the Act and 21 CFR 807.85.

This special control guidance document identifies the classification regulations and product codes for the automated short-term incubation cycle AST system (Refer to Section V – **Scope)**. In addition, other sections of this special control guidance document list the risks to health identified by FDA and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the risks associated with these automated short-term incubation cycle AST systems and lead to a timely premarket notification [510(k)] review and clearance. This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and additional information at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm

Under "**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**[1]," a manufacturer may submit a Traditional 510(k) or has the option of submitting either an Abbreviated 510(k) or a Special 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly once a special controls guidance document has been issued. Manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

III. The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to comply with the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe there is a less burdensome way to address the issues, you should follow the procedures outlined in the **A Suggested Approach to Resolving Least Burdensome Issues** document. It is available on our Center web page at:
http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceProvisionsofFDAModernizationAct/ucm136685.htm

IV. Scope

The scope of this document is limited to the following device as described in 21 CFR 866.1645(a), Fully automated short-term incubation cycle antimicrobial susceptibility device.

> A fully automated short-term incubation cycle antimicrobial susceptibility system is a device that incorporates concentrations of antimicrobial agents into a system for the purpose of determining *in vitro* susceptibility of bacterial pathogens isolated from clinical specimens. Test results obtained from short-term (less than 16 hours) incubation are used to determine the antimicrobial agent of choice to treat bacterial diseases.

The product code for these devices is LON. This document does not apply to devices intended for testing anti-mycobacterial, anti-viral, or anti-fungal agents or devices intended for testing the susceptibility of fastidious organisms for which there is no CLSI standard reference method for testing.

Devices classified in section 21 CFR 866.1640, Antimicrobial susceptibility test powder (product codes shown below) are not subject to this special control guidance. However, information in this document may be useful to manufacturers of these devices.

- LRG - instrument for auto reader & interpretation of overnight susceptibility systems
- JWY - manual antimicrobial susceptibility test systems

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1 http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments

- LTT - panels, test, susceptibility, antimicrobial
- LTW - susceptibility test cards, antimicrobial

This document does not apply to devices detecting genomic features that confer antimicrobial resistances, which are also classified in section 21 CFR 866.1640, Antimicrobial susceptibility test powder, i.e. *mec*A gene for MRSA (product code NQX), *van*A and *van*B genes for VRE (product code NIJ), etc.

This document does not address antimicrobial disks for the disk diffusion method classified in section 21 CFR 866.1620. These devices are addressed in the guidance, "**Review Criteria for Assessment of Antimicrobial Susceptibility Test Discs**," http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM094102.pdf.

V. Device Description

Identify your device by regulation and product code and a legally marketed predicate device. 21 CFR 807.87(a),(f).

In order to help FDA quickly view all the aspects of your device compared with the predicate, you should include a table that outlines the similarities and differences between the predicate and your device.

VI. Risks to Health

In the table below, FDA has identified the risk to health generally associated with the use of the automated short-term incubation cycle AST system addressed in this document. The measures recommended to mitigate the identified risk are given in this guidance document, as shown in the table below. You should also conduct a risk analysis, prior to submitting your premarket notification, to identify any other risks specific to your device. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address the risk identified in this guidance document, or have identified risks additional to those in the guidance, provide sufficient detail to support the approach you have used to address that risk.

Identified risk	Recommended mitigation measures
administration of an inappropriate antimicrobial agent to a patient	Sections IX, X, XI

VII. Device History

This guidance document ensures well-standardized, reliable, and reproducible performance evaluation for AST devices. Clinically, results from AST devices are useful for therapeutic guidance whenever the susceptibility of a bacterial pathogen may be unpredictable or when the infecting organism belongs to a species that may be resistant to antimicrobial agents of choice. Additionally, susceptibility testing is useful for monitoring development of new or emerging resistance to antimicrobial agents.

/ucm080187.htm

A determination of substantial equivalence to a legally marketed predicate device is based on intended use, design, energy used or delivered, materials, performance, safety, effectiveness, labeling, and other applicable characteristics. FDA believes performance of this type device is best established by comparison to the CLSI standard reference methods (Ref. 1, 2) for each antimicrobial agent.

Laboratory procedures used for determining susceptibility of bacteria to antimicrobial agents have been developed and standardized over the past five decades. Historically, there have been two general procedures applied to susceptibility testing, i.e., dilution and diffusion. Other manual testing methods are based on modifications and refinements of older techniques such as gradient diffusion. Voluntary consensus standards on methodology and interpretive categories were implemented for susceptibility testing results that are antimicrobial agent, organism, or methodology dependent. CLSI is the major organization in the United States that establishes voluntary standards and guidelines for standardizing and maintaining performance of laboratory susceptibility tests. A system has been established for continual assessment and upgrading of recommendations and addition of test criteria for new antimicrobial agents and older agents particularly when emerging resistance is recognized. A separate subcommittee was established in 1986 to standardize methods (Ref. 1, 2) for developing *in vitro* susceptibility testing criteria. These methods are also used by the pharmaceutical industry for developing new antimicrobial agents.

The CLSI standard reference methods use 16-24 hours incubation for aerobic bacteria and 48 hours for anaerobic bacteria. Because shorter incubation times 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 are accepted as standards for evaluating methods with a shortened incubation for the following reasons:

- All accepted reference and standard tests use 16 to 24-hour incubations for rapidly growing aerobic bacteria.
- The knowledge and experience for laboratory-clinical correlation has been based on 16 to 24-hour incubation tests.
- Where discrepancies have occurred, they have most often involved failure of shortened incubation procedures to detect bacterial resistance. (Ref. 4)

CLSI has an Approved Standard M7 *"Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically"* (Ref. 1) that recommends a reference method for non-fastidious organisms. Other organisms that will not grow satisfactorily in (or on) unsupplemented Mueller-Hinton medium within 24 hours are considered fastidious organisms and maybe included in CLSI approved standards but generally with a different medium recommended for testing. If the FDA approved pharmaceutical antimicrobial agent package insert includes fastidious organisms (e.g., *Streptococci, Haemophilus*) with interpretive criteria and there is a CLSI standard methodology, the recommendations for performance assessment are similar, but the numbers necessary for review may vary. See Table 1 for recommendations.

A susceptibility result may suggest that an uncomplicated bacterial infection can be effectively treated if AST device results indicate that the bacterial isolate is susceptible to the antimicrobial agent selected. The inability of a new device to produce a susceptible result for an organism that is susceptible to an antimicrobial agent by

the reference method is considered a "major discrepancy." In this case, if a new device yields a resistant result for an organism, the antimicrobial agent may not be made available for treatment when in fact it could be an effective choice. Such major discrepancies can lead to utilization of broad-spectrum agents and needlessly accentuate the pressure for selection of resistant flora. Conversely, the inability to detect resistance is assessed by the "very major discrepancy rate," since therapy with that antimicrobial agent may lead to treatment failure, particularly for serious infections or altered host conditions. Accurate detection of resistance is important for clinical effectiveness and for monitoring emergence of resistance in the community.

Resistance to antimicrobial agents can generally be classified into four basic mechanisms:

- production of antimicrobial-inactivating enzymes
- substitution of antimicrobial-insensitive targets
- alteration in the target site
- decreased drug entry.

The time needed for expression of resistance varies with different combinations of antimicrobial agents and organisms that have different mechanisms of resistance. 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 times have adopted a variety of strategies to bring these results as close to conformity as possible when compared with results using the CLSI standard reference methods. Examples of these strategies include:

- the use of higher concentrations of bacteria in the inoculum
- adjusting media to optimize resistance detection
- the use of sophisticated optical scanning devices with computer assisted reading determinations.

Other devices can detect resistance by the presence or absence of a genotype associated with *in vitro* resistance.

VIII. Study Design

Table 1 outlines in tabular form, FDA's recommendations for the number of sites, and type and numbers of organisms for testing.

Generally, FDA recommends that you establish the performance characteristics of your AST device by agreement with the CLSI standard reference method for each antimicrobial agent and the organisms intended for testing. Because variations in test procedures can affect performance, we believe you should conduct agreement studies on all of the procedural options included in the directions for use section of the package insert. Such procedural options include, but are not limited to, inoculation preparation methods and reading of results, for example:

- growth inoculation preparation method

- direct colony suspension inoculation method
- visual reading
- automated readings.

