

# Draft Guidance for Industry and Food and Drug Administration Staff

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## Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for *Chlamydia trachomatis* and/or *Neisseria gonorrhoea*: Screening and Diagnostic Testing

### *DRAFT GUIDANCE*

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Center for Devices and Radiological Health  
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Division of Microbiology Devices

## **Preface**

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*This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic.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 number listed on the title page of this guidance.*

### I. Introduction

FDA is issuing this draft guidance to provide industry and agency staff with recommendations for studies to establish the analytical and clinical performance of *in vitro* diagnostic devices (IVDs) intended for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* screening and diagnostic testing using nucleic acid based assays. These devices are used to aid in the diagnosis of urogenital *C. trachomatis* and/or *N. gonorrhoeae* infection. They include devices that detect one specific organism, as well as devices that may detect both organisms with or without further differentiation.

This guidance provides detailed information on the types of studies FDA recommends to support Class I and Class II premarket submissions for these devices. The guidance includes a list of *C. trachomatis* and *N. gonorrhoeae* strains recommended for analytical sensitivity studies and a list of microorganisms recommended for analytical specificity studies. This document also addresses recommendations for fulfilling labeling requirements applicable to all *in vitro* diagnostic devices intended to screen for, or aid in the diagnosis of *C. trachomatis* and/or *N. gonorrhoeae* directly from human specimens.

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This document is limited to studies intended to establish the performance characteristics of devices that detect chlamydial and/or gonococcal nucleic acid. It does not address detection of serological response from the host to bacterial antigens, nor does it address establishing performance of non-chlamydial or non-gonococcal components of multi-analyte or multiplex devices.

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.

## **II. Background**

This document recommends studies for establishing the performance characteristics of *in vitro* diagnostic devices for *C. trachomatis* and/or *N. gonorrhoeae* screening and diagnostic testing from human specimens. FDA believes that these recommended studies will be relevant for Class I and Class II premarket submissions (e.g., 510(k) or de novo classification petition) that may be required for a particular test.

A manufacturer who intends to market an *in vitro* diagnostic device for *C. trachomatis* and/or *N. gonorrhoeae* screening and diagnostic testing must conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) and obtain premarket clearance or approval prior to marketing the device (sections 510(k), 513, 515 of the Act; 21 U.S.C. 360(k), 360c, 360e)

This document is intended to supplement 21 CFR 807.87 (information required in a premarket notification) and other FDA resources such as CDRH's "Device Advice" webpage on "Premarket Notification 510(k)," <http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/default.htm>. Guidance on the content and format for abbreviated and traditional 510(k) s is available in the guidance entitled "Format for Traditional and Abbreviated 510(k)s" found at: <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084396.pdf>.

Further information on device testing can be found in the guidance entitled "In Vitro Diagnostic (IVD) Device Studies – Frequently Asked Questions" at <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071230.pdf>, and the guidance entitled "Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" at <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071265.pdf>.

### **III. Scope**

As previously described, this document recommends studies for establishing the performance characteristics of *in vitro* diagnostic devices for *C. trachomatis* and/or *N. gonorrhoeae* screening and diagnostic testing in human specimens. This document is limited to studies intended to establish the performance characteristics of devices that detect chlamydial and/or gonococcal nucleic acids in the urogenital tract (i.e., endocervical and vaginal swabs from women, urethral swabs from men, and urine from both men and women). This guidance does not address detection of serological response from the host to the bacterial antigen. Nor does it address establishing performance of non-chlamydial or non-gonococcal components of multi-analyte or multiplex devices.

The recommendations in this document apply to devices classified by the regulations indicated below. Note that the devices expressly covered by this guidance are *in vitro* diagnostic devices (IVDs) intended for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* screening and diagnostic testing using nucleic acid based assays. They are listed under classifications for serological reagents even though they are not serological reagents.

The classification regulation for *Chlamydia trachomatis* IVD reads:

#### **Sec. 866.3120 Chlamydia serological reagents.**

a) Identification.

Chlamydia serological reagents are devices that consist of antigens and antisera used in serological tests to identify antibodies to chlamydia in serum. Additionally, some of these reagents consist of chlamydia antisera conjugated with a fluorescent dye used to identify chlamydia directly from clinical specimens or cultured isolates derived from clinical specimens. The identification aids in the diagnosis of disease caused by bacteria belonging to the genus *Chlamydia* and provides epidemiological information on these diseases. Chlamydia are the causative agents of psittacosis (a form of pneumonia), lymphogranuloma venereum (a venereal disease), and trachoma (a chronic disease of the eye and eyelid).

b) Classification. Class I (general controls).

The devices cleared under 21 CFR 866.3120 that are covered by this guidance have the product codes:

MGM – *C. trachomatis* (Chlamydia group)  
MKZ – DNA probe, nucleic acid amplification, Chlamydia  
LSK – DNA reagents, Chlamydia

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Although devices classified in 21 CFR 866.3120 are Class I devices, which are generally exempt from premarket notification, a premarket notification may be required for some tests purported to fall within this type of device to the extent the device meets the limitations on exemption defined in 21 CFR 866.9:

Under 21 CFR 866.9(c)(6), an IVD that is intended for use in *identifying or inferring the identity of a microorganism directly from clinical material* is not exempt from premarket notification requirements. An IVD that is intended to detect *C. trachomatis* and/or *Neisseria* spp. directly from a human specimen falls within this provision.

In addition, an IVD to detect *C. trachomatis* and/or *Neisseria* spp. may trip the limitations in 21 CFR 866.9(a) if the new device is *intended for a use different from the intended use of a legally marketed device* classified under 21 CFR 866.3120; or may trip the limitations in 21 CFR 866.9(b), if it *operates using a different fundamental scientific technology* from existing tests in that classification.

The classification regulation for *Neisseria gonorrhoea* IVD reads:

**Sec. 866.3390 *Neisseria* spp. direct serological test reagents.**

(a) Identification. *Neisseria* spp. direct serological test reagents are devices that consist of antigens and antisera used in serological tests to identify *Neisseria* spp. from cultured isolates. Additionally, some of these reagents consist of *Neisseria* spp. antisera conjugated with a fluorescent dye (immunofluorescent reagents) which may be used to detect the presence of *Neisseria* spp. directly from clinical specimens. The identification aids in the diagnosis of disease caused by bacteria belonging to the genus *Neisseria*, such as epidemic cerebrospinal meningitis, meningococcal disease, and gonorrhea, and also provides epidemiological information on diseases caused by these microorganisms. The device does not include products for the detection of gonorrhea in humans by indirect methods, such as detection of antibodies or of oxidase produced by gonococcal organisms.

(b) *Classification*. Class II (performance standards)

The devices cleared under 21 CFR 866.3390 that are covered by this guidance have the product code :

LSL DNA- Reagents, *Neisseria gonorrhoea*

The recommendations contained in this guidance reflect the agency' current thinking concerning the devices described above; however, these recommendations may also apply to future *C. trachomatis* and/or *N. gonorrhoeae* diagnostic devices that fall under other classification regulations. For example, this guidance may apply to *in vitro* diagnostic devices for *chlamydia trachomatis* and/or *neisseria gonorrhoea* initially classified under section 513(f)(2) of the act ("*de novo* classification"), and subsequent devices that seek determinations of substantial equivalence to *de novo* cleared devices.

