

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k122062

B. Purpose for Submission:

To obtain clearance for the APTIMA[®] Trichomonas vaginalis Assay (PANTHER[®] System)

C. Measurand:

Trichomonas vaginalis ribosomal RNA (rRNA)

D. Type of Test:

Nucleic acid amplification assay

E. Applicant:

Gen-Probe Incorporated

F. Proprietary and Established Names:

APTIMA[®] Trichomonas vaginalis Assay (PANTHER[®] System)

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3860

2. Classification:

Class II

3. Product code:

OUY - *Trichomonas vaginalis* nucleic acid amplification test system

4. Panel:

83 - Microbiology

H. Intended Use:

1. Intended use:

The APTIMA *Trichomonas vaginalis* Assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Trichomonas vaginalis* to aid in the diagnosis of trichomoniasis using the PANTHER System.

The assay may be used to test the following specimens from symptomatic or asymptomatic women: clinician-collected endocervical swabs, clinician-collected vaginal swabs, and specimens collected in PreservCyt Solution.

2. Indications for use:

The APTIMA *Trichomonas vaginalis* Assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Trichomonas vaginalis* to aid in the diagnosis of trichomoniasis using the PANTHER System.

The assay may be used to test the following specimens from symptomatic or asymptomatic women: clinician-collected endocervical swabs, clinician-collected vaginal swabs, and specimens collected in PreservCyt Solution.

3. Special conditions for use statement:

For prescription use only

4. Special instrument requirements:

PANTHER System

I. Device Description:

The ATV assay is a nucleic acid amplification test intended for the *in vitro* qualitative detection of ribosomal RNA from *T. vaginalis* in clinician collected vaginal swabs, endocervical swabs and ThinPrep Pap Test specimens collected in Cytoc Preservcyt solution. The assay may be used to test specimens from symptomatic and asymptomatic women to aid in the diagnosis of trichomoniasis using the PANTHER System automated analyzer. This ATV Assay is similar to the ATV Assay originally cleared (ref: K102911) for use on the TIGRIS System, except for the formulation of the TCR. The TCR is a HEPES-buffered solution containing lithium salts and derivatized magnetic beads. The ATV Assay uses Target Capture (TC), Transcription Mediated Amplification (TMA), and Hybridization Protection Assay (HPA) technologies to qualitatively detect ribosomal RNA (rRNA) from *Trichomonas vaginalis*.

The ATV Assay kit is comprised of 3 boxes:

- Refrigerated Box contains the Amplification Reagent, Enzyme Reagent, Probe Reagent and Target Capture Reagent-B
- Room Temperature Box contains Amplification Reconstitution Solution, Enzyme Reconstitution Solution, Probe Reconstitution Solution, Selection Reagent and Target Capture Reagent
- Controls Box contains the Negative and Positive Controls

The ATV Assay on PANTHER utilizes three specimen collection kits. These collection kits were cleared for use with the ATV Assay on the TIGRIS and other commercialized APTIMA Assays.

- APTIMA Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens
- APTIMA Vaginal Swab Specimen Collection Kit
- APTIMA Specimen Transfer Kit

J. Substantial Equivalence Information:

1. Predicate device name:

APTIMA Trichomonas vaginalis Assay on the TIGRIS DTS System

2. Predicate 510(k) number:

K102911

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Device Class	II	same
Qualitative /Quantitative Assay	Qualitative	same
Intended Use	NAAT test for detection of <i>Trichomonas vaginalis</i> ribosomal RNA (rRNA)	same
Technology	Target Capture (TC) Transcription Mediated Amplification (TMA) Hybridization Protection Assay (HPA)	same

Differences		
Item	Device	Predicate
Platform	Assay performed on the PANTHER Instrument	Assay performed on the TIGRIS Instrument
Reagent Formulation	Original formulation plus additional oligo in TCR	Original formulation
Specimen types	Three female specimen types: <ul style="list-style-type: none"> • Vaginal swab • Endocervical swab • ThinPrep in PreservCyt solution 	Four female specimen types: <ul style="list-style-type: none"> • Urine • Vaginal swab • Endocervical swab • ThinPrep in PreservCyt solution

K. Standard/Guidance Document Referenced:

EN 13640:2002 – Stability Testing of In-Vitro Diagnostic Medical Devices

EP5-A2, 2004 – Evaluation of Precision Performance of Quantitative Measurement Methods, CLSI Approved Guideline

EP15-A2, 2006 – User Verification of Performance for Precision and Trueness, CLSI Approved Guideline

EP07-A2, 2005 – Interference Testing in Clinical Chemistry, Approved Guideline

Format for Traditional and Abbreviated 510(k)s – Guidance for Industry and FDA Staff, August 2005

General Principles of Software Validation, Final Guidance for Industry and FDA Staff, January 2002

L. Test Principle:

The APTIMA *Trichomonas vaginalis* Assay involves the technologies of target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solution in these tubes releases the rRNA target and protects it from degradation during storage. When the APTIMA *Trichomonas vaginalis* Assay is performed in the laboratory, the target rRNA is isolated from the specimens by the use of a specific capture

oligomer and magnetic microparticles in a method called target capture. The capture oligomer contains a sequence complementary to a specific region of the target molecule as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific region of the capture oligomer binds to a specific region of the target molecule. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction 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, including the captured target molecule bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The GEN-PROBE TMA reaction amplifies a specific region of the small ribosomal subunit from *T. vaginalis* via DNA and RNA intermediates and generates RNA amplicon molecules. Detection of the rRNA amplification product sequences is achieved using nucleic acid hybridization (HPA). A single-stranded chemiluminescent DNA probe, which is complementary to a region of the target amplicon, is labeled with an acridinium ester molecule. The labeled DNA probe combines with amplicon to form stable RNA:DNA hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled RNA:DNA hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU).

