EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Aptima Mycoplasma genitalium Assay

DECISION SUMMARY

A. DEN Number:

DEN180047

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Aptima Mycoplasma genitalium Assay.

C. Measurands:

Ribosomal RNA from *Mycoplasma genitalium*

D. Type of Test:

Nucleic acid amplification test (NAAT)

E. Applicant:

Hologic, Inc.

F. Proprietary and Established Names:

Aptima Mycoplasma genitalium Assay

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.3393

2. Classification:
   Class II (Special Controls)

3. Product code:
   QEP

4. Panel:
   83 - Microbiology

H. Indications For Use:

1. Indications for use:

   The Aptima Mycoplasma genitalium assay is an *in vitro* nucleic acid amplification test (NAAT) for the qualitative detection of ribosomal RNA (rRNA) from *Mycoplasma genitalium* on the fully automated Panther system. It is intended for use as an aid in the diagnosis of *M. genitalium* urogenital infections in male and female patients suspected of *M. genitalium* infection.

   The assay may be used to test the following specimens: clinician-collected and self-collected vaginal swabs (in a clinical setting), clinician-collected endocervical swabs, female and male urine, clinician-collected male urethral swabs, and self-collected penile meatal swabs (in a clinical setting).

   For females, a vaginal swab is the preferred specimen type due to higher clinical sensitivity for detecting *M. genitalium* than other specimen types; however, female urine or clinician-collected endocervical swabs may be used as alternative specimens when vaginal swab specimens are not available. If female urine or clinician-collected endocervical swab specimens test negative, testing with a vaginal swab may be indicated, if *M. genitalium* infection is suspected.

2. Special conditions for use statement(s):

   For *in vitro* diagnostic use

   For prescription use only

3. Special instrument requirements:

   The Aptima Mycoplasma genitalium Assay is performed on the Panther System.

I. Device Description:

The Aptima Mycoplasma genitalium Assay is a nucleic acid amplification test to qualitatively detect ribosomal RNA from *Mycoplasma genitalium*. The assay is performed using the Panther System and includes three main processing steps: target capture, transcription-mediated amplification (TMA), and detection by nucleic acid hybridization. The Aptima Mycoplasma genitalium Assay oligonucleotides are designed to specifically capture, amplify, and detect a highly conserved region of *Mycoplasma genitalium* and the internal control (IC).

The Aptima Mycoplasma genitalium Assay is provided as a 100-test kit. Following are the reagents and materials required to perform the assay on the Panther System:

Aptima Mycoplasma genitalium Assay Kit

- Amplification Reagent
- Enzyme Reagent
- Probe Reagent
- Internal Control Reagent

- Selection Reagent
- Target Capture Reagent
- Amplification Reconstitution Solution
- Enzyme Reconstitution Solution
- Probe Reconstitution Solution

Aptima Mycoplasma genitalium Calibrators Kit

- Negative Calibrator
- Positive Calibrator

Materials required but sold separately:

- Aptima Assay Fluids Kit
- Aptima Auto Detect Kit
- Aptima Multitest Swab Specimen Collection Kit
- Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- Aptima Urine Specimen Collection Kit

J. Standard/Guidance Document Referenced (if applicable):

- EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.
- FDA Guidance – Design Considerations for Pivotal Clinical Investigations for Medical Devices, 2013.

K. Test Principle:

The Aptima Mycoplasma genitalium Assay is comprised of three main processing steps that take place in a single tube on the Panther System: target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA).

A collected specimen is transferred into an appropriate specimen transport tube containing transport solution that helps release the rRNA target and protects it from degradation during storage. The target rRNA, if present, is isolated by the use of a specific capture oligomer and magnetic microparticles in a method called target capture. During this step, the sequence-specific region of the capture oligomer binds to a specific region of the target molecule. The resulting capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature, which allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles are then captured using magnets and are washed to remove residual specimen matrix that may contain amplification inhibitors. The target capture process isolates and purifies the rRNA from clinical specimens, which is then ready for amplification.

The TMA reaction amplifies a specific region of the small ribosomal subunit from *Mycoplasma genitalium* via DNA and RNA intermediates and generates multiple copies of

RNA amplicon.

Detection of the amplicon is achieved by HPA. During this step, a single-stranded chemiluminescent DNA probe labeled with an acridinium ester molecule combines with amplicon to form stable RNA:DNA hybrids. The light emitted from the labeled RNA:DNA hybrids is measured as photon signals called relative light units (RLU) in a luminometer. Assay results for each patient sample are determined based on the analyte signal-to-cutoff (S/CO) ratio.

Internal Control (IC) is added to each reaction via the Target Capture Reagent. The IC monitors nucleic acid capture, amplification, detection, and operator or instrument error.

L. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Within Laboratory Precision:
The within laboratory precision study was conducted with two Panther Systems, two operators, and three lots of reagents over 12 days. The precision panel was prepared using two specimen matrices, urine and simulated vaginal swab samples, and included *Mycoplasma genitalium* negative, low positive, and moderate positive samples. The low and moderate positive panel members were prepared by spiking *Mycoplasma genitalium* into two specimen matrices at 1.5X Limit of Detection (LoD) and 3X LoD, respectively. Two operators tested five replicates of each panel member. The variability between instruments, operators, reagent lots, days, and between and within runs is shown in Table 1 below. Percent coefficient of variation (%CV) was calculated for all replicates based on S/CO values. Percent agreement was calculated based on the expected result (detected or not detected) for each panel member. Testing of all precision panel members generated acceptable results.