## IV. Risks to Health

*Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted infection in the United States. Several important sequelae can result from untreated *C. trachomatis* infection in women; the most serious of which include pelvic inflammatory disease (PID), ectopic pregnancy, infertility, and chronic pelvic pain. *Chlamydia trachomatis* infection during pregnancy leads to infant conjunctivitis and pneumonia and maternal postpartum endometritis. Since the majority of *C. trachomatis* infections are subclinical, annual screening of all sexually active women under the age of 26 has been recommended by the Centers for Disease Control and Prevention (CDC). (Ref. 1)

Failure of devices for detection of *C. trachomatis* to perform as expected or failure to correctly interpret results may lead to incorrect patient management decisions and inappropriate public health responses. In the context of individual patient management, a false negative report could lead to delays in providing (or failure to provide) definitive diagnosis and appropriate treatment and infection control and prevention measures. A false positive report could lead to unnecessary or inappropriate treatment or unnecessary control and prevention actions. Therefore, establishing the performance of these devices and understanding the risks that might be associated with the use of these devices is critical to their safe and effective use. As of December 2000, *C. trachomatis* cases are supposed to be reported to the CDC. *C. trachomatis* infections are the most commonly reported notifiable disease in the United States.

*Neisseria gonorrhoeae* is a very common infectious agent of the urogenital tract (i.e. endocervical and vaginal swabs from women, urethral swabs from men, and urine from both men and women). Gonorrhea is the second most commonly reported notifiable disease in the United States as of 2008. (Ref. 2). CDC estimates that more than 700,000 persons in the U.S. get new gonorrheal infections each year. It is estimated that only about half of these infections are reported to CDC. (Ref. 2).

Untreated gonorrhea can cause serious and permanent health problems in both women and men. In women, *N. gonorrhoeae* infection is often asymptomatic, and if untreated, can lead to PID, chronic pelvic pain, tubal infertility, and ectopic pregnancy, which is life-threatening. *N. gonorrhoeae* neonatal infection can cause severe conjunctivitis, which can result in blindness if left untreated, as well as sepsis with associated meningitis, endocarditis, or arthritis.

In men, gonorrhea usually causes symptomatic urethritis and occasionally results in epididymitis, which may lead to infertility if left untreated.

*N. gonorrhoeae* can also disseminate, causing an acute dermatitis tenosynovitis syndrome, which may be complicated by arthritis, meningitis, or endocarditis. If left untreated, disseminated *N. gonorrhoeae* can be life threatening.

## V. Establishing Performance Characteristics

### A. Controls

When conducting the performance studies described below we recommend that you run appropriate external controls every day of testing for the duration of the analytical and clinical studies. Examples of appropriate external controls include strains of *C. trachomatis* commonly found in the United States population such as strain D or H which have been recently grown and titered. An *N. gonorrhoeae* reference strain such as ATCC 49226 is an appropriate external control for testing. Alternatively, characterized positive patient samples may be utilized. You may contact the Division of Microbiology Devices within the Office of *In Vitro* Diagnostic Device Evaluation and Safety (OIVD) at FDA for further information regarding controls.

### B. Performance Studies

The following studies are recommended to support a submission:

#### **1a. Analytical Sensitivity for Known *C. trachomatis* Strains**

##### *Limit of Detection*

1. We recommend that you determine the limit of detection (LoD) using limiting dilutions of regrown and retitered *C. trachomatis* stocks. The study should include serial dilutions of at least two strains representative of types commonly found in the United States (please see Table 1 for suggested *C. trachomatis* serovars) using at least 20 measurements for each dilution. This study should include the following: sources of variability at different days (at least 3-5 days), different runs (at least 2 runs per day), and different replicates (at least 2 replicates per run). We suggest that you refer to the Clinical Laboratory Standards Institute (CLSI) document EP17-A [Ref. 4] for the basic concepts, design and statistical analysis of your LoD studies. You may also use the approach described in CLSI EP17-A, and by Linnet and Kondratovich [Ref.5] to estimate the LoD using the standard deviations of samples with very low concentrations. Alternatively, the LoD can be estimated using hit rates (percent of detected) using Probit analysis as long as the determined hit rates cover a larger part of the range of detection 90%-100%]. You should report the LoD as the level of elementary bodies (EB) or inclusion forming units (IFU) per ml that gives a 95% detection rate. The LoD should be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the *C. trachomatis* strains were detected 95% of the time.
2. The LoD for the remaining *C. trachomatis* serovars should be determined using the following procedure. Each of the remaining serovars should be diluted to

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approximately the LoD determined above. Twenty (20) measurements of the target dilutions should be used to confirm the LoD.

We recommend that you determine the LoD for each specimen matrix (e.g., urine, swabs tested by the device).

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**Table 1. Chlamydia Serovars Recommended for Analytical Sensitivity (LoD) Studies.**

Chlamydia Serovars	Comments
A	Ocular isolates from patients with clinical trachoma from regions where trachoma is endemic. These strains lead to blinding Trachoma, a chronic follicular conjunctivitis that leads to scarring in the conjunctiva and cornea.
B	
Ba	
C	
D	Genital tract disease. The commonest causes of urethritis and mucopurulent cervicitis in females and nongonococcal urethritis in males.
E	
F	
G	
H	
I	
J	
K	
L1, L2, L2a, L3	Rare, sexually transmitted biovars, although the eye may also act as the portal of entry. Infection is associated with a suppurative adenitis, usually of the inguinal or perirectal nodes, as well as systemic symptoms. The disease is most commonly seen in tropical and subtropical areas

**1b. Analytical Sensitivity for *N. gonorrhoea* Strains**

*Limit of Detection*

1. We recommend that you determine the limit of detection (LoD) using limiting dilutions of regrown and reitersed stocks of an *N. gonorrhoeae* reference strain such as ATCC 49226. The study should include serial dilutions of two reference strains using at least 20 measurements for each dilution. The study should include the following: sources of variability at different days (at least 3-5 days), different runs (at least 2 runs per day), and different replicates (at least 2 replicates per run). We suggest that you refer to the Clinical Laboratory Standards Institute (CLSI) document EP17-A (Ref. 3) for the basic concepts, design, and statistical analysis of your LoD studies. You may use the approach described in CLSI EP17-A, and by Linnet and Kondratovich (Ref. 4) to estimate the LoD using the standard deviation of samples with very low concentrations. Alternatively, the LoD can be estimated using hit rates (percent of bacteria per ml detected) using Probit analysis as long as the determined hit rates cover a large part of the range of detection (0%-100%). You should report the LoD as the level of bacterial colonies or cells per ml that give a 95% detection rate. The LoD should be confirmed by preparing at least 20 additional replicates at the LoD concentration and demonstrating that the *N. gonorrhoeae* strain was detected 95% of the time.

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2. The LoD for an additional 30 to 50 clinical isolates should be determined using the following procedure. Each of the clinical isolates should be diluted to approximately the LoD determined above. Twenty measurements of the target dilutions should be used to confirm the LoD.