M. Performance Characteristics:

1. Analytical performance:

a. *Reproducibility*

Reproducibility of the APTIMA *Trichomonas vaginalis* Assay was evaluated on the PANTHER System at two external US laboratories and at Gen-Probe.

Reproducibility panel members were created by using pooled TV-negative PreservCyt Solution liquid Pap specimens and specimen transport medium (STM). The positive panel members were created by spiking the PreservCyt Solution liquid Pap matrix with the appropriate amount of *T. vaginalis* lysate. Final *T. vaginalis* concentrations were .003 TV/mL for the high negative panel, .02 TV/mL for the moderate positive panel member (expected positivity: 100%), and 1.00 TV/mL for the high positive panel member (expected positivity: 100%). The fourth panel member contained TV-negative PreservCyt specimen matrix and STM only.

Testing was performed using two lots of assay reagents and a total of six operators (two at each site). At each site, testing was performed over at least 6 days. Two operators performed testing at each site with only one operator performing testing

each day. At each site, each operator performed two runs per day, each run using a different lot of reagents. Each run included three replicates of each panel member.

The following table presents, for each panel member, RLU data in terms of mean, standard deviation (SD), and coefficient of variation (CV) between sites, between operators, between lots, between runs, within runs, and overall (Total). Percent agreement with expected results is also shown. Samples with valid results were included in the analyses. In summary, the ATV assay on the PANTHER demonstrated high agreement with the expected results in the negative panel member and panel members containing target levels at or above the assay LoD. The results indicate reproducible performance within and across all three sites for all panel members tested when compared to the expected outcome.

APTIMA *Trichomonas vaginalis* Assay Reproducibility Study

Conc	Target Conc	N	Agmt (%)	Mean RLU	Between Sites		Between Operators		Between Lots		Between Runs		Within Runs		Totals	
					SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Neg	N/A	108	99.1	23.5	0.0	0.0	2.7	11.6	0.0	0.0	0.0	0.0	37.5	159.7	37.6	160.1
HNeg	0.003	108	90.7	69.3	5.0	7.3	4.5	6.5	6.1	8.8	14.8	21.4	16.0	23.1	23.6	34.1
MPos	0.02	108	97.2	348.1	30.3	8.7	33.1	9.5	33.1	9.5	77.0	22.1	62.9	18.1	114.0	32.8
HPos	1.00	108	100	1185.5	0.0	0.0	17.0	1.4	0.0	0.0	28.0	2.4	34.2	2.9	47.4	4.0

Agmt = agreement, Conc = concentration, CV = coefficient of variation, HNeg = high negative, HPos = high positive,

MPos = moderate positive, Neg = negative, RLU = relative light units, SD = standard deviation.

Note: The RLU value reported by the software is the total measured RLU divided by 1000 with the digits after the decimal point truncated.

Variability from some factors may have been numerically negative. This occurred if the variability due to those factors was very small. In these cases, SD and CV are shown as 0.

b. Precision

The precision of the ATV Assay on PANTHER was evaluated in-house by multiple operators who performed multiple runs over multiple days with three different reagent lots on three different PANTHER Systems. A 4-member reproducibility panel was prepared in PreservCyt (ThinPrep) liquid cytology specimen-STM matrix. Prior to sample preparation, PreservCyt sample pools were screened with the ATV Assay to confirm that *T. vaginalis* was not present. The pools were then spiked with *T. vaginalis* lysate to produce high negative (expected positivity: >0% and <100%), moderate positive (expected positivity: 100%) and high positive (expected positivity: 100%) panel members. Unspiked PreservCyt pools served as the negative panel members. Each panel member was tested in triplicate by three operators, in

two runs per day using three reagent lots on three instruments. Testing was performed over 13 days with 162 replicates per panel member tested.

The negative and positive panel members yielded $\geq 99\%$ agreement with the expected results as shown by the following chart. The results from this study demonstrate that the ATV Assay can be performed reproducibly on the PANTHER System.

Description	TV/ mL	Valid N	% Positive	Percent Agreement
Negative	0	162	0.0	100% (97.7 - 100)
High Neg	0.003	162	11.1	N/A
Moderate	0.02	162	99.4	99% (96.6 -99.9)
High Pos	1	162	100.0	100% (97.7 - 100)

N/A = not applicable, expected positivity was 5% to 95%.

N= 162 for each panel member

c. Linearity/assay reportable range:

The ATV Assay (PANTHER System) test results are automatically interpreted by the PANTHER System APTIMA Trichomonas vaginalis software. A test result may be negative, positive or invalid as determined by the total Relative Light Units (RLU) in the detection step. A test result may be invalid due to RLU values outside the normal expected ranges. Initial invalid results should be retested.