Table 1: Precision of the Aptima Mycoplasma genitalium Assay

Panel	N	% Detected[1]	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Days		Between Runs		Within Runs		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Urine:UTM	240	100[2]	0.0	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
1.5X LoD Urine:UTM	240	100	24.7	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.81	0.47	1.88	2.08	8.43	2.14	8.68
3X LoD Urine:UTM	240	100	25.2	0.08	0.33	0.16	0.62	0.00	0.00	0.27	1.05	0.19	0.76	1.36	5.38	1.41	5.58
Negative SVM	240	100[2]	0.0	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
1.5X LoD SVM	240	98.3	23.7	0.00	0.00	0.00	0.00	0.36	1.51	0.13	0.54	0.00	0.00	3.83	16.13	3.84	16.21

3X LoD SVM	240	100	24.8	0.14	0.58	0.45	1.84	0.00	0.00	0.46	1.87	0.63	2.56	1.18	4.75	1.49	6.02

CV = coefficient of variation, LoD, limit of detection, S/CO = signal to cutoff, SD = standard deviation, SVM = simulated vaginal matrix, UTM = urine transport medium.

[1] Detected defined as S/CO > 1.0.

[2] 100% *Mycoplasma genitalium* negative.

Reproducibility:

The Aptima Mycoplasma genitalium Assay reproducibility was evaluated at three U.S. sites, by two operators at each site. Each operator performed one run per day over five days. Each run tested three replicates of each reproducibility panel member. Each testing site was provided with a 6-member reproducibility panel that included four panel members positive for *Mycoplasma genitalium* and two panel members negative for *Mycoplasma genitalium*. Positive panel members were created by spiking *Mycoplasma genitalium* into two different specimen matices: urine transport medium (UTM) and simulated vaginal swab matrix.

The variability between sites, between operators, between days, between runs, and within runs was calculated separately for each panel member and is presented in Table 2 below.

Table 2: Reproducibility of the Aptima Mycoplasma genitalium Assay

Panel	N	Mean S/CO	Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
UTM Negative	90	0.00	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
UTM Low Pos	90	24.64	0.45	1.82	0.00	0.00	0.43	1.74	0.43	1.74	2.38	9.67	2.59	10.51
UTM Mod Pos	90	25.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.20	4.71	1.41	5.51
SVM Negative	90	0.00*	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC	0.00	NC
SVM Low Pos	90	24.05	0.00	0.00	0.48	1.98	0.00	0.00	0.00	0.00	1.85	7.67	2.12	8.83
SVM Mod Pos	90	25.14	0.00	0.00	0.48	1.91	0.56	2.25	0.56	2.25	1.14	4.53	1.65	6.58

CV = coefficient of variation, Mod = moderate, NC = not calculable, Pos = positive, S/CO = signal to cutoff, SD = standard deviation, SVM = simulated vaginal matrix, UTM = urine transport medium.

Note: In case variability from some factors may be numerically negative, SD and CV are shown as 0.00.

*1.1% (1 out of 90) results had S/CO value of 0.03 and 98.9% (89 out of 90) results had S/CO value of 0.

Agreement with the expected result, along with the corresponding 2-sided 95% score confidence interval, was also calculated for each panel member; agreement with the expected results was 100% (95% CI: 95.9-100) for all panel members.

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Internal Control:
An internal control consisting of a non-infectious RNA transcript is added to each reaction via the Target Capture Reagent. The IC monitors nucleic acid capture, amplification, detection, and operator or instrument error. During sample processing, IC acceptance criteria are automatically verified by the Panther System software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result is retested.

External Controls:
External controls are not provided with the Aptima Mycoplasma genitalium Assay; however, testing of external positive and negative controls are recommended in the assay labeling. External controls should be tested in conformance with local, state, and/or federal regulations or accreditation requirements and each laboratory's standard quality control procedures.

Calibrators:
A positive and negative calibrator are run in duplicate each time a reagent kit is loaded on the Panther System. Assay calibration is required to generate valid results. The Aptima Mycoplasma genitalium Assay calibration is valid for up to 48 hours. Software on the Panther System alerts the operator when a new calibrator set should be run.

During sample processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther System. If two replicates are invalid for either the positive or negative calibrator, the software automatically invalidates the run. Samples in an invalidated run are retested using a freshly prepared set of calibrators.

Specimen Stability:
The specimen stability study indicated that it is acceptable to store swab and urine specimens at the following conditions prior to testing:

Swab specimens

- After collection, swab specimens in transport tubes can be stored at 2ºC to 30ºC for up to 60 days.
- If longer storage is needed, swab specimens in transport tubes can be stored at -20°C or -70°C for up to an additional 90 days.

Urine specimens

- After collection, urine specimens in the primary collection container can be stored at 2ºC to 30ºC for up to 24 hours before urine is transferred to the transport tube.
- Processed urine in the transport tube can be stored at 2ºC to 30ºC for up

to 30 days (after transfer).

- If longer storage is needed, processed urine in the transport tube can be stored at -20°C or -70°C for up to an additional 90 days (after transfer).

