

Draft Guidance for Industry and FDA Staff

Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens

DRAFT GUIDANCE

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For questions regarding this document contact Roxanne Shively 240-276-0496 ext 113.

This draft document replaces “Review Criteria for Nucleic Acid Amplification-based in vitro Diagnostic Devices for Direct Detection of Infectious Microorganisms” dated June, 1993.



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

Office of In Vitro Diagnostics Device Evaluation and Safety
Division of Microbiology Devices

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Preface

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Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. Introduction

This draft guidance document is intended to provide guidance on the types of information and data we consider during premarket review of nucleic acid-based in vitro diagnostic devices for the detection of microbial pathogens. These device test systems are used to determine the presence (and absence) of recognized microbial pathogens directly in human specimens by detecting nucleic acid sequences or regions that are unique to each organism and that discriminate a particular microbial pathogen from other organisms. Results from these device test systems may be used to diagnose or manage infectious diseases.

This draft document replaces a previously issued document, “Review Criteria for Nucleic Acid Amplification-based in vitro Diagnostic Devices for Direct Detection of Infectious Microorganisms” (June, 1993). The current draft reflects changes in the technologies available for nucleic acid detection, and expanded use in clinical laboratories. The recommendations within this draft apply broadly to premarket review of these in vitro diagnostic devices for detecting microbial pathogens. Enzymatic amplification may or may not be part of the applied technology.

This draft document is intended to provide a basic framework for the types of information and data that we believe should be addressed in the premarket review of a nucleic acid-based device, for detecting microbial pathogens. The recommendations contained in this draft document are purposefully general, as each testing system may have an associated unique set of concerns. We encourage you to identify and discuss unique aspects of your device with the Division of

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Microbiology Devices in the Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD) early in your development process.

This draft document focuses on information to describe your device and to support its performance in a premarket review. The appropriate types of information will depend on the following specific factors regarding your device:

- intended use and indications for use (including any unique claims for use)
- technological characteristics
- benefits and risks for use (new issues of safety or effectiveness)
- incorporated risk control elements

The depth and extent of the premarket review depends on classification of your particular device. Depending on the classification, a nucleic acid based microbial pathogen device could be reviewed as a 510(k), or as a PMA (see sections 513 and 515 of the Federal Food, Drug, and Cosmetic Act (Act) (21 U.S.C. 360c, 360e)). We recommend that you consult with OIVD for advice on device classification. We also suggest you consider early interaction with OIVD when you are developing plans for clinical evaluations to assure appropriate methods are incorporated into your evaluation protocols. You are also responsible for complying with 21 CFR Part 820, Quality System Regulation, which includes Design Controls and Corrective and Preventive Action.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

The Least Burdensome Approach

This draft guidance document reflects our careful review of what we believe are the relevant issues for in vitro diagnostic devices designed to detect microbial pathogens using nucleic acid-based test systems and what we believe would be the least burdensome way of addressing these issues. If you have comments on whether there is a less burdensome approach, however, please submit your comments as indicated on the cover of this document.

2. Scope

The scope of this document is limited to those devices whose intended uses are described within 21 CFR Part 866, Subparts B, C, and D. Nucleic acid-based IVD devices may be Class I, II, or III. Because these devices infer the identity of a microorganism directly from clinical specimens, none of these devices would be exempted from premarket review (see Limitations to Exemptions, 21 CFR 866.9(b)(6)).

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Specific recommendations for detection of particular microbial pathogens causing particular infectious diseases are not addressed in this document. We anticipate that developers and manufacturers will incorporate known information about specific pathogens, their pathogenesis and disease manifestation into these broad recommendations. Neither are our recommendations intended to be all-inclusive or to apply in total to all applied technologies. Issues may be identified during the review process, unique to the particular intended use, technology under review, or the specific configuration of your device test system that will need to be addressed on a case-by-case basis. You are encouraged to refer to available resources for specific information on molecular technologies and the chemistries that have been applied to laboratory testing for infectious diseases.¹

The recommendations within this draft are directed toward devices for single organism detection using technologies that enzymatically amplify nucleic acid target regions. All recommendations may not be applicable to every device type. These broad recommendations would also generally apply to technologies that detect nucleic acid targets without enzymatic amplification, or to devices that identify specific microbial pathogens from cultured material. FDA believes that additional or different recommendations would be needed for test systems that:

- detect and identify multiple organisms (genera, species, or strains);
- apply amplification of broadly conserved regions (e.g., ribosomal DNA);
- are designed for use outside a clinical laboratory setting (e.g., point of care use);
- are designed to quantify the amount of nucleic acid detected;
- detect newly emerging microbial pathogens;
- genotype organisms; or
- identify antimicrobial resistance.

You should consult the Division of Microbiology Devices in the Office of In Vitro Diagnostic Device Evaluation and Safety for specific advice on such devices.

Assay systems intended to screen blood and plasma donors, which are licensed tests under the Public Health Service Act, are reviewed by FDA's Center for Biologics Evaluation and Research (CBER). In addition, CBER regulates non-donor HIV diagnostics. These products are not covered by this guidance document. For issues related to donor screening assays and HIV diagnostics, please contact CBER's Office of Communication, Training and Manufacturers Assistance at 1401 Rockville Pike, HFM-40, Rockville, MD 20852-1448, telephone: 1-800-835-4709 or 301-827-2000.

Dedicated instrumentation is critical to the design of many nucleic acid-based detection devices and these device design features (assay-specific instrumentation and related software) are part of a premarket review. However, specific recommendations for describing or supporting these

¹ An example is “*Molecular Diagnostic Methods for Infectious Diseases*”; Approved Guideline (2005), CLSI document MM3-2A, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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features are not part of this document; relevant additional resources are cited where applicable. The guidance document, *Replacement Reagent and Instrument Family Policy*, (available at <http://www.fda.gov/cdrh/oivd/guidance/950.pdf>) does not apply to reagents or to instrumentation that are part of these device test systems, because we believe that interchanging different reagents with different instrument systems would significantly affect the safety and effectiveness of the device.