We recommend that you determine the LoD for each specimen matrix type (e.g., urine, swab tested by the device).

## 2. Analytical Specificity for *C. trachomatis* and/or *N. gonorrhoea* Assays

### *Cross-reactivity*

We recommend that you test for potential cross-reactivity with non-chlamydial and non-gonococcal pathogens and other microorganisms that frequently colonize and/or infect the genital tract. We recommend that you test medically relevant levels of viruses and bacteria (usually 10<sup>6</sup> cfu/ml or higher for bacteria and 10<sup>5</sup> pfu/ml or higher for viruses). We recommend that you confirm virus and bacteria identities and titers prior to use in the study. The microorganisms recommended for cross-reactivity studies are listed in Table 2.

**Table 2. Microorganisms Recommended for Analytical Specificity (cross-reactivity) Studies.**

Organism	Organism	Organism
<i>Achromobacter xerosis</i>	<i>Escherichia coli</i>	<i>Neisseria mucosa</i> (3)
<i>Acinetobacter calcoaceticus</i>	<i>Flavobacterium meningosepticum</i>	<i>Neisseria sicca</i> (3)
<i>Acinetobacter Iwoffii</i>	<i>Fusobacterium nucleatum</i>	<i>Neisseria subflava</i> (14)
<i>Actinomyces israelii</i>	<i>Gardnerella vaginalis</i>	<i>Neisseria perflava</i>
<i>Actinomyces pyogenes</i>	<i>Gemella haemolysans</i>	<i>Neisseria polysaccharea</i>
<i>Aerococcus viridans</i>	<i>Haemophilus ducreyi</i>	<i>Paracoccus denitrificans</i>
<i>Aeromonas hydrophila</i>	<i>Haemophilus influenzae</i>	<i>Peptostreptococcus anaerobius</i>
<i>Agrobacterium radiobacter</i>	Herpes simplex virus I	<i>Peptostreptococcus productus</i>
<i>Alcaligenes faecalis</i>	Herpes simplex virus II	<i>Plesiomonas shigelloides</i>
<i>Bacillus subtilis</i>	Human papilloma virus 16	<i>Propionibacterium acnes</i>
<i>Bacteriodes fragilis</i>	<i>Kingella dentrificans</i>	<i>Proteus mirabilis</i>
<i>Bacteriodes ureolyticus</i>	<i>Kingella kingae</i>	<i>Proteus vulgaris</i>
<i>Bifidobacterium adolescentis</i>	<i>Klebsiella oxytoca</i>	<i>Providencia stuartii</i>
<i>Bifidobacterium brevis</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>
<i>Branhamella catarrhalis</i>	<i>Lactobacillus acidophilus</i>	<i>Pseudomonas fluorescens</i>
<i>Brevibacterium linens</i>	<i>Lactobacillus brevis</i>	<i>Pseudomonas putida</i>
<i>Campylobacter jejuni</i>	<i>Lactobacillus jensonii</i>	<i>Rahnella aquatilis</i>
<i>Candida albicans</i>	<i>Lactobacillus lactis</i>	<i>Rhodospirillum rubrum</i>
<i>Candida glabrata</i>	<i>Legionella pneumophila</i> (2)	<i>Saccharomyces cerevisiae</i>
<i>Candida parapsilosis</i>	<i>Leuconostoc paramensenteroides</i>	<i>Salmonella minnesota</i>
<i>Candida tropicalis</i>	<i>Listeria monocytogenes</i>	<i>Salmonella typhimurium</i>
<i>Chlamydia pneumoniae</i>	<i>Micrococcus luteus</i>	<i>Serratia marcescens</i>

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<i>Chlamydia psittaci</i> (2)	<i>Moraxella lacunata</i>	<i>Staphylococcus saprophyticus</i>
<i>Chromobacterium violaceum</i>	<i>Moraxella osloensis</i>	<i>Staphylococcus aureus</i>
<i>Citrobacter freundii</i>	<i>Morganella morganii</i>	<i>Staphylococcus epidermidis</i>
<i>Clostridium perfringens</i>	<i>Mycobacterium smegmatis</i>	<i>Streptococcus agalactiae</i>
<i>Corynebacterium genitalium</i>	<i>Mycoplasma genitalium</i>	<i>Streptococcus bovis</i>
<i>Corynebacterium xerosis</i>	<i>Mycoplasma hominis</i>	<i>Streptococcus mitis</i>
<i>Cryptococcus neoformans</i>	<i>N. meningitidis</i> Serogroup A	<i>Streptococcus mutans</i>
<i>Cytomegalovirus</i>	<i>N. meningitidis</i> Serogroup B	<i>Streptococcus pneumoniae</i>
<i>Deinococcus radiodurans</i>	<i>N. meningitidis</i> Serogroup C (4)	<i>Streptococcus pyogenes</i>
<i>Derxia gummosa</i>	<i>N. meningitidis</i> Serogroup D	<i>Streptococcus salivarius</i>
<i>Eikenella corrodens</i>	<i>N. meningitidis</i> Serogroup Y	<i>Streptococcus sanguis</i>
<i>Enterobacter aerogenes</i>	<i>N. meningitidis</i> Serogroup W135	<i>Streptomyces griseinus</i>
<i>Enterobacter cloacae</i>	<i>Neisseria cinerea</i> (4)	<i>Trichomonas vaginalis</i>
<i>Enterococcus avium</i>	<i>Neisseria dentrificans</i>	<i>Ureaplasma urealyticum</i>
<i>Enterococcus faecalis</i>	<i>Neisseria elongata</i> (3)	<i>Vibrio parahaemolyticus</i>
<i>Enterococcus faecium</i>	<i>Neisseria flava</i>	<i>Yersinia enterocolitica</i>
<i>Erwinia herbicola</i>	<i>Neisseria flavescens</i> (2)	
<i>Erysipelothrix rhusiopathiae</i>	<i>Neisseria lactamica</i> (9)	

**Interference**

We recommend that you conduct a comprehensive interference study using medically relevant concentrations of the interferent and at least two strains of *C. trachomatis* and *N. gonorrhoeae* to assess the potentially inhibitory effects of substances encountered in swab and/or urine specimens. See Table 3. Substances should be diluted into a swab and/or urine matrix containing *C. trachomatis* or *N. gonorrhoeae* levels near the LoD. We also recommend that you evaluate each interfering substance at its potentially highest concentration (“the worst case”). If no significant clinical effect is observed, no further testing is necessary. You should refer to the CLSI document EP7-A2 (Ref. 5) for additional information.

**Table 3. Substances Recommended for Interference Studies**

<b>Swab</b>	<b>Urine</b>
Blood ( $\leq 60\%$ )	Blood ( $\leq 1\%$ )
Seminal Fluid	Seminal fluid
Mucus	Mucus
Over-the-counter vaginal products and contraceptives	Antibiotics
Hemorrhoidal cream	Analgesics
Prescription vaginal treatments	Over The Counter deodorant sprays and powders
Leukocytes ( $1 \times 10^6$ cells/mL)	Hormones
$1 \times 10^6$ EB/mL <i>Chlamydia trachomatis</i>	Leukocytes
Intravaginal hormones	Albumin $< 1$ mg/mL

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Blood (> 60%)	Glucose Acidic urine (pH 4.0) Alkaline urine (pH 9.0) Bilirubin 1x10 <sup>6</sup> EB/mL <i>Chlamydia trachomatis</i> Organisms associated with Urinary Tract Infections Not applicable
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### **3. Precision (for both *C. trachomatis* and *N. gonorrhoeae*)**

#### *Within-Laboratory Precision/Repeatability*

We recommend that you conduct within-laboratory precision studies for devices that include instruments or automated components. You may perform these studies in-house (i.e., within your own company).

