Class II Special Controls Guideline: Nucleic Acid Amplification Assays for the Detection of *Trichomonas vaginalis*

Guideline for Industry and Food and Drug Administration Staff

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For questions regarding this document contact Himani Bisht, Ph.D. Division of Microbiology Devices at 301-796-6180 or by email at himani.bisht@fda.hhs.gov

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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I. Introduction

This special controls guideline was developed to support the classification of a *Trichomonas vaginalis* nucleic acid assay into class II (special controls).

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) premarket notification for a *Trichomonas vaginalis* nucleic acid assay 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 equivalent assurance of safety and effectiveness.

II. *Trichomonas vaginalis* – Background

Trichomoniasis is a common sexually transmitted disease (STD) that is caused by infection with a protozoan parasite, *Trichomonas vaginalis* (*T. vaginalis*). In the United States, an estimated 3.7 million people have the infection, but only about 30% develop any symptoms of trichomoniasis.[1]

[1] http://www.cdc.gov/std/trichomonas/STDFact-Trichomoniasis.htm

III. Special Controls - 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 a *Trichomonas vaginalis nucleic acid assay*. Thus, 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] (2) comply with the special controls identified in this guideline or use alternative mitigation measures, but demonstrate to the Agency's satisfaction that those alternative measures identified by the firm will provide at least equivalent assurance of safety and effectiveness., and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

IV. Scope

The scope of this document is limited to the following device described in 21 CFR 866.3860:

21 CFR 866.3860 – *Trichomonas vaginalis* nucleic acid assay

(a) *Identification*. A *Trichomonas vaginalis* nucleic acid assay is a device that consists of primers, probes, enzymes and controls for the amplification and detection of trichomonas nucleic acids in endocervical swabs, vaginal swabs, and female urine specimens, from women symptomatic for vaginitis, cervicitis, or urethritis and/or to aid in the diagnosis of trichomoniasis in asymptomatic women. The detection of trichomonas nucleic acids, in conjunction with other laboratory tests, aids in the clinical laboratory diagnosis of trichomoniasis caused by *Trichomonas vaginalis*. This classification currently consists of the following product codes:

OUY [*Trichomonas Vaginalis* Nucleic Acid Amplification Test System]

The special controls set forth in this guideline applies to all devices classified under 21 CFR 866.3860, including those falling under product codes not yet established.

This special controls document applies to *in vitro* diagnostic devices (IVDs) for the detection of *T. vaginalis* nucleic acids in human urogenital specimens. This guideline is not intended to address devices that utilize detection mechanisms other than nucleic acid based approaches, such as detection of serological response in the host against the protozoan antigens, nor does it address the devices that are intended to screen donors of blood, and blood components, and donors of human cells, tissues, and cellular and tissue-based products.

[2] For additional information regarding 510(k) submissions, refer to 21 CFR 807.87 and the Center for Devices and Radiological Health (CDRH) Device Advice (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm)

V. Risks to Health

FDA has identified four risks to health associated with the use of the device. The risks are false negative test results, false positive test results, failure of the test to perform properly, and failure to properly interpret the test results. The measures to mitigate these identified risks are summarized in the table below.

FDA has identified the risks of false negative test and false positive test results, both of which can lead to individual and/or public health consequences, as issues of safety and effectiveness associated with this device that require special controls. A false positive test result for an individual may lead to unnecessary or inappropriate antibiotic use, unnecessary patient distress and possibly a less thorough laboratory evaluation for the true cause of illness. A false negative result may lead to disease progression due to a delay in the treatment and thereby increasing the risk of spreading the infection and of acquiring other complications, for example, it may result in preterm delivery in pregnant women or may increase the risk of getting or spreading other sexually transmitted infections, such as HIV[3] [Ref. 1]. Failure of *T. vaginalis* nucleic acid assay to perform as indicated or an error in interpretation of the results may lead to misdiagnosis with significant implications to patient management.

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 and Mitigation Measures

Identified Risks	Mitigation Measures
A false positive test result may lead to inappropriate use of antibiotics for treatment.	• Section VI (Specific Device Description Requirements) • Section VII (Performance Characteristics • Section VIII (Labeling)
A false negative test result for an individual may lead to a potential delay in treatment.	• Section VI (Specific Device Description Requirements) • Section VII (Performance Characteristics) • Section VIII (Labeling)

[3] http://www.cdc.gov/std/trichomonas/trich-fact-sheet-aug-2012.pdf

Failure of the test to perform properly.	• Section VIII (Labeling)
Failure to properly interpret test results.	• Section VIII (Labeling)

VI. Specific Device Description Requirements

In your 510(k) submission, you must provide, as discussed more fully below, certain detailed information regarding the intended use of your device, test methodology, instrumentation, ancillary reagents, specimen collection and handling, and interpreting test results and reporting.

VI(A). Intended Use

Your 510(k) must include labeling that describes the intended use of your product. The intended use statement must clearly specify that the device is an aid in the diagnosis of trichomoniasis. You must specify the name of the organism, the nature of the analyte (RNA or DNA), the specimen type(s) (e.g., vaginal swabs), whether the swab specimens are collected by clinician or self-collected, and the name of the test methodology/technology. You must clearly state the clinical indications for which the test is to be used, the specific population(s) for which the test is intended, and any limitations on the device use. The intended use statement must state that the test is qualitative and any specific conditions of use. Additional qualifications may be required based on the results of the clinical studies.