3. Risks to Health

Failure of a device test system to perform as indicated, or error in interpretation of test results may lead to improper patient management or, for some microbial pathogens, an inappropriate public health response.

A failure to detect specific microbial nucleic acid sequences when a patient is infected (a false negative result) could result in clinical misdiagnosis, withholding appropriate treatment, and not instituting prevention/control efforts in the case of a transmissible disease or one with a common community source. Missed infections in pregnant women and newborns, or other unique populations, may entail additional risk due to limited treatment opportunities (e.g., for preventing consequences of congenital infection).

Detecting specific microbial nucleic acid sequences when a patient is not infected (a false positive result), could lead to unnecessary treatment with potentially toxic drugs or failure to appropriately diagnose and treat the actual disease condition. False positives may be devastating to an individual's social and psychological health, particularly for the diagnosis of a sexually transmitted infection. A false positive result can also lead to unnecessary isolation procedures or contact tracing.

In the table below, FDA has identified the broad types of risk to health generally associated with the use of an IVD based on nucleic acid detection for microbial pathogens. General measures to mitigate or manage these risks are shown in the table below. We encourage you to identify risks specific to your device's technological characteristics and to design features to mitigate these risks. You should provide sufficient detail to support the approach you have used to address risks.

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Identified Risks	Mitigation measures
<ul style="list-style-type: none">• Improper Patient Management• Inappropriate Public Health Response	<ul style="list-style-type: none">• Assure specimen adequacy and integrity• Design elements and controls for false positives due to contamination, carry-over, and assay non-specificity• Design elements and controls for false negatives due to inefficient recovery, inappropriate specimen volume, and inhibition• Account for role of non-viable organisms in an infectious process.

4. Device Descriptive Characteristics

Key issues for a device review are the specific intended use (the microbial nucleic acid marker detected and the indications for use) and the technological characteristics of your device. We recommend that you describe each of these device elements in detail. We encourage you to reference appropriate peer-reviewed literature that supports the use of your device for its intended diagnostic use and the specific nucleic acid detection principles incorporated into the device design.

Information to Support the Intended Use

The intended use encompasses the nucleic acid region detected (analyte) of the targeted organism, the patient population to be tested using the device, disease(s) or other conditions caused by the organism that may be diagnosed, and specimen types for which testing will be indicated. Settings in which specimens will be collected, and the type of facility where testing with the device will be done are also part of the intended use for an in vitro diagnostic device. The intended use must also be stated in your labeling (21 CFR 807.87(e)). You should ensure that all aspects are stated clearly (see Section 6, Labeling).

We recommend that you provide the following types of information to support the specific intended use of your device and assist FDA in assessing benefits and risks associated with use of your device (see Section 3, Risks to Health), relevant design elements of your device, and the performance data provided:

- Specific microorganism(s) that will be detected and identified, and the level of identification certainty (i.e., presumptive or definitive).
- Disease(s)/syndrome(s) associated with infection by a microbial pathogen.
- Epidemiology, prevalence rates, and individuals at risk for infection.

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- How the device test results will be used in a diagnostic algorithm along with other clinical or laboratory testing that would be clinically needed for a specific disease diagnosis.
- Type(s) of acceptable test specimens (matrix) and any unique device requirements for collection, handling and transport. When you indicate an alternate specimen type, for convenience or reduced invasiveness, you should provide data to support that use of the alternate specimen type provides reliable diagnostic results.
- Microbial target region (genomic or plasmid DNA, genomic RNA in viruses, transcripts such as mRNA, rRNA, cDNA) and rationale for using. We also recommend that you detail the specific sequence(s) targeted by primers and probes: size (number of nucleotide bases); location of the region or sequence on the genome, plasmid, or RNA; property encoded; expected number of target copies per organism; method of estimating copy number; applicable reference, if sequence(s) have been published; degree of conservation of the target region and specific sequences detected.

Note: In this document, the analyte is considered the nucleic acid target region detected (e.g., *C. trachomatis* cryptic plasmid DNA).

We also recommend that you provide the following descriptive information related to the use of the device (as applicable):

- A discussion of diagnostic criteria for the disease(s) attributed to the specific microbial pathogen detected and identified by the device. This discussion should include clinical presentation (signs and symptoms), supportive laboratory findings, definitive criteria for pathogen identification, and other diagnostic testing (e.g., radiography, histopathology) used to clinically establish a disease diagnosis. You should also include the criteria that may be used to rule out a particular disease etiology.
Note: for certain diseases, the recovery of a recognized pathogen from a particular specimen type is laboratory evidence for infection (e.g., *M. tuberculosis* from lower respiratory specimens is laboratory evidence of active pulmonary disease when clinical signs and symptoms are compatible. However, not recovering *M. tuberculosis* from a single specimen does not rule out pulmonary tuberculosis when clinically suspected). For other diseases, detection of the pathogen nucleic acids does not necessarily correlate with disease (e.g., HCV RNA detected in an individual with normal liver enzymes).
- Other applied laboratory detection and identification approaches (using either commercial test systems or standardized laboratory procedures).
- A comparison (similarities and differences; strengths and weaknesses) of the new device to a previously marketed device(s) for the same intended use. Note: this comparison is a required element for a 510(k) submission.
- The recognized laboratory methods, if available, for detecting and identifying the microbial pathogen.
- The medical and public health impact of a positive and a negative result for the indicated patient group(s), including consideration for variations in disease prevalence.

We recommend use of well-recognized sources for this descriptive information (e.g., professional practice guidelines when available; infectious disease, microbiology, and clinical

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laboratory resources)². These types of information should be concisely summarized in labeling (Summary and Explanation section) and also used in designing your studies.

