Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures

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Guideline for Industry and Food and Drug Administration Staff

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostics and Radiological Health
Division of Microbiology Devices

Preface

Public Comment

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1. Introduction

This special controls guideline was developed to support the classification of multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures devices into class II (special controls). A multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures is a qualitative in vitro device intended to simultaneously detect and identify microorganism nucleic acids from blood cultures that test positive by Gram stain or other microbiological stains. The device detects specific nucleic acid sequences for microorganism identification as well as for antimicrobial resistance. This device aids in the diagnosis of bloodstream infections when used in conjunction with other clinical and laboratory findings. However, the device does not replace traditional methods for culture and susceptibility testing.

This guideline identifies measures that FDA believes will mitigate the risks to health associated with these devices and provide a reasonable assurance of safety and effectiveness. Firms submitting a 510(k) for a multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures device will need either to (1) comply with the particular mitigation measures set forth in the special controls guideline or (2) use alternative mitigation measures, but demonstrate to the Agency's satisfaction that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.

2. Bloodstream Infections (Bacteremia) – Background

Bacteremia is the presence of bacteria in the blood, which is normally a sterile body fluid. Bacteremia is often a complication of infections (e.g., pneumonia, urinary tract infection (UTI), and is also a common nosocomial infection caused by indwelling catheter lines). Untreated bacteremia in itself can lead to more serious complications such as sepsis, shock, and deep-seated infection (e.g., endocarditis), which have a relatively higher mortality rate. Therefore, earlier diagnosis and treatment is beneficial to the patient.

Traditional methods of diagnosis include blood cultures obtained from patients suspected of having a blood stream infection. When using an automated blood culture monitoring system, the patient specimen is added to culture media and incubated using a blood culture instrument until the growth of bacteria is detected via an instrument signal. A Gram stain is performed on the positive blood culture material, results recorded, and the microorganisms from the blood specimen are subsequently cultured on a variety of media to support the growth of microorganisms. The physical and biochemical characteristics of the microorganism isolates are recorded. Further testing utilizing a variety of biochemical reactions to give the final identification of the microorganism and its susceptibility pattern is then performed. This process may take 2-3 days.

Generally, after blood is collected for culture, patients suspected of having blood stream infections are managed with broad spectrum antibiotic therapy to cover both Gram-negative and Gram-positive microorganisms as well as to address potentially resistant microorganisms. The most readily available information to the physician (within ~12-24 hours) is the Gram stain from the positive blood culture bottle. The antibiotic may be changed based on the Gram stain results (either Gram positive or Gram negative microorganisms). Definitive microorganism targeted therapy can be delayed for an additional 1-2 days while the final identification of the microorganism and antimicrobial susceptibility is completed.

Initial empiric therapy is broad and may or may not cover resistant microorganisms depending on the physician's level of suspicion and the prevalence of resistance at a particular institution. Broad spectrum antibiotic therapy has drawbacks (e.g., overly broad spectrum antibiotic therapy can lead to emergence of more resistant bacteria, and/or *Clostridium difficile* colitis and other antibiotic toxicities or side effects). The addition of methods for earlier identification of microorganisms and associated resistance markers to traditional identification methods would be beneficial in managing patients suspected of having bloodstream infection because more definitive microorganism targeted therapy may be started earlier.

3. Premarket Notifications – Background

FDA concludes that special controls, when combined with the general controls of the Federal Food, Drug & Cosmetic Act (the FD&C Act), are necessary to provide reasonable assurance of the safety and effectiveness of multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures. A manufacturer who intends to market a device of this type must (1) conform to the general controls of the FD&C Act, including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific

issues of safety and effectiveness identified in this guideline, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This guideline identifies the classification regulation for multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures. In addition, other sections of this guideline list the risks to health and describe mitigation measures that, if followed by manufacturers and combined with the general controls, will address the risks associated with these devices and will generally lead to a timely premarket notification (510(k)) review. This document will supplement other FDA documents regarding the specific content requirements of a premarket notification submission for multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures. For additional information regarding 510(k) submissions, refer to 21 CFR 807.87 and the Center for Devices and Radiological Health (CDRH) Device Advice: Comprehensive Regulatory Assistance.[1]

4. Scope

The scope of this document is limited to devices classified under 21 CFR 866.3365.

21 CFR 866.3365 – Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures

(a) *Identification*:
A multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures is a qualitative in vitro device intended to simultaneously detect and identify microorganism nucleic acids from blood cultures that test positive by Gram stain or other microbiological stains. The device detects specific nucleic acid sequences for microorganism identification as well as for antimicrobial resistance. This device aids in the diagnosis of bloodstream infections when used in conjunction with other clinical and laboratory findings. However, the device does not replace traditional methods for culture and susceptibility testing.
(b) *Classification*. Class II (special controls). The special control is the FDA's guideline entitled "Class II Special Controls Guideline Document: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures." See §866.1(e) for the availability of the guideline document.

5. Risks to Health

FDA has identified the risks of false negative and false positive test results, both of which can lead to individual and/or public health consequences, and error in interpretation of results as risks to health associated with this device that require special controls. Failure of the device to detect and identify a targeted microorganism when such microorganism is present in the specimen (false negative result) may lead to a delay in finding the true cause of the bloodstream

[1] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

infection/bacteremia, unnecessary treatment or to inappropriate antibiotic use. An incorrect positive test result (false positive result) also may lead to unnecessary or ineffective antibiotic therapy and delay in determining the true cause of the patient's illness.

Failure of the device to detect a targeted gene associated with resistance when such gene is present in the detected microorganism (e.g., false negative results for *mec*A, *van*A, *van*B) may lead to treatment with ineffective antibiotics and lapses in infection control measures. An incorrect positive result for a resistance marker (false positive result) may also lead to inappropriate antibiotic therapy (frequently overly broad) to cover resistant microorganisms that are not present. The more potent antibiotics may have more side effects (e.g., renal toxicity), and may lead to unnecessary and often costly implementation of infection control measures.