We recommend that you test sources of variability (such as operators, days, runs, etc.) over 12 days (preferably not consecutive), with 2 runs per day per operator, and 2-3 replicates of each sample per run. You can also include different lots or consider a separate precision study for evaluation of lot-to-lot precision.

The test panel should consist of 3-6 samples (for each of the *C. trachomatis* and/or *N. gonorrhoeae* strains) at three levels of *C. trachomatis* and/or *N. gonorrhoeae* load that include:

- A “negative” sample (truly negative, zero concentration of analyte): a sample with an analyte concentration such that results of repeated tests of this sample are negative almost 100% of the time.
- A “high negative” sample (C<sub>5</sub> concentration): a sample with an analyte concentration below the cut-off such that results of repeated tests of this sample are negative approximately 95% of the time (and results are positive approximately 5% of the time).
- A “low positive” sample (C<sub>95</sub> concentration): a sample with a concentration of analyte just above the cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A “moderate positive” sample: a sample with a concentration of analyte such that one can anticipate positive results approximately 100% of the time (e.g., approximately two to three times the concentration of the cut-off).
- A “high positive” sample: a sample with concentration of analyte close to the upper limit of the range of the assay
- Controls samples should also be included in the precision study.

When the limit of blank (LoB) is used as a cutoff, then the concentration C<sub>95</sub> is the same as the limit of detection (LoD) and the zero concentration (no analyte present in sample) is C<sub>5</sub> (see CLSI EP17-A). CLSI documents EP5-A2 (Ref. 6) and EP12-A2 (Ref. 7) contain further information about designing and performing precision studies.

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For an ultrasensitive test for which the clinical cut-off has not been established based on the truly negative samples (zero concentration), it may be difficult to obtain  $C_5$  as truly negative samples almost always have negative results (type I error is close to zero). If the percent of subjects from the intended use population who have Positive Patient Infected Status (PIS) and test results less than LoD is less than 10% of all patients with Positive PIS, then concentration  $C_5$  does not need to be included in the precision study for these ultrasensitive tests.

If the percent of subjects from the intended use population who have Positive PIS and test results less than LoD is greater than or equal to 10% of all patients with Positive PIS, then the following additional concentration levels should be tested in the precision/reproducibility study in place of the  $C_5$  concentration recommended above:

- A “negative” sample: a sample with an analyte concentration such that results of repeated tests of this sample are negative almost 100% of the time.
- A sample from the range ( $C_{20}$  to  $C_{80}$ ): a sample with a concentration of analyte such that results of repeated tests of this sample are positive approximately 20% to 80% of the time.

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

*Lot-to-Lot Precision*

You should also provide an evaluation of lot-to-lot precision using different reagent and calibrators lots (at least 3 reagent lots). You may conduct either a separate precision study for estimation of lot-to-lot precision or include this source of variation in either a within-laboratory precision study or a reproducibility study.

*Reproducibility*

The protocol for the reproducibility study may vary slightly depending on the assay format. As a general guide, we recommend the following protocol:

- Evaluate the reproducibility of your test at 3 testing sites (for example, two external sites and one in-house site).
- Use a five day testing protocol, including a minimum of two runs per day per operator, (unless the assay design precludes multiple runs per day) and three replicates of each panel member per run.
- Each day, have at least 1 operator at each facility perform the test.
- Use the same sample panel as described in the within-laboratory precision study above.

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For each panel member in the reproducibility study, we recommend you present the mean value with components of variance (standard deviation and percent CV), for each site separately and for all sites combined, as repeatability (within-run), between-run, between-day, between-operator, etc.. Information about total precision (which components of variance were included) should be also provided. In addition, you should include the percent of values above and below the cutoff for each site separately and for all sites combined. For details of appropriate statistical analysis of the precision data, you should follow the CLSI document EP5-A2.

*Additional Reproducibility for Combination C. trachomatis and N. gonorrhoeae Assays*

Reproducibility of the system should be evaluated at three clinical sites on one system per site. A panel of specimens composed of *C. trachomatis* and *N. gonorrhoeae* organisms seeded into the appropriate matrix, e.g., swab diluent, negative urine, should be prepared using combinations of negative, low, medium, and high analyte concentrations. Using 3 replicates per sample the assay should be run for 5 days, using 2 operators per day each completing 2 runs per day. In a request by industry, we were asked how they might provide this information. Table 4 is a format that you can use as an aide.

**Table 4. Combination *C. trachomatis* and *N. gonorrhoeae* Reproducibility Study**

Panel Member Concentration						
Panel Member <i>C. trachomatis</i> and <i>N. gonorrhoeae</i>	<i>C. trachomatis</i> EB/ml*	<i>N. gonorrhoeae</i> Cells/ml**	% Correct for Site 1	% Correct for Site 2	% Correct for Site 3	% Correct for 3 Sites Combined
Neg/Neg						
Neg/High						
Neg/Medium						
Neg/Low						
Neg/LoD						
High/Neg						
Medium/Neg						
Low/Neg						
LoD/Neg						
High/High						
High/Low						
Low/High						
Low/Low						

\*EB/ml or IFU/ml      \*\*Cells/ml or CFU/ml

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In addition, present mean value with variance components (standard deviation and percent CV) for each panel member *C. trachomatis* and *N. gonorrhoeae* for each site separately and for all sites combined.

#### **4. Specimen Storage and Shipping Conditions**

If you recommend specimen storage conditions, you should demonstrate that your device generates equivalent results for the stored specimens at several time points throughout the duration of the recommended storage and at both ends of your recommended temperature range. If a transport medium (TM) is recommended for storage or shipping, you should conduct appropriate studies to demonstrate that the device will perform as described when the specimen is preserved in TM. (Ref. 8)

#### **5. Clinical Performance Studies**

We recommend that you conduct prospective clinical studies to determine the performance of your device for all the specimen types you claim in your labeling. The assay's performance should be judged versus the Patient Infected Status (PIS). The PIS is determined using two FDA cleared nucleic acid amplification test (NAAT) assays detecting different target sequences to define whether a patient is infected or not.

The reasons for use of a PIS rather than a single assay or culture are multiple. Culture is not typically used for routine urethral and cervical/vaginal samples because of the cost of processing, the dearth of facilities equipped to perform the test, and the handling requirements of the samples. (Ref. 9). Perhaps most important is the low sensitivity of culture, generally around 60-85%. Additionally, it is known that in some infected women, *C. trachomatis* can be found only in the endocervix, while in others it can be detected only in the urine specimen. (Ref. 10) Therefore, exclusive use of multiple swab specimens or multiple urine specimens can significantly bias performance estimates of a new test. The use of two NAAT tests, each analyzing a swab and urine specimen, results in a more precise evaluation of clinical truth.