VI(B). Test Methodology

You must describe in detail the methodology underlying your device in your 510(k). Examples of elements that must be described in detail, as applicable to your device, include:

- The specific test methodology (e.g., real-time polymerase chain reaction (PCR), strand displacement amplification, transcription-mediated amplification, etc.) and whether the test is performed manually or utilizes an instrument.
- Information and rationale for the selection of specific targets, and the methods used to design detection elements (e.g., primers and probes including the sequences).
- Specificity of capture and detection reagents for nucleic acid sequences of interest.
- Specimen types (e.g., swabs, etc.), collection methods and handling methods.
- All pre-analytical methods and instrumentation for collection, stabilization, and concentration of specimens.
- Assay procedural steps (e.g., pipetting, incubation, washing, and mixing).

- Reagent components provided or recommended for use, and their function within the system (e.g., buffers, enzymes, fluorescent dyes, oligonucleotides, chemiluminescent reagents, substrates, conjugates, and other signaling/amplification reagents).
- Instrumentation required for your device, including the components and their function within the system.
- Type of output generated by the device and system parameters (e.g., measurement ranges, units, when applicable).
- 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, and a detailed description of non-standard equipment or methods.

When applicable, you must describe in detail design control specifications for your device that address or mitigate risks associated with nucleic acid-based procedures for detecting *T. vaginalis*, such as the following:

- Positive, negative, and internal controls to ensure accurate test results.
- Minimization of false positives due to amplicon or carryover contamination
- Developing or recommending validated methods for nucleic acid extraction and purification that yield suitable quality and quantity of nucleic acid for use in the test system with your reagents. You must address suitable validated extraction method(s) for different specimen types claimed in its intended use.
- Optimizing your reagents and test procedure for recommended instruments.

In your 510(k), you must provide performance information that supports the conclusion that design requirements have been met.

You must provide in your 510(k) submission a detailed description of the principles of operation of your device. You must specifically describe testing conditions, procedures, and controls designed to provide safeguards for conditions that can cause false positive and false negative results, or that may present a biosafety hazard. These include, but are not limited to:

- Description of, or recommendations for, any external controls and/or internal controls (e.g., sample negative controls and/or internal controls that monitor assay performance).
- Overall design of the testing procedure, including control elements incorporated into the recommended testing procedures.
- Features and additional controls that monitor procedural errors or factors (e.g., degradation of reagents) that adversely affect assay performance and detection.

You must include a description for all additional procedures, methods, and practices incorporated into the directions for use (See Section VIII - Labeling) that mitigate risks associated with *T. vaginalis* testing.

VI(C). Instrumentation – Hardware and Software

In your 510(k) submission, you must provide software documentation, including:

- A clear description of how raw signals are converted into a result, including adjustment to the background signal for normalization. In addition, you must describe software controls for identifying and managing anticipated problems.

- Information dependent on the level of concern for your type of software (Minor, Moderate, or Major). The level of concern must be driven by a hazard analysis in the absence of mitigations (i.e., the hazard analysis must be performed as though none of the individual hazard mitigations were present). The level of concern of nucleic acid-based IVDs for the detection of *T. vaginalis* in urogenital specimens is expected to typically be moderate. The level of concern is based on how the operation of the software associated with the functioning of the device could affect the patient or operator and is defined below.
    - **Major -** The level of concern is Major if (1) a failure or latent flaw could directly result in death or serious injury to the patient or operator and/or (2) if a failure or latent flaw could indirectly result in death or serious injury of the patient or operator through incorrect or delayed information or through the action of a care provider.
    - **Moderate –** The level of concern is Moderate if (1) a failure or latent design flaw could directly result in minor injury to the patient or operator and/or (2) if a failure or latent flaw could indirectly result in minor injury to the patient or operator through incorrect or delayed information or through the action of a care provider.
    - **Minor -** The level of concern is Minor if failures or latent design flaws are unlikely to cause any injury to the patient or operator.

See Table 2 below for the software documentation required in the 510(k) submission dependent on the level of concern associated with the subject device.

Table 2 - Required Documentation Based on Level of Concern

SOFTWARE DOCUMENTATION	MINOR CONCERN	MODERATE CONCERN	MAJOR CONCERN
Level of Concern	A statement indicating the Level of Concern and a description of the rationale for that level.		
Software Description	A summary overview of the features and software operating environment.		
Device Hazard Analysis	Tabular description of identified hardware and software hazards, including severity assessment and mitigations.		

Software Requirements Specification (SRS)	Summary of functional requirements from SRS.	The complete SRS document.	
Architecture Design Chart	No documentation is necessary in the submission.	Detailed depiction of functional units and software modules. May include state diagrams as well as flow charts.	
Software Design Specification (SDS)	No documentation is necessary in the submission.	Software design specification document.	
Traceability Analysis	Traceability among requirements, specifications, identified hazards and mitigations, and Verification and Validation (V&V) testing.		
Software Development Environment Description	No documentation is necessary in the submission.	Summary of software life cycle development plan, including a summary of the configuration management and maintenance activities.	Summary of software life cycle development plan. Annotated list of control documents generated during development process. Include the configuration management and maintenance plan documents.
Verification and Validation Documentation	Software functional test plan, pass / fail criteria, and results.	Description of V&V activities at the unit, integration, and system level. System level test protocol, including pass/fail criteria, and tests results.	Description of V&V activities at the unit, integration, and system level. Unit, integration and system level test protocols, including pass/fail criteria, test report, summary, and tests results.
Revision Level History	Revision history log, including release version number and date.		
Unresolved Anomalies (Bugs or Defects)	No documentation is necessary in the submission.	List of remaining software anomalies, annotated with an explanation of the impact on safety or effectiveness, including operator usage and human factors.	

- Configuration of the hardware and software components must be very similar or identical to that anticipated for the final version of the device before beginning clinical studies. If any significant changes are made to the hardware or software after the completion of the clinical studies but before the clearance and distribution of the device, you must perform a risk assessment and include it in your 510(k) submission.