The specimen stability study evaluated vaginal swabs, penile meatal swabs, and male and female urine. For vaginal and penile meatal swabs, 20 pools of each of the swab types were spiked with *Mycoplasma genitalium* at 3X LoD and stored at the recommended storage conditions. For male and female urine, 20 pools of male urine and 20 pools of female urine, initially spiked with *Mycoplasma genitalium* at ~90 genome equivalents (GE)/mL, were diluted to < 2 GE/mL and tested at the recommended storage conditions. An aliquot from each pool was removed from the storage at specific time intervals and nine replicates of each pool (per temperature condition) were tested. An additional female urine stability study provided the support for longer storage, i.e., storage of female urine at -20°C or -70°C for up to an additional 90 days (following 31 days storage at 30°C). The concentration tested in this additional study was higher than the analytical LoD; however, the tested concentration is at the low range of the *Mycoplasma genitalium* concentration observed in the clinical samples.

d. *Detection limit:*

The Limit of Detection (LoD), is the concentration at which the Aptima Mycoplasma genitalium Assay has positive results at least 95% of the time. The LoD of the Aptima Mycoplasma genitalium Assay was determined in pooled male urine, female urine, vaginal swab, and penile meatal swab specimens. Dilutions of two strains of *Mycoplasma genitalium* were tested in the study with at least (b) (4) eplicates per concentration, per reagent lot, using two reagent lots for a tot f at least (b) (4) replicates per strain. Testing was performed on two Panther Systems. The results of the LoD study are summarized below in Table 3:

Table 3: Limit of Detection of the Aptima Mycoplasma genitalium Assay

Specimen Type	*Mycoplasma genitalium* LoD (GE/mL)	
	Strain 1	Strain 2
Vaginal Swab	0.04	0.10
Female Urine	0.04	0.12
Penile meatal swab	0.05	0.10
Male Urine	0.03	0.16

e. *Analytical Reactivity (Inclusivity):*

A study was performed to verify that the Aptima Mycoplasma genitalium Assay is able to detect various *Mycoplasma genitalium* strains. Nine *Mycoplasma genitalium* strains (b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4)(b) (4) were evaluated in male and female urine, vaginal swabs, and penile meatal swabs in triplicates. Seven of the nine strains were detected at ≥95% positivity at ≤0.29-0.49 GE/mL in all four specimen types. One strain was

≥95% positive at 0.85-1.46 GE/mL in each of the four specimen types. The remaining strain was detected at 100% positivity at 1.16 and 1.46 GE/mL in vaginal and penile meatal swabs respectively, 100% positivity at 3.47 GE/mL in female urine, and 100% positivity at 8.50 GE/mL in male urine.

f. Analytical specificity:

Cross-reactivity

Cross-reactivity study was performed using a panel of 56 microorganisms, including common flora of the genitourinary tract and closely related microorganisms. The microorganisms were spiked into 3 clinical matrices: vaginal swabs, penile-meatal swabs, and female urine. Microorganisms were tested at $2.5x10^5$ $TCID_{50}$/mL for viruses, $1x10^6$ CFU/mL for fungi and bacteria, $1x10^5$ cells/mL for protozoa, or the highest concentration possible if these concentrations could not be achieved. All microorganisms were tested with three lots of reagents with three replicates per lot of reagent. The Aptima Mycoplasma genitalium Assay did not generate a false positive test result with any microorganism listed in Table 4.

In addition, an *in silico* analysis was performed to determine if the oligonucleotides of the Aptima Mycoplasma genitalium Assay could amplify and detect nucleic acid sequences from Human papillomavirus (HPV) type 31, HPV type 35, HPV type 54, *Mycobacterium smegmatis*, *Chlamydia trachomatis* serovars L1, L2, L3, and *Treponema pallidum*. No significant interactions were detected in the *in silico* analysis.

Table 4: Microorganisms Tested in the Aptima Mycoplasma genitalium Assay

Microorganism	Test concentration	Microorganism	Test concentration
Acinetobacter lwoffii	$1x10^6$CFU/mL	HPV type 18 (HeLa cells)	$1x10^4$ cells/mL
Actinomyces israelii	$1x10^6$ CFU/mL	HPV type 58	$1x10^4$ copies/mL
Alcaligenes faecalis	$1x10^6$ CFU/mL	HPV type 39	$1x10^4$ copies/mL
Atopobium vaginae	$1x10^9$ rRNA copies/mL	HPV type 51	$1x10^4$ copies/mL
Bacteroides fragilis	$1x10^6$ CFU/mL	*Klebsiella pneumoniae*	$1x10^6$ CFU/mL
Bifidobacterium adolescentis	$1x10^6$ CFU/mL	*Lactobacillus acidophilus*	$1x10^6$ CFU/mL
Campylobacter jejuni	$1x10^6$ CFU/mL	*Lactobacillus crispatus*	$1x10^6$ CFU/mL
Candida albicans	$1x10^6$ CFU/mL	*Leptotrichia buccalis*	$1x10^5$ CFU/mL
Chlamydia trachomatis	$1x10^4$ IFU/mL	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Clostridium difficile	$1x10^6$ CFU/mL	*Megasphaera* type 1	$1x10^9$ copies/mL
Chromobacterium violaceum	$1x10^6$ CFU/mL	*Mobiluncus curtisii*	$1x10^6$ CFU/mL
Corynebacterium genitalium	$1x10^6$ CFU/mL	*Mobiluncus mulieris*	$1x10^6$ CFU/mL
Cryptococcus neoformans	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^9$ copies/mL
Cytomegalovirus	$2.5 x10^4$ TCID50/mL	*Mycoplasma pneumoniae*	$1x10^6$ CFU/mL
Elizabethkingia meningosepticum	$1x10^6$ CFU/mL	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Enterobacter cloacae	$1x10^6$ CFU/mL	*Pentatrichomonas hominis*	$1x10^5$ cells/mL
Enterococcus faecalis	$1x10^6$ CFU/mL	*Prevotella bivia*	$1x10^6$ CFU/mL