Failure to correctly interpret test results in the context of other clinical and laboratory findings may lead to inappropriate or delayed treatment. For example, positive assay results do not rule out viral or other bacterial co-infections. Therefore, additional testing (e.g., bacterial sub-culture) is needed in order to obtain a final diagnosis. Additionally, a concurrently detected microorganism and resistance gene does not establish a definitive link between the detected microorganism and the resistance gene.

It is likely that the results of these devices will strongly influence attributing the cause of illness to the identified microorganism/resistance marker. However, testing with this device does not replace traditional positive blood culture testing, which once available, would be used to inform therapy choices.

In Table 1 below, FDA has identified the risks generally associated with the use of a multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures that require special controls. The measures to mitigate these identified risks are in this guideline, as shown in Table 1 below, in combination with subsection 21 CFR 866.3365. Under this guideline, manufacturers who intend to market a device of this type must conduct a risk analysis prior to submitting a premarket notification to identify any other risks specific to their device. The premarket notification must describe the risk analysis method used. If you elect to use an alternative approach to mitigate a particular risk identified in this guideline, or if you or others identify additional potential risks from use of a device of this type, you must provide sufficient detail regarding the approaches used to mitigate these risks and a justification for your approach.

Table 1 – Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
False Negative Result	Device Description Containing the Information Specified in the Special Control Guideline (Section 6) Performance Characteristics (Section 7) Labeling (Section 8)
False Positive Result	Device Description Containing the Information Specified in the Special Control Guideline (Section 6)

	Performance Characteristics (Section 7) Labeling (Section 8)
Errors in Interpretation	Device Description Containing the Information Specified in the Special Control Guideline (Section 6) Performance Characteristics (Section 7) Labeling (Section 8)

6. Device Description

In your 510(k) submission, you must include a device description that meets the requirements of 21 CFR 807.87(a) and (f) and you must identify the legally marketed predicate device as required by 21 CFR 807.92(a)(3). Furthermore, you must also identify the applicable regulation and the product code(s) for your device; you must include a table that outlines the similarities and differences between the predicate device (or another legally marketed device for the same intended use) and your device. You may reference appropriate peer-reviewed articles that support the use of your device for its intended diagnostic use and the specific test principles incorporated into the device design. You must describe each of these device elements in detail.

In addition, you must include the following descriptive information to adequately characterize your multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures.

a. Intended Use

The intended use must specify the specific populations(s) and specimen type(s) for which the test is intended (e.g., positive blood cultures), specific analytes that are detected by your device (microorganism, resistance marker), the nature of the analyte (e.g., DNA), and the clinical indication(s) for which the test is to be used. The intended use must be qualitative and state whether analyte detection is presumptive (e.g., not to be replacing traditional methods of culture and susceptibility testing) and any specific conditions of use.

In your 510(k), you must clearly describe the following information related to the intended use of your product:

- The identity of the microorganisms and resistance markers that your device is designed to detect.
- How the device test results will be used to aid in laboratory identification of microorganisms and microorganism antimicrobial resistance from patients with positive blood cultures.

b. Test Methodology

You must describe in detail the methodology used by your device. This must include describing the following elements as applicable to your device:

- Test platform (e.g., Real-time PCR, hybridization assay, bead arrays).

- Specificity of the primers/probes and targets for the bacteria/resistance markers in question.
- Information regarding the rationale for the selection of specific targets.
- Limiting factors of the assay (e.g., saturation level of hybridization, maximum cycle number).
- Device design to minimize false positives due to contamination or carryover.
- Sample types (e.g., specific types of blood culture bottles).
- Reagent components provided or recommended for use, and their function within the system (e.g., buffers, enzymes, enzymes, fluorescent dyes, chemiluminescent reagents, other signaling/amplification reagents).
- The potential for specific and non-specific probe cross-hybridization.
- Description of any internal controls and external controls specific to use with the device.
- Instrumentation and software involved in the use of your device, including the components and their function within the system.
- The computational path from raw data to the reported result (e.g., how raw signals are converted into a value), if appropriate. This would include sufficient software controls for identifying and dealing with obvious problems in the dataset. It would also include adjustment for background and normalization, if applicable.
- Illustrations or photographs of non-standard equipment or methods, as appropriate and provide a detailed description.

When applicable, you must describe design control specifications for your device that address or mitigate risks associated with primers, probes and controls used in nucleic-acid based multiplex test procedures to detect microorganism nucleic acid, such as the following:

- Minimization of false positives due to contamination or carryover of a sample.
- Prevention of probe cross-contamination for multiplexed tests in which many probes are handled during the manufacturing process.
- Correct placement and identity of assay features (e.g., probes).
- Use of multiple probes to enable detection of strain or resistance gene marker variants.
- Development of recommendations regarding validated methods for nucleic acid extraction and purification that yield suitable quality and quantity of nucleic acid from Gram stain positive blood cultures for use in the test system with your reagents, if appropriate.
- Optimization of your reagents and test procedure for the recommended instrument(s).

In your 510(k), you must provide performance information that supports the conclusion that your design control specifications have been met. You must also provide information to verify the design of your reagents (e.g., rationale for selection of specific primers/probes). See Section 7 – Performance Characteristics.

c. Specimen Storage Conditions

If you recommend specimen storage conditions, you must demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage period. Each recommended storage temperature must be evaluated, and for wide temperature ranges, both ends of the range must be evaluated (e.g., test both 15ºC and 30ºC for a room temperature claim of 15-30ºC).

d. Ancillary Reagents

Ancillary reagents are reagents specified in device labeling as "required but not provided" in order to carry out the assay as indicated in its instructions for use and to achieve the test performance claimed in labeling for the assay. For example, if your device labeling specifies the use of a specific reagent (e.g., 'Brand X Extraction Buffer' or other buffers shown to be equivalent), and use of any other extraction buffer may alter the performance characteristics of your device from that reported in your labeling, then Brand X Extraction Buffer or any other buffers shown to be equivalent are ancillary reagents of concern for the purposes of this document. Even if you establish that one or more alternative ancillary reagents may be used in your assay, each of those named alternatives may still be an ancillary reagent of concern. You may consult with FDA if you are unsure whether this aspect of the special controls applies to your device.

