510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K143329

B. Purpose for Submission:

To obtain clearance for a new device, Amplivue® Trichomonas Assay

C. Measurand:

A conserved multi-copy sequence of *Trichomonas vaginalis* genomic DNA

D. Type of Test:

Nucleic acid amplification assay (Helicase-dependent Amplification, HDA)

E. Applicant:

Quidel Corporation

F. Proprietary and Established Names:

Amplivue® Trichomonas Assay

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(s):

   The AmpliVue® Trichomonas Assay is an in vitro diagnostic test, uses isothermal amplification technology (helicase-dependent amplification, HDA) for the qualitative detection of *Trichomonas vaginalis* nucleic acids isolated from clinician-collected vaginal swab specimens obtained from symptomatic or asymptomatic females to aid in the diagnosis of trichomoniasis.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

The AmpliVue® Trichomonas Assay is a self-contained disposable amplicon detection device that uses an isothermal amplification technology named Helicase-Dependent Amplification (HDA) for the detection of *Trichomonas vaginalis* in clinician-collected vaginal swabs from symptomatic and asymptomatic women. The assay targets a conserved multi-copy sequence of the *T. vaginalis* genomic DNA.

The vaginal swab is eluted in a lysis tube, and the cells are lysed by heat treatment. After heat treatment, an aliquot of the lysed specimen is transferred into a dilution tube. An aliquot of this diluted sample is then added to a reaction tube containing a lyophilized mix of HDA reagents including primers specific for the amplification of a conserved DNA sequence only found in *T. vaginalis*. The assay includes an internal control for monitoring the integrity of the assay reagents and detection cassette as well as for monitoring HDA-inhibitors that may be present within the clinical specimens. The HDA reaction is asymmetric generating an excess of single-stranded DNA amplicons. The sequence specific capture probes as well as a biotinylated detection probe shared by both target and internal control bind to the corresponding single-stranded amplicons, forming dual labeled probe-amplicon hybrid.

After completion of the HDA reaction, the reaction tube is transferred to a cassette for rapid detection and test result display. The dual-labeled probe-amplicon hybrid is detected by the lateral flow strip within the cassette. The bottom line captures the *T. vaginalis* amplicon and the top line captures the control amplicon. The biotin label binds the streptavidin-conjugated color particles for visualization and the test result is shown as a visible colored lines.

The cassette is comprised of two individual components: an amplicon cartridge that holds the running buffer and a single 0.2 mL thin wall reaction tube containing the amplified product; and the detection chamber which houses the amplicon cartridge and a vertical-flow DNA detection strip embedded into the cassette. The DNA detection strip is coated with different anti-hapten antibodies that serve as the *T. vaginalis* test (T) line and the control (C) line in the assay. A razor blade and a plastic pin located at the bottom of the detection chamber open the HDA reaction tube and the running buffer bulb when the handle of the cassette is closed. The mixture flows through a fiberglass paper connected to the DNA detection strip containing a fiberglass pad pre-loaded with streptavidin-conjugated color particles for color visualization. Detection of *T. vaginalis* DNA is reported whenever the T2 (Test line 2) is visible through the detection window of the cassette. The presence of T1 line is an invalid result for this assay and the test should be repeated with the lysed specimen. The presence of the C line is not required for positive results. No detection of *T. vaginalis* DNA is reported when only the C line is displayed. The assay is regarded as invalid when neither line is displayed.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   APTIMA Trichomonas vaginalis Assay (PANTHER® System)

2. Predicate 510(k) number(s):

   K122062

3. Comparison with predicate:

Similarities		
Item	Device **AmpliVue® Trichomonas Assay**	Predicate **APTIMA Trichomonas vaginalis Assay (PANTHER® System) (K122062)**
Intended Use	The AmpliVue® Trichomonas Assay is an in vitro diagnostic test, uses isothermal amplification technology (helicase-dependent amplification, HDA) for the qualitative detection of *Trichomonas vaginalis* nucleic acids isolated from clinician-collected vaginal swab specimens obtained from symptomatic or asymptomatic females to aid in the diagnosis of trichomoniasis.	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.
Assay Results	Qualitative	Qualitative

Differences		
Item	Device **AmpliVue® Trichomonas Assay**	Predicate **APTIMA Trichomonas vaginalis Assay (PANTHER® System) (K122062)**
Sample Types	Clinician-collected Vaginal Swabs	Clinician-collected Vaginal Swabs, Endocervical Swabs, ThinPrep in PreservCyt solution
Target Sequence Detected	Repeated DNA fragment located in *T. vaginalis* genome	*T. vaginalis* ribosomal RNA (rRNA)
Amplification Technology	Helicase-dependent amplification (HDA)	Transcription Mediated Amplification (TMA) Hybridization Protection Assay (HPA)
Self-Contained System Assay after sample preparation	No	Yes
Detection Technique	Manual	Automated
Instrument	None	PANTHER System

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

None

L. Test Principle:

The AmpliVue® Trichomonas Assay uses HAD to detect *T. vaginalis* genomic DNA in vaginal swab specimens. The vaginal swab is eluted in a lysis tube, and the cells are lysed by heat treatment. An aliquot of the lysed specimen is transferred into a dilution tube which is then added to a reaction tube containing a lyophilized mix of HDA reagents including primers specific for the amplification of a conserved DNA sequence of *T. vaginalis*. The reaction tube also includes an internal control to confirm the integrity of the assay reagents and cassette detection as well as to monitor for HDA-inhibitors that may be present within the clinical specimens. The sequence specific capture probes as well as a biotinylated detection probe are shared by *T. vaginalis* target sequences and the internal control bind to the corresponding single-stranded amplicons (product of HDA reaction), forming a dual labeled probe-amplicon hybrid.