Test Interpretation	Total RLU (x 1000)
Negative	0* to <100
Positive	100 to <2400
Invalid	0* or \geq 2400

*If the RLU measured on the PANTHER System is between 0 and 999, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid

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

Specimen Stability Studies: Data to support the recommended shipping and storage conditions for vaginal swab and PreservCyt liquid Pap specimens was generated with negative clinical specimens spiked with *T. vaginalis*. For vaginal swab specimens (100% of replicates) studies demonstrated stability of samples for storage up to 60 days at 30°C followed by storage up to two years at -20°C. For PreservCyt specimens (>98%), studies demonstrated stability of samples up to 30 days stored at 30C followed by storage up to 2 years frozen at -20°C. For either sample type, there was no statistical difference in RLU values from initial testing at T₀ to various time

points during storage confirming the validity of the claimed maximum storage times and temperatures.

Quality Control Results and Acceptability: The APTIMA Negative Control for *Trichomonas* and APTIMA Positive Control for *Trichomonas* act as controls for the target capture, amplification and detection steps of the assay. The Positive Control contains non-infectious *Trichomonas vaginalis* rRNA.

The ATV Assay Controls must produce the following test results:

Control	Total RLU (x1000)	<i>Trichomonas vaginalis</i> Result
Negative Control	0* and <20	Negative
Positive Control	>/=500 and < 2400	Positive

*If the RLU measured on the PANTHER System in between 0 and 999, a result of “0” is reported in the “Total RLU (000s)” column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

e. Detection limit:

The limit of detection (LOD) for the APTIMA ATV Assay on PANTHER was determined by testing quantified (TV/mL) strains of *T. vaginalis* lysates diluted into pooled TV-negative residual PreservCyt liquid Pap specimen and swab matrices. Sensitivity panels were prepared with two strains of *T. vaginalis* (one Metronidazole-susceptible strain and one Metronidazole-resistant strain).

For each strain and dilution, twenty replicates of each panel member were tested with each of three reagent lots on different PANTHER instruments over 3 days for a total of 60 replicates. The LOD was defined as the lowest TV concentration at which > 95% of replicates tested positive.

Greater than 97% positivity for both strains was observed at a concentration of 0.003 TV/mL for swab samples and 100% positivity was observed for both strains at 0.01 TV/mL for PreservCyt specimens. The LOD results are summarized in the table below.

Positivity by *T. vaginalis* Strain

Panel Concentration (TV/mL)	PreservCyt Panel		Swab panel	
	TV Strain: ATCC #30236	TV Strain: ATCC #50138	TV Strain: ATCC #30236	TV Strain: ATCC #50138
0.1	100% (94-100)	100% (94-100)	100% (94-100)	100% (94-100)
0.03	100% (94-100)	100% (94-100)	100% (94-100)	100% (94-100)
0.01	97% (89-99)	100% (94-100)	100% (94-100)	100% (94-100)
0.003	23% (14-35)	65% (52-76)	100% (94-100)	100% (94-100)
0.001	0% (0-6)	0% (0-6)	0% (0-6)	3% (1-11)
0.0003	0% (0-6)	0% (0-6)	0% (0-6)	0% (0-6)
0	0% (0-6)		0% (0-6)	

N=60 for each TV strain and matrix

f. Analytical specificity:

Analytical Specificity of the ATV Assay on the PANTHER System was evaluated by testing 42 microorganisms of the genitourinary tract, opportunistic organisms, and closely related organisms. Organisms tested included bacteria, fungi, yeast, and viruses (see table below).

Panels were prepared in Specimen Transport Media (STM, representative of vaginal and endocervical samples) and PreservCyt specimens diluted 1:2.9 in STM. Panel members were prepared by spiking each of the potentially interfering microorganisms in both sample matrices at $\geq 10^6$ CFU/mL for bacteria and parasites and $\geq 10^5$ TCID₅₀/mL for viruses. Controls for each sample matrix, with and without TV were also prepared.

Testing of 25 replicates of each isolate was conducted in both matrices. No false positive results were observed with any of the organisms tested. Samples with *P. hominis* and *T. tenax* yielded slightly elevated RLUs although these results were negative and far below the assay cut-off. In summary, no cross-reactivity or significant effect on ATV Assay specificity was observed with any of the organisms tested.