By contrast, if your device relies on the use of 95% ethanol and any brand of 95% ethanol will allow your device to achieve the performance characteristics provided in your labeling, then 95% ethanol is not an ancillary reagent of concern for the purposes of this document.

If the instructions for use of your device specify one or more ancillary reagents of concern, you must provide a plan that addresses how you will ensure that the results of testing with your device and these ancillary reagents, in accordance with your instructions, will be consistent with the performance established in your premarket submission. Your plan may include application of quality systems approaches, product labeling, and other measures.

FDA will evaluate whether your plan will help to mitigate the risks presented by the device to offer reasonable assurance of the safety and effectiveness of the device and establish its substantial equivalence. In order to address this aspect of the guideline, your 510(k) submission must address the elements described below.

1. You must include in your 510(k) a risk assessment addressing the use of ancillary reagents, including risks associated with management of reagent quality and variability, risks associated with inconsistency between instructions for use provided directly with the ancillary reagent and those supplied by you with your assay, and any other issues that could present a risk of obtaining incorrect results with your assay.
2. Using your risk assessment as a basis for applicability, you must describe in your 510(k) how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These may include, where applicable:

- User labeling to assure appropriate use of ancillary reagents (see "Labeling" for further discussion).
- Plans for assessing user compliance with labeling instructions regarding ancillary reagents.
- Material specifications for ancillary reagents.
- Identification of reagent lots that will allow appropriate performance of your device.
- Stability testing.
- Complaint handling.
- Corrective and preventive actions.
- Plans for alerting users in the event of an issue involving ancillary reagents that would impact the performance of the assay.
- Any other issues that need to be addressed in order to assure safe and effective use of your test in combination with named ancillary reagents, in accordance with your device's instructions for use.

In addition, you must provide testing data to establish that the quality controls you supply or recommend are adequate to detect performance or stability problems with the ancillary reagents.

If you have questions regarding identification, use, or control of ancillary reagents, please contact the Division of Microbiology Devices to obtain advice or information regarding your planned study.

e. Controls

Controls must approximate the composition and nucleic acid concentration of a sample in order to adequately challenge the system, as well as address reproducibility around the cut-off. You must run appropriate controls every day of testing for the duration of the analytical and clinical studies. This includes any positive and negative controls provided with your assay as well as appropriate external controls recommended but not necessarily provided with the assay.

You must describe the following concerning quality control and calibration:

- The nature and function of the various controls that you include with, or recommend for, your system. These controls must enable the user to determine if all steps and critical reactions have proceeded properly without contamination or cross-hybridization.
- Your methods for value assignment (relative or absolute) and validation of control and calibrator material, if applicable.
- The control parameters that could be used to detect failure of the instrumentation to meet required specifications.

Controls must provide information about (1) sample quality, (2) nucleic acid quality, and (3) process quality. You may contact the Division of Microbiology Devices when designing specific controls for your device. Generally, you must include the following types of controls:

(1) Negative Controls

Blank or no template control

The blank, or no-template control, contains buffer or sample transport media and all of the assay components except nucleic acid. This control is used to rule out contamination with target nucleic acid or increased background in the amplification reaction. It may not be applicable for assays performed in single-test, disposable cartridges or tubes.

Negative sample control

The negative sample control contains non-target nucleic acid or if used to evaluate extraction procedures, it contains whole microorganism(s) not targeted by the assay. It reveals non-specific priming or detection and indicates that signals are not obtained in the absence of target sequences. This control must be tested daily in the analytical and clinical studies. Examples of acceptable negative sample control materials include:

- Negative blood culture bottle
- Samples containing a non-target microorganism (e.g., blood culture bottle spiked with non-target microorganism)
- Surrogate negative control (e.g., extracted DNA)

(2) Positive Controls

External positive control for complete assay

The positive control is designed to mimic a positive blood culture specimen, contains target nucleic acids, and is used to control the entire assay process, including nucleic acid extraction, amplification, and detection. Acceptable positive assay control materials include blood culture media spiked with blood and whole microorganism(s) targeted by the assay. This positive control must be run daily during the analytical and clinical studies. When applicable, representative analytes may be tested in a rotating manner using a pre-defined schedule.

Positive control for amplification and detection

The positive control for amplification/detection can contain purified target nucleic acid at or near the limit of detection for a qualitative assay. It controls for the integrity of the reaction components and instrument when negative results are obtained. It indicates that the target is detected if present in the sample.

Internal control

The internal control is a non-target nucleic acid sequence that is co-extracted and co-amplified with the target nucleic acid. It controls for integrity of the reagents (e.g., polymerase, primers), equipment function (e.g., thermal cycler), and the presence of inhibitors in the samples. An

example of acceptable internal control is a non-specific target microorganism such as *Bacillus subtilis*. The need for this control is determined on a device case-by-case basis through agency feedback. You may refer to Clinical and Laboratory Standards Institute (CLSI) document MM3-A2, *Molecular Diagnostic Methods for Infectious Disease* [REF. 1], for additional information.

f. Interpreting Test Results/Reporting

In your 510(k), you must describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they are interpreted. Interpretative algorithms must be clearly explained. In your 510(k) submission, you must indicate the cut-off values for all outputs of the assay.

- You must provide the cut-off value for defining a negative result of the assay. If the assay has only two possible output results (negative/positive), this cut-off also defines a positive result of the assay.
- If the assay has an invalid result, you must describe how an invalid result is defined. If internal controls are part of the determination of invalid results, you must provide the interpretation of each possible combination of control results for defining the invalid result. You must provide recommendations for how to follow up any invalid result (i.e., whether the result is reported as invalid or whether re-testing is recommended). If re-testing is recommended, you must provide information similar to that for the re-testing of equivocal results (i.e., whether re-testing must be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen).
- Resistance markers must be reported only if a microorganism that is known to carry that marker is detected by the device.

7. Performance Characteristics:

a. General Study Recommendations

Your 510(k) submission must include detailed descriptive information regarding the studies that you conducted to establish each of the performance characteristics outlined below.