You should also address all possible combinations of these procedural options. For example, when you add manual or automated inoculation, and/or visual or automated reading methods, you should perform additional testing such as agreement studies, challenge, QC, reproducibility; and demonstrate acceptable performance on each procedural option. You should present this data with your 510(k) submission for each new method that users will be instructed to use. However, if you designate a specific method as "secondary", you should include testing only for QC, Reproducibility and Challenge panels.

You should have a testing protocol describing testing procedures for both the reference method and new device. The protocols should include the exact procedure to follow for the reference and new device.

We recommend that you include your testing protocol in your 510(k). The protocol should describe your study design and contain the type of quality control recommended and the procedures for the reference and test method. The procedures should include:

- method(s) of inoculation
- media used
- incubation conditions
- recommendations for the selection of organisms.

Submissions for antimicrobial susceptibility testing (AST) systems should include only one drug, one method of reading, and one method of inoculation. However, you may bundle gram-negative and gram positive claims (provided the same methods of reading and inoculation are used for both). For more information, refer to the FDA guidance, **Guidance for Industry and FDA Staff: Bundling Multiple Devices or Multiple Indications in a Single Submission**, http://www.fda.gov/cdrh/mdufma/guidance/1215.html.

For a valid comparison, FDA believes you should not deviate from the CLSI standard reference method procedure. As stated above, you should include testing procedures for the new device with all procedural options, or possible combinations of these options that are included in the instructions of the package insert. This is especially important for certain organism-antimicrobial agent combinations that are affected by variations in inoculum and have growth patterns that may be interpreted differently when read visually or automatically. Your study design should include options for different methods of inoculation or additional dilutions of the inoculum suspension for certain groups of organisms (e.g., *Proteus sp*.), if any options are in the instructions for use.

Where appropriate to the device design and instructions for use, performance data using alternate methods of reading and/or inoculation procedures should include test results for all challenge, quality control, and reproducibility studies.

A. Reference Method

The reference method plates should contain two-fold dilutions of the antimicrobial agent for which FDA clearance is sought. The selection of dilutions should include the FDA and CLSI interpretive standards with one two-fold dilution above the resistant threshold and several below the susceptible threshold to provide a range for evaluating the results and for detecting emerging resistance or trending. For example, if interpretive standards are: $\leq$ 1, susceptible (S); 2, intermediate (I); $\geq$ 4, resistant (R), then the reference plate should include serial two-fold dilutions between 0.25 μg/mL and 8 μg/mL. Including one concentration above the resistant threshold provides data for essential agreement (EA) evaluations. We believe including concentrations more than two dilutions above the resistant concentration provides little evaluable data. Including dilutions below the susceptible category provides more results that are on-scale, and therefore, available for inclusion in the calculations of the EA of evaluable results. The table format samples, Table 5 and 5A show our recommendations for determining graphically whether a result is evaluable or not.

The reference method performed at the clinical sites may produce errors when testing clinical organisms. Manufacturers may avoid this problem with the challenge and reproducibility assessments by comparing the new device results to a pre-determined expected value, instead of to the results of the reference method performed at the sites. If expected values are not used for the challenge organisms and there is a concern about the variability of the reference method, FDA suggests performing the initial reference test in triplicate for all clinical and challenge organisms. This will help reduce any potential bias. Special care should be taken in the preparation of all reference plates, since the reference result will be used in the final analysis.

B. New Device

Both the new device and the reference panel should include a sufficient number of serial two-fold dilutions around the susceptible and resistant thresholds. For a quantitative minimal inhibitory concentration (MIC) device the concentrations tested should include at least five two-fold dilutions that surround the susceptible and resistant thresholds of the antimicrobial agent as described above.

C. Organism Selection

You should select organisms for the comparative study that represent the clinical indications of the antimicrobial agent and are within its spectrum of activity according to the Microbiology and Indication and Usage Sections of the FDA approved pharmaceutical antimicrobial agent package insert, and the most recent CLSI M100 (Ref. 3) Informational Supplement. (See CLSI M100 Table 1 "Suggested Groupings of U.S. FDA-Approved Antimicrobial Agents That Should be Considered for Routine Testing and Reporting of Non-fastidious Organisms by Clinical Microbiology Laboratories" and Table 1A for fastidious organism recommendations). You should include organisms for which clinical efficacy and *in vitro* activity have been demonstrated.

A 50% susceptible, 50% resistant distribution within species would be ideal, but such a distribution may be rare when sequential clinical isolates are tested. You should avoid using the same organism from multiple sources and repeat isolates obtained less than three days apart from the same patient. Organisms with known mechanisms of clinically significant resistance should be included in the comparison study as either fresh or selected stock and challenge organisms.

The following example may help to clarify the types of organisms that we recommend you test. If the antimicrobial agent has been shown to be active against *Enterobacter cloacae, Klebsiella oxytoca*, and *Citrobacter* spp., both *in vitro* and in clinical infections, then all *Enterobacteriaceae* routinely isolated would be relevant for testing.

We recommend you avoid testing organisms that are not included in the Microbiology and Indication and Usage Sections of the FDA approved pharmaceutical antimicrobial agent package insert, because we believe this does not provide useful information. If, for example, *Pseudomonas* spp. are not indicated, they should not be selected for testing.

There are situations where the spectrum of activity (i.e., resistance) of the antimicrobial agent for certain organisms has not been demonstrated in bacteriological or clinical studies. In this instance, the FDA approved pharmaceutical antimicrobial agent package insert has only a susceptible interpretation. Since only organisms in the susceptible category are available, your labeling should recommend that results other than susceptible be referred to a reference laboratory for further analysis. In the event that resistant strains become available, they can be evaluated later, after which you may need to submit a new 510(k).

FDA discourages testing rare organisms for which antimicrobial agents are approved for use, since sufficient data for the rare organism is usually difficult to acquire in a clinical setting. Refer to Tables 1 and 1A in the CLSI Approved Standard (Ref. 3), for suggested organisms to include or exclude in an evaluation.

1. Fresh clinical organisms

You should include organisms isolated from routine cultures processed in the clinical laboratory study site in the 7 days preceding testing. You should also include all isolates in the appropriate testing group as indicated for testing with the antimicrobial agent in the test device. You should perform the reference method in parallel with the new device.

2. Clinical stock organisms

Generally each site has its own collection of infrequently isolated or less common organisms. These are organisms saved because of their unique growth or resistance patterns. This selection may be used to enhance the clinical isolates, but should not comprise more than 50% of any group of organisms or the total number tested. You should include these in the study as necessary to incorporate a wider variety of genus and species and also to augment the number of resistant organisms tested.

3. Challenge organisms

You should select challenge organisms from the organisms listed in the Microbiology and Indication and Usage Sections of the FDA approved pharmaceutical antimicrobial agent package insert. The challenge organisms should fall within the spectrum of activity of the antimicrobial agent. The selection of these isolates should favor resistant strains and include organisms for which the antimicrobial agent's MIC is on-scale. If interpretive criteria are ≤ 4 (S), 8 (I), > 8 (R), then we believe organisms with known results in all dilutions between 0.25 and 32 μg/mL are appropriate selections. These challenge organisms are meant to demonstrate whether a device can reliably detect

intermediate and resistant organisms. These organisms may be available from the Centers for Disease Control and Prevention (CDC) or a reference laboratory that collects and characterizes strains based on their resistance patterns or particular uniqueness. You may add a selection of organisms that were not used in the developmental stages of the antimicrobial agent algorithm for susceptibility testing, if they are clinically indicated organisms for *in vitro* testing as stated in the FDA approved pharmaceutical antimicrobial agent package insert.

If the organisms have been characterized phenotypically with repeated CLSI standard reference method testing, these consensus results can be used as the "expected results." If the "expected result" is not known, you should perform MIC testing using the reference method before using them in the evaluation. You may do so internally or at an outside site. Only the reference method results should be used to determine the expected results.

If the challenge organisms have known expected values, reproducibly obtained using the CLSI standard reference method, the clinical site need only perform testing with the new device. This will reduce the burden at the clinical site. You should code the set with the expected results, mask it and send it to one site for performance testing on the new device. Alternatively, you could conduct performance testing of the challenge organisms on the new device and the reference method at the same time.

D. Quality Control

We recommend that you conduct the following quality control testing for both the reference method and the new device as well as with any procedural options given in the labeling of the new device:

- daily testing of all quality control organisms recommended by FDA and CLSI
- periodic inoculum colony counts
- purity check of all organisms.