Technological Characteristics

We recommend that you provide a thorough explanation of all technological aspects of the device test system including reagents, procedures, and instrumentation that enable specimen extraction/stabilization, target amplification, hybridization, and detection. In addition to a general description of the product to be distributed (e.g., reagent test kit components needed to perform 50 tests), we recommend providing a complete description of the following (as appropriate):

Reagent components

- a. For those reagents with primers or probes, relevant descriptive characteristics can include:
 - Sequence length, type (DNA or RNA), source (synthetic, clone, or genomic), purity, GC content, and melting temperatures.
 - Sequence and accession number if registered with GenBank or other data banks, licensure or patent information.
 - The method of probe or primer production: instrumentation used, purification, and acceptance specifications.
 - Internal secondary structures, and complementarities with other primers or probes in assay reagents.
 - Verification of composition (e.g., nucleotide sequence analysis, restriction endonuclease mapping, DNA fingerprinting, etc.), purity,³ and potency (functional efficiency) along with procedures that will be used to accept new lots.
 - Detailing of the function of multiple probes or primers.
 - For conjugates or attachments that allow for capture or detection, the method of attachment and the stability of attachment, along with acceptance criteria. Also include acceptance specifications for coating and for any solid phase components (e.g., beads, plates).
 - Results of a database search to determine homology to known sequences of DNA. Note: not finding homologous sequences does not assure specificity or preclude evaluation with known near-neighbors, other pathogens, commensals, and host nucleic acids expected in the sample type for which the device is designed to be used.

- b. For those reagents with enzymes: source, specifications (thermostability, purity, etc.), and function. Define the specific activity and acceptance criteria based on functional testing.

² These can include but are not limited to: Infectious Disease Society of America Guidelines; American Society for Microbiology; Center for Disease Control and Prevention information resources; World Health Organization.

³ Current ICH guidelines describe validated techniques (e.g., reverse phase high performance liquid chromatography, ion exchange HPLC) for assessing purity.

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- c. For all other reagents and those above: quantities or concentrations of reagent ingredients, using standardized measurements. Define the component specifications required for suitable use.
- d. For those calibrators provided or required for instrument standardization: describe the calibrator source (e.g., biological, physical and/or chemical properties), concentration, and method of preparation, as applicable.
- e. For controls that verify acceptable assay performance: provide specifications for each control, source, method of preparation, and validation of inactivation (as applicable). You should specify value limits for your controls based on statistical parameters from design and optimization experiments. Control material should be separate from calibrators, and similar to the specimen type. A low positive control (e.g., within 2-3X the lowest amount of organism or nucleic acid detectable in the test system) allows for monitoring of nucleic acid detection at the low level. You should include or recommend negative control material (non-target organism and organism-free blanks). You should explain the nature and function of controls that you incorporated into the device design.

Instrumentation and software components:

You should describe instrumentation required to perform testing with the specific physical and chemical reagent properties of your device. You should also include specifications (e.g., type of reaction vessel and volume capacity, thermocycling speed, limits for well to well temperature variation, sample capacity, optics, excitation sources and filters) or a specific instrument model. Note: we recommend including instrument manuals in your information package. If an instrument is non-dedicated, you should cite the premarket notification (510k) submission, or PMA number of the instrument.

You should describe the assay-specific software, or programmable assay settings; the mathematical method(s) applied to normalize, interpolate or otherwise process the signal generated; and the range of output values.

Other component specifications:

You should describe specifications for any other reagents, materials and equipment required for proper function and performance of the device test system. In cases where you do not supply all of these materials, you should include sufficient information (e.g., specifications) so that device users can purchase or prepare all necessary components of the test system.

Safety aspects for performing the assay:

You should specify at what procedural step the testing material is non-infectious. You should also specify the recommended biosafety precautions that should be used for earlier steps, along with handling and disposal precautions to minimize contamination. You should describe the methods used to verify non-infectivity. Note: the level of concern for

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infectivity depends on the nature of specimens used and the design of your device test system.

Functional performance requirements:

You should detail the performance requirements you established for subsequent lot production and the testing that will be done to assure product uniformity. When copy numbers are used to define functional performance, you should provide the method used for estimating copy number. Materials used for defining functional performance should be quantified according to standard measures (e.g., fg/mL). If these materials are not traceable to reference material, you should describe the source or method of preparation. Note: although it is common practice during development to quantify measures by the volume used in the analytic phase of the test system, you should translate this measure to input specimen volume (e.g., fg/mL).

Specimen collection/transport devices:

You should detail the method(s) that will be used to collect specimens for testing. You should also specify the types and volume (if applicable) of all specimens acceptable for testing with the device and ways laboratories can assure that specimens were collected and transported appropriately. Discuss the effects of testing inadequate or inappropriate specimens. CLSI MM13-P⁴ and other resources may be referenced as appropriate.

Note: We recommend that you cite the 510(k) numbers for collection and transport devices required for use with your device. If these were not previously cleared, and are a necessary component of the test system, you should provide relevant information concerning specifications and use of the device [e.g., swab specifications, materials, design considerations (e.g., biocompatibility, sterilization methods as applicable)].

5. Performance

You should provide performance data for your device to support descriptive characteristics that are not sufficiently precise, to assure product reliability for its specified diagnostic use, and to define those factors that may affect quality of test system results using the device. Studies to estimate diagnostic performance parameters and to determine analytical parameters are also critical to establish device performance characteristics for labeling (see Section 6. Labeling). In this document, diagnostic performance refers to data used to support diagnostic accuracy (see also the “Clinical Evaluations” section on page 19), whereas analytical performance refers to those data that support functional performance of the device.

⁴ “Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods”; Proposed Guideline (2005), CLSI document MM13-P, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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We recommend you completely describe your studies (objective, procedures, materials, source and preparation of materials tested, analyses and conclusions). We recommend that, along with the complete description of the study, you present relevant data with analyses and conclusions that the findings meet acceptance criteria consistent with the intended use. Whenever you use acceptance criteria for conclusions, you should describe your rationale and basis for setting those acceptance criteria.

You should include all controls that will be part of the final test system in your studies (including those not included in your device test system but recommended for use; e.g., extraction controls and controls to monitor for contamination during handling). We also recommend including explanations for unexpected results and any additional testing performed (or measures taken to mitigate unacceptable results). You should include charts (scattergrams, histograms, receiver operator curves, etc.) and tables as part of the analyses and conclusions when appropriate.