Microorganisms tested for Potential Cross Reactivity

Microorganism	Concentration	Microorganism	Concentration
<i>Acinetobacter lwoffii</i>	1x10 ⁸ CFU/mL	HPV 16	2.5x10 ⁶ copies/mL
<i>Actinomyces israelii</i>	1x10 ⁸ CFU/mL	HPV 6	2.5x10 ⁶ copies/mL
<i>Atopobium vaginae</i>	1x10 ⁸ CFU/mL	<i>Klebsiella pneumoniae</i>	1x10 ⁶ CFU/mL
<i>Bacteroides fragilis</i>	1x10 ⁸ CFU/mL	<i>Lactobacillus acidophilus</i>	1x10 ⁶ CFU/mL
<i>Bifidobacterium adolescentis</i>	1x10 ⁸ CFU/mL	<i>Lactobacillus crispatus</i>	1x10 ⁶ CFU/mL
<i>Campylobacter jejuni</i>	1x10 ⁸ CFU/mL	<i>Listeria monocytogenes</i>	1x10 ⁶ CFU/mL
<i>Candida albicans</i>	1x10 ⁸ CFU/mL	<i>Mobiluncus curtisii</i>	1x10 ⁶ CFU/mL
<i>Chlamydia trachomatis</i>	1x10 ⁸ CFU/mL	<i>Mycoplasma genitalium</i>	2.5 x10 ⁶ copies/mL
<i>Clostridium difficile</i>	1x10 ⁸ CFU/mL	<i>Mycoplasma hominis</i>	1x10 ⁶ CFU/mL
<i>Corynebacterium genitalium</i>	1x10 ⁸ CFU/mL	<i>Neisseria gonorrhoeae</i>	1x10 ⁶ CFU/mL
<i>Cryptococcus neoformans</i>	1x10 ⁸ CFU/mL	<i>Pentatrichomonas hominis</i>	1x10 ⁶ cells/mL
Cytomegalovirus	2x10 ⁶ TCID ₅₀ /mL	<i>Peptostreptococcus magnus</i>	1x10 ⁶ CFU/mL
<i>Dientamoeba fragilis</i>	1x10 ⁸ CFU/mL	<i>Prevotella bivia</i>	1x10 ⁶ CFU/mL
<i>Enterobacter cloacae</i>	1x10 ⁸ CFU/mL	<i>Propionibacterium acnes</i>	1x10 ⁶ CFU/mL
<i>Enterococcus faecalis</i>	1x10 ⁸ CFU/mL	<i>Proteus vulgaris</i>	1x10 ⁶ CFU/mL
<i>Escherichia coli</i>	1x10 ⁸ CFU/mL	<i>Pseudomonas aeruginosa</i>	1x10 ⁶ CFU/mL
<i>Gardnerella vaginalis</i>	1x10 ⁸ CFU/mL	<i>Staphylococcus aureus</i>	1x10 ⁶ CFU/mL
<i>Haemophilus ducreyi</i>	1x10 ⁸ CFU/mL	<i>Staphylococcus epidermidis</i>	1x10 ⁶ CFU/mL
Herpes simplex virus I	2x10 ⁶ TCID ₅₀ /mL	<i>Streptococcus agalactiae</i>	1x10 ⁶ CFU/mL
Herpes simplex virus II	2x10 ⁶ TCID ₅₀ /mL	<i>Trichomonas tenax</i>	1x10 ⁶ cells/mL
HIV-1	2.5x10 ⁶ copies/mL	<i>Ureaplasma urealyticum</i>	1x10 ⁶ CFU/mL

The ATV assay on PANTHER was also evaluated in STM and PreservCyt matrices by testing the organisms listed in the table above in TV-positive samples. Samples containing each potentially interfering organism were spiked with TV lysate at 0.01 TV/mL with the exception of *Trichomonas tenax* and *Pentatrichomonas hominis* which were spiked at 2.5 TV/mL. Twenty-five replicates were tested. All results were positive as expected with the exception of samples containing *Trichomonas tenax* and *Pentatrichomonas hominis*, two organisms closely related to *T. vaginalis*. For these two organisms, false negative results were observed when testing at concentrations of 10⁶ CFU/mL. Further testing was performed at lower concentrations of the two organisms and TV positivity was recovered at 300,000 CFU/mL. The following limitation is included in the package insert regarding the potential interference of TV detection in the presence of *T. tenax* or *P. hominis*:

"A negative result does not preclude a possible infection because the presence of *Trichomonas tenax* or *Pentatrichomonas hominis* in a specimen may affect the ability to detect *T. vaginalis* rRNA."

In summary, no significant interference was observed for detection of TV in the presence of high concentrations of the potentially interfering organisms tested with the exception of the closely related *P. hominis* and *T. tenax* organisms, both of which are rarely found in the genitourinary tract.

g. *Interference*

The effect of endogenous and exogenous substances that may be present in urogenital specimens on the sensitivity and specificity of the APTIMA *Trichomonas vaginalis* (ATV) Assay on the PANTHER System was evaluated in an interference study.

The table below describes the substances that were individually spiked into STM and PreservCyt in STM sample matrices. Testing included 25 replicates containing each potentially interfering substance alone as well as 25 replicates containing each substance spiked with *T. vaginalis* lysate to a final concentration of 0.01 TV/mL.

Interfering Substances Tested

Product Category	Product Brand or Type	Concentration	V/V or W/V
Control	No substance	N/A	N/A
Lubricant	KY Warming Liquid KY Sensitive Touch Target Lubricant Liquid Astroglide KY Sensitive Jelly	1%	V/V
Spermicide	Gynol II Jelly Max Strength Conceptrol VCF Vaginal Contraceptive Film Encare Inserts	1%	W/V
Anti-fungal	Monistat 3 Combo CVS Clotrimazole 3 Target Vagicaïne Cream Vagisil Maximum Strength Target Miconazole 7	1%	W/V
Deodorant Spray/Powder	Summer's Eve Powder Vagisil Powder Summer's Eve Spray New Freshness Spray FDS Spray	1%	W/V
Intra-vaginal Hormones	Estrace Vaginal Cream (estradiol) CrinoneGel (progesterone) Endometrin tablets (progesterone) Vagifem tablets (estradiol)	1%	W/V
Seminal Fluid	Samples from 25 subjects	1%	V/V
Mucus	Porcine Mucin	1%	W/V
Whole Blood	N/A	10%	V/V
Glacial Acetic Acid**	N/A	10%	V/V

*Panels were prepared in both STM and PreservCyt solution diluted 1:2.9 in STM (Thinprep)

** used to treat PreservCyt liquid cytology specimens contaminated with blood

V/V = volume/volume

W/V = weight/volume

Study results yielded no false positive results for all substances tested (100% specificity) in both specimen matrices.