For additional information on how FDA believes the level of concern is to be determined as well as additional descriptions of software documentation, see FDA's guidance entitled "The Content of Premarket Submissions for Software Contained in Medical Devices" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm).

The following references may aid in the development and maintenance of a new device under good software life cycle practices consistent with FDA regulations:

- The guidance entitled "General Principles of Software Validation; Final Guidance for Industry and FDA Staff" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085281.htm).
- The guidance entitled "Off-the-Shelf Software Use in Medical Devices" (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073779.pdf).
- The guidance entitled "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm).
- 21 CFR 820.30 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

For instruments and systems that measure multiple signals, and other complex laboratory instrumentation, please consult with the Division of Microbiology Devices in the Office of In Vitro Diagnostics and Radiological Health (OIR).

VI(D). Ancillary Reagents

Ancillary reagents are those reagents that a manufacturer specifies 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 the labeling. For the purposes of this document only, ancillary reagents of concern are those that must be specified according to manufacturer and catalog or product number, or other specific designation, in order for your device to achieve its labeled performance characteristics. For example, if the device labeling specifies the use of Brand X nucleic acid amplification enzyme, and

use of any other nucleic acid amplification enzyme may alter the performance characteristics of the device from that reported in the labeling, then Brand X nucleic acid amplification enzyme is an ancillary reagent of concern for purposes of this document.[4]

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

If the instructions for use of the device specify ancillary reagents of concern, you must include in your submission a description of how you will ensure that the results obtained by testing with the device and these ancillary reagents will be consistent with the performance described in your premarket submission. Every effort must be made to bring the ancillary reagents under your quality system by recommending use of only those ancillary reagents that have been determined to meet your quality standards for the test. This may include application of quality systems approaches, product labeling, and other appropriate measures.

In order to address this aspect of the special control, your 510(k) submission must include the information described below. FDA will evaluate the information submitted is sufficient to support a demonstration that your device is at least as safe and effective, that is, substantially equivalent, to a legally marketed device..

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. This assessment must include 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 with particularity in your 510(k) how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These include, where applicable:

    - User labeling to assure appropriate use of ancillary reagents (see Section VIII – 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.

[4] 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. If you are unsure whether this aspect of the special controls applies to your device, consult with the Division of Microbiology Devices in the Office of In Vitro Diagnostics and Radiological Health (OIR)

- 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 must 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, you may contact the Division of Microbiology Devices in OIR to obtain advice or information regarding your planned study.

VI(E). Specimen Collection and Handling

In your 510(k) submission, you must specify the specimen type(s) to be used with your device. A specimen has to be collected from the appropriate anatomical site or source with the recommended specimen collection procedure. The quality and quantity of the target analyte can be highly dependent on factors such as specimen source, collection method, and handling (e.g., transport and storage times and temperatures). Testing results you provide in your 510(k) must validate that the device maintains acceptable performance (e.g., accuracy, reproducibility) under all the conditions recommended in your labeling. You must state your acceptance criteria for all specimen collection and handling conditions and stability parameters.

If your device is to be used with a dedicated collection kit, you must include detailed information about that product. In addition, a collection kit for urogenital specimens must include detailed instructions on how to perform the collection. If the collection is to be performed by the patient (self-collection), additional instructions written in an easy-to-understand language, accompanied by diagrams, must be included.

Follow all applicable state and federal biosafety guidelines for collecting and handling specimens for pathogen identification. For standard precautions in handling of specimens, refer to the most current editions of the related Clinical and Laboratory Standards Institute (CLSI) documents [Ref. 2].

VI(F). Interpreting Test Results/Reporting

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

You must provide the specific cut-off value (e.g., signal intensity level) for defining a negative result of the assay. If the assay has only two possible output results (e.g., positive and negative), then this cut-off will also define a positive result of the assay.

If the assay has an equivocal zone, you must provide ranges (limits) for the equivocal zone and recommendations for how the user must follow up the equivocal results. If your interpretation of the initial equivocal results requires retesting, your 510(k) must address:

- A recommendation whether re-testing must be done by the same assay or a different method.
- A recommendation whether re-testing must be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen.
- An algorithm for defining a final result by combining the initial equivocal result and the results after retesting. This algorithm must be developed before the pivotal clinical studies that evaluate the clinical performance 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 recommendations on 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 must be reported as invalid or whether retesting is recommended). If retesting is recommended, you must provide information similar to that for retesting of equivocal results (i.e., whether retesting must be repeated from a new aliquot of the same sample or a new patient specimen).

VII. Performance Characteristics

VII(A). General Study Principles

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

You must evaluate your assay performance with each specimen type that you intend to be used with your assay.

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 during our review. When referring to Clinical and Laboratory Standards Institute (CLSI) protocols or guidelines, you must indicate which specific aspects of the protocols or guidelines were followed. Relevant findings in published literature may also be cited.

Please contact the Division of Microbiology Devices in OIR prior to initiating clinical studies to obtain feedback regarding planned studies and to confirm that these studies will support the proposed intended use for the device.

VII(B). Controls

When conducting the performance studies described below, you must run appropriate controls every day of testing for the entire duration of the analytical and clinical studies. You must test the controls described below and include the results in your 510(k) submission, if applicable for your device. You must define the supplier(s) of the controls and have a plan to provide for their continued availability. You may contact the Division of Microbiology Devices in OIR for further information regarding appropriate controls.

VII(B)(1). Negative Controls

Blanks or no template control
The blank, or "no-template" control, contains buffer or sample transport media and all of the assay components except the 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 needed for assays performed in single test disposable cartridges or tubes.