Optimal assay performance with male specimens is demonstrated when the result of a male urethral swab or a urine specimen from the new test are compared with the patient infected status generated by results from two different NAAT tests detecting different target sequences using a swab and urine specimen in each test. However, given the difficulty in obtaining multiple urethral swab specimens from males, the slightly lower sensitivity and specificity in performance characteristics using 1 urethral swab and 2 urine specimens is a reasonable alternative.

Frozen archived specimens may be useful for developing pre-clinical data but are not recommended for studies to calculate clinical sensitivity or specificity. Freeze-thawing can change the characteristics of specimens, such that they are no longer representative of the fresh specimens for which the test is intended to be used, possibly affecting assay performance.

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In general, when the number of specimens available for clinical testing is very low (e.g., newly emerging strains), the available evidence for FDA's premarket review may, of necessity, contain data from frozen in addition to fresh samples. In this circumstance, it is acceptable to use fresh and frozen samples.

Please consult the Division of Microbiology Devices prior to using frozen specimens in clinical studies supporting a 510(k) submission.

*Study Protocol*

We recommend that, in your premarket submission, you provide a detailed study protocol that takes into account variances to prevent data bias in its patient inclusion and exclusion criteria, sample type, directions for use, statistical analysis plan, and any other relevant components of a detailed study protocol.

We encourage sponsors to contact the Division of Microbiology Devices to request a review of their proposed studies and selection of specimen types. This is referred to as the pre-IDE process.

*Specimen Type(s)*

The specimen types recommended for females are endocervical or vaginal swabs (either clinician or self collected) or urine and liquid based Pap test samples. For males, the specimen types are urethral swab and urine samples. The total number of samples you should include in your study for substantiating a claim for detection of chlamydia and/or gonorrhoeae will depend on the prevalence of the bacterium and on assay performance. In general, a minimum of 100 positive samples from both symptomatic and asymptomatic patients and for each specimen type are sufficient for chlamydia assays while a lesser number may be generated for *N. gonorrhoeae* as the prevalence of gonococcal infection is lower.

*Order of Collection of Specimens*

In your clinical study, the same subject should be used for collection of multiple specimen types in order to establish the patient infected status and to obtain a claim for specific types of specimens for use on the device under investigation. Table 5 shows the suggested order of specimen collection if swab specimens are collected from a woman in the clinical study.

**Table 5. Specimen Collection Order for Female Swab Specimens**

Order of Specimen	Type	Purpose
1	swab	NAAT1
2	swab	NAAT2
3	swab	Device under investigation

Table 6 shows the suggested order of specimen collection if you are testing a specimen for a liquid based cytology test and swabs are collected from a female patient in the

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clinical study. If you are collecting a specimen for a liquid based cytology test and swabs are collected from a female patient, the following order of collection is recommended. The cytology sample from a patient to generate a cytology Pap test result should always be taken first, so that this result (and subsequently, the health of the patient) is not compromised:

**Table 6. Specimen Collection Order for Female Liquid Based Cytology Test and Swabs**

Order of Specimen	Type	Purpose
1	LB pap test	device under investigation
2	swab	NAAT1
3	swab	NAAT2

One urine specimen should be aliquoted into 3 samples: two aliquots for NAAT1 and NAAT2 testing and one aliquot for testing by the device under investigation.

*Cytology Sample Aliquoting*

Sponsors intending to make claims for *C. trachomatis* and *N. gonorrhoeae* testing from cytology samples should consider, when designing their studies, whether they should be testing from pre-aliquoted cytology samples (aliquots taken prior to slide processing) or working from residual cytology samples (aliquots taken after slide processing). Pre-aliquoting of cytology samples can only occur if the cytology collection system has been approved for aliquot removal prior to cytology slide processing. This will ensure that patient cytology test results are not compromised by off-label processing of their cytology specimens.

Alternatively, sponsors who work from residual cytology samples will need to analytically assess the effects of carryover during cytology slide processing (see Carry-over and Cross-contamination Studies in the Ancillary Studies Section).

If you have questions regarding the choice of appropriate specimen type(s) and numbers, please contact the Division of Microbiology Devices, OIVD.

We recommend that all chlamydia and gonorrhoeae detecting devices demonstrate a sensitivity and specificity with a point estimate of at least 95% and a lower bound of the 95% (two-sided) confidence interval exceeding 90%.

*Study Sites*

We recommend that you conduct your studies at a minimum of three separate testing facilities, one of which may be in-house. Clinical investigations of unapproved and uncleared *in vitro* diagnostic devices, including diagnostic devices for *C. trachomatis* and *N. gonorrhoeae* are subject to the investigational device exemption (IDE) provisions of Section 520(g) of the FD&C Act (21 U.S.C. 360j) and the implementing regulations. You should consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to 21 CFR part 50 (informed consent), and 21 CFR part 56 (institutional review board review) for other applicable requirements.

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*Study Population*

We recommend that you conduct your studies on individuals both symptomatic and asymptomatic for both *C. trachomatis* and *N. gonorrhoeae*. Current CDC *C. trachomatis* recommendations suggest annual testing for all sexually active women 26 years of age and younger. An annual screening test also is recommended for older women with risk factors for *C. trachomatis* (a new sex partner or multiple sex partners) and all pregnant women should have a screening test for *C. trachomatis*. The patient populations tested in the study should reflect these parameters. However, as these recommendations are subject to revisions, the most current guidelines should be consulted.

*Patient Infected Status*

We recommend that you compare results obtained with your device to the following PIS algorithm.

The current algorithm for demonstrating the clinical performance of any *C. trachomatis* assay uses two FDA cleared NAAT assays to determine the patient infected status. NAAT1 and NAAT2 should have different target regions. *N. gonorrhoeae* assays may use the same algorithm with or without the use of culture. In the case of a positive culture result, that result will supersede the NAAT findings.

For specimens from female (may be either endocervical or vaginal from the cleared assays) and male patients (swab and urine) the algorithm is as follows.

NAAT 1		NAAT 2	
Swab	Urine	Swab	Urine

One or more positives in each assay (target) designates the PIS as positive. Any other combination of results defines the PIS as negative. See Table 7 below:

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**Table 7. Patient Infected Status Possible Outcomes**

		NAAT 1 Swab	Pos	Pos	Pos	E	E	E	Neg	Neg	Neg
		NAAT 1 Urine	Pos	E	Neg	Pos	E	Neg	Pos	E	Neg
NAAT 2 Swab	NAAT 2 Urine										
Pos	Pos		I	I	I	I	NI	NI	I	NI	NI
Pos	E		I	I	I	I	NI	NI	I	NI	NI
Pos	Neg		I	I	I	I	NI	NI	I	NI	NI
E	Pos		I	I	I	I	NI	NI	I	NI	NI
E	E		NI	NI	NI	NI	NI	NI	NI	NI	NI
E	Neg		NI	NI	NI	NI	NI	NI	NI	NI	NI
Neg	Pos		I	I	I	I	NI	NI	I	NI	NI
Neg	E		NI	NI	NI	NI	NI	NI	NI	NI	NI
Neg	Neg		NI	NI	NI	NI	NI	NI	NI	NI	NI

Key: Pos = Positive result  
 Neg = Negative result  
 E = Equivocal result  
 I = Infected Patient Status  
 NI = Not Infected Patient Status

Alternative algorithms for male patients may be based on one swab and multiple urine samples as follows.