No interference with the detection of *T. vaginalis* ($\geq 95\%$ sensitivity) was observed with any of the substances tested with the exception of Astroglide personal lubricant, porcine gastric mucus, and glacial acetic acid. Astroglide personal lubricant and glacial acetic acid interfered with the detection of *T. vaginalis* when tested at a concentration of 0.01 TV/mL, however all specimens were positive as expected at concentrations of 0.3 TV/mL. Porcine gastric mucus did not yield any false negative results with the detection of *T. vaginalis* when tested at a concentration of 1 TV/mL (100% sensitivity), however the RLU signal was suppressed. The package insert includes the following limitation regarding potential interference from the presence of mucus:

"TV-positive mucoid samples may exhibit decreased RLU values. To ensure proper endocervical sampling, excess mucus should be removed."

h. Assay cut-off:

The ATV Assay on the PANTHER System is designed for and validated on the PANTHER System. The assay test results are automatically interpreted by the PANTHER System APTIMA Trichomonas vaginalis software. A test result may be negative, positive or invalid as determined by the total Relative Light Units (RLU) in the detection step. A test result may be invalid due to RLU values outside the normal expected ranges. Initial invalid results should be retested.

Test Interpretation	Total RLU (x 1000)
Negative	0* to <100
Positive	100 to <2400
Invalid	0* or ≥ 2400

*If the RLU measured on the PANTHER System is between 0 and 999, a result of "0" is reported in the "Total RLU (000s)" column in the run report. Measured RLU values less than 690 are reported as invalid. RLU values between 690 and 999 are reported as valid.

i. Carryover Study

Two studies were conducted to assess PANTHER instrument carry-over with the ATV Assay. In the first study, high-titer *T. vaginalis* samples were prepared by spiking TV lysate into specimen transport media (STM) to a final concentration of 10,000 TV/mL. The negative samples were comprised of STM. Testing included four runs on each of three different PANTHER instruments. The first run was comprised of all negative samples. The remaining runs were comprised of approximately 30% TV-positive samples (n=78) interspersed amongst the negative samples (n=170) in various patterns. In the second study, high positive and negative samples were similarly prepared as described above. This study included runs

consisting of ~50% alternating TV-positive and negative samples in a checkerboard pattern. Each of the studies yielded no false positive results and therefore a 0% carryover contamination rate.

2. Comparison studies:

a. *Method comparison with predicate device:*

Agreement between APTIMA *Trichomonas vaginalis* Assay results on the PANTHER System and the TIGRIS DTS System was assessed with prospectively collected specimens from asymptomatic subjects. Women were enrolled from six US clinical sites, including obstetrics and gynecology, family planning, and STD clinics. One vaginal swab, one endocervical swab, and one PreservCyt Solution liquid Pap specimen were collected from each subject. All specimens were clinician-collected. PreservCyt liquid Pap specimens were collected with a broom-type device or a spatula and cytobrush.

APTIMA *Trichomonas vaginalis* Assay testing was conducted in accordance with the package insert instructions. PANTHER System testing was conducted at three sites (two external laboratories and internally at Gen-Probe). TIGRIS DTS System testing was conducted at Gen-Probe.

Eighteen APTIMA *Trichomonas vaginalis* Assay runs were initiated on the PANTHER System; all were valid. A total of 227 vaginal swab, 227 endocervical swab, and 227 PreservCyt Solution liquid Pap specimens were tested. Of these specimens, one vaginal swab specimen (0.4%, 1/227) had an initial invalid result due to hardware error. The specimen with an initial invalid result was retested and had a valid result.

Of the samples with final valid APTIMA *Trichomonas vaginalis* Assay results on the PANTHER System, 227 vaginal swabs, 227 endocervical swabs, and 226 PreservCyt Solution liquid Pap specimens had valid, paired results on the TIGRIS DTS System.

The following table shows positive and negative percent agreements of APTIMA *Trichomonas vaginalis* Assay results on the PANTHER System and the TIGRIS DTS System in each specimen type for asymptomatic subjects.

Agreement between APTIMA Trichomonas vaginalis Assay Results on the PANTHER System and the TIGRIS DTS System in Asymptomatic Subjects

Specimen Type	n	TIGRIS + PANTHE R +	TIGRIS - PANTHE R +	TIGRIS - PANTHE R -	TIGRIS + PANTHE R -	TIGRIS Positivity	% Positive Agreement (95% CI) ²	% Negative Agreement (95% CI) ²
CVS ¹	227	29	5	191	2	13.7	93.5 (79.3-98.2)	97.4 (94.2-98.9)
ES	227	28	1	198	0	12.3	100 (87.9-100)	99.5 (97.2-99.9)
PCyt	226	26	1	199	0	11.5	100 (87.1-100)	99.5 (97.2-99.9)

+ = positive, - = negative, CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, PCyt = PreservCyt Solution liquid Pap,

¹The 2 vaginal swab samples with positive APTIMA Trichomonas vaginalis Assay results on the TIGRIS DTS System and negative results on the PANTHER System were from subjects whose other samples had negative results on both the PANTHER System and the TIGRIS DTS System.