Escherichia coli	$1x10^6$ CFU/mL	*Propionibacterium acnes*	$1x10^6$ cells/mL
Finegoldia magna	$1x10^9$ copies/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Fusobacterium nucleatum	$1x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Gardnerella vaginalis	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Haemophilus ducreyi	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Herpes simplex virus type 1	$2.5 x10^3$ TCID50/mL	*Staphylococcus saprophyticus*	$1x10^6$ CFU/mL
Herpes simplex virus type 2	$2.5 x10^3$ TCID50/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
HIV-1	$1x10^6$ copies/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
HPV type 6	$1x10^6$ copies/mL	*Trichomonas vaginalis*	$1x10^5$ cells/mL
HPV type 11	$1x10^8$ copies/mL	*Ureaplasma parvum*	$1x10^9$ rRNA copies/mL
HPV type 16 (SiHa cells)	$1x10^4$ cells/mL	*Ureaplasma urealyticum*	$1x10^9$ rRNA copies/mL

Microbial Interference

The same organisms that were tested in the cross reactivity study were evaluated in the microbial interference study. Each sample tested contained *Mycoplasma genitalium* at a concentration of 3X LoD and microorganism concentration indicated above in Table 4. Once prepared, each sample was tested in triplicate. No microbial interference with the Aptima Mycoplasma genitalium Assay was observed, except in the presence of *Mycoplasma pneumoniae*, which is commonly found in the lower respiratory tract. A limitation has been added to the Aptima Mycoplasma genitalium Assay's package insert to mitigate this finding.

Interfering Substances

A study was performed to evaluate the performance of the Aptima Mycoplasma genitalium Assay in the presence of gynecological products, feminine hygiene products, whole blood, leukocytes, and other potentially interfering substances. Assay results were evaluated to determine if the presence of potentially interfering substances in *Mycoplasma genitalium*-negative or *Mycoplasma genitalium*-positive samples had an effect on the assay performance. Potentially interfering substances were diluted into relevant corresponding clinical matrices (penile meatal swab, vaginal swab, and female urine) and tested in the absence or presence of *Mycoplasma genitalium* at 3X LoD. A minimum of nine replicates of each panel member were tested with three reagent lots (three replicates per reagent lot). No interference was observed with the interfering substances at the concentration listed in Table 5 (i.e., all positive replicates tested produced positive Aptima Mycoplasma genitalium Assay results and all negative replicates tested produced negative Aptima Mycoplasma genitalium Assay results).

Table 5: Interfering Substances Tested in the Aptima Mycoplasma genitalium Assay

Potential Interfering Substance	Target Concentration
(b) (4)	

Interference in assay results was observed when mucus at a final concentration of 0.3% w/v was added to clinical specimen matrix. Interference was not observed when mucus at a final concentration of 0.03% w/v was added to clinical specimen matrix. A limitation has been added to the Aptima Mycoplasma genitalium Assay's package insert to reflect this finding.

g. *Carryover Contamination:*

A series of alternating *Mycoplasma genitalium* negative and positive samples were processed using the Aptima Mycoplasma genitalium Assay. The positive samples consisted of simulated vaginal swab samples spiked with $6.1x10^6$ GE/mL of *Mycoplasma genitalium* and the negative samples consisted of simulated vaginal swab samples without *Mycoplasma genitalium*. The checkerboard arrangement was tested on three Panther instruments, four runs/instrument, 40 negative, and 40 positive samples per run with one reagent lot. All samples tested produced expected results with all positive samples producing positive Aptima Mycoplasma genitalium Assay results, and all negative samples producing negative Aptima Mycoplasma genitalium Assay

results. No carryover was observed.

h. Assay cut-off:

Assay result from each patient is determined by a cutoff based on the total RLU. A test may be negative, positive, or invalid, as determined by the IC RLU and Signal to Cutoff (S/CO) ratio for the detection of *Mycoplasma genitalium* rRNA. Table 6 below describes the criteria associated with each possible assay result.

Table 6: Result Interpretation

Assay Result	**Criteria**
Negative	Analyte S/CO < 1.0 IC $\geq$ IC Cutoff IC $\leq$ 1,200,000 RLU
Positive	Analyte S/CO ≥ 1.0 IC $\leq$ 1,200,000 RLU Analyte $\leq$ 3,000,000 RLU
Invalid	Analyte S/CO < 1.0 and IC < IC Cutoff Or IC > 1,200,000 RLU Or Analyte > 3,000,000 RLU

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

Simulated Vaginal Swab Matrix Equivalency Study:
A study was performed to demonstrate matrix equivalency between simulated vaginal swab matrix and natural vaginal swab matrix via a head-to-head LoD study. Self-collected natural vaginal swab samples were collected using the Aptima Multi-test Swab Collection Kit. The simulated vaginal swab matrix consists of Specimen Transport Media (STM) combined with a simulated vaginal fluid (SVF).

The LoD was determined by testing eight dilutions of *Mycoplasma genitalium* lysate prepared in both the natural vaginal swab and simulated vaginal swab matrices. A minimum of 20 replicates were tested for each panel member across three reagent lots. Probit analysis was used to determine the 95% LoD for *Mycoplasma genitalium* in each sample matrix. The LoDs of the Aptima Mycoplasma genitalium Assay for *Mycoplasma genitalium* in simulated vaginal swab matrix and natural vaginal swab matrix were found to be similar (~0.04 GE/mL).