Generally, prospective clinical studies are required to determine the performance of your device in conditions similar to the intended use. In general, for both clinical studies and reproducibility studies, you must conduct testing at sites consistent with where you intend to market the device (e.g., clinical laboratory sites).

You must provide appropriate specific information in your 510(k) submission describing the protocols used during your assay development in order for FDA to accurately interpret acceptance criteria and data summaries contained in your application. This information is also important to aid users in understanding the information in your labeling. When referring to CLSI protocols or guidelines, you must indicate whether all aspects of the guideline were followed or, if not, which specific aspects of the protocols or guidelines were followed.

In your 510(k), you must describe in detail the study design you used to evaluate each of the performance characteristics identified in Section 7. You may contact the Division of Microbiology Devices prior to initiating your clinical study to obtain feedback regarding your planned studies and the intended uses that are planned for inclusion in your 510(k) submission.

b. Preanalytical Factors

Consideration of preanalytical factors is critical for these devices. In your 510(k), you must address the following issues regarding preanalytical factors.

(1) Specimen Collection and Handling

You must specify the specific blood culture bottle type(s) for which your assay is intended to be used.

If you recommend specimen storage and/or shipping conditions, you must demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage. Each claimed temperature range must also be evaluated. You must state your acceptance criteria for all specimen stability parameters.

CLSI document MM13-A, *Collection, Transport, Preparation and Storage of Specimens for Molecular Methods* [REF 2], and CLSI document H18-A4, *Procedures for Handling and Processing of Blood Specimens* [REF3], and CLSI document M29-A2, *Protection of Laboratory Workers from Occupationally Acquired Infections* [REF 4] contain additional information regarding this topic.

(2) Fresh versus Frozen Samples (Stability)

Performance for positive blood cultures may be different for fresh versus frozen samples. If you use any frozen samples in your studies, or are claiming the use of frozen samples for testing with your device, you must assess the effect of freezing samples prior to testing and the effect of multiple freeze/thaw cycles on the performance of your device. You must test representative analytes with a minimum of 60 samples, with most of the samples containing levels close to the Limit of Detection (LoD), and the rest of the samples containing concentrations throughout the clinically relevant analyte concentration range. The study must demonstrate positive agreement of at least 95% with a lower bound of a 95% (two-sided) confidence interval exceeding 90%.

(3) Nucleic Acid Extraction

Different extraction methods may yield nucleic acids of varying quantity and quality, and therefore the extraction method can be crucial to a successful result. Therefore you must evaluate your assay's analytical and clinical performance characteristics for each targeted microorganism using the entire pre-analytical process (including extraction procedures) that you recommend for use with your device.

If you recommend multiple extraction methods, the LoD of your device must be determined separately for each method. Additionally, the reproducibility study design must allow for evaluation of each extraction procedure. Provided the LoD results are equivalent for the different extraction methods, it is acceptable for different methods to be used at different testing sites in the reproducibility study.

If LoD and reproducibility study results demonstrate equivalent performance using the different extraction procedures, it may be acceptable for clinical study testing sites to use different extraction procedures. Furthermore, data may be pooled in the final analysis if clinical performance of the device is equivalent for each extraction method.

You must provide information on how your device controls for extraction efficiency (e.g., the presence of an internal control added to each tested sample).

(4) Cut-off Determination

You must explain how the cut-off for each target/analyte was initially determined as well as how it was validated. The cut-off must be determined using appropriate statistical methods. The performance of your device using the pre-determined cut-off must then be validated in an independent set of positive blood cultures. Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of the pilot studies with clinical specimens. For details about ROC analysis, you may refer to CLSI document EP24-A2 *Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristics Curves*) [REF 5].

c. Analytical Performance

(1) Limit of Detection (LoD)

The LoD is defined as the lowest concentration (CFU/ml) of analyte that can be detected in a positive blood culture sample approximately 95% of the time. The LoD must be determined for each targeted microorganism and resistance marker in the most challenging blood culture media claimed for use with your device. The number of strains tested may vary by analyte, but in general you must determine the LoD for a minimum of two strains for each microorganism and for each resistance marker targeted by your device.

LoD determination can be accomplished by preparing serial dilutions of positive blood cultures into negative simulated blood culture matrix (e.g., blood culture media with human blood.) Tested sample concentrations must be confirmed by plating and subsequent colony counts. Initially the LoD can be estimated by testing a small number of replicates at each dilution. Confirmation of the LoD can then be performed by testing a minimum of 20 replicates at the lowest concentration that can produce a positive result greater than 95% of the time.

You may refer to CLSI document EP17-A2, *Evaluation of Detection Capability for Clinical Laboratory Measurements* [REF 6] when designing your LoD studies.

(2) Analytical Reactivity (Inclusivity)

You must demonstrate reactivity for a comprehensive variety of strains of each microorganism and resistance marker that your device is designed to detect. For each microorganism detected by your device, you must include multiple well-characterized strains that are clinically relevant and represent temporal and geographical diversity (e.g., for MRSA, test multiple strains representing the various Pulse-Field Gel Electrophoresis (PFGE) types: USA 100, 200, 300 and 400, with emphasis on the USA 300 strains). For each resistance marker that your device detects, you must include multiple strains of each microorganism detected by your device and known to carry the specific marker. Strains appropriate to be tested in the inclusivity studies will vary depending on the device's targeted analytes. Samples must contain low concentrations of each challenge strain (e.g., near the assay LoD). All isolate identities and concentrations must be confirmed.

If appropriate, *in silico* results based on target sequence alignment identity can be used to guide the selection of strains that need to be "wet tested." *In silico* analyses must include clinically relevant strains and represent temporal, geographical, and phylogenetic diversity for each claimed target. With this approach, increasing numbers of representative microorganisms selected from groups with decreasing levels of identity to the target region can be selected for further laboratory testing. You must provide a clear rationale for the inclusion of the selected strains, the metrics used to assess inclusivity, and a clear presentation of the sequence alignment in the specific regions of interest for each microorganism evaluated. In addition, you must provide information regarding primer and probe sequences and any sequence differences in the target region for each species tested along with calculated percent homology. You may contact the Division of Microbiology Devices for study design guidance.