²Score confidence interval.

b. Matrix comparison:

Not applicable

3. Clinical studies:

Clinical performance of the ATV Assay on the PANTHER System was evaluated using leftover specimens collected from consenting subjects during a previous, prospective, multicenter clinical study of the ATV Assay on the TIGRIS DTS System. Symptomatic and asymptomatic women were enrolled from nine US clinical sites, including obstetrics and gynecology, family planning, and STD clinics. Patients ≥ 14 years of age were enrolled. Three vaginal swabs, one endocervical swab, and one PreservCyt Solution liquid Pap specimen were collected from each subject. All specimens were clinician-collected.

PreservCyt liquid Pap specimens were collected with a broom-type device or a spatula and cytobrush. Two of the vaginal swab specimens were tested with a commercially available culture system and wet mount microscopic examination to establish infected status. The remaining specimens were prepared for APTIMA Trichomonas vaginalis Assay testing in accordance with the appropriate APTIMA specimen collection kit package insert instructions. PANTHER System testing with the APTIMA Trichomonas vaginalis Assay was conducted at three sites (two external laboratories and Gen-Probe) in accordance with package insert instructions.

Performance characteristics of the APTIMA Trichomonas vaginalis Assay were estimated by comparing results to a patient infected status algorithm. In the algorithm, the designation of a subject as being infected or non-infected with *T. vaginalis* was based on results from vaginal swab specimens tested by culture and/or wet mount microscopic examination. At least one of the reference test results was required to be positive to

establish an infected patient status. Both reference tests were required to be negative to establish a non-infected patient status.

Twenty-three APTIMA *Trichomonas vaginalis* Assay runs were initiated on the PANTHER System. Of these 23 runs, 1 (4.3%, 1/23) was aborted due to a fatal hardware error that led to a software failure. Specimens tested in the aborted run were retested. A total of 689 vaginal swab, 737 endocervical swab, and 791 PreservCyt Solution liquid Pap specimens were tested in the 22 valid runs. Of these specimens, 12 vaginal swab (1.7%, 12/689), 24 endocervical swab (3.3%, 24/737), and 29 PreservCyt Solution liquid Pap (3.7%, 29/791) specimens had initial invalid results due to hardware or software errors. Specimens with initial invalid results were retested. Eleven vaginal swab (1.6%, 11/689), 24 endocervical swab (3.3%, 24/737), and one PreservCyt Solution liquid Pap (0.1%, 1/791) specimens had final invalid results due to hardware or software errors; these specimens were excluded from the analyses.

The following table shows the sensitivity, specificity, PPV, and NPV of the APTIMA *Trichomonas vaginalis* Assay on the PANTHER System and the prevalence of *T. vaginalis* (based on the infected status) in each specimen type by symptom status and overall. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms. Prevalence was higher in symptomatic women.

Performance Characteristics of the APTIMA *Trichomonas vaginalis* Assay by Symptom Status

Specimen Type	Symptom Status	n	TP	FP ¹	TN	FN	Prev %	Sensitivity % (95% CI) ²	Specificity % (95% CI) ²	PPV % (95% CI) ³	NPV % (95% CI) ³
CVS	Asymptomatic	274	12	7 ^a	255	0	4.4	100 (75.8-100)	97.3 (94.6-98.7)	63.2 (45.8-80.9)	100 (98.8-100)
	Symptomatic	393	57	4 ^b	332	0	14.5	100 (93.7-100)	98.8 (97.0-99.5)	93.4 (84.9-98.1)	100 (98.9-100)
	All	667	69	11 ^c	587	0	10.3	100 (94.7-100)	98.2 (96.7-99.0)	86.3 (77.9-92.6)	100 (99.4-100)
ES	Asymptomatic	309	16	5 ^d	288	0	5.2	100 (80.6-100)	98.3 (96.1-99.3)	76.2 (58.1-90.8)	100 (98.9-100)
	Symptomatic	391	51	7 ^e	333	0	13.0	100 (93.0-100)	97.9 (95.8-99.0)	87.9 (78.1-94.7)	100 (99.0-100)
	All	700	67	12 ^f	621	0	9.6	100 (94.6-100)	98.1 (96.7-98.9)	84.8 (76.3-91.5)	100 (99.4-100)
PCyt	Asymptomatic	333	19	2 ^g	312	0	5.7	100 (83.2-100)	99.4 (97.7-99.8)	90.5 (72.6-98.7)	100 (98.9-100)
	Symptomatic	441	64	8 ^h	369	0	14.5	100 (94.3-100)	97.9 (95.9-98.9)	88.9 (80.4-94.9)	100 (99.1-100)
	All	774	83	10 ⁱ	681	0	10.7	100 (95.6-100)	98.6 (97.4-99.2)	89.2 (82.0-94.5)	100 (99.5-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, PCyt = PreservCyt Solution liquid Pap, Prev = prevalence, TN = true negative, TP = true positive.