3. Clinical studies:

Prospective Study:
A prospective, multi-center clinical study was conducted to establish the clinical performance characteristics of the Aptima Mycoplasma genitalium Assay on the Panther System. The clinical samples were collected at 21 US sites with testing performed at three sites. A total of 3393 subjects were enrolled, of which 93 were excluded (subjects withdrawn and unknown patient infected status). Of the 3300 remaining subjects, 1737 were women and 1563 were men. Four subjects were 15 to 17 years of age, 242 were 18 to 20 years of age, 483 were 21 to 24 years of age, 1954 were 25 to 44 years of age, 572 were 45 to 64 years of age, and 45 were ≥65 years of age. The study included collection of the following specimens in a clinical setting from symptomatic and asymptomatic subjects:

From males: self-collected penile meatal swab, clinician-collected urethral swab, and self-collected first catch urine.

From females: Self-collected first catch urine, self-collected vaginal swab, clinician-collected vaginal swab, and clinician collected endocervical swab.

The clinical performance of the Aptima Mycoplasma genitalium Assay was evaluated against patient infected status (PIS) determined using a composite comparator algorithm comprised of three validated transcription mediated amplification (TMA) based assays for *Mycoplasma genitalium* (please see section O for validation of the comparator TMA assays). The results of the comparator TMA assays from self-collected vaginal swabs and male urethral swabs were used to define the PIS. Two of the three comparator TMA assays had to be positive or negative to establish the infected or not-infected PIS respectively. The third TMA assay was run, as a tie-breaker, if the results of the first two TMA assays were discordant.

Of the specimens collected, 11,827 were tested in valid Aptima Mycoplasma genitalium Assay runs. Of these, 11,774 (99.6%) had final valid Aptima Mycoplasma genitalium Assay results and 53 (0.4%) had final invalid results and were excluded from the analysis. Of 11,774 specimens, 11,557 had valid PIS results. Therefore, for the 3300 evaluable subjects, the following specimens were included in the analyses: 1709 clinician-collected vaginal swab, 1724 self-collected vaginal swab, 1715 endocervical swab, 1733 female urine, 1563 urethral swab, 1554 self-collected penile meatal swab, and 1559 male urine samples.

Performance in Female and Male Specimens based on PIS:
The sensitivity, specificity, PPV, and NPV of the Aptima Mycoplasma genitalium assay for *Mycoplasma genitalium* detection are shown for female and male specimens in Tables 7 and 8.

Table 7: Performance Characteristics Based on PIS

Specimen Type	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI) [1]	Specificity % (95% CI) [1]	PPV % (95% CI) [2]	NPV % (95% CI) [2]
CVS	1709	160	30	1505	14	10.2	92.0 (86.9-95.1)	98.0 (97.2-98.6)	84.2 (79.1-88.6)	99.1 (98.5-99.5)
PVS	1724	173	24	1525	2	10.2	98.9 (95.9-99.7)	98.5 (97.7-99.0)	87.8 (83.1-91.7)	99.9 (99.5-100)
ES	1715	141	26	1565	32	10.1	81.5 (75.1-86.6)	98.3 (97.5-98.8)	84.4 (78.9-89.1)	97.9 (97.2-98.5)
FU	1733	137	16	1541	39	10.2	77.8 (71.1-83.3)	99.0 (98.3-99.4)	89.5 (84.3-93.6)	97.5 (96.8-98.2)
US	1563	162	6	1392	3	10.6	98.2 (94.8-99.4)	99.6 (99.1-99.8)	96.4 (92.7-98.6)	99.8 (99.4-100)
PM	1554	145	30	1360	19	10.6	88.4 (82.6-92.5)	97.8 (96.9-98.5)	82.9 (77.4-87.6)	98.6 (97.9-99.1)
MU	1559	149	9	1386	15	10.5	90.9 (85.5-94.4)	99.4 (98.8-99.7)	94.3 (90.0-97.2)	98.9 (98.3-99.4)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, FU = female urine, MU = male urine, NPV = negative predictive value, PM = penile meatal swab, PPV = positive predictive value, Prev = prevalence, PVS = patient-collected vaginal swab, TN = true negative, TP = true positive, US = male urethral swab.

[1] Score CI.

[2] PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio.

Table 8: Performance Characteristics Based on PIS by Symptom Status

Specimen Type	Symptom Status	N	TP	FP	TN	FN	Prev (%)	Sensitivity % (95% CI) [1]	Specificity % (95% CI) [1]	PPV % (95% CI) [2]	NPV % (95% CI)[2]
CVS	Sym	1040	112	22	898	8	11.5	93.3 (87.4-96.6)	97.6 (96.4-98.4)	83.6 (77.3-88.8)	99.1 (98.3-99.6)
	Asym	669	48	8	607	6	8.1	88.9 (77.8-94.8)	98.7 (97.5-99.3)	85.7 (75.8-92.9)	99.0 (98.0-99.6)
PVS	Sym	1047	121	18	908	0	11.6	100 (96.9-100)	98.1 (96.9-98.8)	87.1 (81.1-91.9)	100 (99.6-100)
	Asym	677	52	6	617	2	8.0	96.3 (87.5-99.0)	99.0 (97.9-99.6)	89.7 (80.4-95.7)	99.7 (98.9-100)
ES	Sym	1046	101	17	909	19	11.5	84.2 (76.6-89.6)	98.2 (97.1-98.9)	85.6 (79.1-90.8)	98.0 (97.0-98.7)
	Asym	669	40	9	607	13	7.9	75.5 (62.4-85.1)	98.5 (97.2-99.2)	81.6 (70.3-90.2)	97.9 (96.8-98.8)
FU	Sym	1051	97	15	914	25	11.6	79.5 (71.5-85.7)	98.4 (97.4-99.0)	86.6 (80.0-91.8)	97.3 (96.3-98.2)
	Asym	682	40	1	627	14	7.9	74.1 (61.1-83.9)	99.8 (99.1-100)	97.6 (88.7-99.9)	97.8 (96.7-98.7)
US	Sym	866	102	1	761	2	12.0	98.1 (93.3-99.5)	99.9 (99.3-100)	99.0 (94.9-100)	99.7 (99.1-100)
	Asym	697	60	5	631	1	8.8	98.4 (91.3-99.7)	99.2 (98.2-99.7)	92.3 (84.0-97.3)	99.8 (99.2-100)