We recommend that you obtain performance data for establishing diagnostic accuracy (e.g., sensitivity and specificity, positive and negative predictive values with prevalence) from studies using your device test system with intended use specimens. Within this draft guidance, the term “reference standard” is defined as: “The best available method for establishing the infected status (and ‘not infected status’) of the patient”. The reference standard could be a combination of laboratory methods and clinical procedures (e.g., radiography) including clinical follow-up of tested subjects.”⁵ Note: criteria for ‘not infected status’ may need to be expanded compared to those for infected status, depending on the particular disease entity (e.g., for pulmonary tuberculosis, negative acid fast bacilli smears and negative cultures of two to three successive days’ sputa from untreated patients is considered not tuberculosis when an alternate cause of signs and symptoms is identified, while any one positive culture may be evidence for infection).

In this document, we designate within-laboratory precision as an analytical performance parameter. We describe inter-laboratory reproducibility as a clinical performance parameter because we believe that such performance should be assessed with specimen-based samples, in laboratory settings typical of where the device test system will likely be used and encompass pre-analytic factors (specimen handling and extraction). We also believe that you should support performance claims for your labeling using a final form of the device with the procedures finalized in your product labeling.

Analytical Data (Pre-Clinical)

You should establish specific performance parameters of importance to the operation and successful use of the device prior to conducting diagnostic evaluations. This testing may be done in-house or at a designated laboratory facility as part of the test development and optimization phases.

Your experiments should use clinical samples obtained from individuals who have previously been shown to have, or not have an infectious process attributed to a specific pathogen, if this will be relevant to your conclusions. In some experiments, you may use stable sample materials

⁵ Bossuyt PM et al. *The STARD Statement for Reporting Studies of Diagnostic Accuracy: Explanation and Elaboration*. 2003 *Clinical Chemistry* 49:1, 7-18.

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designed to approximate matrix and expected organism levels in clinical specimens to define reagent specifications, supporting equipment calibration, assessing interference, homologous interactions with other organisms, and reactivity with relevant species or strain variations. Artificial or contrived samples can be used when it is reasonable to assume that culture-maintained organisms would simulate organisms detected in an in vivo infectious process. Infected cell lines may be utilized for obligate intracellular organisms. We also recommend that replicates of a low positive sample (e.g., within 2-3x the concentration of analyte detected at the LoD) be included in test panels for all analytical studies performed.

You should incorporate scientific principles and relevant statistical analysis into the design of these studies. You should use the same numeric output values in your analyses that are utilized by your test system, as appropriate. For example, test systems that report output values as relative light units (RLUs) should use RLU values in their statistical analyses, even if a qualitative result is ultimately deduced from the numeric value.

Specific parameters and the types of data that may verify that your device is functionally performing as expected are listed below. Many of these data types may be generated concurrently in studies designed to meet the specified objectives.

A. Microbial variants

You should demonstrate the ability of the device to detect genotypic and phenotypic organism variants in matrices indicated for testing. The range and numbers appropriate will depend on the documented diversity of the target organism (e.g., serovars, serotypes, auxotypes, genotypes, clades, drug resistant strains). You should characterize the organisms (e.g., serotype, geographic origin) you tested and justify that organisms selected represent species or genus diversity (both geographic and temporal). We recommend that you include recent clinical isolates in addition to archived strains or strains from culture collections.

We recommend assessing two levels of these target organism variants. One level should be consistent with the lowest clinically relevant level (or near the detection limit of your device test system). The other should be a higher, clinically relevant level. When the analyte (nucleic acid target region) is plasmid DNA, you should support (and demonstrate as necessary) that plasmid-free strains or plasmid transfer to non-target organisms is not a risk factor for reliable diagnostic use.

B. Limits of detection (LoD)

You should determine the LoD with specimen material using approaches described in CLSI EP17-A.⁶ Detection limits may be predicted early during product development using ‘clean’ samples (e.g., purified nucleic acids from target organisms); this approach is helpful to optimize assay parameters, using standardized sample control materials. However, we recommend that you establish the LoD that you represent as a performance characteristic,

⁶ “*Protocols for Determination of Limits of Detection and Limits of Quantitation*”; Approved Guideline (2004), CLSI document EP17-A, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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following CLSI EP17-A . We recommend that you estimate the LoD by using preparations quantitated by referenced methods (CFU, PFU, or IFU/mL; microscopically visualized units, etc.). You may wish to also provide nucleic acid equivalents (e.g., fg/mL).

CLSI EP17-A recommends using individual samples from different subjects rather than pooled specimens or a single specimen spiked and tested repeatedly. You should also verify that clinically significant strain variants have similar LoDs (e.g., each of the nine Group B Strep serotypes). When reporting your results, we recommend that you use the model presented in CLSI EP17-A.

C. Non-target organisms

You should demonstrate that non-target organisms (i.e., near-neighbors, other pathogens, and commensals reasonably expected to be present in anatomical sites to be sampled for testing) do not have homologous interactions in the device test system. You should include a concentration of these organisms consistent with the densities of organisms in relevant clinical samples (10^8 cfu/mL has been suggested for most bacteria because this level may be seen for certain specimen types, e.g., feces, vaginal fluids, broth cultures). This type of testing can also be incorporated into interference experiments to demonstrate that the presence of other organisms along with the target organism does not suppress detection.

D. Interference

You should demonstrate that you can specifically detect the target organism in the presence of relevant interferents (including other organisms). These studies should be performed in each specimen matrix indicated for use. We recommend assessing two levels of the target organism, one of which is consistent with the lowest clinically relevant levels (or near the detection limit of your device test system). The nature of the experimental design will depend on the technological characteristics and design of your device test system. We recommend using experimental designs and data analysis methods described in CLSI EP7-A.⁷ For those test systems that incorporate a purification or nucleic acid isolation step, you should demonstrate the effectiveness of that process for recovering microbial nucleic acid in each specimen type (see pre-clinical section below). If that process is shown to remove potential interfering substances, then interference testing can be reduced for the downstream system testing.