Negative sample control
The negative sample control is used to evaluate the complete assay procedure, including extraction. Negative results for this control confirm that signals are not obtained in the absence of target sequences (e.g., due to non-specific priming or detection). Examples of acceptable negative sample control materials are:

- Patient specimens from a non-*T. vaginalis* infected individual
- Samples containing a non-target organism (e.g., cell line infected with non-*T. vaginalis* protozoa)

VII(B)(2). Positive Controls

Positive control for complete assay
The positive control contains target nucleic acids and is used to control the entire assay process, including nucleic acid extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate sample, concurrently with patient specimens, at a frequency determined by a laboratory's Quality System (QS). Examples of acceptable positive assay controls include:

- Inactivated whole *T. vaginalis*
- Patient specimens positive for *T. vaginalis*
- Pooled negative specimens spiked with whole *T. vaginalis*

Positive control for amplification and detection
The positive control for amplification and detection contains 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 the procedural steps of the reaction. It also indicates that target can be detected if it is present in the sample. Example of this type of control includes a non-infectious plasmid containing the target sequence.

VII(B)(3). 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, etc.), equipment function, and the presence of inhibitors in the specimen. Examples of acceptable internal control materials include human nucleic acids co-extracted with the *T. vaginalis* and primers amplifying human housekeeping genes (e.g., RNaseP, β-actin). Alternatively, the internal control can be a non-infectious plasmid containing the non-target nucleic acid that is added to each clinical specimen before any pre-analytical steps and is analyzed simultaneously with the clinical targets.

VII(C). Analytical Performance Studies

The analytical studies appropriate for a device of this type depend on the underlying technology, principles of operation, and available scientific evidence specific to the new device. Samples for the LoD, interference, and specimen stability studies must be prepared in natural clinical matrix. Non-clinical matrix, for example, specimen transport medium can be used for your other analytical studies (e.g., inclusivity, reproducibility, and cross-contamination if your molecular test includes nucleic acids extraction and purification step). You must demonstrate in a study that analytical performance of your assay is equivalent using the proposed non-clinical matrix and the natural clinical matrix containing *T. vaginalis*. The equivalence study includes a comparison of natural clinical matrix with non-clinical matrix spiked with *T. vaginalis* at a concentration near the LoD (1-2X). This study can be conducted in-house and include a limited number of samples (e.g., 60 samples per analyte) with the majority of the samples containing analyte levels close to the LoD, and the rest of the samples distributed 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%. Alternatively, equivalence between the matrices can be established by determining that the LoD levels are comparable in natural clinical matrix and non-clinical matrix.

You must always establish the following performance characteristics for your *T. vaginalis* assay in your 510(k); however, please note that additional analytical studies may be necessary depending on the specific device characteristics:

VII(C)(1). Analytical Sensitivity

Limit of Detection
The limit of detection (LoD) is defined as the lowest concentration of target analyte that can be consistently detected in ≥95% of sample measurements. The LoD of your test system must be determined for each distinct specimen type that your device utilizes considering the entire test system from sample preparation to the detection. This can be accomplished by limiting dilutions of regrown and retitered *T. vaginalis* stocks (quantified in trichomonads/per mL). The initial study must include serial dilutions of at least two strains (one metronidazole sensitive and one metronidazole resistant) representative of *T. vaginalis* types commonly found in the United States and at least 5 measurements

(replicates) for each dilution to establish the preliminary range. The preliminary LoD must be confirmed for each strain, testing at least 20 additional replicates demonstrating that *T. vaginalis* at this concentration was detected 95% of the time.

You may refer to Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 for examples of the study design [Ref. 3]. Probit analysis may also be used to establish LoD as long as the study is appropriately designed.

Additionally, you must demonstrate that your test can detect other clinically relevant *T. vaginalis* strains representing temporal and geographical diversity. The strains must be tested in triplicate at or near the LoD in pooled *T. vaginalis* negative human clinical specimens. All strains must be retitered to ensure that the tested dilutions are at or near the LoD predetermined in pooled clinical matrix. The procedure for quantification must be provided in the 510(k) submission.

VII(C)(2). Analytical Specificity

Cross-Reactivity
You must test your device for potential cross-reactivity with other relevant microorganisms, including common flora of the genitourinary tract, opportunistic and closely related organisms. In particular, you must characterize performance of the test in the presence of whole microorganisms that may present similar clinical symptoms and may be confused with *T. vaginalis* infection. You must test three (3) replicates of each microorganism at medically relevant levels (usually 10^6 CFU/ml or higher for bacteria and 10^5 PFU/ml or higher for viruses). The titers of these microorganisms must be confirmed prior to use in the study. Any one positive result represents cross-reactivity. Relevant microorganisms for cross-reactivity testing include, but are not limited to, the microorganisms listed in Table 3.

Table 3 – Microorganisms for Analytical Specificity (Cross-Reactivity) Studies

Acinetobacter lwoffi	*Enterobacter aerogenes*	*Mobiluncus curtisii*
Actinomyces israelii	*Enterobacter cloaceae*	*Mycoplasma genitalium*
Atopobium vaginae	*Enterococcus fecalis*	*Mycoplasma hominis*
Bacteroides fragilis	*Escherichia coli*	*Neisseria gonorrhoeae*
Bifidobacterium adolescentis	*Fusobacterium nucleatum*	*Pentatrichomonas hominis*
Campylobacter jejuni	*Gardnerella vaginalis*	*Peptostreptococcus anaerobius*
Candida albicans	*Haemophilus ducreyi*	*Prevotella bivia*
Candida glabrata	Herpes simplex virus I	*Propionibacterium acnes*
Candida parapsilosis	Herpes simplex virus II	*Proteus mirabilis*
Candida tropicalis	HIV-1	*Pseudomonas aeruginosa*
Chlamydia trachomatis	HPV	*Staphylococcus aureus*
Clostridium difficile	*Klebsiella oxytoca*	*Staphylococcus epidermidis*
Clostridium perfringens	*Lactobacillus acidophilus*	*Streptococcus pyogenes*
Corynebacterium genitalium	*Lactobacillus jensenii*	*Streptococcus agalactiae*
Cryptococcus neoformans	*Lactobacillus vaginalis*	*Trichomonas tenax*