	NAAT 1		NAAT 2		NAAT3
	Swab	Urine	Urine		Urine

One or more positives in at least two assays (target) designates the PIS as positive. Any other combination of results defines the PIS as negative.

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*Clinical Performance Evaluation*

a) The clinical performance of a qualitative test with two outcomes (positive and negative) is described by its clinical sensitivity and specificity, and by its positive and negative predictive values along with the prevalence of the target condition in the intended use population. The device performance should be evaluated separately for male, female, symptomatic, asymptomatic, type of specimen, and site as well as pooled. In a request by industry, we were asked how they might provide this information. Table 8 is a format that you can use as an aide for displaying each subgroup.

**Table 8. Device Performance by Subgroup**

		PIS		Total
		Infected	Not Infected	
Device	Pos	A	B	A+B
	Neg	C	D	C+D
Total		A+C	B+D	N

The clinical performance of the device should be evaluated as follows:

Sensitivity =  $A/(A+C)$ ;

Specificity =  $D/(B+D)$

PPV =  $A/(A+B)$

NPV =  $D/(C+D)$

Prevalence =  $(A+C)/N$

If your assay has an equivocal result as an interpretation of assay results outcome you should provide information on how the cutoffs for the equivocal zone were established and the recommendation of how to follow up with the equivocal result, (e.g., whether the equivocal result should be reported as such, or whether repeat testing is necessary).

In a request by industry, we were asked how they might provide this information if the test has three outcomes (Positive, Equivocal, Negative). Table 9 is a format that you can use as an aide in that situation.

**Table 9. Report of Results**

		PIS		Total
		Infected	Not Infected	
Device	Pos	A	B	A+B
	Equivocal	C	D	C+D
	Neg	E	F	E+F

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Total	A+C+E	B+D+F	N
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The clinical performance of the device should be evaluated as follows:

Sensitivity =  $A/(A+C+E)$ ;

Specificity =  $F/(B+D+F)$

PPV =  $A/(A+B+D)$

NPV =  $F/(E+F+C)$

Prevalence =  $(A+C+E)/N$

You should provide percent of equivocal results in the clinical study as  $(C+D)/N$  along with the 95% confidence interval (provide also percents of equivocals for Infected and Not Infected separately).

The estimation of sensitivity and specificity should be provided along with 95% two-sided confidence intervals. For the 95% confidence intervals for sensitivity and specificity, a score method is recommended (for more details about score confidence intervals, see the Statistical Methods Appendix).

In addition, you should provide the percent of invalid (final, after re-testing if applicable) results along with 95% confidence interval.

c) You should calculate positive predictive value and negative predictive value for hypothetical prevalence for the test with estimates of sensitivity and specificity for all combined data of the clinical study for *C. trachomatis* and *N. gonorrhoeae* separately.

PPV is calculated as  $(\text{Sensitivity} \times \text{Prevalence}) / (\text{Sensitivity} \times \text{Prevalence} + \text{Specificity} \times (1 - \text{Prevalence}))$ .

NPV is calculated as  $(\text{Specificity} \times (1 - \text{Prevalence})) / (\text{Specificity} \times (1 - \text{Prevalence}) + (1 - \text{Sensitivity}) \times \text{Prevalence})$

In a request by industry, we were asked how they might provide this information. Table 10 is a format that you can use as an aide.

**Table 10. Calculation of Results**

Prevalence (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
2				
5				
10				
20				
30				

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Prevalence (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
40				
50				

d) You should provide frequency distributions of signals according to the patient infected status for each type of specimen with male and female results shown separately. You should present frequency distributions in graphic and tabular formats.

e) You should provide information about clinical performance of your test based on detailed information of the NAAT assays results used in establishing the patient infected status. See Table 11.

**Table 11. Clinical Performance**

PIS	NAAT1		NAAT2		Device Under Investigation			Symptomatic Status		total
	Swab	Urine	Swab	Urine	Endocervical swab	Urine	Vaginal swab	A	S	
	+	+	+	+	+	+	+	a <sub>1</sub>	b <sub>1</sub>	a <sub>1</sub> +b <sub>1</sub>
	+	+	+	+	+	+	-	a <sub>2</sub>	b <sub>2</sub>	a <sub>2</sub> +b <sub>2</sub>
	+	+	+	+	+	-	+	a <sub>3</sub>	b <sub>3</sub>	a <sub>3</sub> +b <sub>3</sub>
	+	+	+	+	-	+	+	a <sub>4</sub>	b <sub>4</sub>	a <sub>4</sub> +b <sub>4</sub>
	+	+	+	+	+	-	-	a <sub>5</sub>	b <sub>5</sub>	a <sub>5</sub> +b <sub>5</sub>
	....	...	...	...	...	...	...	...	...	...

f) You should provide information about expected results for each collection site separately and for all sites combined. This information should include the expected values using your test for different types of specimens (In a request by industry, we were asked how they might provide this information. Table 12 is an example format for *C. trachomatis* that you can use as an aide.). You should also include information about the number of samples, age, gender, and demographics of the population used to determine the expected values.

**Table 12. Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by % (# positive/#tested)**

Site	Male Urethral	Male Urine	Endocervical Swab	Female Urine	Patient-Collected Vaginal	Clinician-Collected Vaginal	Male Urethral
------	---------------	------------	-------------------	--------------	---------------------------	-----------------------------	---------------

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	Swab				Swab	Swab	Swab
1							
2							
3							

g) Line data should also be included in the submission. In a request by industry, we were asked how they might provide this information. Table 13 is a format for female swabs that you can use as an aide.

**Table 13 Line Listing for Female Swabs**

		Pat. ID	Age	Gender	Collection Site	Date Collected	Test Site	Date Tested	NAAT1 Result <sup>1</sup>		NAAT2 Result <sup>1</sup>		Patient Infected Status	New Test Result
									Swab	Urine	Swab	Urine		
Female	Swab								P <sup>2</sup>	N <sup>3</sup>	N <sup>3</sup>	P <sup>2</sup>	I <sup>4</sup>	P <sup>2</sup> (Ct)

<sup>1</sup> The assay name can be used if desired

<sup>2</sup>P denotes a positive result

<sup>3</sup>N denotes a negative result

<sup>4</sup>I denotes an infected Patient Status

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### **C. CLIA Waiver**

If you are seeking waiver for your device under the Clinical Laboratory Improvement Amendments of 1988 (CLIA),<sup>1</sup> we recommend that you consult with the Division of Microbiology Devices staff regarding the design of specific studies to support the CLIA waiver application for your device. The draft guidance for industry and FDA staff, “Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications,” is available at <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm070890.pdf>.

### **D. Ancillary Studies**

This section complements the recommendations for performance studies described earlier in this document.

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<sup>1</sup> See 42 U.S.C. § 263a(d)(3).