1. Selection of Quality Control Organisms

Please refer to the appropriate CLSI Approved Standard (Ref. 1, 2, 3) for recommended methods and quality control organisms. The FDA approved pharmaceutical antimicrobial agent package insert will also provide the expected quality control range for each organism.

The selection of antimicrobial concentrations should include a minimum of one two-fold dilution below the lowest dilution and one two-fold dilution above the highest dilution for the recommended quality control organism. For example, if the expected range is 1 - 4 µg/mL, the reference plate should include 0.5 - 8 µg/mL.

In instances where the quality control organisms' expected results are significantly above or below the expected ranges, on-scale results may not be possible. In this case, alternative quality control organisms that are appropriate for the specific drug-bug combination should be selected. These alternative quality control (QC) organisms should be well characterized with established QC ranges. Additionally, these organisms should be recognized in the FDA approved drug label for the antibiotic for which clearance is sought or recommended by the CLSI as appropriate for use for the specific

antibiotic for which clearance is being sought. On-scale results for at least one of these QC organisms should be demonstrated.

In the event that the QC organism with expected ranges yields a result of no growth with the new device, this may indicate that the new device does not support the growth of specific organisms. In this case, the no growth result is an invalid result. Quality control testing should not be continued until the root cause of the error has been identified. Please refer to CLSI M23-A3 for guidance or reassessment of QC ranges.

If multiple quality control strains are used and one strain has results outside of the expected range on any given day, you should repeat the quality control strain with the out of range result. When the results are interpreted the next day and the repeat testing is within the expected quality control range, the study data from the previous test day is acceptable and may be included in the comparative summary. However, if the repeated quality control result is still outside the expected range, the data from the previous day's testing is invalid.

If multiple quality control strains are tested and there are results for more than one strain that are outside the expected results in the reference method on any test day, you should not include test data from that day. You should repeat quality control strains with the out or range results with the reference method. If quality control is still out of range, conduct an investigation to determine the cause of the aberrant result(s). Do not continue testing until the problem has been resolved.

We believe that you should perform quality control testing with the selected organisms on the reference plate daily to ensure that the reference method and reference plates are in control for each day of comparative testing. We recommend that you perform quality control testing with these same organisms on the new device a sufficient number of times to demonstrate that the user will be able to achieve the same results in the recommended ranges. We recommend a minimum of 20 quality control test results per site.

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 method (broth dilution of approximately 5×10^5 CFU/mL) and the new device. Some antimicrobial agents are affected by variance in the final inoculum and performance may be compromised. You should perform plate counts as recommended in the CLSI M7 Approved Standard (Ref. 1) on all methods of inoculum preparation that are recommended in the package insert of the new device. Ideally, this should include all quality control isolates daily, isolates for reproducibility testing, and 10% of fresh isolates.

In the broth dilution test, you should perform plate counts (colony count study) directly from the inoculated panel to ensure the time period from the initial inoculum adjustment and the final time of inoculation has not adversely affected the inoculum density. For a non-broth device, you should perform a colony count determination immediately before conducting the test.

There may be alternative approaches for this type of quality control if the inoculum method uses a spectrophotometric device. This type of device can be validated separately. You should provide

adequate information to demonstrate that the colony count study described above is not necessary. However, if a non-spectrophotometric method is used, it is also the manufacturer's responsibility to provide adequate information to demonstrate with a study as recommended above that the inoculum is reproducible and in the expected range. The study should demonstrate that the inoculum for the ATCC 25922 *Escherichia coli* is in the expected range of 3-7 x 10^5 CFU/mL. The study should also demonstrate that the inoculum method for the new device provides the same range as the reference method of inoculation with all organism groups. The calculations may be different if the inoculum density for the new device is different from the recommended reference inoculum.

3. Purity check

The purity check is necessary for broth dilution procedures to detect mixed cultures that may cause aberrant results. As recommended in the CLSI M7 (Ref. 1) Approved Standard, you should conduct these checks after inoculation of the new device or reference plate. You should perform purity check plates for all inocula used for the reference method and the new device.

E. Reproducibility

You should test a minimum of 25 selected organisms with known on-scale results for which the antimicrobial agent is indicated. These may be challenge organisms or other organisms with known results. We recommend that you code organisms and send the organisms to three sites for testing, one time at each site on the new device only. Since this study design will not produce variability within sites, you should provide internal summary data to demonstrate this.

An alternative reproducibility assessment may be performed using 10 selected organisms with known results on-scale. You should not use the quality control isolates if they are not on-scale. These 10 organisms should be tested at each site on three separate days in triplicate with a different inoculum prepared for each test (27 results per isolate). Using this study design, you should calculate reproducibility for within-site (intra-site), for each site, and between-sites (inter-site). See also the table format samples for presenting your reproducibility results, Tables 6A and 6B.

Personnel at each site in the study should perform the same reproducibility study for all inoculum preparation methods and/or reading options recommended in the package labeling. See also Table 1 which outlines in tabular form, FDA's recommendations for reproducibility testing.

IX. Data Presentation

We recommend that you provide summary data for comparative performance, reproducibility, and quality control.[2] We have provided several table format samples at the end of this guidance as examples of how to present your results.

[2] In order to meet the requirements of section 21 CFR 807.87(i), you must include in your 510(k), a financial certification or disclosure statement or both, as required by 21 CFR Part 54. Please refer to **Guidance for Industry: Financial Disclosure by Clinical Investigators**, issued 03/20/2001, http://www.fda.gov/oc/guidance/financialdis.html.

A. Comparative Performance Data

Tables 5 and 5A are table format samples that summarize performance using:

- Essential Agreement (EA)
- Category Agreement (CA)
- Essential Agreement of evaluable results
- Major Discrepancy (maj)
- Minor Discrepancy (min)
- Very Major Discrepancy (vmj)

The formulas for calculating the percent agreement and the discrepancies listed above are included in Table 5A and in the glossary section.

1. Clinical – fresh and stock

A list of organisms tested should be presented in chart format for each site, identifying the numbers that are stock and fresh for each genus or species. A line listing for all organisms with MIC and/or category result discrepancies between the reference method and the new device should be presented to include genus or species, site, reference method result, test result, type of error, method of inoculation and reading, if applicable. You should submit summary data for all organisms and all study sites combined using Table 5A. This table can also be used to summarize all organisms by site. You should also provide summary data by organism using Table 5.

2. Challenge organisms

You should present results from challenge organisms with the comparison to the expected value or reference result performed at the time of testing. The table format sample in Table 5A is suitable for this purpose. The formulas for calculating the percent agreement and discrepancies are included in Table 5A and in the glossary section.

You should also present all methods of inoculation or reading of results separately. You may wish to use the table format sample in Table 5A for this as well.

We recommend that you also present a line listing of all discrepancies, including the name of the organism, site, reference method result, test result, type of error, and method of inoculation and reading, where applicable.

3. Challenge plus clinical organisms

We recommend that you also present summary data for the challenge data combined with the clinical data. You may wish to use the table format sample Table 5A for this purpose. If there appears to be trending for a particular group of organisms (e.g. *Staphylococcus* spp.*, Pseudomonas* spp.) such as that observed in the clinical data presented in Table 5 of this guidance, you may present these groups separately. See also Table 3 for an additional table format sample you may wish to use for presenting the EA, CA, discrepancies, and evaluable results in a concise manner for both challenge and clinical

B. Quality Control

Table 4 gives an example of how you should present quality control strain results. We recommend a minimum of 20 test results per site for each method of inoculation and/or reading included in the package insert. You should present both initial and repeat quality control results with an explanation of the action taken for all out-of-range test results.

C. Reproducibility

You should present reproducibility data for all procedural options. If you used the 25-organism study design, you may wish to use the table format sample in Table 6 for this purpose. You should also provide a summary of the internal studies demonstrating the variability across repetitions of the same organism. If you used the 10-organism study design, you may wish to use the table format sample in Table 6A and 6B for this purpose.

With multiple procedural options, the presentation of data is the same for each option.

X. Evaluating the Results of your Study

The following are recommendations for evaluating the results of your study. The quality control and reproducibility results should also be considered when assessing comparative performance of the reference method and the new device.