You should identify reasonably anticipated interferents that may contribute to non-specificity. These may be due to assay components (e.g., residual alkaline phosphatase) or the nature of the sample being tested. Interference can also occur due to competitive inhibition when other organisms have homologies, or amplification kinetics between an internal control and the target are unbalanced. You should provide the data from your studies to demonstrate that potentially interfering substances (or conditions) encountered in specific specimen type(s) do not affect assay results. You should include an appropriate limitation in the package insert when interference is demonstrated or cannot be discounted.

⁷ “*Interference Testing in Clinical Chemistry*”; Approved Guideline (2002), CLSI document EP7-A, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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The following are examples of substances (or conditions) that may interfere with nucleic acid amplification methods:

- 1) Endogenous substances likely to be present in patients' specimens (e.g., blood, mucus, pyuria). These can also include hemolysis, lipemia, and icterus when serum is sampled.
- 2) Possible exogenous substances present in specimens or used in the specimen collection process (e.g., anticoagulants, medications, local antiseptics or anesthetics, spermicides, and specimen preservatives), and tubes and media or other materials (e.g., swabs) used for and during collection.
- 3) Competitive inhibition from other pathogens or commensals that may be present in the specimen.
- 4) Conditions such as freeze-thaw cycles, heat exposure, light exposure.

E. Amplification carry-over prevention

You should demonstrate the effectiveness of any amplification carry-over prevention measures incorporated into your assay. Dilutions of 10^3 - 10^{11} amplicons/ μL may be used in the test system, comparing samples that are treated to those untreated with the carryover protection measure.

F. Within-laboratory precision

You should establish the within-laboratory precision for your assay according to CLSI EP5-A2⁸, which provides guidance for the design of precision protocols. Estimates of within-laboratory precision should include an analysis of within-run and between-day variance in a single laboratory. We recommend a full 20 day evaluation, including multiple operators and instruments in these studies to appropriately estimate precision (for details on incorporating multiple factors, see Section 11.2 of CLSI EP5-A2). These studies can use the same preparation or extraction of DNA (in matrix) using fresh aliquots for each day. At a minimum, you should test (a) 3 levels of the target with at least one being a low level (consistent with low amounts expected in clinical samples), and (b) 1-2 samples containing a close-neighbor non-target organism, blanks (no target or non-target added)*, and device test system controls. CLSI EP5-A2 also provides guidance for statistical analyses and reporting in labeling.

* Note: CLSI EP17-A defines a blank as a sample that does not contain the analyte of interest, or has a concentration at least an order of magnitude less than the lowest level of interest. In this document, (unless in the context of CLSI EP17-A), we define a blank sample as one that contains all test system reagents (including specimen transport medium, when applicable) to which no test substance has been added.

G. Effectiveness of controls for monitoring critical assay conditions

⁸ "Evaluation of Precision Performance of Clinical Chemistry Devices"; Approved Guideline-Second Edition (2004), CLSI document EP5-A2, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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You should validate that controls included in your device design will fall outside designated measurement regions when critical assay conditions are outside acceptable limits. When you do not provide such validation, or when the device controls are not able to monitor for changes in critical assay conditions, your labeling should warn laboratories that the controls are designed for monitoring catastrophic reagent or assay failures, and may not monitor for either errors that can cause altered test performance, or for the accuracy and precision of test performance over time.

If the assay system also includes an internal control (recommended by CLIA for test systems incorporating amplification to detect inhibition),⁹ you should verify that its use could monitor for inhibition that would prevent detection of low organism levels. You could spike dilutions of known inhibitors into samples with low levels of target organism, for this purpose. You should also demonstrate that the internal control itself does not interfere with detection of low levels of target organism. Note: a sample other than the control should be used as the indicator of failure in these experiments.

- H. Verification of calibration stability
You should validate recommended intervals for calibration over appropriate time intervals. We recommend referring to available resources (e.g., applicable ISO documents).
- I. Nucleic acid stability
You should establish stability of target nucleic acids in specimen preparations and in processed samples following extraction. We encourage you to provide appropriate graphical representations. We recommend approaches shown in CLSI EP9-A2 to compare differences.¹⁰
- J. Kit and component stability
We recommend that you provide the protocol you will use for establishing real time stability of reagents and controls for both storage and shipping temperatures. You may refer to EN13640:2002¹¹ for additional information. We also recommend using definitions for commonly specified conditions tested (e.g., those definitions from the U.S. Pharmacopeia).¹²
- K. Instrumentation and software function

⁹ 42 CFR Part 493 1256(d)(3)(v); Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications, January 24, 2003.

¹⁰ “*Method Comparison and Bias Estimation Using Patient Samples*”; Approved Guideline - Second Edition (2002), CLSI document EP9-A2, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

¹¹ “*Stability testing of in vitro diagnostic reagents*”; (2001) EN 13640:2002, Brussels: European Committee for standardization.

¹² Definitions from USP are available at <http://www.usp.org/patientSafety/newsletters/qualityReview/qr401994-06-01c.html> (USP Quality Review No. 40, Revised 6/94 Storage Definitions)

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You should validate dedicated equipment or specifications for generic type equipment that will be used in the assay system.

You should also provide documentation for the software components of your instrumentation, detailed in accordance with the level of concern (See: *Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices*¹³). You should determine the Level of Concern prior to the mitigation of hazards. 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 result in injury (through the action or inaction of a health care provider). We consider the following points to be important elements in preparing software documentation for FDA review:

- You should fully describe the software design. You should not include software utilities specifically designed to support uses beyond those of the legally marketed assays identified in your 510(k). You should also consider privacy and security issues in your design. Information on some of these issues may be found at the following website regarding the Health Insurance Portability and Accountability Act (HIPAA) <http://aspe.os.dhhs.gov/admnsimp/index.shtml>
- You should submit a hazard analysis based on critical thinking about the device design and the impact of any failure of subsystem components, such as signal detection and analysis, data storage, system communications and cybersecurity in relation to incorrect patient reports, instrument failures, and operator safety.
- You should complete verification and validation (V&V) activities for the version of software that will be utilized for the submission in demonstrating substantial equivalence.
- If the information you include in the 510(k) is based on a version other than the release version, you should identify all differences and detail how these differences (including any unresolved anomalies) impact the safety and effectiveness of the device.