PM	Sym	865	92	17	745	11	11.9	89.3 (81.9-93.9)	97.8 (96.5-98.6)	84.4 (77.5-90.0)	98.5 (97.6-99.2)
	Asym	689	53	13	615	8	8.9	86.9 (76.2-93.2)	97.9 (96.5-98.8)	80.3 (70.8-88.1)	98.7 (97.7-99.4)
MU	Sym	866	93	7	755	11	12.0	89.4 (82.0-94.0)	99.1 (98.1-99.6)	93.0 (86.9-96.9)	98.6 (97.6-99.3)
	Asym	693	56	2	631	4	8.7	93.3 (84.1-97.4)	99.7 (98.9-99.9)	96.6 (89.0-99.5)	99.4 (98.5-99.8)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, PM = penile meatal swab, PPV=positive predictive value, Prev=prevalence, PVS=Patient-collected vaginal swab, Sym=symptomatic, TN=true negative, TP=true positive, US=male urethral swab

[1] Score CI

[2] PPV 95% CI computed from the exact 95% CI for the positive likelihood ratio, NPV 95% CI computed from the exact 95% CI for the negative likelihood ratio.

Specimen-specific Agreements:

Specimen-specific agreements were calculated by comparing the Aptima Mycoplasma genitalium Assay results and the composite comparator results, comprised of testing results from the earlier mentioned three validated TMA assays for the same specimen type. The sample was considered positive when two out of three TMA assay results were positive; the sample was considered negative when two out of three TMA assay results were negative. Of the 11,827 specimens from 3300 evaluable subjects, 11,665 specimens that had specimen-specific composite comparator results available were included in the specimen-specific agreement analysis. The positive (PPA) and negative (NPA) percent agreement of the Aptima Mycoplasma genitalium Assay for *Mycoplasma genitalium* detection are shown for female and male specimens in Tables 9 and 10.

Table 9: Specimen-Specific Agreements

Specimen Type	N	Composite comparator+/ Aptima+	Composite comparator-/ Aptima+	Composite comparator-/ Aptima-	Composite comparator+/ Aptima-	PPA (95% CI)[1]	NPA (95% CI)[1]
CVS	1729	175	17	1534	3	98.3 (95.2-99.4)	98.9 (98.3-99.3)
PVS	1724	173	24	1525	2	98.9 (95.9-99.7)	98.5 (97.7-99.0)
ES	1734	163	7	1559	5	97.0 (93.2-98.7)	99.6 (99.1-99.8)
FU	1774	147	9	1609	9	94.2 (89.4-96.9)	99.4 (98.9-99.7)
US	1563	162	6	1392	3	98.2 (94.8-99.4)	99.6 (99.1-99.8)
PM	1563	162	14	1379	8	95.3 (91.0-97.6)	99.0 (98.3-99.4)
MU	1578	159	2	1413	4	97.5 (93.9-99.0)	99.9 (99.5-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NPA = negative percent agreement, PM = penile meatal swab, PPA = positive percent agreement, PVS = patientcollected vaginal swab, US = male urethral swab

[1] = Score 95% CI

Table 10: Specimen-Specific Agreements by Symptom Status

Specimen Type	Symptom Status	N	Composite comparator+/ Aptima+	Composite comparator-/ Aptima+	Composite comparator-/ Aptima-	Composite comparator+/ Aptima-	PPA (95% CI)[1]	NPA (95% CI)[1]
CVS	Sym	1050	123	12	913	2	98.4 (94.4-99.6)	98.7 (97.7-99.3)
	Asym	679	52	5	621	1	98.1 (90.1-99.7)	99.2 (98.1-99.7)
PVS	Sym	1047	121	18	908	0	100 (96.9-100)	98.1 (96.9-98.8)
	Asym	677	52	6	617	2	96.3 (87.5-99.0)	99.0 (97.9-99.6)
ES	Sym	1057	115	4	935	3	97.5 (92.8-99.1)	99.6 (98.9-99.8)
	Asym	677	48	3	624	2	96.0 (86.5-98.9)	99.5 (98.6-99.8)
FU	Sym	1074	106	7	955	6	94.6 (88.8-97.5)	99.3 (98.5-99.6)
	Asym	700	41	2	654	3	93.2 (81.8-97.7)	99.7 (98.9-99.9)
US	Sym	866	102	1	761	2	98.1 (93.3-99.5)	99.9 (99.3-100)
	Asym	697	60	5	631	1	98.4 (91.3-99.7)	99.2 (98.2-99.7)
PM	Sym	870	101	8	756	5	95.3 (89.4-98.0)	99.0 (97.9-99.5)
	Asym	693	61	6	623	3	95.3 (87.1-98.4)	99.0 (97.9-99.6)
MU	Sym	874	99	2	770	3	97.1 (91.7-99.0)	99.7 (99.1-99.9)
	Asym	704	60	0	643	1	98.4 (91.3-99.7)	100 (99.4-100)

Asym = asymptomatic, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FU = female urine, MU = male urine, NPA = negative percent agreement, PM = penile meatal swab, PPA = positive percent agreement, PVS = patient-collected vaginal swab, Sym = symptomatic, US = male urethral swab.