A. Fresh, Stock, and Challenge Organisms

Using the table format sample given in Table 5 will help you and FDA visualize discrepancies and trending by organism. However you choose to present the results, you should include only those organisms that would be routinely tested for the antimicrobial agent. For example, you should not include *Pseudomonas* spp. results for antimicrobial agents that have indications only in the *Enterobacteriaceae* group, or for *Enterococcus*, if the indications only include *Staphylococcus* spp. An additional purpose for the recommendation to use these tables is to identify the on-scale or evaluable test results based on the interpretative criteria of the antimicrobial agent and the concentrations tested on both the reference method and new device.

You should pay particular attention to the organisms with clinical utility and within the spectrum of activity of the antimicrobial agent as shown in the Microbiology and Indication and Usage Sections of the FDA approved pharmaceutical antimicrobial agent package insert. If the EA and CA that you obtain for the organisms listed in the FDA approved antimicrobial agent labeling are below 90%, we recommend that you add a limitation statement to your labeling and consider conducting a future study to support acceptable performance.[3] This kind of limitation statement is not necessary for organisms (genus or

[3]We believe revising this limitation in a legally marketed AST device significantly affects the safety and effectiveness of the device. Therefore, you will need to submit the results of this future study to FDA as a new 510(k) to support modifying this limitation according to section 21 CFR 807.87(g).

species) for which the antimicrobial agent has no clinical utility or is inactive and has not been approved for use by FDA (e.g., cefdinir with *Enterococcus* and *Pseudomonas* sp.).

You should also evaluate the overall performance of the AST device in the clinical studies for the ability to detect resistance. The use of challenge and stock organisms may be of particular importance in selecting organisms with resistance.

Tables 8 and 9 show the discrepancy rate and minimum acceptable EA rate we recommend. Discrepancy rates will vary depending on the proximity of the MIC of the organisms tested to the interpretative categories. FDA considers the following to be acceptable performance for the clinical data for AST devices for all organisms appropriate for testing:

- Percent essential and category agreement > 89.9 %. A CA of < 90% may be acceptable under certain circumstances (e.g., very good EA of the evaluable test results with the majority of the discrepancies as minor discrepancies).

- A maj rate of $\leq$ 3% based on the number of susceptible organisms tested.

- A vmj rate based on the number of resistant organisms tested. Table 8 lists the numbers of very major discrepancies as a function of the total number of resistant organisms tested with proposed statistical criteria for acceptance that include an upper 95% confidence limit for the true vmj rate of $\leq$ 7.5% and the lower 95% confidence limit for the true vmj $\leq$ 1.5%.

- Growth failure rates in the system < 10% for any genus or species tested.

B. Quality Control

Test results on the new device for the recommended quality control isolates should be within the expected range 95% of the time. In rare events, the expected result with the new device may not agree with the CLSI recommended ranges for an antimicrobial agent. In this case, you should submit additional data following CLSI recommendations in M23 "Development of In vitro Susceptibility Testing Criteria and Quality Control Parameters." (Ref. 5). These data should demonstrate the reproducibility of the newly submitted range, plus supportive data showing that all parameters of the test method are in control. These data should include all quality control parameters (e.g., inoculum density check). You should include a statement in the product insert that alerts the user to your unique quality control range. Quality control results that are frequently out of the recommended range will also require a closer scrutiny of the other data to determine if there is a similar trend that might affect clinical results.

If one procedural option provides quality control results that are not accurate for any particular antibiotic while another procedural option produces accurate results (e.g., inoculum preparation, automated reading), you should include a limitation in the labeling stating that results should not be reported for that antibiotic when this particular procedural option is used.

C. Reproducibility

It is difficult for the FDA to determine substantial equivalence for a device if the results of the overall reproducibility study from all test sites for any antimicrobial agent show < 95% (+/- 1 dilution) agreement

as compared to the mode. If there is a trending-bias or reproducibility problem with a different procedural option (e.g., inoculum preparation, automated reading), you should include a limitation statement similar to that in section **XII. Labeling**, stating that users should not report the results. This type of limitation may apply if some procedural options (method of inoculum, reading method, etc.) were considered unacceptable while another was acceptable.

Observations of trending by a particular organism group or by a procedural option should be investigated further in the other study data to assess the impact on interpretations of patient results.

XI. Labeling

The premarket notification must include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). Although final labeling is not required for 510(k) clearance, final labeling must also comply with the requirements of 21 CFR 809.10 before a medical device is introduced into interstate commerce.[4] Some of these requirements are further discussed below.

A. Intended Use Statement

You must specifiy the product's intended use. 21 CFR 809.10(a)(2), (b)(2). The intended use should be representative of the target populations tested, and the performance characteristics of the assay. The Intended Use statement must indicate:

- which organism groups the device is indicated for testing and
- any instrumentation the device may be used with, if applicable. 21 CFR 809.10(b)(2).

A typical example of an intended use statement is:

"ABC's system is intended for the in vitro qualitative or quantitative determination of antimicrobial susceptibility of rapidly growing aerobic non-fastidious Gram positive and Gram negative organisms on the ABC Instrument."

B. Summary and Explanation of the Test

The summary and explanation of the test section must also include:

- whether the assay is quantitative (MIC) or qualitative (breakpoint devices)
- whether results may be read and reported manually. 21 CFR 809.10(b)(3).

C. Principle of the Method

You must specify the principles of the procedure. 21 CFR 809.10(b)(4). You should include a clear and concise description of the technological features of the specific device and how the device is to be used with patient samples.

[4] In addition, final labeling for prescription medical devices must comply with 21 CFR 801.109.

D. Reagents

You must list antimicrobial agents along with concentration ranges and abbreviations. You may include these in the reagent section of the labeling or on each package container if different for different devices. 21 CFR 809.10(b)(5). To prevent confusion between different drugs with similar generic or trade names, you should use abbreviations recommended by the pharmaceutical manufacturer.

The product insert should be flexible to accommodate additional antimicrobial agents. Charts should be used when possible to facilitate additions of future antimicrobial agents, limitations and performance characteristics.

E. Directions for Use

You must provide a step by step outline of recommended procedures. 21 CFR 809.10(b)(8).

F. Quality Control

The step by step outline of the procedure must include details of kinds of quality control procedures and materials required, as well as details of calibration. 21 CFR 809.10(b)(8)(v) and 21 CFR 809.10(b)(8)(vi). You should ensure that the specifics of calibration and quality control procedures you recommend to users are those necessary to ensure performance claims. You must list all recommended quality control strains whether CLSI or other and the expected results when tested with each antimicrobial agent. 21 CFR 809.10(b)(8)(vi).

G. Reporting of Results

You must provide the interpretive criteria users should use for each antimicrobial agent on the MIC or breakpoint device based on the FDA interpretive standards that you used in the evaluation. 21 CFR 809.10(b)(9).

Automated systems should have the interpretations included in the software but if manual readings are an option, a chart of thresholds to be used for SIR (Susceptible, Intermediate, Resistant) interpretations must be included in the package insert. 21 CFR 809.10(b)(9).

Results should not be reported in instances where performance has not been established either because there are no interpretive criteria for a particular organism group or insufficient numbers of organism groups have been tested. Where feasible, FDA suggests that suppression of results be software driven. Interpretations (i.e., SIR) should not be reported for these groups of organisms. If you report MIC results, they should carry a disclaimer that device performance or antimicrobial agent clinical effectiveness have not been established. MIC results for this type of organism may be useful for antibiogram patterns, but the practice of reporting results should be discouraged when the antimicrobial agent has not been proven to be effective for treating infections caused by these organisms and the performance on your device has not been established.

H. Limitations

You must include a statement of limitations of the procedure. 21 CFR 809.10(b)(10). If the device has software-generated interpretations, these limitations should be incorporated into the software. The following are examples of some limitation statements that may apply to your device:

- You should recommend the use of an alternative method for testing prior to reporting of any results when the spectrum of activity for any antimicrobial agent includes organisms with either unacceptable very major discrepancy or major discrepancy rates.

- If you did not test sufficient resistant organisms with an approved indication for use for the antimicrobial agent, you should include a statement in the labeling similar to this:

  "The ability of the ABC system to detect resistance to [Antimicrobial agent] in [organism(s)] is unknown because resistant organisms were not available at the time of comparative testing."

  However, this limitation may not be necessary if a sufficient number of evaluable results close to the interpretative categories are available and the EA is adequate.