You can also refer to the numerous resources cited in that guidance¹³ to help you develop and maintain your device under good software life cycle practices consistent with FDA regulations.

Feasibility (Pre-clinical) Data

Feasibility (preclinical) analytical studies provide preliminary information on assay performance, allowing further optimization as needed and reducing variables that may confound clinical testing. We recommend that you perform these studies using intended use specimens (received in clinical laboratories for diagnostic testing) whenever possible. However, for those test systems designed for detecting rare or unusual pathogens, performance testing may not be possible with naturally infected specimens. We recommend that you contact the Division of Microbiology Devices if you are designing alternate approaches.

¹³ <http://www.fda.gov/cdrh/ode/guidance/337.pdf>

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A. Proportion of specimens with inhibitors

You should assess presence of inhibitors in specimens by spiking target organism (or infected cell lines) at known low level concentrations into aliquots of specimens prior to processing. You should express the frequency of inhibition with any one specimen type as a percentage of the total number tested, whether or not the specimen contains the analyte of interest. You should select specimens for this study that include the spectrum of expected characteristics (e.g., bacteriuric specimens if urine is an indicated matrix). These data may also be obtained in parallel with your clinical studies if feasible. Note: actual inhibition rates, or the proportion of infected patients whose specimens are falsely negative with your device due to inhibition can be deduced from clinical evaluations (See Clinical Performance section).

B. Cutoff verification

We recommend that you optimize assay cutoffs to minimize false positives and negatives by testing a representative sampling of specimens containing, and not containing, the target organism. We expect that a positive cutoff and a negative cutoff may be needed to define positives and negatives with a high degree of certainty. We believe that an equivocal designation is appropriate for a measurement region that includes a significant proportion of falsely positive or falsely negative results in the intended use population(s) that cannot be explained by additional testing (e.g., culture recovery, amplicon sequencing, and other nucleic acid analyses). This study should include all device test system procedures, including extraction or processing. If you choose to recommend repeat testing for these types of measurements, you should carefully assess the effectiveness of such repeat testing and demonstrate that it improves sensitivity and specificity of the assay test system in your clinical studies.

C. Specimen stability

Prior to clinical evaluations, you should verify stability of specimens to prevent confounding with clinical variables. We recommend that you compare results between fresh specimens and aliquots held under conditions expected during transport and storage of clinical specimens. This specimen stability study should include all device test system procedures, including extraction or processing. We recommend that you include minimally 25-30 specimens with the target organism and an equal number without from representative populations. When infection is rare, you may consider preparing surrogates using appropriate levels of organism or infected cell lines.

D. Instrument and system carry-over

Prior to clinical evaluations, you should assess your device test system for carry-over contamination from instruments or other equipment used in your test system. For such assessments, specimens with naturally occurring high levels of organisms can be used in series, alternating with blank controls in patterns dependent on the operational function of the instrument or other equipment. You should perform this testing over multiple runs (at

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least 5 runs is recommended by the Commission of European Communities).¹⁴ We recommend methods described in appropriate references such as the International Union of Pure and Applied Chemistry.¹⁵

Clinical Evaluations

The design, conduct, and analysis of diagnostic studies are important to avoid bias and allow results to be generalized to appropriate clinical settings. In this context, diagnostic accuracy refers to the comparison of the results between the test system under evaluation and the results from a reference standard (previously defined in this document as the best available methods for establishing the infected status or other condition of the patient; e.g., a combination of methods and procedures). Diagnostic accuracy may be expressed in a number of ways, depending upon your study design, including sensitivity and specificity, positive and negative predictive values with prevalence, likelihood ratios, diagnostic odds ratios and areas under ROC curves.¹⁶ You should include measures of statistical uncertainty (e.g., 95% confidence intervals) for all performance parameters, as well as a qualification of the target condition detected (defined as a particular disease, a disease stage, health status, or other identifiable patient condition). We recommend that you use the checklist in the reference cited (Bossuyt et al.) to ensure that you provide essential information in describing and reporting your study.

A. Diagnostic Accuracy

We expect that all patients (subjects) evaluated for diagnostic accuracy determinations will be from the intended use population(s). The appropriate number of subject specimens to include in a clinical evaluation will vary according to the prevalence and spectrum of the disease, and methods of its analysis. In general, we recommend planning for subgroup analyses in estimating sample size, particularly for unique populations (e.g., pregnant women), for different disease stages or syndrome presentations (e.g., symptomatic vs. asymptomatic; patients hospitalized with pneumonia vs. outpatients), or for demographic groups when disease presentation is unique between genders or age groups. We also recommend multi-center evaluations to assure consistency and generalizability. Note: any one laboratory may perform testing on patient specimens from multiple clinical healthcare settings. At a minimum, three external clinical laboratories should conduct evaluations, and patients whose specimens are tested by these laboratories should encompass the indicated use populations.

You should provide a detailed protocol used at each of your clinical laboratory evaluation sites and detail the methods and procedures used. Both kit controls and other recommended control materials (e.g., whole organism preparations or infected cell lines) should be used with testing done with your assay. We recommend including negative extraction controls

¹⁴ Commission Decision [2002/364/EC](#) of 7 May 2002 on common technical specifications for in vitro-diagnostic medical devices

¹⁵ R. Haeckel. *Proposals for the Description and Measurement of Carry-over Effects in Clinical Chemistry*, 1991. Pure & Appl. Chem, 63:301-306

¹⁶ Bossuyt et al. *The STARD Statement for Reporting Studies of Diagnostic Accuracy: Explanation and Elaboration*. 2003 Clinical Chemistry 49:7-18.