[1] Score 95% CI

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The observed expected values (number of positive results detected by the Aptima Mycoplasma genitalium Assay) in this prospective clinical study were 11.1% in clinician-collected vaginal swab samples, 11.4% in self-collected vaginal swab samples, 9.7% in endocervical swab samples, 8.8% in female urine, 10.7% in male urethral swab samples,

11.3% in self-collected penile meatal swab samples, and 10.1% in male urine samples.

M. Instrument Name:

The Panther System

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X_____ or No _____

   Level of Concern:

   Moderate

   Software Description:

   There are two software components that comprise the Panther System which is required to perform the Aptima Mycoplasma genitalium Assay:

   - System Software: System software includes the Master Controller software and instrument firmware. This software is assay independent and does not include assay specific processing parameters. The System software communicates with the Assay software to access assay specific parameters.

   - Assay Software: Assay software contains information that is specific to a given assay and provides assay-specific configuration information to the instrument. Assay software includes assay specific parameters for reagent volumes, incubation times, incubation temperatures, and other assay parameters, such as the sequence of steps taken.

3. Specimen Identification:

   Specimens are identified via barcode labels on the sample vial.

4. Specimen Sampling and Handling:

   Clinician-collected and self-collected vaginal swab specimens, clinician-collected endocervical swab specimens, female and male urine specimens, clinician-collected male urethral swab specimens, and self-collected penile meatal swab specimens can be tested with the Aptima Mycoplasma genitalium assay. Assay performance has not been evaluated with specimens other than those collected with the following specimen collection kits:

   - Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
   - Aptima Urine Collection Kit for Male and Female Urine Specimens
   - Aptima Multitest Swab Specimen Collection Kit

   For Specimen collection, refer to the appropriate specimen collection kit package insert for specific collection instructions.

   Please refer to section L.1.c. for specimen transport and storage.

5. Calibration:

   The Panther System is calibrated upon installation by Hologic Inc. Field service engineers as well as during preventive maintenance as scheduled.

6. Quality Control:

   Please see section L.1.c.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Comparator TMA Assays validation:
The three comparator TMA assays used to determine the PIS status (for sensitivity and specificity calculation) and composite comparator result (for specimen-specific agreements calculation) detected regions of the rRNA gene different than the region detected by the Aptima Mycoplasma genitalium assay. The validation of these three comparator TMA assays include:

1. LoD study: A complete LoD study was conducted with one strain of *Mycoplasma genitalium* using two Panther Systems with at leas(b) (4) eplicates per dilution in four clinical matrices: vaginal swab, penile meatal swa(b) (4) ale urine, and female urine. The LoD of each TMA assay was found comparable to the Aptima Mycoplasma genitalium assay.

2. Inclusivity: Ten *Mycoplasma genitalium* strains were tested in this study in triplicates using three Panther Systems and one reagent lot for each comparator TMA assay. Each of the three TMA assays and the Aptima Mycoplasma genitalium assay detected the tested *Mycoplasma genitalium* strains at a similar concentration.
3. Cross-reactivit (b)(4) icroorganisms tested in the cross-reactivity study for the Aptima Mycopl (4) genitalium assay were tested in triplicates for each comparator TMA assay. In addition, *in silico* analysis was performed similar to the Aptima Mycoplasma genitalium assay with eight microorganisms. No cross-reactivity was observed and the three comparator TMA assays were found specific by *in silico* analysis.
4. Microbial Interference: (b)(4) microorganisms evaluated for cross-reactivity were also tested for microbial int rence in the presence of *Mycoplasma genitalium* at 3X LoD. Interference was observed with *M. pneumoniae,* commonly found in the lower respiratory tract, similar to what was observed with the Aptima Mycoplasma genitalium assay.
5. Comparator TMA assays were further evaluated using (b)(4) archived clinical specimens. The positive percent agreement between th ma Mycoplasma genitalium assay and each comparator TMA assay ranged from 94.4% to 97.7% and negative percent agreement ranged from 99.8% to 99.9%.

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Risks to Health and Mitigation Measures:

Identified Risks to Health	Mitigation Measures
Risk of false results	General controls and Special Controls (1), (2), (3), and (4)
Failure to correctly interpret test results	General Controls and Special Controls (1), (3)(iii), (3)(iv), and 3(v)
Failure to correctly operate the device	General Controls and Special Controls (1), (3)(i), and (4)

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

There are no other FDA cleared or approved assays currently on the market for the detection of *Mycoplasma genitalium*. The Aptima Mycoplasma genitalium Assay is the first qualitative nucleic acid amplification test (NAAT) to detect *Mycoplasma genitalium* nucleic acids directly from urogenital specimens. Use of the Aptima Mycoplasma genitalium assay will improve detection and treatment of *Mycoplasma genitalium* infections, and may help limit the spread of infection in the community and reduce the sequelae of infection. The performance demonstrated in the prospective clinical study suggests that the Aptima Mycoplasma genitalium assay will be highly sensitive and specific for detection of *Mycoplasma genitalium* in the preferred specimen types.