- If the reproducibility results for any antimicrobial agent using one procedural option are not reproducible while another option is reproducible, you should include a limitation against reporting results, for example:

  "The results of testing (antimicrobial agent) showed < 95% reproducibility when inoculum method [cite which inoculum method] is used. Results should not be reported."

  This applies if any recommended procedural option (method of inoculum, reading method, etc.) was unacceptable while another was acceptable.

- You should recommend an alternate method for any specific organism group that had a "no growth" rate >10%. You should recommend that users not test these organisms because the results might be misleading. If the device is software driven, the device should block the results from being reported.

AST systems may be able to provide results for organisms that may not be appropriate for all of the antimicrobial agents provided on a test panel or system. Therefore we recommend you explain the clinical utility of your interpretive criteria in your labeling. For example:

"There are antimicrobial agents included in this [panel, device, or section] that have not been proven to be effective for treating infections for all organisms tested. Refer to the individual FDA approved pharmaceutical antimicrobial agent package insert for interpreting and reporting results of antimicrobial agents that have shown to be active against organism groups both *in vitro* and in clinical infections."

I. Performance Characteristics

You must include specific performance characteristics of the assay, including the study design, stating the reference method used, number of sites, etc. 21 CFR 809.10(b)(12). You must list the percent EA and/or CA in table format with the CLSI standard reference method for each antimicrobial agent from comparative performance evaluations. 21 CFR 809.10(b)(12). You must also include results of reproducibility studies in either a table format or a summary paragraph describing the type of study and a statement that all reproducibility results were acceptable at $\geq$ 95%. 21 CFR 809.10(b)(12).

J. Updating susceptibility test information for in vitro diagnostic AST devices

For additional information on the procedures AST device manufacturers should follow when the applicable NDA holders update their labeling in response to newly recognized standards, please see Section V ("Updating Susceptibility Test Information For In Vitro Diagnostic AST devices") of the guidance entitled "Updating Labeling for Susceptibility Test Information in Systemic Antibacterial Drug Products and Antimicrobial Susceptibility Testing Devices" and found at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM169359.pdf.

XII. 510(k) Submissions for Expanded Labeling for Legally Marketed AST Devices

For expanded labeling you must submit a new 510(k). 21 CFR 807.81(a)(3). The FDA will request appropriate data and information in a new 510(k) to support an expanded labeling, such as the removal of any limitation included in the labeling that was placed there during the original clinical studies. These types of data are described below and detailed in Table 2. You should refer to the submission in which the limitation statement was made. If you made changes to the device to alter the performance, your additional studies should include all organisms previously tested if these changes significantly affect safety and effectiveness.

A. Performance

If a limitation in the labeling is a result of performance characteristics that are based on EA and/or CA and you wish to modify the device in order to delete the limitation, you should perform a comparative clinical laboratory study. This study should follow the design for the comparative study described in this guidance. The organism mix should be concentrated around those groups that provided the original EA or CA results. You should include all groups that might be affected by modifications to the device. You may wish to report the results using the table format samples provided in this guidance.

B. Insufficient Resistant Strains

If a limitation in the labeling is a result of not testing sufficient resistant strains, you should perform a comparative study to demonstrate that the device can detect resistance in organisms that are included in the Microbiology and Indication and Usage Sections of the FDA approved pharmaceutical antimicrobial agent package insert. 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

organisms may be substituted for fresh isolates. You may wish to report the results using the table format samples provided in this guidance.

C. Reproducibility

If the reproducibility was <95% for a particular procedural option, you should perform a study to verify that this method is now reproducible. This study should include the problematic organism(s) or procedural options (alternate methods of inoculation, alternate reading procedures, etc.) which originally did not demonstrate reproducible results. You should conduct either the 25-organism study, or the 10-organism study at three test sites as described in this guidance. The strains should include organisms for which the antimicrobial agent is intended for testing with known results near the interpretive criteria range. Include organisms you determined to be problematic in the original reproducibility study. You may wish to report the results using the table format samples in Tables 6, 6A, and 6B. You should also include the testing of all quality control organisms. Notable bias or poor reproducibility of an alternate method of inoculation or reading may indicate additional concerns with this particular procedure. Additional challenge data may be needed to resolve these concerns. If you determine that the inoculum was the problem, you should perform an evaluation of colony count data.

D. Quality Control

If alternate methods of inoculation or reading produced quality control values that did not match CLSI acceptable ranges, you should test a minimum of 20 replicates per site with each quality control organism on the test device to verify that the quality control values are now within the acceptable CLSI quality control range. Prepare each quality control organism from a different inoculum suspension. You may wish to report the results using the table format samples in Table 4. You should perform colony counts if you do not use a standardized inoculation method (e.g., photometric device). Colony counts should be done once on each day of testing using the CLSI recommendations for sampling from the inoculated test device. If the device package insert recommends additional methods of inoculation and/or reading, you should test all options. If you elect to propose an alternative range, you should follow a CLSI M23 (Ref. 5) study design. You should explain any affect on clinical isolate results.

XIII. QSR Considerations

Part of the QSR (Quality System Regulation, 21 CFR Part 820) is to ensure that the finished product will be safe and effective and perform as intended. For this reason when AST devices can not reproducibly generate the same results within plus or minus one well (approximately ten fold dilution) of the expected results, FDA is concerned that this may result in a risk to the public health. If the non-reproducible result is around the interpretative criteria cut off for determining susceptibility or resistance a vmj could occur. The patient report would recommend for treatment an antibiotic to which the organism is actually resistant, which could lead to treatment failure, particularly for serious infections or altered host conditions. Another possibility is a major error, which can lead to utilization of broad-spectrum agents and needlessly accentuate the pressure for selection of resistant flora. This may also result in further risk to patients because the selection of treatment antibiotic may now be an antibiotic that could be more toxic to the patient when a less toxic antibiotic is available.

You must consider the reproducibility and stability of components in the design of the device and in the development of release criteria. 21 CFR 820.30(c). All aspects of the final product will have an effect on the

performance, but the lot-to-lot reproducibility and stability studies of the antimicrobial agent should be for performance of the antimicrobial agent only. You must validate the other aspects by other means or at different times. 21 CFR 820.30(g). Testing should be performed internally with design components similar to the clinical trial protocol. You must keep the data on file and available upon request. 21 CFR 820.30(j).

Manufacturers also must use surveillance data and customer complaints as part of design controls (21 CFR 820.100 Corrective and preventive action). The CLSI includes surveillance data in performing continuous assessment of older antimicrobial agents, particularly when new mechanisms of resistance emerge. The manufacturer is responsible for keeping abreast of all information to better reevaluate the product to determine if the change in resistance patterns has affected performance and accuracy of the test.

Although not part of the class II special controls, FDA recommends that you consider the following in your approach to complying with QSRs. The method you use to validate reproducibility and stability should be able to detect a change in potency of at least 50% for each antibiotic. For example, if the organisms you select for such testing have stable on-scale MICs, then a sufficient number of replicates of these organisms could detect shifts in the mode of the test organism. Although FDA acknowledges that the methodology has a +/- one well variability, each antimicrobial agent should be evaluated by you for even slight trending to ensure the product will continue to be safe and effective and perform as intended.

A. Lot to Lot Reproducibility

Your study design should demonstrate that different lots of prepared antimicrobial agents in the final format (minimum 3 lots) will perform with the same accuracy. If all other device components have been previously evaluated, you need only include one lot of these components since this study design is to monitor the antimicrobial agent.

B. Stability

Your study design should verify the shelf life of the antimicrobial agents in their final format for all conditions that are recommended by your labeling. The temperatures at which the product is stored should include the extremes of the range recommended for storage. Include observations of slight trending in one direction over time as part of the evaluation.