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frequently during your clinical evaluations to monitor for contamination between specimens during pre-analytic processing, and to allow for prompt recognition of laboratory error that could adversely affect your performance characteristics. Appropriate controls should also be used with any laboratory tests you include in your reference standard.¹⁷

You should report all missing results, and indeterminate or equivocal results from your device test system, and from any laboratory tests used as part of your reference. You should make every effort to minimize the number of uncertain (e.g., invalid) results from the reference standard.

You may calculate sensitivity for a patient's infected status (or other relevant target condition) as the proportion of patients with a positive output value in your device system who are determined infected by the reference standard. You can calculate specificity as the proportion of patients with a negative output value in your device system who are determined uninfected by the reference standard. You should always represent the proportion calculated along with the fraction (numerator and denominator value), 95% confidence intervals, and the target condition detected (see Labeling, Section 6).

Example: Sensitivity with urine specimens for *N. gonorrhoeae* urogenital infection in asymptomatic males = 80% (8/10); 44.4-97.5%

Because of the variety of organisms that may be detected using nucleic-acid based methods and the range of disease syndromes and specimen types for which detection may be indicated, we encourage you to contact the Division of Microbiology Devices early in your assay development. We would be able to work with you in the design of clinical evaluations that we believe could support effectiveness determinations.

B. Reproducibility

You may demonstrate variability between laboratories using a panel of at least 15-20 samples (or more, as necessary to encompass specimen variables and different matrices). We recommend that you assess reproducibility with specimen-based samples in at least three laboratory settings typical of where the device would likely be used. We recommend these samples include at least 2-3 low organism level samples, with each panel member a distinct matrix based material (minimally 5 uniquely sourced specimens are recommended). At least 30-40% of panel members should be negative for the target organism (also matrix based). The laboratories performing this testing should also include all device test system controls and recommended external controls. Panels (and controls) may be tested over multiple days or runs. You should report inter-laboratory variability based on testing of individually extracted or prepared specimens or samples. For statistical analysis of these data, we recommend that you refer to Section 11.2 of CLSI EP5-A2.

¹⁷ For further information, refer to CLIA regulations under Title 42, Code of Federal Regulations (CFR), Part 493, or contact the State Survey Agency in the state where the laboratory is located or the accrediting organization with which the laboratory is affiliated.

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6. Labeling

The premarket notification should include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e).¹⁸ The following suggestions are aimed at assisting you in preparing labeling that satisfies these requirements and follows the format and content outlined in 21 CFR 809.10(b).

A. Intended Use Statement

The statement should be a concise description of essential information about the product [nucleic acid region detected, target organism, patient population(s) to be tested using the device, disease(s) caused by the organism that may be diagnosed, and specimen types for which testing will be indicated]. Indications for use should describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use Statement.

Note: These conditions for use may be further detailed in the Summary and Explanation, Limitations, and/or Performance Characteristics sections of the package insert.

B. Specimen Collection and Handling

You should state the type(s) of specimen to be collected and the types of collection devices that may be used, along with incorrect or unacceptable collection procedures. Additionally, when suitable standardized collection procedures cannot be referenced, you should describe the collection procedures that should be used (e.g., patient preparation, order of collection). We recommend that you identify interfering substances or conditions, instructions for transport to the laboratory for testing, specify inappropriate transport conditions, and state the specimen storage conditions and stability periods. Whenever possible, you should provide a mechanism by which the laboratory can assure that collection and transport requirements have been followed.

C. Precautions

You should alert laboratories of precautions to follow for handling hazardous substances¹⁹ or specimens.²⁰ We recommend that you also list the special precautions required to avoid or

¹⁸ Although final labeling is not required for 510(k) clearance, final labeling must also comply with the requirements of 21 CFR 801 and 21 CFR 809.10 before a medical device is introduced into interstate commerce. Labeling recommendations in this guidance are consistent with the requirements of part 801 and section 809.10.

¹⁹ Any substance or mixture of substances which is toxic, corrosive, an irritant, a strong sensitizer, flammable or combustible, or generates pressure through decomposition, heat, or other means, if such substance or mixture of substances may cause substantial personal injury or substantial illness during or as a proximate result of any customary or reasonably foreseeable handling or use, including reasonably foreseeable ingestion by children. [16 CFR Part 1500.3 (b)(4)(i)(A)].

²⁰ According to *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition* (<http://www.cdc.gov/od/ohs/biosfty/biosfty.htm>): Biosafety Level 2 is appropriate when

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minimize contamination with carryover nucleic acids from personnel or environmental sources (e.g., using positive displacement pipettors or pipette tips with hydrophobic plugs, using a biological cabinet for preparation phases). You should also caution that personnel performing molecular testing should have specialized experience and training.

D. Directions for Use

You should provide clear and concise instructions for performing the assay that describe the operational steps for using the device to test clinical specimens and controls. You should include temperature and timing tolerances for each procedural step. You should include recommended instructions for collection, transport, and handling of specimens prior to testing in the device assay system. You should also provide recommendations for decontaminating equipment, along with disposal and decontamination instructions for reaction mixtures and waste upon test completion.

E. Quality control

Your labeling should contain sufficient information about control materials and procedures to allow clinical laboratories to perform adequate quality control for your test. CLIA regulations specify that the laboratory is responsible for having control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, FDA-cleared or approved test system (42 CFR Part 493.1256). According to CLIA regulations, these control procedures must:

- detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance.
- monitor over time both the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance.
- include control materials tested in the same manner as patient specimens.

We recommend that you provide the following types of information to enable laboratories to meet CLIA requirements:

- A description of control material included in your kit and other design features that may control testing quality.
- A description of the effectiveness of controls included in the assay kit for monitoring extraction, amplification, hybridization, and detection as appropriate.
- Recommendations for placement of controls and calibrators.
- Recommendations for frequency of controls that are not incorporated into each test.

work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Refer also to: U.S. Department of Labor, Occupational Safety and Health Administration. 1991. Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register **56**:64175-64182.