Dientamoeba fragilis	*Listeria monocytogenes*	*Ureaplasma urealyticum*

Interference

You must conduct a comprehensive interference study with your device. Potentially interfering substances include, but are not limited to, endogenous substances, such as white blood cells, protein, whole blood, and mucus; and exogenous substances, such as over the counter lubricants, spermicides, deodorant sprays/powders, and anti-fungal/anti-itch medications. You must test 2-3 replicates of each potential interferent using samples spiked with *T. vaginalis* at a concentration that challenges the medical decision point of your assay (i.e. C_{95}). You must evaluate each interfering substance at its potentially highest concentration ("the worst case"). You must establish acceptance criteria prior to conducting this study. A sample is considered an interfering substance if *T. vaginalis* is not detected in one or more of the replicates. If interference occurs, you must test decreasing concentrations of the interfering substance until false negatives no longer occur. If no significant clinical effect is observed, no further testing is necessary. You may refer to the CLSI document, "Interference Testing in Clinical Chemistry," EP07-A2 for additional information [Ref. 4]. Examples of potentially interfering substances are presented in Table 4.

Table 4 – Substances for Interference Studies

Swab	Urine Matrix
Whole Blood	Phenazopyridine Hydrochloride
Seminal Fluid	Whole Blood
Mucus	Acidic urine (pH 4.0 or 5.0)
Over the counter vaginal products and contraceptives	Alkaline urine (pH 9.0)
Hemorrhoidal cream	Hormones
Prescription vaginal treatments	Antibiotics
Leukocytes ($1x10^6$ cells/mL)	Bilirubin
Intravaginal hormones	Mucus
	Albumin ($\leq$ 1mg/mL)
	Glucose
	Semen (5% v/v)
	Over the counter deodorant spray and powder
	Leukocytes

VII(C)(3). Cut-off and Equivocal Zone Determination

Your submission must describe the analytical and clinical data utilized to establish the assay cut-off. For example, if you establish a cut-off based on a pre-determined specificity using a pilot study with clinical samples without any trichomonads (zero analyte concentration), you may provide a result distribution (e.g., 95th and 99th percentiles, percent of the positive results), and other statistics. If you select the cut-off based on optimal sensitivity and specificity estimated by Receiver Operating Curve (ROC) analysis of the pilot studies with clinical samples (for details about ROC analysis,

see CLSI document EP24-A2 [Ref. 5]), selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity.

If the assay has an equivocal zone, you must explain how you determined the limits of the equivocal zone.

The performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) must be validated in a pivotal clinical study on a new set of samples collected from individuals that represent the intended use population of your device.

VII(C)(4). Precision

Within-Laboratory Precision
Dependent on your device methodology, a separate within-laboratory precision studies may or may not be required; please contact the Division of Microbiology Devices in OIR regarding the need for precision studies for your device. If separate within-laboratory precision studies are required, you must conduct these studies using the instruments and/or automated components anticipated for use in your clinical study. You may perform these studies in-house (i.e., within your own company facility).

You must evaluate sources of variability (e.g., operators, days, and assay runs) by testing your device for a minimum of 12 days with two operators, each performing two runs per day and at least two replicates of each sample per run. These 12 days are not necessarily consecutive. If your system calibration interval fits within the time frame of the studies then at least two calibration cycles must be included in the study design; otherwise, please provide your rationale for not including an evaluation of the variation introduced due to instrument calibration. The test panel must consist of panel members at the concentration levels that include:

- A "negative" sample: a sample with no analyte such that results of repeated tests of this sample are negative 100% of the time.
- A "high negative" sample (C_5 concentration): a sample with an analyte concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 95% of the time and positive approximately 5% of the time.
- A "low positive" sample (C_{95} concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A "moderate positive" sample (e.g., approximately two to three times the concentration of the clinical cut-off): a sample with a concentration at which one can anticipate positive results approximately 100% of the time.

For ultrasensitive test (e.g., a real-time PCR assay) for which samples with zero analyte concentration almost always have a negative assay result (type I error is close to zero), it may be difficult to prepare a sample with C_5 concentration.

If less than 10% of all subjects positive by the reference method yield test results below LoD, then samples at the C_5 concentration can be excluded from the precision panel. In this case, panel members at the following concentration levels must be tested: True negative, Low positive, and Moderate positive.

If more than 10% of all subjects positive by the reference method yield test results below LoD, then members of precision panel must include the following concentration levels: True negative, Low positive, a sample in the range of C_{20} to C_{80}, and Moderate positive. A sample in the range of C_{20} to C_{80} is a sample with a concentration of analyte just above or below the cut-off such that the results of repeated tests of this sample are positive approximately 20-80% of the time.

Samples must be adequately masked or blinded to the operators performing the testing in these studies to avoid possible bias. Each daily panel must have a different order of samples so the operator does not know what the expected result is. The procedures used for masking and randomization of samples must be described in your submission.

CLSI documents EP05-A3 *Evaluation of Precision of Quantitative Measurement Procedures*, and EP12-A2, *User Protocol for Evaluation of Qualitative Test Performance* [Ref. 6 and 7], contain further information about designing and performing precision studies.