XIV. Glossary

Agar Dilution Susceptibility Test	An antimicrobial susceptibility test method using concentrations of an antimicrobial agent incorporated into agar growth medium plates.
Agreement - Category (CA)	Agreement of interpretive results (SIR) between a new device under evaluation and a standard reference method using FDA interpretive criteria as presented in the FDA approved pharmaceutical antimicrobial agent package insert.
Agreement, Essential (EA)	Agreement within plus or minus, one two-fold dilution of the new device under evaluation with the reference method MIC

	determination. Both the new device and the reference method should test a range of two-fold dilutions that include at least one dilution above and below the interpretive thresholds.
Bias	Measure of whether the new device produces the correct result.
Breakpoint System	Systems similar in design to MIC systems, but with four or fewer concentrations of each antimicrobial agent. These concentrations are the interpretive thresholds (based on the FDA interpretive categorical MIC values for each antimicrobial agent) that provide a qualitative (category) result (SIR). FDA considers these devices qualitative.
Broth Dilution Susceptibility Test	An antimicrobial susceptibility test method using concentrations of an antimicrobial agent in broth growth medium, in either tubes (macrodilution) or wells (microdilution).
Discrepancy	A disagreement between the new device result and the reference method. Either the new device MIC is greater than plus or minus one two-fold serial dilution and/or the interpretive category is different.
Discrepancy - major (maj)	The reference category result is S and the new device result is R. To calculate the major discrepancy rate, use the following formula: $$\text{maj} = \frac{\#\,\text{maj discrepancies}}{\text{Total}\,\#\,\text{susceptible organisms by reference method}}\ \text{X}\,100$$
Discrepancy – minor (min)	The reference category result is R or S and the new device result is I; or the reference result is I and the new device result is R or S. To calculate the minor discrepancy rate, use the following formula: $$\text{min} = \frac{\#\,\text{min discrepancies}}{\text{Total}\,\#\,\text{organism tested}}\ \text{X}\,100$$
Discrepancy – very major (vmj)	The reference category result is R and the new device result is S. To calculate the very major rate, use the following formula: $$\text{vmj} = \frac{\#\,\text{vmj discrepancies}}{\text{Total}\,\#\,\text{resistant organisms by reference method}}\ \text{X}\,100$$
Evaluable Result	When the reference method result is on-scale and the new device result is also on-scale. FDA believes that if the reference result is

	on-scale and the new device result is not on-scale, comparative data may not be evaluable. FDA does not consider evaluable any reference result that falls in the less than or greater than category. However, such results may be part of the EA and/or CA assessments. See Table 5 and 5A for examples. Evaluable (i.e., on-scale) results are those that fall within the test range of the reference method and could also be on-scale with the new device if within the plus/minus one well variability.
Fastidious Organism	Those that require very specialized nutrients and environmental conditions to thrive and remain viable. For the purposes of this document, a fastidious organism is one that will not grow well in (or on) unsupplemented Mueller-Hinton medium within 24 hours.
Genotypic Resistance	The presence of resistance-expressing genes. The presence of resistance-expressing genes can often infer resistance. Absence of these resistance determinants cannot generally exclude resistance by other mechanisms.
In vitro Diagnostic (IVD)	*In vitro* diagnostic products (reagents, instruments, and systems) that are medical devices under the Federal Food, Drug, and Cosmetic Act. The generic product class is intended for use in clinical laboratories for determining *in vitro* susceptibility or resistance of bacterial pathogens to therapeutic agents. (see 21 CFR Section 866.1640).
Inoculum Density Check	Plate counts performed to ensure that the numbers of organism inoculated into the test system are within prescribed ranges. See CLSI M7 Approved Standard (Ref. 1).
Minimal Inhibitory Concentration (MIC)	The lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test.
Minimal Inhibitory Concentration (MIC) Systems	Broth dilution, agar dilution, or other methods or systems that have at least five concentrations of generally two-fold dilutions of antimicrobial agents. These may be in broth, plate, gradient diffusion, or other formats. The antimicrobial concentrations may be frozen, lyophilized, or dehydrated. They should include a minimum of two dilutions below the susceptible threshold in order to assess developing resistance, and to trend and track patterns of resistance. These devices provide quantitative MIC results. They can be manual, semi-automated, or fully automated.
Mode	The most frequently occurring MIC result when one organism is

	repeatedly tested.
On-scale Result	An MIC result from testing a series of dilutions when there is growth in at least one, but not all concentrations tested.
Organism - Challenge	Selected organisms with expected MICs that are near the SIR thresholds or at least on-scale. Testing these challenge organisms enriches the numbers of organisms in the evaluation with evaluable results.
Organism – Clinical stock	An organism isolated from a clinical specimen at a clinical laboratory site, has been retained/stored for more than 7 days, and is used in the comparative study between a new device and the CLSI standard reference method. These are usually retained because they have: known mechanisms of resistance, have an unusual susceptibility pattern to antimicrobial agents in the same class as the antimicrobial agent under evaluation, or are the genus and/or species for which the antimicrobial is indicated but are not commonly isolated, and would likely not be included with fresh organisms used in the evaluation.
Organism - Fresh clinical	Organisms isolated from clinical specimens at a clinical laboratory site during 7 days prior to testing in the comparative evaluation between the new device and the reference method. These organisms should not be frozen or repeatedly subcultured.
Phenotypic Resistance	Observable or measurable *in vitro* growth in the presence of a known antimicrobial concentration.
Predicate	A device that was legally marketed prior to May 28, 1976 (preamendments device), or a device which has been reclassified from Class III to Class II or I, or a device which has been found to be substantially equivalent to such a device through premarket notification.
Procedural Options	Optional methods in the instructions for use (Procedure Section) in the package insert for the new device. Examples of such procedural options are: alternate organism inoculation preparation methods such as direct colony suspension without turbidimetric qualification, visual reading when the system is primarily instrument-read and automated readings.
Purity Check	A quality control procedure to ensure that the growth endpoint for an MIC or breakpoint result is not caused by more than one organism. See CLSI M7 Approved Standard (Ref.1).

Qualitative Susceptibility Result	A category result (S, I or R) obtained with a device containing four or fewer concentrations of an antimicrobial agent.
Quantitative Susceptibility Result	An MIC result obtained with a device containing five or more concentrations of an antimicrobial agent. In addition to reporting a category result of susceptible (S), intermediate (I), or resistant (R), the actual MIC can also be reported.
Reference Method	Standard broth dilution (macrodilution or microdilution) or agar dilution as described in CLSI M7 Approved Standard (Ref. 1).
Reproducibility	Measure of whether the new device produces the same result across different testing conditions.
Resistant Threshold	Highest *in vitro* concentration at which most organisms are no longer considered susceptible. Organisms with an MIC at this concentration, or higher are reported as resistant.
Shortened Incubation	Determinations of growth in less than 16 hours.
SIR	Susceptible, Intermediate, Resistant.
Susceptible Threshold	Lowest *in vitro* concentration at which most organisms are still considered susceptible. Organisms that do not grow at this concentration or at lower concentrations are reported as susceptible.
Trending	An upward or downward change associated with increased resistance (decreased susceptibility) or increased susceptibility (decreased resistance). This type of change may not necessarily be seen with qualitative susceptibility testing. Trending is applied for certain organisms or certain antimicrobials to detect emerging resistance or may be used to compare results between different susceptibility testing methods to assess bias that would not be evident using EA or CA, unless larger numbers of organisms were evaluated.
vmj	See Discrepancy – very major

XV. References

1. CLSI. M7 - *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*; *Approved Standard.* (most recently approved supplement). CLSI; Wayne, Pennsylvania.

2. CLSI. M11 - *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*; *Approved Standard.* (most recently approved supplement). CLSI; Wayne, Pennsylvania.

3. CLSI. M100 - *Performance Standards for Antimicrobial Susceptibility Testing.* (most recent informational supplement). CLSI; Wayne, Pennsylvania.

4. Ferraro MJ, Jorgensen JH. Susceptibility Testing in Instrumentation and Computerized Expert Systems for Data Analysis and Interpretation. In: Murray PR, Baron EJ, Pfaller MA, et al, eds. *Manual of Clinical Microbiology*, 7th Edition, Washington DC: American Society of Microbiology; 1999 1593 – 1600.

5. CLSI. M23 - *Development of In vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline*. (most recently approved supplement). CLSI; Wayne, Pennsylvania.

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XVII. Appendix

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TABLE 1: Recommendations for Antimicrobial Susceptibility Devices[a]

		MIC/BP Formats	Fastidious[b]	Additional methods of Inoculation/Reading[c]
Number of Sites (including 1 in-house)		3	3	3
Organisms	Fresh[d] Clinical/stock[e]	100/site	75/site	0
	CDC Challenge[f]	75/one site	50/one site	75 or 50/one site
Reproducibility[g]		25/site **or** 10x3x3/site	25/site **or** 10x3x3/site	25/site **or** 10x3x3/site
Interpretive Standards		FDA	FDA	FDA
Stability (3 lots)		Real time (on file)	Real time (on file)	Real time (on file)
QC Reference and Test Device Results	CLSI Strains	20 results/site	20 results/site	20 results/site
	(Other Mfg. Recommended)	Optional	Optional	Optional
	On-scale	At least 1	At least 1	At least 1
	Inoculum density check[h]	QC, reproducibility, fresh	QC, reproducibility, fresh	QC, reproducibility
CLSI Reference Method		MIC	MIC	MIC

[a] See Tables 5, 8, and 9 for statistical calculation examples and evaluable results.