(3) Analytical Specificity (Cross-reactivity)

You must determine analytical specificity for your device for a comprehensive number of yeast and aerobic and anaerobic bacteria that are not targeted by your device. You must include microorganisms that are phylogenetically related to the microorganisms detected by your device as well as microorganisms that may be present in blood culture specimens, including those that are commonly considered skin contaminants. This testing must be performed in a blood culture media matrix containing human blood and potential cross-reacting microorganisms at high concentrations (i.e., $10^8 – 10^9$ CFU/mL).

To supplement exclusivity wet-testing, *in silico* evidence must also be included in your submission to support the specificity of the nucleic acid targets for your assay and as an aid in determining the microorganisms to be tested in your cross-reactivity study. You may contact the Division of Microbiology Devices for study design guidance.

Microorganisms that you include in your cross-reactivity study are dependent on the intended use of your device. Table 2 contains examples of the types of microorganisms that might be appropriate to include in an exclusivity study for a Gram positive blood culture panel:

Table 2

Abiotrophia defectiva	*Corynebacterium bovis*	*Leuconostoc carnosum*
Acinetobacter baumanii	*Corynebacterium diphtheriae*	*Leuconostoc mesenteroids*
Acinetobacter haemolyticus	*Corynebacterium flavescens*	*Moraxella catarrhalis*
Acinetobacter lwoffi	*Corynebacterium genitalium*	*Mycobacterium tuberculosis*
Acinetobacter junii	*Corynebacterium glutamicum*	*Mycoplasma pneumoniae*
Acinetobacter ursingii	*Corynebacterium jeikeium*	*Neisseria lactamica*
Aerococcus viridans	*Corynebacterium renale*	*Neisseria meningitidis* (serogroup B)
Arcanobacterium bernardiae	*Corynebacterium striatum*	*Neisseria mucosa*
Arcanobacterium haemolyticum	*Corynebacterium urealyticum*	*Neisseria sicca*
Bacillus cereus	*Cryptococcus neoformans*	*Pasteurella aerogenes*
Bacillus licheniformis	*Enterobacter aerogenes*	*Pediococcus acidilactici*
Bacillus thuringiensis	*Enterobacter cloacae*	*Pediococcus pentosaceus*
Bordetella pertussis	*Enterococcus avium*	*Peptostreptococcus anaerobius*
Candida albicans	*Enterococcus casseliflavis*	*Planococcus citrues*
Candida glabrata	*Enterococcus durans*	*Planococcus kocurri*
Candida krusei	*Enterococcus flavescens*	*Propionibacterium acnes*
Candida parapsilosis	*Enterococcus gallinarum*	*Proteus mirabilis*
Candida tropicalis	*Enterococcus hirae*	*Proteus vulgaris*
Cellulosimicrobium cellulans	*Enterococcus mundtii*	*Providencia stuartii*
Cellumonas turbata	*Enterococcus raffinosus*	*Pseudomonas aeruginosa*
Citrobacter amalonaticus	*Erysipelothrix rhusiopathiae*	*Pseudomonas fluorescens*
Citrobacter braakii	*Escherichia coli*	*Rothia dentocariosa*
Citrobacter farmeri	*Haemophilus influenzae*	*Rothia mucilaginosa*
Citrobacter freundii	*Klebsiella oxytoca*	*Salmonella enterica* (serovar Enteritidis*)*
Citrobacter gillenii	*Klebsiella pneumoniae*	*Salmonella enterica* (serovar Typhimurium*)*
Citrobacter koseri	*Kocuria kristinae*	*Serratia marescens*
Citrobacter murliniae	*Kytococcus sedentarius*	*Serratia odorifera*
Citrobacter rodentium	*Lactobacillus acidophilus*	*Shigella sonnei*
Citrobacter werkmanii	*Lactobacillus crispatus*	*Yersinia enterocolitica*
Citrobacter youngae	*Lactobacillus rhamnosus*	
Corynebacterium amycolatum	*Legionella pneumophilia*	

(4) Competitive Inhibition Studies

Competitive inhibition studies must challenge your assay with combinations of analytes that are commonly found in mixed positive blood cultures as well as analytes mixed with non-target microorganisms including common skin contaminants (e.g., *Corynebacterium* spp., *Propionibacterium acnes)*. In these studies, you must demonstrate that a high concentration of

one microorganism does not inhibit detection of a targeted microorganism that is present at concentrations near the LoD and vice versa. You may reference current published literature to support your selection of the most appropriate microorganism combinations to be included in these studies.

(5) Interference Testing

(a) Interfering Substances

In order to assess the inhibitory effects of substance encountered in blood and blood culture media, you must conduct an interference study using relevant concentrations of potential interferents. These substances may include but are not limited to hemoglobin, triglycerides, conjugated and unconjugated bilirubin, gamma-globulin, high concentrations of white cells, and sodium polyanetholesulfonate. In designing your studies, you may refer to CLSI document EP07-A2, *Interference Testing in Clinical Chemistry*; Approved Guideline. [REF 7].

(b) Testing of Blood Culture Bottle Types

To demonstrate that different types of blood culture media do not interfere with your device, you must conduct additional interference testing for different types of blood culture media. You must evaluate the majority of blood culture bottle types currently used by clinical microbiology laboratories for blood culture testing. This analytical evaluation of blood culture bottle types can be conducted using a blood culture spiking model.

Contrived positive blood cultures can be prepared by spiking each targeted microorganism into blood culture bottles containing sterile human blood. Bottles are then incubated on the appropriate blood culture system until microbial growth is detected and signaled as positive by the automated blood culture instrument ("bottle ring"). The concentration of spiked microorganism must be representative of microorganism concentrations that are present in blood at the time a blood specimen is drawn [REF 8]. Each positive bottle must be confirmed by Gram stain prior to testing on your device.