¹*T. vaginalis* NAAT results from a previous study (# positive results / # samples tested): a: 4/7, b: 3/4, c: 7/11, d: 1/5, e: 2/7, f: 3/12, g: 0/2, h: 3/8, i: 3/10.

²Score confidence interval.

³PPV 95% confidence interval computed from the exact 95% confidence interval for the positive likelihood ratio, NPV 95% confidence interval computed from the exact 95% confidence interval from the negative likelihood ratio.

The following table shows the sensitivity, specificity, PPV, and NPV of the APTIMA *Trichomonas vaginalis* Assay on the PANTHER System and the prevalence of *T. vaginalis* (based on the infected status) in each specimen type by collection site. For each specimen type, performance was similar across collection sites.

Performance Characteristics of the APTIMA *Trichomonas vaginalis* Assay by Collection Site

Site	Specimen Type	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI) ¹	Specificity (95% CI) ¹	PPV % (95% CI) ²	NPV % (95% CI) ²
1	CVS	52	8	1	43	0	15.4	100 (67.6-100)	97.7 (88.2-99.6)	88.9 (60.2-99.7)	100 (93.7-100)
	ES	53	9	2	42	0	17.0	100 (70.1-100)	95.5 (84.9-98.7)	81.8 (56.9-97.4)	100 (93.5-100)
	PCyt	59	11	0	48	0	18.6	100 (74.1-100)	100 (92.6-100)	100 (75.6-100)	100 (93.9-100)
2	CVS	52	3	1	48	0	5.8	100 (43.9-100)	98.0 (89.3-99.6)	75.0 (28.5-99.2)	100 (95.8-100)
	ES	56	4	1	51	0	7.1	100 (51.0-100)	98.1 (89.9-99.7)	80.0 (40.5-99.4)	100 (95.6-100)
	PCyt	68	5	0	63	0	7.4	100 (56.8-100)	100 (94.3-100)	100 (58.3-100)	100 (98.0-100)
3	CVS	12	2	0	10	0	16.7	100 (34.2-100)	100 (72.2-100)	100 (32.1-100)	100 (85.6-100)
	ES	16	2	0	14	0	12.5	100 (34.2-100)	100 (78.5-100)	100 (31.5-100)	100 (89.3-100)
	PCyt	17	2	1	14	0	11.8	100 (34.2-100)	93.3 (70.2-98.8)	86.7 (19.9-98.8)	100 (89.5-100)
4	CVS	41	7	1	33	0	17.1	100 (64.6-100)	97.1 (85.1-99.5)	87.5 (57.3-99.6)	100 (92.2-100)
	ES	41	7	0	34	0	17.1	100 (64.6-100)	100 (89.8-100)	100 (66.7-100)	100 (92.2-100)
	PCyt	43	7	1	35	0	16.3	100 (64.6-100)	97.2 (85.8-99.5)	87.5 (57.2-99.6)	100 (92.6-100)
5	CVS	145	1	0	144	0	0.7	100 (20.7-100)	100 (97.4-100)	100 (6.4-100)	100 (99.3-100)
	ES	162	1	0	161	0	0.6	100 (20.7-100)	100 (97.7-100)	100 (6.4-100)	100 (99.4-100)
	PCyt	167	1	0	166	0	0.6	100 (20.7-100)	100 (97.7-100)	100 (6.4-100)	100 (99.4-100)
6	CVS	67	10	2	55	0	14.9	100 (72.2-100)	96.5 (88.1-99.0)	83.3 (59.2-98.2)	100 (94.8-100)
	ES	80	13	4	63	0	16.3	100 (77.2-100)	94.0 (85.6-97.7)	76.5 (57.1-92.2)	100 (95.3-100)
	PCyt	92	20	3	69	0	21.7	100 (83.9-100)	95.8 (88.5-98.6)	87.0 (70.4-97.0)	100 (95.5-100)
7	CVS	173	18	3	152	0	10.4	100 (82.4-100)	98.1 (94.5-99.3)	85.7 (67.7-96.7)	100 (97.9-100)
	ES	161	12	3	146	0	7.5	100 (75.8-100)	98.0 (94.2-99.3)	80.0 (58.3-95.4)	100 (97.9-100)
	PCyt	194	18	4	172	0	9.3	100 (82.4-100)	97.7 (94.3-99.1)	81.8 (64.1-94.3)	100 (98.1-100)
8	CVS	80	10	2	68	0	12.5	100 (72.2-100)	97.1 (90.2-99.2)	83.3 (59.0-98.2)	100 (95.8-100)
	ES	83	9	2	72	0	10.8	100 (70.1-100)	97.3 (90.7-99.3)	81.8 (56.3-97.4)	100 (96.1-100)
	PCyt	86	9	0	77	0	10.5	100 (70.1-100)	100 (95.2-100)	100 (71.4-100)	100 (96.2-100)
9	CVS	45	10	1	34	0	22.2	100 (72.2-100)	97.1 (85.5-99.5)	90.9 (65.7-99.7)	100 (91.9-100)
	ES	48	10	0	38	0	20.8	100 (72.2-100)	100 (90.8-100)	100 (74.0-100)	100 (92.5-100)
	PCyt	48	10	1	37	0	20.8	100 (72.2-100)	97.4 (86.5-99.5)	90.9 (65.6-99.7)	100 (92.5-100)

CI = confidence interval, CVS = clinician-collected vaginal swab, ES = endocervical swab, FN = false negative, FP = false positive, PCyt = PreservCyt Solution liquid Pap. Prev = prevalence, TN = true negative, TP = true positive.
¹Score confidence interval.
²PPV 95% confidence interval computed from the exact 95% confidence interval for the positive likelihood ratio, NPV 95% confidence interval computed from the exact 95% confidence interval from the negative likelihood ratio.