For each panel member in the within-laboratory precision study, you must provide the mean value with variance components (standard deviation and percent coefficient of variation (CV)) as repeatability (within-run), between-run, between-day, etc. Information about total precision and components of variance that were included must be also provided. In addition, you must include the percent of values above and below the cutoff for each panel member.

Between-Laboratory Precision/Reproducibility
The protocol for the reproducibility study may vary slightly depending on the assay format although the sample panel must be the same as described for within-laboratory studies cited above. In general, the protocol must:

- Evaluate the reproducibility of your test at three testing sites. This may include two external sites and one in-house site or three external sites. A minimum number of 90 observations must be provided for each test panel member across the three sites (with at least 30 observations per site for each test panel member).
- Use a five day testing protocol, including a minimum of two runs per day, (unless the assay design precludes multiple runs per day), and three replicates of each panel member per run.
- Have at least two operators at each facility perform the test. The same operator must perform two runs on the same day to allow for run-to-run and operator-to-operator variance calculations. You must provide training only to the same extent that you intend to train users after marketing the test.

For each panel member in the reproducibility study, you must provide the mean value with each variance component estimate (standard deviation and percent CV) as well as total variance, for each site separately and for all sites combined. For example, for a combined site data analysis, if a reproducibility study is performed at three sites over five days using two operators and three replicates per run, provide the mean value, standard deviation, and percent CV for total variance and variance components for site-to-site, day-to-day, operator-to-operator, run-to-run, and replicate-to-replicate. In addition, you must include the percent of values above and below the cutoff and the percent of invalid results for each site separately and for all sites combined. You may refer to the CLSI document EP05-A3 and EP15-A3 for additional information on reproducibility study design [Ref. 6 and 8].

VII(C)(5). Specimen Storage and Shipping Conditions

If you recommend specimen storage conditions, you must demonstrate that your device generates equivalent results for the fresh and stored specimens at several time points throughout the duration of the recommended storage and at both ends of your recommended temperature range. Note that this study must include at least one additional data point beyond the time point you are claiming. The specimen shipping and storage studies must include samples close to the cut-off and additional samples across the clinically relevant range. In addition to the analysis of qualitative results, you must provide analysis of the raw signals, if applicable. If a transport medium (TM) is recommended for storage or shipping, you must conduct appropriate studies to demonstrate that the device will perform as described when the specimen is preserved in TM.

CLSI document MM13-A, *Collection, Transport, Preparation and Storage of Specimens for Molecular Methods* [Ref. 9], contains additional information specific to this topic.

VII(C)(6). Device Shipping and Device Storage Studies

You must evaluate the performance of your device after exposing the device to various shipping and storage conditions described in your product labeling.

VII(C)(7). Carry-Over and Cross-Contamination Study

For multi-sample assays and devices that require instrumentation, you must demonstrate that carry-over and cross-contamination does not occur with your device. In a carry-over and cross-contamination study, you must perform an assay run with high positive samples alternating with negative samples in patterns dependent on the operational function of the device. You must perform at least five runs alternating high positive and negative samples. The concentration of *T. vaginalis* in the high positive samples must be high enough to exceed 95% or more of the positive results obtained from specimens of actively infected patients in the intended use population. Negative samples must be samples with no analyte such that results of repeated tests of this sample are negative 100% of the time.

The carry-over and cross-contamination effect can then be estimated by the percent of negative results for the negative samples that are adjacent to high positive samples in the carry-over study compared to the percent of negative results in the absence of adjacent high positive samples (i.e. when the same source of negative samples are run alone on a separate plate). For additional details, see Haeckel [Ref. 10].

VII(D). Clinical Studies

The clinical study for your *T. vaginalis* detection device must be designed to support your proposed intended use. The clinical performance (i.e., sensitivity and specificity) of your device must be established in a prospective clinical study (or studies) testing all the specimen types claimed in your labeling collected from individuals representing the intended use population.

Please contact the Division of Microbiology Devices in OIR to request a review of your proposed studies as part of the pre-submission review process prior to the initiation of the studies.

In general, the following clinical studies principles must be followed:

- Clinical samples must be collected from a minimum of three geographically diverse locations.
- Laboratory testing using your device must be performed at a minimum of three different sites representing settings where the device is intended for use. Laboratory testing sites may be the same as the clinical enrollment sites. One laboratory testing site may be the manufacturer's laboratory.
- Reference method testing may be conducted at a centralized laboratory.
- The collection, transport, and testing of specimens using the investigational device must be performed by individuals with training equivalent to that anticipated for users of the marketed device.

VII(D)(1). Study Protocol

Clinical study protocol(s) must be finalized prior to the study initiation. At a minimum, protocol(s) must include complete patient inclusion and exclusion criteria, study procedures, a description of where the tests will be performed, specimen transport procedures, storage conditions, maximum storage times (if appropriate), documents supporting compliance with human subject protection regulations, description of the reference method, blinding procedures, as applicable to your study and your specific device, and other components as appropriate. The protocol must also describe safety precautions for the collection, handling, processing, and testing of specimens that will be tested during the study.

Study case report forms must capture any time-sensitive steps (e.g., the amount of time a specimen is stored if not tested immediately).

Also, you must develop and provide a detailed statistical analysis plan that includes the statistical analysis methods to be used and justification of the study sample size. Any planned monitoring of the study data or examinations of study progress must be described in the clinical protocol/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 sponsors to contact FDA to request a review of their proposed study protocols and the selection of specimen type as part of the pre-submission review process. This is particularly recommended in a situation where different intended uses of the test may be studied or sponsors are planning to submit a 510(k) submission for the first time. For guidance related to the clinical protocol, you may also refer to FDA's guidance entitled "Design Considerations for Pivotal Clinical Investigations for Medical Devices" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm373750.htm).