Alternatively, if blood culture instruments are not available for all bottle types, blood culture bottles containing negative blood may be incubated in a standard laboratory incubator for a period of time (e.g., 24 hours) in order to mimic the incubation time of the blood culture system before the appropriate concentration of microorganism can be spiked. Contrived positive bottles can then be prepared by spiking microorganisms into each of the blood culture bottle types containing negative blood, at the concentration level that is equivalent to the levels present when a bottle is flagged as positive by the blood culture system.

Depending on the breadth of commercially-available blood culture bottle types evaluated and assay performance with each bottle type tested, it may be acceptable for intended use claims to be non-specific for blood culture bottle types. Labeling must clearly specify which bottles were evaluated clinically as well as those that were only evaluated analytically.

(6) Precision (In-house within laboratory/repeatability)

The need for an in-house precision study will be determined on a case by case basis for each individual device. Contact the Division of Microbiology Devices for guidance specific to your device.

If performing a within-laboratory precision study is deemed necessary for your device, you must test sources of variability such as operators, days, and assay runs. Testing must be performed over a minimum of 12 days (not necessarily consecutive) with replicates of each blood culture sample per run.

Samples must be prepared by spiking individual blood culture bottles containing human blood with low concentrations of each targeted microorganism/resistance gene. Bottles must then be incubated on an automated blood culture instrument until "bottle ring". For each targeted microorganism/resistance gene, we recommend that you test a minimum of two different concentrations of targeted microorganisms. In addition, a blood culture sample positive with a non-targeted microorganism must be included in the panel (e.g., skin contaminant such as *Propionibacterium acnes* or *Corynebacterium* spp.). At a minimum, you must include the following panel members in the precision study:

- Positive blood culture at "bottle ring"
- Positive blood culture at "bottle ring" plus an additional eight hours incubation on the automated blood culture instrument
- Positive blood culture with non-target microorganism at high concentration levels (e.g., greater than $1x10^8$ CFU/mL).

Your precision study report must include the following information: number of days and runs, number of operators, and the acceptance criteria applied to your studies. In general for qualitative tests, component of variation must be assessed individually as well as in total. For qualitative tests that have an underlying quantitative output, components of variation must be numerically analyzed (e.g., mean, standard deviation, and percent coefficient of variation).

CLSI documents EP05-A2 *Evaluation of Precision Performance of Quantitative Measurement Methods* [REF 9], and EP12-A2, *User Protocol for Evaluation of Qualitative Test Performance* [REF 10], contain further information about designing and performing repeatability studies.

(7) Multi-Site Reproducibility Study

The protocol for the reproducibility study may vary slightly depending on the assay format although the panel of samples must be prepared in the same manner and generally will consist of the same panel members described for in-house precision studies above.

If your device targets a large number of analytes, it may be reasonable for the reproducibility study to include a subset of representative targeted microorganisms. You may contact the Division of Microbiology Devices for guidance specific to your device.

As a general guide, you must use the following approach to the reproducibility studies:

- Evaluate the reproducibility of your test at three testing sites (one of which may be in-house).
- Use a five day testing protocol, including a minimum of two runs per day (unless the assay design precludes multiple runs per day), three replicates of each panel member per run, with at least two operators at each facility perform the test each day. A minimum of 90 replicates must be tested for each analyte and each concentration.
- You must provide training only to the same extent that you intend to train users after marketing your device.

Your reproducibility study report must include the following information: number of days and runs, number of operators, and acceptance criteria used in determining device performance. In general for qualitative tests, component of variation must be assessed individually as well in total. For qualitative tests that have an underlying quantitative output, components of variation must be numerically analyzed as well (e.g., mean, standard deviation, and % coefficient of variation). In addition, you must provide the percentage of invalid results for each site separately and for all sites combined.

CLSI document EP15-A2, *User Verification of Performance for Precision and Trueness* [REF 11], contains additional information on reproducibility study design.

(8) Carryover Studies and Cross-contamination Studies (for multi-sample assays and devices that require instrumentation)

For multi-sample assays and devices that require instrumentation, you must demonstrate that carryover and cross-contamination do not occur when using your device. In a carryover and cross-contamination study, positive blood culture samples must be tested in a series alternating with negative blood culture samples (or blood culture samples containing a non-targeted microorganism). The study must include a minimum of five runs in which alternating positive and negative samples are performed. Positive samples must consist of representative analytes targeted by your device.

d. Clinical Performance

Clinical study protocols must be completed and reviewed by the investigators prior to the study's initiation. At a minimum, protocols must include complete patient inclusion and exclusion criteria, the type and number of specimens needed, study procedures, and a detailed statistical analysis plan. Copies of the original study protocols, protocol modifications, and any other relevant study information must be included in your 510(k) submission.

We encourage you to contact Division of Microbiology Devices to request a review of your proposed studies through the pre-submission review process. You must conduct prospective clinical studies to determine the performance of your device for the specific intended use. You must develop a detailed study protocol that includes the specific patient inclusion and exclusion criteria, the type and number of specimens needed, the directions for use, and a statistical

analysis plan that accounts for variances to prevent data bias. You must include a description of how the studies support the proposed intended use. The protocol for the clinical study must be included in the 510(k) submission. We encourage manufacturers to contact the Division of Microbiology Devices to request a review of proposed studies prior to study initiation.

Clinical investigations of unapproved and uncleared *in vitro* diagnostic devices are subject to the investigational device exemption (IDE) provisions of Section 520(g) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360j) and the implementing regulations. You must consider how 21 CFR part 812 (IDEs) applies to your particular study, and refer to 21 CFR part 50 (protection of human subjects) and 21 CFR part 56 (institutional review boards) for other applicable requirements.

The following issues must be addressed during the design of your clinical trials:

(1) Reference Assays

You must compare the performance of your device to established reference methods of culture, microorganism identification, and resistance determination tests. Positive blood culture bottles must be subcultured onto appropriate media and incubated for microorganism growth.

- For microorganism identification, cultured isolates may be identified using automated and manual conventional biochemical methods (FDA-cleared or CLSI standard methods). It may be acceptable to use alternative reference methods for identification such as PCR followed by bi-directional sequencing. You may contact the Division of Microbiology Devices for further information regarding the use of any alternate reference methods.