The following table shows the sensitivity, specificity, PPV, and NPV of the APTIMA *Trichomonas vaginalis* Assay on the PANTHER System and the prevalence of *T. vaginalis* (based on the infected status) in PreservCyt Solution liquid Pap specimens by cervical collection device. For PreservCyt Solution liquid Pap specimens, performance was similar across collection devices.

Performance Characteristics of the APTIMA Trichomonas vaginalis Assay in PreservCyt Solution Liquid Pap Specimens by Collection Device Type

Collection Device	n	TP	FP	TN	FN	Prev %	Sensitivity (95% CI) ¹	Specificity (95% CI) ¹	PPV % (95% CI) ²	NPV % (95% CI) ²
Broom-type Device	414	54	5	355	0	13.0	100 (93.4-100)	98.6 (96.8-99.4)	91.5 (82.4-97.1)	100 (99.0-100)
Spatula/Cytobrush	360	29	5	326	0	8.1	100 (88.3-100)	98.5 (96.5-99.4)	85.3 (71.5-94.7)	100 (99.0-100)

CI = confidence interval, FN = false negative, FP = false positive, Prev = prevalence, TN = true negative, TP = true positive.

¹Score confidence interval.

²PPV 95% confidence interval computed from the exact 95% confidence interval for the positive likelihood ratio, NPV 95% confidence interval computed from the exact 95% confidence interval from the negative likelihood ratio.

The distribution of the RLU values for the APTIMA Trichomonas vaginalis Negative Control and the APTIMA Trichomonas vaginalis Positive Control from all valid APTIMA Trichomonas vaginalis Assay runs performed during the clinical performance study of the APTIMA Trichomonas vaginalis Assay on the PANTHER System is presented in the table below.

RLU Distribution of APTIMA Trichomonas vaginalis Negative and Positive Controls

Control	Statistic	Total RLU (x1000)
Negative	N	22
	Mean	1.3
	SD	0.99
	Median	1.0
	Minimum	0
	Maximum	5
	CV%	75.5
Positive	N	22
	Mean	1262.3
	SD	45.89
	Median	1276.0
	Minimum	1168
	Maximum	1322
	CV%	3.6

4 Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

A summary of the prevalence of *T. vaginalis*, by specimen type, as determined by the APTIMA Trichomonas vaginalis Assay during the PANTHER System clinical study is shown in the following table.

Prevalence of *T. vaginalis* as Determined by the APTIMA Trichomonas vaginalis Assay by Specimen Type and Collection Site

Specimen Type	% (# positive / # tested)									
	All Sites	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
CVS	11.8 (80/678)	17.0 (9/53)	7.7 (4/52)	16.7 (2/12)	19.5 (8/41)	0.7 (1/145)	16.0 (12/75)	12.0 (21/175)	15.0 (12/80)	24.4 (11/45)
ES	11.2 (80/713)	20.4 (11/54)	8.9 (5/56)	12.5 (2/16)	17.1 (7/41)	0.6 (1/162)	20.2 (18/89)	9.1 (15/164)	13.3 (11/83)	20.8 (10/48)
PCyt	11.8 (93/790)	18.3 (11/60)	7.4 (5/68)	17.6 (3/17)	18.6 (8/43)	0.6 (1/167)	22.1 (23/104)	11.2 (22/197)	10.5 (9/86)	22.9 (11/48)

CVS = clinician-collected vaginal swab, ES = endocervical swab, PCyt = PreservCyt Solution liquid Pap.

N. Instrument Name:

The PANTHER System

O. System Descriptions:

1. Modes of Operation:

Batch, random access

2. Software:

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

Yes or No

3. Specimen Identification:

By handheld barcode reader and positional checks

4. Specimen Sampling and Handling:

Fully automated

5. Calibration:

Gen-Probe Field Service Engineers perform a luminometer calibration on the PANTHER System every 12 months as part of the Preventive Maintenance. Also, there are process controls and calibration checks on all of the dispensers, thermal devices, and the vacuum system.

6. Quality Control:

In addition to the assay controls that are specific to each assay, the PANTHER System contains process controls that employ both hardware and software components. The process controls include, but are not limited to:

- Verification that the sequence of assay processing steps is correct for each reaction.
- Verification that the reaction incubation times and temperatures are correct.
- Verification that reagents and fluids are appropriately dispensed.

P. ~~Other Supportive Instrument Performance Characteristics Data Not Covered in the~~ “Performance Characteristics” Section above:

Not Applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.