Summary of the Assessment of Risk

The risks associated with the device are those related to the risk of false test results, failure to correctly interpret test results, and failure to correctly operate the instrument. False-positive results and false-negative results are the primary risks associated with the use of the Aptima Mycoplasma genitalium assay. A false-positive result may lead to inappropriate or unnecessary antibiotic therapy with the potential for side effects and increasing antibiotic resistance, although the specificty of the assay suggests that false positives will be uncommon. A false-negative result may lead to a delayed or missed diagnosis with the potential for adverse reproductive health outcomes in women and urethritis in men. The lower performance in some claimed specimen types is mitigated by an additional statement included in the Indications for Use (IFU) and product labeling. The IFU statement emphasizes the higher risk of false-negative results in endocervical swab and female urine specimens and identifies vaginal swabs as the preferred specimen type. It also indicates that follow up testing could be necessary, if negative results are obtained with endocervical swabs or female urine and *Mycoplasma genitalium* infection is suspected. However, general controls alone are not sufficient to mitigate the risks described. The special controls include a statement that the labeling must include, for example, a detailed description of the device, detailed description of the test procedure and interpretation of test results, and appropriate limiting statements, and they include requirements for assay design verification and validation.

Summary of the Assessment of Benefit-Risk

The probable benefits of the Aptima Mycoplasma genitalium Assay outweigh the potential risks, given the proposed special controls and applicable general controls. The Aptima Mycoplasma genitalium Assay is the first qualitative NAAT to detect *Mycoplasma genitalium* in urogenital specimens and is likely to benefit patients by allowing a rapid diagnosis of an emerging sexually transmitted infection. The performance demonstrated during the clinical study suggest that errors will be uncommon and will be mitigated by the proposed special controls.

S. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

T. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.3393. FDA believes that the special controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QEP

Device Type: Device to detect nucleic acids from non-viral microorganism(s) causing sexually transmitted infections and associated resistance marker(s)

Class: II (special controls)

Regulation: 21 CFR 866.3393

(a) Identification. A device to detect nucleic acids from non-viral microorganism(s) causing sexually transmitted infections and associated resistance marker(s) is an *in vitro* diagnostic device intended for the detection and identification of nucleic acids from non-viral microorganism(s) and their associated resistance markers in clinical specimens collected from patients suspected of sexually transmitted infections. The device is intended to aid in the diagnosis of non-viral sexually transmitted infections in conjunction with other clinical and laboratory data. These devices do not provide confirmation of antibiotic susceptibility since mechanisms of resistance may exist that are not detected by the device.

(b) Classification: Class II (special controls). A device to detect nucleic acids from non-viral microorganism(s) causing sexually transmitted infections and associated resistance marker(s) must comply with the following special controls:

(1) The intended use for the 21 CFR 809.10 labeling must include a detailed description of targets the device detects, the results provided to the user, the clinical indications appropriate for test use, and the specific population(s) for which the device is intended.

(2) Any sample collection device used must be FDA-cleared, -approved, or -classified as 510(k) exempt (standalone or as part of a test system) for the collection of specimen types claimed by this device; alternatively, the sample collection device must be cleared in a premarket submission as a part of this device.

(3) The 21 CFR 809.10(b) labeling must include:

(i) A detailed device description, including reagents, instruments, ancillary materials, all control elements, and a detailed explanation of the methodology, including all pre-analytical methods for processing of specimens;

(ii) Detailed discussion of the performance characteristics of the device for all claimed specimen types based on analytical studies, including, but not limited to, Limit of Detection, inclusivity, cross-reactivity, interfering substances, competitive inhibition, carryover/cross contamination, specimen stability, with-in lab precision, and reproducibility, as appropriate;

(iii) Detailed descriptions of the test procedure, the interpretation of test results for clinical specimens, and acceptance criteria for any quality control testing.

(iv) Limiting statements indicating that:

(A) a negative test result does not preclude the possibility of infection;

(B) the test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician;

(C) reliable results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results; and

(D) if appropriate (e.g., recommended by CDC, by current well-accepted clinical guidelines, or by published peer reviewed research), that the clinical performance is inferior in a specific clinical subpopulation or for a specific claimed specimen type.

(v) If the device is intended to detect antimicrobial resistance markers, limiting statements, as appropriate, indicating that:

(A) negative results for claimed resistance markers do not indicate susceptibility of detected microorganisms, as resistance markers not measured by the assay or other potential mechanisms of antibiotic resistance may be present;

(B) detection of resistance markers cannot be definitively linked to specific microorganisms and the source of a detected resistance marker may be an organism not detected by the assay, including colonizing flora;

(C) detection of antibiotic resistance markers may not correlate with phenotypic gene expression; and

(D) therapeutic failure or success cannot be determined based on the assay results, since nucleic acid may persist following appropriate antimicrobial therapy.

(4) Design verification and validation must include:

(i) Detailed device description documentation, including, but not limited to, methodology from obtaining sample to result, design of primer/probe sequences, rationale for target sequence selection, and computational path from collected raw data to reported result (e.g., how collected raw signals are converted into a reported result).

(ii) Detailed documentation of analytical studies including but not limited to, Limit of Detection, inclusivity, cross-reactivity, microbial interference, interfering substances, competitive inhibition, carryover/cross contamination, specimen stability, with-in lab precision, and reproducibility, as appropriate.

(iii) Detailed documentation and performance results from a clinical study that includes prospective (sequential) samples for each claimed specimen type and, when determined to be appropriate by FDA, additional characterized clinical

samples. The study must be performed on a study population consistent with the intended use population and compare the device performance to results obtained from FDA accepted comparator methods. Documentation from the clinical studies must include the clinical study protocol (including a predefined statistical analysis plan) study report, testing results, and results of all statistical analyses.

(iv) A detailed description of the impact of any software, including, but not limited to, software applications and hardware-based devices that incorporate software, on the device's functions.