- For resistance markers, you must include phenotypic antimicrobial susceptibility testing methods to assess microorganism resistance in addition to genotypic methods to assess the presence of specific resistance genes. For example:

    - For the *mec*A gene, you must follow the accepted phenotypic reference method which is testing with cefoxitin (disc diffusion or broth microdilution) as described in the current version of CLSI M100, *Performance Standards for Antimicrobial Susceptibility Testing* [REF 12]. If the microorganism is not recovered, discordant evaluation may be performed by using the genotypic methods described below.

    - For specific vancomycin-resistance gene markers such as *van*A and *van*B, phenotypic susceptibility testing must be performed for each *Enterococcus faecalis* or *Enterococcus faecium* isolate. All vancomycin-resistant isolates must then be confirmed to contain the specific vancomycin resistance gene target (e.g., *van*A or *van*B) via PCR followed by bi-directional sequencing.

    - For other resistance markers, please contact the Division of Microbiology Devices regarding phenotypic reference testing.

Genotypic reference methods include PCR followed by bi-directional sequencing or another FDA-cleared assay that detects the specific resistance gene directly from the positive blood culture specimen and directly from blood culture isolates. Ideally, reference assays would target different genomic regions from the regions targeted by your device. If it is necessary for the reference assay to target the same genomic region, the primer pair of the reference assay must be different from and not overlap or align between the primer pair of your device. Bi-directional sequencing must be performed on both strands of the amplicon and demonstrate that the generated sequence is at least 200 base pairs of an acceptable quality (e.g., a quality score of 20 or higher as measured by PHRED or similar software packages).

Sequencing analysis must demonstrate that the results match the reference or consensus sequence. You must provide published literature or laboratory data in your premarket submission in support of your PCR/sequencing reference assay validation for detection of specific resistance determinant sequences.

We recommend that you refer to CLSI document MM18-A, *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing* [REF 13] and CLSI document MM-9, *Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine* [REF 14].

(2) Study Sites

You must conduct your clinical study at a minimum of three different geographical sites representing testing environments where the device will ultimately be used (e.g., clinical laboratories) and by laboratory personnel likely to perform the testing in clinical practice. At least two of the study sites must be in the United States. Testing sites can be independent from specimen collection sites. However, your studies must include a minimum of three geographically diverse collection sites.

(3) Study Population

You must conduct your studies using samples from your proposed target population. In order to preserve the true prevalence of your target analytes in your clinical study patient population, patients previously enrolled in the clinical study must not be allowed to re-enter the same clinical study. History of patients' antibiotic use must be collected and recorded if it is available and reliable. Appropriate patient demographics must be collected (e.g., age).

(4) Study Design

Sample size for a prospective study for estimating sensitivity is determined by the estimated prevalence of clinical disease and the expected prevalence for each analyte. We consider a prevalence of $\leq 2\%$ to be a rare analyte. You must present a detailed summary substantiated by literature references of the expected prevalence for each analyte in your study.

Clinical studies must primarily include testing of fresh prospectively-collected and positive blood culture specimens, each from a unique patient. In addition a portion of the total tested specimens may consist of prospectively collected and archived frozen blood culture specimens. Prospectively collected archived specimens must be collected sequentially from all patients

meeting study inclusion criteria and representing the assay intended use population and must be collected between two predetermined dates, so there is no bias and the prevalence of the analyte is preserved. In the event that some targeted analytes are "rare" (prevalence of ≤ 2%), it is acceptable to supplement with retrospectively tested blood cultures that are known to be positive for specific analytes.

Results from testing of frozen archived specimens are acceptable for inclusion in support of test performance provided that studies are performed to demonstrate that freezing specimens does not alter the performance of the device in comparison to testing of fresh specimens.

In addition, inclusion of contrived specimens may also be an acceptable enrichment approach. Microorganisms used for contrived sample testing must represent different clinical isolates and not multiple isolates of the same microorganisms from the same patient. Contrived specimens must be prepared by spiking blood culture bottles containing sterile human blood with low numbers of microorganisms. Bottles can then be incubated on the appropriate blood culture instrument until signaled as positive.

Retrospective known positive and contrived blood culture specimens must be tested in a blinded manner and randomly distributed with testing performed at a minimum of three clinical testing sites.

If multiple blood culture systems are used in your clinical study, you must evaluate the poolability of data (e.g., equivalence across systems) from the claimed blood culture instruments and their respective claimed blood culture bottle types.

(5) Presentation of Clinical Study Results

You must present sensitivity and specificity (or positive and negative agreement) with 95% confidence intervals separately for each targeted microorganism and resistance gene that your device identifies. Also, you must present (1) the results of your test for the specimens which have more than one analyte as determined by the reference method, and (2) the results for the reference method for the specimens which have more than one microorganism as determined by your device.

All blood culture samples tested in your clinical study must be tested as described in the instructions for use for your device. For example, if the samples with initial invalid results must be re-tested according to the assay instructions, then these samples must be re-tested in the clinical study and the final results for these samples must be used in your statistical analysis. You must provide the percentage of re-tested samples due to equivocal results (if applicable) and due to invalid results for each individual analyte and for all combined.

Performance for prospectively collected fresh specimens must be presented separately in your 510(k) submission. Additionally, separate analyses must be presented for retrospective and contrived blood culture specimens.

e. Instrumentation and Software

For instruments and systems that measure and sort multiple signals, and other complex laboratory instrumentation that have not been previously cleared, refer to the document "Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems," for details on the types of data that must be provided to support instrument clearance.

If your system includes software, you must submit software information detailed in accordance with the level of concern associated with your software. (See the guidance entitled "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices" found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm for information on how FDA believes the level of concern should be determined.) You must determine the level of concern prior to determining how to mitigate the hazards associated with your software. *In vitro* diagnostic devices of this type are typically considered a moderate level of concern, because software flaws could indirectly affect the patient and potentially results in injury when the healthcare provider and patient do not get accurate information.

Below are additional references to consider in developing and maintaining your device under good software life cycle practices consistent with FDA regulations.