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

[c] Minimal data to establish performance should be presented for each procedural option of the method of inoculation (growth, direct colony suspension etc.), reading of results (visual vs. automated), or combinations of options.

[d] Fresh clinical - an organism isolated from a clinical specimen which has been on an agar plate for less than 7 days and not frozen.

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

[f] Challenge - CDC or reference laboratory source with known results (preferably on-scale) to be tested on the test system. Organisms that are intended for the testing with the antimicrobial agent as stated in the FDA approved pharmaceutical antimicrobial agent package insert (microbiology section), should be selected for testing on the test device.

[g] All on-scale results.

[h] Inoculum density check should be performed daily on the QC isolates, on reproducibility isolates, and 10% of fresh isolates. Alternate approaches may be substituted if the inoculum method uses a spectrophotometric device.

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

Items		Performance	Insufficient Resistant Strains	Quality Control[b]	Reproducibility
Number of Sites[c]		3	1	3	3
Organisms	Fresh or Stock Clinical	100/site	75[d]	0	NA
	Challenge	75/one site	as needed	0	
Reproducibility[e]		NA	NA	NA	25/site **or** 10x3x3/site
Quality Control		Daily	Daily	20	Daily

[a] For Statistically evaluable numbers see Tables 5, 8 and 9
[b] To be used to demonstrate that the QC ranges are now in the same ranges as the FDA/CLSI
[c] One may be in-house
[d] A minimum of 75 organisms either resistant or clustered near the susceptible threshold.
[e] On-scale

Note:
1. If changes have been made to the device to alter the overall performance, the testing should include all organisms previously tested.
   Refer to Table 1.
2. Perform testing for all procedural options.

TABLE 3: Presentation of Summary Data for Both Challenge and Clinical Data

Clinical Data

Organism Group	Total Tested	# EA	% EA	Total Evaluable	# EA of Evaluable	%EA of Evaluable	# CA	% CA	# R	# vmj	# maj	# min
K. pneumoniae	79	74	93.7	64	62	92.2	70	88.6	20	0	1	8
P. aeruginosa	96	90	93.8	91	85	93.4	88	91.7	40	0	1	7
C. freundii	26	21	80.8	18	14	72.2	23	88.5	10	0	0	3
E. aerogenes	22	21	95.5	21	21	95.2	21	95.5	2	0	0	1
E. cloacae	57	53	93	50	48	92	51	89.5	13	1	1	4
M. morganii	15	14	93.3	12	12	91.7	15	100	7	0	0	0
P. mirabilis	34	26	76.5	34	29	76.5	34	100	33	0	0	0
E. coli	92	85	92.4	62	59	88.7	87	94.6	43	2	1	2
S. marcescens	50	49	98	48	48	100	47	94	32	0	0	3
Acinetobacter spp	41	40	97.6	29	28	96.6	34	82.9	14	0	0	7
TOTAL	512	473	92.4	429	406	94.6	470	91.8	214	3	4	35

Challenge

K. pneumoniae	10	10	100	10	10	100	10	100	6	0	0	2
P. aeruginosa	20	18	90	17	15	88.2	19	95	15	1	0	0
C. freundii	10	10	100	9	9	100	9	90	2	0	1	0
E. aerogenes	5	4	80	4	3	75	5	100	0	0	1	1
E. cloacae	5	5	100	2	2	100	5	100	4	0	0	1
Aeromonas sp.	5	4	80	4	4	100	5	100	0	0	0	0
P. mirabilis	10	9	90	8	6	75	9	90	4	0	0	2
S. marcescens	10	10	80	9	9	100	9	90	2	0	0	1
Acinetobacter spp	10	8	80	5	5	100	8	80	2	1	0	2
TOTAL	85	78	91.8	68	63	92.6	79	92.9	35	2	2	9

Clinical and Challenge Combined

All organisms	597	551	92.3	497	469	94.4	549	92.0	249	5	6	44

GRZAN

TABLE 4: Example of Reporting Format for Quality Control Data

Antimicrobial agent: _____

QC Organism	Expected Result		Reference Result Frequency			New Device Frequency		
			Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
E. coli ATCC 25922	0.25 - 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.
- To be used for all procedural options.

TABLE 5: Sample Table Format for Device Performance

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	≤ 0.25[a]	0.5	1	2 **S**	4 **R**	8	≥ 16
≤ 0.25	6[a]	[a]	1				[a]
0.5	10[2]	100	21	2			[a]
1	[a]	10	8	1			[a]
2 **S**	[a]	6	11	1			[a]
4 **R**	[a]						[a]
≥ 8	[a]				[a]	[a]	31[a]
Evaluable Results		116	41	4			

Organism: Staphylococcus aureus

Evaluation	
Overall EA[b] 199/208	95.7%
EA based on evaluable results[a b] 152/161	94.4%
CA based on interpretation[c]	100%

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	≤ 0.25 **S**	0.5 **R**	1	2	4	8	≥ 16
≤ 0.25 **S**	29[a]	[a]					[a]
0.5 **R**	[a]						[a]
1	[a]						[a]
2	[a]		1	1		1	[a]
4	[a]				1		[a]
≥ 8	[a]			7	12[a]	12[a]	52[a]
Evaluable Results			1	8	1	1	

Organism: Staphylococcus epidermidis

Evaluation	
Overall EA[b] 108/116	93.1%
EA based on evaluable results[a b] 3/11	27.3%
CA based on interpretation[c]	100%

Antimicrobic agent: Oxacillin

Test Results	Reference Results						
	≤ 0.25 **S**	0.5 **R**	1	2	4	8	≥ 16
≤ 0.25 **S**	25[a]	1[a] vmj					1[a]
0.5 **R**	[a]	8	3				[a]
1	[a]						1[a]
2	[a]						[a]
4	[a]						[a]
≥ 8	1[a] (maj)				2[a]	3[a]	26[a]
Evaluable Results		8	3				

Organism: other CNS

Evaluation	
Overall EA[b] 67/70	95.7%
EA based on evaluable results[a b] 11/11	100%
CA based on interpretation[c] 67/70	95.7%

see footnotes on Table 5A

Present all fresh and stock results for organisms in a genus/species in a separate diagonal. For example all Staphylococcus aureus results from all sites would appear in the first diagonal.

TABLE 5A: Sample Table Format for Device Performance, continued

Antimicrobic agent: **Ciprofloxacin** **Organism:** All

Test Results	**Reference Results**						
	≤ 0.12	0.25	0.5	1 **S**	2 **I**	4 **R**	≥ 8
≤ 0.12	259 [a]	[a]					[a]
0.25	[a]	4	2				[a]
0.5	6[a]	4	2				[a]
1 **S**	[a]		10	10	5	1	[a]
2 **I**	[a]		2	9	10	11	[a]
4 **R**	[a]				8	10	[a]
≥ 8	[a]					1[a]	53[a]
Evaluable Results		8	16	19	23	22	

Evaluation		
Overall EA[b]		398/407 97.8%
EA based on evaluable results[a b]		85/88 96.6%
CA based on interpretation[c]		371/407 91.2%
CA	Minor[d]	35/407 8.6%
	Major[e]	0 0%
	Very major[f]	1/76 1.3%

[a] Results are not considered evaluable if they fall in this box

[b] $$\text{Essential Agreement (EA)} = \frac{\#\text{ exact agreement or within } \pm \text{ one two - fold dilution of the reference method}}{\text{Total \# organisms tested}} \text{ X } 100$$

[c] $$\text{Category Agreement (CA)} = \frac{\#\text{ with interpretive agreement to the reference method}}{\text{Total \# organisms tested}} \text{ X } 100$$

[d] $$\text{Minor Discrepancy (min)} = \frac{\#\text{ min discrepancies based on interpretation}}{\text{Total \# organisms tested}} \text{ X } 100$$

[e] $$\text{Major Discrepancy (maj)} = \frac{\#\text{ maj discrepancies based on interpretation}}{\text{Total \# susceptible organisms by reference method}} \text{ X } 100$$

[f] $$\text{Very Major Discrepancy (vmj)} = \frac{\#\text{ vmj discrepancies based on interpretation}}{\text{Total \# resistant organisms by reference method}} \text{ X } 100$$

To be used for:

- All fresh and stock organisms for each site presented in one 2A diagonal.
- All fresh and stock organisms for all sites combined presented in one 2A diagonal.
- All challenge organisms presented in one 2A diagonal chart.
- All challenge organisms presented in one 2A diagonal chart for each method variation.
- All organisms (challenge, fresh, stock) combined and presented in one 2A diagonal chart. Do not combine different method variations.