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**1. Carry-Over and Cross-Contamination Studies (for multi-sample assays and devices that require instrumentation.)**

We recommend that you demonstrate that carry-over and cross-contamination do not occur with your device. In a carry-over and cross-contamination study, we recommend that high positive samples be used in series alternating with high negative samples in patterns dependent on the operational function of the device. At least 5 runs with alternating high positive and high negative samples should be performed. We recommend that the high positive samples in the study be high enough to exceed 95% or more of the results obtained from specimens of diseased patients in the intended use population. We recommend that the high negative samples contain the analyte concentration below the cut-off such that repeat testing of this sample is negative approximately 95% of the time. The carry-over and cross-contamination effect can then be estimated by the percent of negative results for the high negative sample in the carry-over study compared with 95%. For details, see reference 11.

**2. Controls for Nucleic Acid-based *C. trachomatis* and *N. gonorrhoeae* Assays**

We recommend that you use quality control material for verification of assay performance in analytical and clinical studies. We recommend that you consult with FDA when designing specific controls for your device. Devices based on nucleic acid technology should include the following types of controls:

*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 nucleic acid. These controls are 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 contains non-target nucleic acid or, if used to evaluate extraction procedures, it contains whole organism samples. It reveals non-specific priming or detection and indicates that signals are not obtained in the absence of target sequences. Examples of acceptable negative sample control materials include:

- Patient specimen from a non- *C. trachomatis* infected individual
- Samples containing a non-target organism (e.g., cell line infected with non- *C. trachomatis* organism)

*Positive Controls*

*Positive control for complete assay*

The positive control contains target nucleic acids, and is used to control the entire assay process, including DNA extraction, amplification, and detection. It is designed

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to mimic a patient specimen and is run as a separate assay concurrently with patient specimens, at a frequency determined by a laboratory’s Quality System (QS).  
Examples of acceptable positive assay control materials include:

- Patient specimens positive for *C. trachomatis*
- Pooled negative specimens spiked with whole *C. trachomatis* organisms

*Positive control for amplification/detection*

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

*Internal Control*

The internal control is a non-target nucleic acid sequence that is co-extracted and co-amplified with the target nucleic acid. It controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of inhibitors in the samples. Examples of acceptable internal control materials include human nucleic acid co-extracted with the *C. trachomatis* organism and primers amplifying human housekeeping genes (e.g., RNaseP,  $\beta$ -actin). The need for this control is determined on a device case-by-case basis [10].

A table of the distribution of device results for the assay Positive and Negative Controls in the clinical study for each specimen type should be presented. The following information about a distribution should be provided: total number, maximum, 95<sup>th</sup> percentile, median, mean, 5<sup>th</sup> percentile, and minimum. In a request by industry, we were asked how they might provide this information. Table 14 is a format that you can use as an aide.

**Table 14. Distribution of Assay Control Results**

	Statistic	Endocervical Swab	Vaginal Swab	Urine
Positive Control	n			
	Maximum			
	95 <sup>th</sup> Percentile			
	Median			
	Mean			
	5 <sup>th</sup> Percentile			
	Minimum			

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Negative Control	n			
	Maximum			
	95 <sup>th</sup> Percentile			
	Median			
	Mean			
	5 <sup>th</sup> Percentile			
	Minimum			

## **VI. Labeling**

*C. trachomatis* and *N. gonorrhoeae* assay systems, like other devices, are subject to statutory requirements for labeling (sections 502(a) and 201(n) of the FD&C Act (21 U.S.C. § 352(a) and 321(n))). These IVD devices must provide adequate directions for use and adequate warnings and precautions. (Section 502(f) of the FD&C Act (21 U.S.C. 352(f)).) Specific labeling requirements for all IVD devices are set forth in 21 CFR 809.10. See also 21 CFR § 801.119 (IVDs labeled in accordance with 21 CFR 809.10 are deemed to satisfy section 502(f)(1) of the FD&C Act (352 U.S.C. 352(f)(1)).)

Although final labeling is not required for 510(k) clearance, final labeling for *in vitro* diagnostic devices must comply with the requirements of 21 CFR section 809.10 before an *in vitro* diagnostic device is introduced into interstate commerce or you will be in violation of 301(a) of the FD&C Act.

To ensure compliance with section 502 of the FD&C Act and 21 CFR section 809.10, FDA recommends that labeling for these devices should include the items identified below. These labeling recommendations also help to mitigate the risks identified previously in this guidance to ensure safe and effective use of these devices.

Your labeling should clearly describe the identity, phylogenetic relationship, or other recognized characterization of Chlamydia that your device is designed to detect, and the associated clinical aspects of human infection.

### *Intended Use*

In addition to specific elements that describe the analyte detected, your intended use should specify indications for testing, specimen type, and whether swab collection is clinician or self collected. The intended use should specify that the device should be used in conjunction with other laboratory testing and clinical observations.

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*Device Description*

In the device description, you should briefly describe the test methodology used in this type of device. You should describe the target chromosomal sequences used in your NAAT assay.

*Procedure*

This section should include a general description of the entire analysis procedure, from physician sampling to result reporting.

*Directions for Use*

You should provide clear and concise instructions that delineate the procedures for using the device and types of controls that will minimize risks of inaccurate results. Instructions should encourage use of additional control measures and testing of external control materials to ensure use in a safe and effective manner.

If your reagents will not include reagents for extraction and preparation, you should recommend and list the nucleic acid extraction method(s) that demonstrated safety and effectiveness with your device. You should provide the name and catalogue number of each method you recommend.

For test systems that call for ancillary reagents of concern you:

- Should emphasize through conspicuous labeling that proper product performance requires use of specific ancillary reagents as directed. This labeling should include warnings against use of the test system if specified ancillary reagents are not available.
- Must identify which ancillary reagents are suitable for use with your test. 21 CFR 809.10(b)(8)(ii). For example, if only certain lots of a named ancillary reagent are appropriate for use, the labeling for your test system must identify those lots by number. 21 CFR 809.10(b)(8)(ii).

When your labeling calls for ancillary reagents that are supplied with instructions for use or other warnings or limitations by the ancillary reagent manufacturer, you should ensure that users of your test system will understand which instructions they should follow when using those ancillary reagents in your test system. If there is a conflict between the directions and warnings provided by the manufacturer of the ancillary reagent and the instructions for use that you supply with your test system, you should assess and address the risk that users may mistakenly follow the labeling provided directly with the ancillary reagent and consequently obtain invalid results with your test system. We note that in some circumstances, statements in the labeling of the test system may not be sufficient to address the risks created by this conflict.

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*Quality Control*

Quality control recommendations should include types of procedures and materials that can be used as additional quality control measures, and the expected results for acceptability of control testing.

If controls are included with your device, the specifications for control materials, including level of bacteria, source of that bacteria, method of inactivation and method for determining non-infectiousness should be provided, if applicable.