VII(D)(2). Study Sites

The clinical samples must be collected from at least three geographically diverse facilities, representing both high prevalence settings (e.g., STD clinics) and low prevalence settings (e.g., public health and family planning clinics, prenatal clinics, and OB/GYN clinics) of the *T. vaginalis* infection. There must be at least three clinical testing sites, of which one can be an internal site (i.e., in-house). The testing sites must represent environments where the device would be used (e.g., clinical laboratories) and laboratory personnel with training similar to those likely to perform the test in laboratory settings. Testing sites must document all quality control results and all repeat tests for runs with quality control values out-of-range.

VII(D)(3). Study Population

Samples from individuals both symptomatic and asymptomatic for *T. vaginalis* infection must be collected and tested in the clinical studies. For guidance on specific patient populations and prevalence rates of *T. vaginalis* infections refer to CDC recommendations.[5] However, as these recommendations are subject to revisions, the most current guidelines/recommendations must be consulted.

You must collect all relevant demographic, clinical, and laboratory information available for your clinical study participants. This must include age, sex, signs and symptoms, time since onset of symptoms, indications for testing, any medications taken or administered, and a final diagnosis if available.

[5] http://www.cdc.gov/std/default.htm

VII(D)(4). Specimens

Urogenital specimens are appropriate specimen matrices for this device. If other sample types will be studied, please contact the Division of Microbiology Devices in OIR before beginning your study. You must conduct your clinical studies with all specimen types and matrices claimed in the intended use to demonstrate that correct results can be obtained from each type of clinical material. Please note that specimens requested for the standard care of patients and reference testing must be collected first. Contact FDA if you have an alternative approach or order for specimen collection. You must indicate the types of collection devices, such as swabs and transport media (TM), used for the collection of clinical specimens and for establishing the performance claims stated in the package insert. The clinical studies must include testing of all claimed swab types and transport media. If the swabs are not provided with the device, the package insert must contain information about the commercial source and swab specifications (e.g., size, shape, fiber, and shaft type) required for collection. If the recommended TM in the labeling is different from the one used in the clinical study or if more than one TM is recommended in the labeling, you must demonstrate equivalency in performance through analytical studies (e.g., LoD, stability, etc.).

The performance of your device will be evaluated for symptomatic and asymptomatic patients separately. Therefore, the study must be designed to collect sufficient number of positive samples for each specimen type and for each symptomatic status (symptomatic and asymptomatic). The expected sensitivity (point estimate) for each specimen type is 95% or greater with the lower bound of the two-sided 95% CI of at least 85%. Consideration may be given to devices demonstrating an expected sensitivity point estimate with the lower bound of the 95% confidence interval less than 85% when the submission includes an appropriate scientific or statistical justification related to the difficulty in procuring low prevalence specimens that is acceptable to FDA. The expected point estimate for the specificity is at least 95% with the lower bound of the two-sided 95% CI greater than or equal to 90%. In general, a minimum of 100 positive specimens from both symptomatic and asymptomatic individuals for each specimen type will be required. Exception may be made to allow a lesser number of specimen types from asymptomatic individuals if the submission includes an appropriate justification that is acceptable to FDA (e.g., the prevalence is too low to accommodate this requirement). You may contact the Division of Microbiology Devices in OIR with related questions regarding the sample size.

VII(D)(5). Reference Method

You must assess the performance of your device by comparing the results to a "Patient Infected Status" (PIS) algorithm where a subject is designated as being infected or non-infected with *T. vaginalis* based on the results of a composite reference method. At least one of the reference test results (part of the composite reference method) must be positive to establish an infected patient status. Both reference tests must be negative to establish a non-infected patient status.

The following are the acceptable composite reference methods to determine the PIS for *T. vaginalis*:

- *T. vaginalis* culture derived from vaginal swab samples and wet mount; or
- An FDA-cleared molecular assay or a validated molecular assay (if FDA cleared assay is not available) and either culture of urogenital swab or wet mount ; or
- Two FDA-cleared molecular assays or two validated molecular assays (if FDA cleared assays are not available) testing urogenital swab or urine samples. In this case the performance will be presented as percent agreement.

Reference method testing in urine may affect your results as (in females) this sample type can have lower sensitivity compared to vaginal swabs. Please note that the FDA-cleared assay(s) used for reference testing be used for testing only cleared specimen types.

For any other options please contact the Division of Microbiology Devices in OIR.

VII(D)(6). Presentation of Study Data and Results

All study data including the clinical line data and the analytical data sets must be included in the 510(k) submission in an acceptable electronic format that supports independent data analyses (e.g., Excel spreadsheet). Data files must include all primary and derived variables, with appropriate annotations or separate codebooks as necessary.

The data must include the following information for each sample tested:

- Specimen type
- Patient ID
- Age
- Sex
- Collection site
- Date of collection
- Testing site
- Date of testing
- Results (separate) for each of the reference tests used in the comparator algorithm
- Final PIS
- Result from your investigational device

You must calculate and present sensitivity, specificity (or positive and negative percent agreement), and positive and negative predictive values (PPV and NPV) with 95% two-sided confidence intervals along with the prevalence of the target condition in the intended use population. The results must be presented in a tabular format by symptom status (symptomatic and asymptomatic), by specimen type, for each site separately as well as for all sites combined. Additionally, you must provide the percent of invalid results along with 95% confidence interval.

You must provide frequency distribution of signals (results) from your device for the samples in your clinical study according to the PIS determined by the composite reference method for each specimen type. You must present frequency distributions in graphic and tabular formats. Description of the statistical methods applied to the data set must be sufficiently detailed to allow the Agency to reproduce the results reported in the submission.