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- Discussion of unacceptable measurement ranges for controls included in your assay design. Your recommendations should also include that each laboratory should verify these ranges (using statistical parameters).
- A statement similar to the following: "If controls do not behave as expected, results are invalid and patient results should not be reported."
- When your test system fails to include control material that can detect errors in the extraction phase, or when control material is not tested in the same manner as patient specimens, you should alert laboratories that additional controls may be required. You may recommend sourcing or preparation of those controls, if you demonstrated during your device evaluations that these controls were able to monitor effectiveness of extraction (or other processing procedures).

You may also provide troubleshooting instructions when controls are unacceptable.

F. Interpretation of Results

We recommend that you explain the rationale for interpretation of your test system output, and describe cut-off and decision levels as appropriate. You should recommend terminology consistent with the intended use for reporting results. The report for a non-reactive or negative result could be "no analyte (specify the nucleic acid target) detected; presumed negative for the target organism (specify organism)." The term "negative," used alone, should be avoided. You should use a similar approach for results above the cut off. An example is given below.

Example for consolidating interpretation of results for patient specimens, are shown:

Output Value	Result Report	Relevant Interpretation
≥ 100 signal	Positive - <i>C. trachomatis</i> cryptic plasmid DNA detected	Likely positive for <i>C. trachomatis</i> infection Note: potential for falsely positive results particularly for patients from low prevalence settings, or when <i>a priori</i> likelihood of infection is low
50-99 signal	Equivocal	Inconclusive; insufficient certainty to predict presence or absence of <i>C. trachomatis</i> ; repeat patient testing from a new specimen is recommended
<50 signal	Negative - <i>C. trachomatis</i> cryptic plasmid DNA not detected	Likely negative for <i>C. trachomatis</i> Note: infection cannot be precluded. Falsely negative results may occur particularly when specimen collected is inadequate or sub-optimal
IC failed	Indeterminate	Inconclusive; testing inhibition could prevent detection of <i>C. trachomatis</i> DNA if present. Repeat patient testing from a new specimen is recommended

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You may also recommend follow-up action for equivocal or otherwise inconclusive results if appropriate (e.g., "If result is equivocal on repeat testing, obtain a new specimen and request follow-up testing").

When clinical evaluations support uncertainty for interpreting results, you should provide guidance to laboratories for additional testing that was shown to reduce uncertainty in your studies.

G. Limitations

List the important limitations and known contraindications of the assay. We recommend that you include available published references. You should describe any interfering substances, conditions, or other factors that can affect the performance reliability of the device test system. For these types of devices, limitations can include (but are not limited to):

- False negative reports may result from improperly collected, transported, or handled specimens, procedural errors, amplification inhibitors in the specimen, or inadequate numbers of organisms for amplification.
- False positive reports may result from cross-contamination of target organisms, their nucleic acids, and amplified product, or non-specific signal in the assay.
- Specimen adequacy cannot be assessed.
- Therapeutic success or failure cannot be determined as nucleic acid target may persist independent of organism viability following appropriate therapy.
- For genus-specific devices, multiple species of the genus present in the specimen cannot be distinguished.

H. Expected Values

We recommend that you represent expected values graphically (e.g., histogram or table) for your device from your clinical evaluations showing cutoff(s) and the equivocal region, as applicable. You should designate values for infected and uninfected patient status (or other target condition) for each specimen type and population, as applicable.

You should also indicate the expected prevalence of the disease in diverse patient populations or clinical settings with the caveats applicable to the intended use (e.g., geographical location, age, gender of population studied, type of test employed, specimen collection and handling procedures, clinical and epidemiological history of individual patients, etc.).

I. Performance Characteristics

For clinical evaluations, you should describe the evaluation design and method for assigning patient status (e.g., infected, not infected, or uncertain) or other patient condition as appropriate. You should provide summaries of the diagnostic performance data for your device, (e.g., diagnostic accuracy estimates), with relevant subgroup analyses. Positive and

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negative predictive values with prevalence should be based on specific populations sampled for each disease syndrome or condition. We also recommend that you represent the prevalence at each laboratory evaluation site for relevant subgroups (using your device) and describe the effect of prevalence on positive and negative predictive values in different test populations. You should also clearly represent all equivocal and indeterminate, invalid or otherwise inconclusive results. You can discuss discrepancies between your device results and reference results, along with additional findings that would clarify potential sources of error with either the reference status or your device results.

We recommend that you represent performance point estimates (e.g., sensitivity, specificity, positive and negative predictive values with prevalence), based on the total number of true positive and true negative specimens determined by the reference standard with fractions and 95% confidence limits.

Example: Sensitivity of testing urine specimens for *N. gonorrhoeae* urogenital infection in asymptomatic males = 80% (8/10); 44.4-97.5%

You should summarize reproducibility findings by laboratory evaluation site and by sample levels.

You should also represent the following analytical performance parameters and provide a brief study design summary, including the following:

- A listing or description of organisms and number of strains of target and non-target organisms evaluated for reactivity in your assay; specify all positive, negative, and inconclusive (e.g., borderline, equivocal, indeterminate, or invalid) results.
- Limits of detection for species or strain variants, and matrices as appropriate. Include findings for number of copies and number of organisms (CFU, PFU, etc.) detected. We recommend using the format for reporting described in CLSI EP17-A.
- Interferences and sources of inhibition evaluated and your findings. We recommend using the format for reporting described in CLSI EP7-A.
- A description of the specimen stability studies used to support recommendations for transport and storage conditions.
- A description of testing done to support calibration interval recommendations.
- A summary of precision for each testing condition. We recommend using the format for reporting described in CLSI EP5-2A.
- A summary of conditions and operator factors evaluated for effect on controls and a representation of the control ranges documented during your studies to assess device accuracy and precision. You should also include a representation of findings with other controls (any internal controls, or external control preparations recommended).