TABLE 6: Presentation of Reproducibility Results[a] by Organism

	Difference in the number of wells between test result and test mode										
	Off-scale	**-2**	**-1**	**0**	**+1**	**+2**	**Off-scale**	**Site 1 result**	**Site 2 result**	**Site 3 result**	**Test Mode[b]**
P. aeruginosa				3				0.5	0.5	0.5	0.5
P. aeruginosa				2	1			4	2	2	2
P. aeruginosa			1	2				16	32	32	32
P. aeruginosa				2	1			8	16	8	8
E. coli				3				2	2	2	2
E. coli			1	2				8	16	16	16
E. coli				2	1			4	2	2	2
E. coli				3				1	1	1	1
E. coli			1	2				2	4	4	4
E. coli				3				32	32	32	32
M. morganii			1	2				16	16	8	16
C. diversus				2	1			0.5	0.5	1	0.5
C. freundii				2	1			1	2	1	1
C. freundii				3				16	16	16	16
E. cloacae				2	1			16	32	16	16
E. cloacae				2		1		0.5	0.5	2	0.5
E. cloacae				3				4	4	4	4
P. mirabilis				3				8	8	8	8
P. mirabilis				3				2	2	2	2
S. marcescens				2	1			2	1	1	1
S. marcescens				2	1			32	16	16	16
S. marcescens				2	1			4	2	2	2
K. pneumoniae				2			1	32	32	>32	32
K. pneumoniae				2	1			0.25	0.5	0.25	0.25
P. stuartii		1		2				16	16	4	16
Total		1	4	58	10	1	1				
Between-site reproducibility[c]	73/75 = **97.3%[d]** 72/75 = **96%[e]**										

[a] Results in the table are occurrences of the difference in the number of wells between the test result and the test mode. The study is based on 25 on-scale organisms, tested at 3 sites.

[b] Most frequent new test result. If there is no mode, the median should be used.

[c] Total number of results that fall within 1 well (+/-1) of the mode result divided by total number of results. This should be calculated for the best and worst case if some of the values are off-scale while some are on-scale. If all three results are off-scale they should still be included in the calculation as part of the best-worst case calculations. For this study the denominator would always be 75 whether the results are off or on-scale.

[d] Best case calculation for reproducibility assuming the off-scale result is within one well from the mode.

[e] Worst case calculation for reproducibility assuming the off-scale result is greater than one well from the mode.

TABLE 6A: Presentation of Reproducibility Results[a] by Organism and Site

	Difference in the number of wells between new test result and test mode							
	Off-scale	**-2**	**-1**	**0**	**+1**	**+2**	**Off-scale**	**Test Mode[b]**
				Site 1				
E. coli 1			6	3				0.5
E. coli 2				9				2
E. coli 3			6	3				2
Pseudomonas 4				3	6			16
Pseudomonas 5			3	5			1	32
Klebsiella 6				8		1		8
Enterobacter 7				8	1			8
Serratia 8				4	5			4
Serratia 9				5	4			16
Proteus 10			5	4				1
Total			20	52	16	1	1	
Within-Site Reproducibility[c]	89/90 = **98.9%**[d] 88/90 = **97.8%**[e]							
				Site 2				
E. coli 1			3	6				0.5
E. coli 2				9				2
etc…								

[a] Results in the table are occurrences of the difference in the number of wells between the test result and the test mode. The study is based on 10 on-scale organisms, tested in triplicate on 3 separate days at 3 sites.

[b] Most frequent test result.

[c] Total number of results that fall within 1 well (+/-1) of the mode result divided by total number of results. This should be calculated for the best and worst case if some of the values are off-scale while some are on-scale. If all results are off-scale they should still be included in the calculation. For this study the denominator would be ninety whether they are all on scale or not.

[d] Best case calculation for reproducibility assuming the off-scale result is within one well from the mode.

[e] Worst case calculation for reproducibility assuming the off-scale result is greater than one well from the mode.

TABLE 6B: Presentation of Reproducibility Results[a] by Organism, Pooled Across Sites

	Difference in the number of wells between new test result and test mode							
	Off-scale	**-2**	**-1**	**0**	**+1**	**+2**	**Off-scale**	**Test Mode[b]**
All Sites								
E. coli 1			9	18				0.5
E. coli 2				25	2			2
E. coli 3		3	9	12	3			2
Pseudomonas 4			6	12	9			16
Pseudomonas 5			2	17			8	32
Klebsiella 6			7	14	5	1		8
Enterobacter 7			5	14	8			8
Serratia 8			4	15	8			4
Serratia 9			3	20	4			16
Proteus 10			3	14	10			1
Total		3	48	161	49	1	8	
Between-site reproducibility[c]	266/270 = **98.5%[d]** 258/270 = **95.6%[e]**							

[a]Results in the table are occurrences of the difference in the number of wells between the test result and the test mode. The study is based on 10 on-scale organisms, tested in triplicate on 3 separate days at 3 sites.
[b] Most frequent test result.
[c]Total number of results that fall within 1 well (+/-1) of the mode result divided by Total number of results. This should be calculated for the best and worst case if some of the values are off-scale while some are on-scale. If all results are off-scale they should be included in the calculation with a denominator of 270 for all calculations.
[d] Best case calculation for reproducibility assuming the off-scale result is within one well from the mode.
[e] Worst case calculation for reproducibility assuming the off-scale result is greater than one well from the mode.

TABLE 7: Report Format for Inoculum Density

ORGANISM[a]	NUMBER TESTED	SOURCE	METHOD[b]	MEAN	MINUMUM	MAXIMUM	STD. DEV.
S. aureus ATCC #	20	QC ATCC	Reference	6 X 10^5	2 x 10^5	9 x 10^5	
S. aureus ATCC #	20	QC ATCC	Direct inoculum	5 x 10^5	2 x 10^5	8 x 10^5	
S. aureus ATCC #	20	QC ATCC	Growth inoculum	5 x 10^5	2 x 10^5	6 x 10^5	
MRSA	13	Reproducibility, clinical	Direct inoculum	7 x 10^5	4 x 10^5	6 x 10^6	
MRSA	13	Reproducibility, clinical	Growth inoculum	6 X 10^5	2 x 10^5	8 x 10^6	
MSSE	3	Reproducibility	Direct inoculum	8 x 10^5	5 x 10^5	7 x 10^5	
MSSE	3	Reproducibility	Growth inoculum	7 x 10^5	4 x 10^5	12 x 10^5	
MRSE	19	Reproducibility	Direct inoculum	6 X 10^5	2 x 10^5	8 x 10^6	
MRSE	19	Reproducibility	Growth inoculum	7 x 10^5	5 x 10^5	7 x 10^5	
Enterococcus	4	Clinical	Direct inoculum			9 x 10^5	
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 unless a standardized method (photometric device) is used.

TABLE 8: Number of VMJ Discrepancies as a Function of the Number of Resistant Organisms Tested

Number of Resistant Organisms	Acceptable Number of Discrepancies	Estimated Rate[a]	95% Confidence Interval[b] for True VMJ 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)
500	13	2.60	(1.39, 4.41)
600	15	2.50	(1.40,4.09)
700	19	2.43	(1.42,3.86)

[a] Est. Rate = estimated vmj rate = number of vmj discrepancies divided by number of resistant organisms.
[b] Exact confidence intervals based on the binomial distribution.

TABLE 9: Essential Agreement as Function of the Number of Evaluable Organisms Tested

Number of Evaluable[a] Organisms	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 (e.g. on-scale) organisms are those that fall within the test range of the reference and have the opportunity for a result on the test method that could also be on-scale. Any 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 organisms that are evaluable.

[c] Exact confidence intervals based on the binomial distribution.