VIII. Labeling

Your labeling for your *T. vaginalis* nucleic acid assay must include the information described below to help to ensure that users understand the appropriate uses of the device.

VIII(A). Intended Use

The intended use statement must clearly specify that the device is an aid in the diagnosis of trichomoniasis. You must specify the name of the organism, the nature of the analyte (RNA or DNA), the specimen type(s) (e.g., vaginal swabs), whether the swab specimens are collected by clinician or self-collected, and the name of the test methodology/technology. You must clearly state the clinical indications for which the test is to be used, the specific population(s) for which the test is intended, and any limitations on the device use. The intended use statement must state that the test is qualitative and any specific conditions of use. Additional qualifications may be required based on the results of the clinical studies.

VIII(B). Device Description

The device description must describe with particularity the assay methodology and rationale used in this type of device.

VIII(C). Procedure

This section must include a detailed description of the entire analysis procedure from the collections of patient samples to result reporting.

VIII(D). Directions for Use

You must provide clear and concise instructions that systemically describe the procedures for using the device, and the types of controls that will minimize risks of inaccurate results. This section of the labeling must include guidance for biosafety precautions with specimen handling and testing procedures and must clearly specify at which procedural step the test is rendered non-infectious.

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 your device and any reagents or other components.

For devices that involve the use of ancillary reagents of concern (see also Section VI(D) of this document), you must:

- Emphasize prominently that proper device performance involves use of specific ancillary reagents as directed. This may include warnings against device use if specified ancillary reagents are not available.
- Ensure that users can clearly identify which ancillary reagents are suitable for use with your device.
- Ensure that users of your device will understand which instructions for use they must follow when using ancillary reagents that are supplied with instructions for use or other warnings or limitations by the ancillary reagent manufacturer. If there is a conflict between the directions and warnings provided by the manufacturer of the ancillary reagents and the instructions for use that you supply with your device, you must assess and address the risk that users may mistakenly follow the labeling provided directly with the ancillary reagent manufacturer and possibly obtain invalid or inaccurate test results with your device. We note that in some circumstances, statements in the labeling of your device may not be sufficient to address the risks created by this conflict.

VIII(E). Quality Control

Your 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 your device, the 510(k) submission must include the specifications for control materials.

VIII(F). Warnings, Precautions and Limitations

In addition to any other limitations and warnings that are relevant to your specific assay, you must include statements such as the following under Limitations, as applicable:

- Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this insert may result in erroneous results.
- The effects of tampon use, douching, and specimen collection variables have not been assessed for their impact on the detection of *T. vaginalis*.
- *T. vaginalis*-positive mucoid samples may exhibit decreased output. To ensure proper endocervical sampling, excess mucus must be removed.
- Urine, vaginal swab and PreservCyt Solution liquid Pap specimen sampling is not designed to replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, or vaginal infections due to other causes or concurrent infections with other agents.
- This assay has been tested using only the specimen types indicated. Performance with other specimen types has not been evaluated.

- Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. See *Specimen Collection and Storage* for instructions. For detailed information, refer to the appropriate instructions for use.
- Therapeutic failure or success cannot be determined with the assay since nucleic acid may persist following appropriate antimicrobial therapy.
- Results must be interpreted in conjunction with other clinical data available to the clinician.
- A negative result does not preclude a possible infection because results are dependent on adequate specimen collection. Test results may be affected by improper specimen collection, technical error, specimen mix-up, or target levels below the assay limit of detection.

VIII(G). Specimen Collection

Your must state how specimens are to be collected, stored, and transported, and that inadequate or inappropriate specimen collection, storage, number of freeze/thaw cycles, and transport are likely to yield false negative test results.

VIII(H). Interpretation of Test Results

The interpretation of test results section in the package insert must list all possible assay outputs and determinations of the presence or absence of *T. vaginalis* nucleic acid and the expected result of the assay controls. If internal controls are part of the determination of valid positive and negative results, you must provide the interpretation of each possible control result and a recommendation for how to follow up any invalid or indeterminate result.

If your assay has an equivocal zone, you must provide the interpretation and the recommendation for how to follow up the equivocal result. (e.g., whether the equivocal result must be reported as such, or whether testing must be repeated). If your interpretation of the results requires repeat testing of an invalid or equivocal result, you must provide the recommendation whether testing must be repeated and how repeat testing must be performed (e.g., on the same or a different specimen from the same patient).

Depending on test performance or other device-specific factors, additional qualification may be necessary.

VIII(I). Expected Values

This section must provide information about the expected results for each specimen type and for each collection site. You must summarize the information about the number of samples, age, sex, and demographics of the population used to determine the expected values. Additionally, you must calculate PPV and NPV for different hypothetical

prevalence rates using the sensitivity and specificity estimates per specimen type from the clinical performance study.

VIII(J). Performance Characteristics

Your labeling must include a summary of the study designs and study results described in Sections VI and VII of this document that would aid the user in interpreting test results and understanding device performance. This section must include descriptions of both clinical and analytical study results.

IX. References

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2. Clinical and Laboratory Standards Institute. 2005. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Third Edition. CLSI document M29-A3. Clinical and Laboratory Standards Institute Wayne, PA.

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4. Clinical and Laboratory Standards Institute. 2005. Interference Testing in Clinical Chemistry; Approved Guideline. EP07-A2. Clinical and Laboratory Standards Institute, Wayne PA.

5. Clinical and Laboratory Standards Institute. 2012. Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; Second Edition. EP24-A2. Clinical and Laboratory Standards Institute, Wayne PA.

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9. 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.

10. Haeckel R. (1991) Proposals for the description and measurement of carry-over effects in clinical chemistry. Pure Appl. Chem. 63:302-306.