*Precautions, Warnings, and Limitations*

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

- *A trained health care professional should interpret assay results in conjunction with the patient’s medical history, clinical signs and symptoms, and the results of other diagnostic tests.*
- *Analyte targets (chlamydial sequences) may be present persistently in vivo, independent of bacteria viability. Detection of Chlamydial DNA does not imply that the Chlamydia organism is infectious.*
- *The detection of Chlamydial sequences is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.*
- *There is a risk of false positive values resulting from cross-contamination by target organism, its nucleic acids or amplified product, or from non-specific signals in the assay.*
- *There is a risk of false negative values due to the presence of Chlamydial sequence variants in the assay’s bacterial target.*
- *Assay performance was not established in immunocompromised patients or pregnant women.*
- *If positive or negative interference has been reported for any commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing, you should advise users of the possibility of false negative or false positive results due to such interference.*

*Specimen Collection*

We recommend that you state that inadequate or inappropriate specimen collection, storage, and transport are likely to yield false negative test results.

***Contains Nonbinding Recommendations***  
***Draft – Not for Implementation***

*Results*

We recommend that you incorporate into the Results section directions for reporting results that include statements such as the following, as applicable:

*Report negative test results as “CT nucleic acid not detected or NG nucleic acid not detected.*

*Report positive test results as “CT DNA (or RNA) detected or NG DNA detected. This result does not rule out co-infections with other pathogens.”*

*Interpretation of Results*

The interpretation of results section in the package insert should list all possible assay outputs and determinations of the presence or absence of *C. trachomatis* or *N. gonorrhoeae* and assay controls.

If internal controls are part of the determination of valid positive and negative results, you should provide the interpretation of each possible control result and a recommendation for how to follow up with any invalid or indeterminate result.

If your assay has an equivocal zone, you should provide the interpretation and the recommendation of how to follow up with the equivocal result, (e.g., whether the equivocal result should be reported as such, or whether repeat testing is necessary).

If the interpretation of results requires repeat testing of an invalid or equivocal result, you should provide the recommendation whether testing should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen.

If your assay performance (i.e., sensitivity) demonstrated a lower bound of the two-sided 95% CI less than 90%, negative results may need to be interpreted as presumptively prompting a recommendation for confirmation by an alternate method. You should append to the interpretation of results table information similar to that in the following footnotes. The CDC guidelines in the first footnote come from Reference 5.

<sup>1</sup>According to CDC guidelines, “consideration should be given to routine additional testing for persons with positive *C. trachomatis* or *N. gonorrhoeae* screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., <90%).” Refer to CDC guidelines for details on additional testing and patient management after a positive screening test.

<sup>2</sup>Refer to Table for signal distribution of results. The magnitude of the signal is not indicative of the level of organism in the specimen.

***Contains Nonbinding Recommendations***  
***Draft – Not for Implementation***

*Expected Values*

This section should include the expected values using your test and the explanation of the result. In a request by industry, we were asked how they might provide this information. Table 15 is an example format for *C. trachomatis* that you can use as an aide. It should also include the number of samples, age, gender, and demographics of the population used to determine the expected values (In a request by industry, we were asked how they might provide this information. Table 16 is an example format for *C. trachomatis* that you can use as an aide.).

**Table 15. *C. trachomatis* Hypothetical Positive and Negative Predictive Values**

<b>Prevalence (%)</b>	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>	<b>PPV (%)</b>	<b>NPV (%)</b>
2				
5				
10				
20				
30				
40				
50				

**Table 16. Prevalence of *C. trachomatis* by Clinical Site and Overall as Determined by % (# positive/#tested)**

<b>Site</b>	<b>Male Urethral Swab</b>	<b>Male Urine</b>	<b>Endocervical Swab</b>	<b>Female Urine</b>	<b>Patient-Collected Vaginal Swab</b>	<b>Clinician-Collected Vaginal Swab</b>	<b>Male Urethral Swab</b>
1							
2							
3							

***Contains Nonbinding Recommendations***  
***Draft – Not for Implementation***

*Performance Characteristics*

You should include in the package insert a summary of the study designs and the results that would aid users in interpreting test results. This includes clinical and analytical performance characteristics. Clinical performance characteristics typically comprise prospective clinical study results summarizing performance (sensitivity, specificity, 95% confidence intervals) by your assay. In cases where some retrospective clinical samples were also used, these results should be presented separately from the prospective clinical study results, as positive and negative agreement. Analytical performance characteristics contain descriptions of the results and methodology used for the studies outlined in Section V.

We recommend that the Performance Characteristics section describe the population(s) (i.e., geographical location, specimen types, and age groups) used to establish the performance characteristics of the device. You should also include:

- a table containing the Devices Ranges for False Negative, False Positive, True Negative, and True Positive Results;
- a Table of the Distribution of Device Results for the Assay Positive and Negative Controls
- an analysis of CT Positive/Negative Specimens from each subject by specimen type and whether symptomatic or asymptomatic based on patient infected status should be included; and
- for devices which generate a numerical value for results, a graph illustrating the frequency distribution of these numbers.

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## VII. References

1. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines 2006. MMWR 2006;55(No. RR-11).
2. Chlamydia and Gonorrhea — Two Most Commonly Reported Notifiable Infectious Diseases in the United States  
<http://www.cdc.gov/Features/dsSTDData/>
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9. Mahto M, Mallinson H. Sex Transm Infect. 2007 Jul;83(4):335-6
10. Martin, DH, et.al., (2004) J. Clin. Micro. 42 4749-4758.
11. Haeckel R. Proposals for the description and measurement of carry-over effects in clinical chemistry. *Pure Appl. Chem.* 1991; 63:302-306.

## Statistical Analyses Appendix

### Calculating Score Confidence Intervals for Percentages and Proportions

The following are additional recommendations for performing statistical analyses of percentages or proportions. There are several different methods available. We suggest that either a score method described by Altman, et al. (Altman D.A., Machin D., Bryant T.N., Gardner M.J. eds. *Statistics with Confidence*. 2<sup>nd</sup> ed. British Medical Journal; 2000) or a Clopper-Pearson Method (Clopper CJ, Pearson E. *Biometrika* 1934; 26:404-413) be used. The advantages with the score method are that it has better statistical properties and it can be calculated directly. Score confidence bounds tend to yield narrower confidence intervals than Clopper-Pearson confidence intervals, resulting in a larger lower confidence bound. Thus when n=100 samples and 96/100=96%, the score lower confidence bound is 90.2%. In contrast, the Clopper-Pearson lower confidence bound is 90.1%. In this document, we have illustrated the reporting of confidence intervals using the score approach. For convenience, we provide the formulas for the score confidence interval for a percentage.

A two-sided 95% score confidence interval for the proportion of A/B is calculated as:  $[100\%(Q_1 - Q_2)/Q_3, 100\%(Q_1 + Q_2)/Q_3]$ , where the quantities  $Q_1$ ,  $Q_2$ , and  $Q_3$  are computed from the data using the formulas below. For the proportion of A/B:

$$Q_1 = 2 \cdot A + 1.96^2 = 2 \cdot A + 3.84$$

$$Q_2 = 1.96 \sqrt{1.96^2 + 4 \cdot A \cdot (B - A) / B} = 1.96 \sqrt{3.84 + 4 \cdot A \cdot (B - A) / B}$$

$$Q_3 = 2 \cdot (B + 1.96^2) = 2 \cdot B + 7.68$$

In the formulas above, 1.96 is the quantile from the standard normal distribution that corresponds to 95% confidence.