- The guidance entitled "General Principles of Software Validation" found at http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM085371.pdf.
- The guidance entitled "Off-the-Shelf Software Use in Medical Devices" found at http://www.fda.gov/ MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073778.htm.
- 21 CFR 820.30[9] Subpart C – Design Controls of the Quality System Regulation.
- ISO 14971-1; Medical devices - Risk management - Part 1: Application of risk analysis.
- AAMI SW68:2001; Medical device software - Software life cycle processes.

8. Labeling

Multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures devices, like other devices, are subject to statutory requirements for labeling (including sections 201(n) and 502(a) of the FD&C Act; 21 U.S.C. 321(n) and 352(a)). These *in vitro* diagnostic (IVD) devices must provide adequate directions for use and adequate warnings and precautions (Section 502(f) of the FD&C Act; 21 USC § 352(f)). Specific labeling requirements for all IVD devices are set forth in 21 CFR Parts 801 and 809.

Your 21 CFR 809.10(b) compliant labeling for your multiplex nucleic acid assay for identification of microorganisms and resistance markers from positive blood cultures device must also include the information described below. This labeling information helps to mitigate the risks identified previously in this guideline to ensure safe and effective use of these devices. All requirements in 21 CFR 809.10 must be addressed in device labeling even if not mentioned below.

a. Intended Use

The intended use statement must clearly specify the intended use of the device, the specific populations(s) and specimen type(s) for which the test is intended (e.g., positive blood cultures), and other significant aspects of use as appropriate (e.g., whether the test must be used in conjunction with culture and/or Gram stain). The intended use must clearly state that the device is to serve only as an aid in diagnosis of bloodstream infections. Additional qualifications may be appropriate based on the results of the clinical studies.

b. Device Description

The Device Description must briefly describe the test methodology used by the device.

c. Procedure

This section must include a description of the entire testing process from patient sampling to result reporting.

d. Directions for Use

The Directions for Use must present clear instructions that systematically describe the procedures for using the device and the types of control measures that will minimize risks of inaccurate results. Instructions must encourage use of additional control measure and testing of control materials to ensure use in a safe and effective manner.

Device handling and storage instructions must be included as well as a description of the expiration dating for both open and closed storage conditions for the device and any reagents or other components.

e. Quality Control

Quality control recommendations in the package insert must include a clear explanation of what controls must be used with the assay and the expected results for the control material.

If controls are included with the device, the 510(k) submission must include the specifications for control materials.

f. Warnings, Contraindications, Precautions, and Limitations

All warnings, contraindications, precautions, and limitations relevant to the specific device must be included in the device labeling. At a minimum, you must include a discussion of certain populations in which device performance may differ or in which the device has not been studied (e.g., pediatrics). In addition, the following statements must be included as limitations:

- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- The detection of bacterial nucleic acid is dependent on proper specimen collection, handling, transport, storage, and preparation, including extraction. Failure to observe proper procedures in any of these steps could lead to incorrect results.
- Isolation on solid media is needed to differentiate mixed growth with other microorganisms and to identify positive blood cultures yielding a negative device result.
- False negative results may occur from improper specimen collection, handling or storage, technical error, sample mix-up, target concentration below the analytical sensitivity of the test, or below the concentration at bottle positivity, which might be caused by the growth of other microorganism(s).
- A negative result for targeted microorganisms or resistance genes should not be used as the sole basis for diagnosis, treatment or patient management decisions.
- The listing of specific blood culture bottle types tested in the analytical and clinical studies.

A statement that pediatric blood culture bottles were not tested in the clinical studies [2]

g. Specimen Collection

You must provide instructions for specimen collection. If you recommend that positive blood culture specimens can be stored for later testing, you must provide information for validated storage conditions.

h. Interpretation and Reporting of Assay Results

You must describe how the operator must interpret each of the possible device results, e.g., positive, equivocal, and negative. You must also describe the recommendations for retesting or reporting of specimens that are equivocal (if this is a possible device output) or where specimen processing fails (e.g., whether another aliquot of the same specimen or a fresh specimen is necessary). See also Section 6(f) of this document entitled "Interpreting Test Results/Reporting." If appropriate, you must include photographs and/or diagrams to indicate how to interpret results for tests with a qualitative result.

i. Performance Characteristics

Labeling must include a summary of the study designs and study results described in Section 7 of this document that would aid the user in interpreting test results and understanding device performance; this must include descriptions of both the clinical and analytical study results.

[2] This limitation statement is needed when applicable to the specific device.

9. References

1. Clinical and Laboratory Standards Institute. 2006. Molecular Diagnostic Methods for Infectious Disease; Proposed Guideline. MM3-A2. Clinical and Laboratory Standards Institute, Wayne PA.

2. Clinical and Laboratory Standards Institute. 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. MM13-A. Clinical and Laboratory Standards Institute, Wayne PA.

3. Clinical and Laboratory Standards Institute. 2010. Procedures for Handling and Processing of Blood Specimens; Approved Guideline. H18-A4. Clinical and Laboratory Standards Institute, Wayne, PA.

4. Clinical and Laboratory Standards Institute. 2005. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. M29-A3. Clinical and Laboratory Standards Institute, Wayne PA.

5. Clinical and Laboratory Standards Institute. 2011. Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristics Curves; Approved Guideline. EP24-A2. Clinical and Laboratory Standards Institute, Wayne PA.

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11. Clinical and Laboratory Standards Institute. 2006. User Verification of Performance for Precision and Trueness; Approved Guideline. EP15-A2. Clinical and Laboratory Standards Institute, Wayne PA.

12. Clinical and Laboratory Standard Institute 2012. Performance Standard for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.

13. Clinical and Laboratory Standards Institute. 2008. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline. MM18-A. Clinical and Laboratory Standards Institute, Wayne PA.

14. Clinical and Laboratory Standards Institute. 2004. Nucleic Acid Sequencing Methods in diagnostic Laboratory Medicine; Approved Guideline; CLSI document MM9-A. Clinical and Laboratory Standards Institute, Wayne PA.