After completion of the HDA reaction, the reaction tube is transferred to a cassette for rapid detection. The test result is displayed in the window of the cassette as test and/or control colored lines visible to the naked eye.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   With-in laboratory Precision

   With-in laboratory precision for AmpliVue® Trichomonas Assay was determined via a study, where a four-member panel (3x LoD (Moderate positive), 1x LoD (Low positive), 1/9x LoD (High negative, C_{20} to C_{80}), and a negative sample) was tested at one site in a random manner by two operators, three samples per concentration, twice a day for 12 days.

   All negative samples generated negative results for *T. vaginalis*. The percent agreement with positive results for High negative samples is 39% (within the target range of 20 to 80%). A 100% agreement was observed with expected results for Low positive and Moderate positive samples.

Concentration	Operator #1			Operator #2			Overall Percent Agreement		95% Confidence Interval
	#expected results/# tested	*% Agreement*	95% Confidence Interval	*#expected results/# tested*	*% Agreement*	95% Confidence Interval			
High Negative* (34 trophozoites /mL)	11/36	31%	18.0% to 46.9%	17/36	47%	32.0% to 63.0%	28/72	39%	28.5% to 50.4%
Low Positive (307 trophozoites /mL)	36/36	100%	90.4% to 100%	36/36	100%	90.4% to 100%	72/72	100%	94.9% to 100%
Moderate Positive (921 trophozoites /mL)	36/36	100%	90.4% to 100%	36/36	100%	90.4% to 100%	72/72	100%	94.9% to 100%
Negative	36/36	100%	90.4% to 100%	36/36	100%	90.4% to 100%	72/72	100%	94.9% to 100%
Positive Control	36/36	100%	90.4% to 100%	36/36	100%	90.4% to 100%	72/72	100%	94.9% to 100%
Negative Control	36/36	100%	90.4% to 100%	36/36	100%	90.4% to 100%	72/72	100%	94.9% to 100%

* The expected detection rate for High Negative sample was 20% to 80%.

Reproducibility

In order to evaluate the reproducibility of the AmpliVue® Trichomonas Assay a blinded and randomized study was performed at three test sites (one in-house laboratory and two clinical sites) with four-member panel containing *T. vaginalis* positive samples: 3× LoD (Moderate positive), 1× LoD (Low positive), 1/9× LoD (C_{20} to C_{80}, High negative), and a negative sample. The reproducibility panel and Assay Controls were tested by two operators for five days in triplicate at each site. The AmpliVue® Trichomonas Assay generated reproducible results presented in the table below.

Concentration	SITE									Overall Percent Agreement		95% CI
	Site #1			Site #2			Site #3					
	#expected results /# tested	*% Agreement*	95% CI	*#expected results /# tested*	*% Agreement*	95% CI	*#expected results/ # tested*	*% Agreement*	95% CI			
High Negative* (34 trophozoites	9/30	30%	16.7% to 47.9%	8/30	27%	14.2% to 47.9%	19/30	63%	45.5% to 78.1%	36/90	40 %	30.5% to 50.3%

Concentration	SITE									Overall Percent Agreement		95% CI
	Site #1			Site #2			Site #3					
	#expected results /# tested	*% Agreement*	95% CI	*#expected results /# tested*	*% Agreement*	95% CI	*#expected results/ # tested*	*% Agreement*	95% CI			
/mL)												
Low Positive (307 trophozoites /mL)	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%
Moderate Positive (921 trophozoites /mL)	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%
Negative	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%
Positive Control	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%
Negative Control	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%

* The expected detection rate for High Negative sample was 20% to 80%.

b. Linearity/assay reportable range:

Not applicable

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

Specimen Stability:
Two transport media (Stuart Transport Medium and Amies Transport Medium) were tested in this study. *T. vaginalis* strain G3 was inoculated into the above mentioned transport media containing negative vaginal matrix at 2× LoD and 100× LoD, and stored at either 2-8°C or at room temperature (25±2°C) for up to 8 days. Each run tested 20 specimens at 2× LoD levels, two specimens at 100× LoD levels at each time point as well as assay positive and negative controls. Based on the data generated by this study, specimens may be stored in both transport media between 2° to 8°C for 7 days and up to 2 days at room temperature (25±2°C) prior to testing.

Processed Specimen Stability:
This study was performed to determine the stability of the sample eluted in Lysis Buffer of AmpliVue® Trichomonas Assay at room temperature and 2-8°C.
Panel members were prepared by inoculating *T. vaginalis* into Stuart Transport Medium containing negative vaginal matrix at 1× LoD and 2× LoD. Three samples of each panel members were added to Lysis buffer in duplicate; one set was tested and

stored at room temperature and one set was tested and stored at 2-8°C. Each set was further processed, amplified and detected at times 0, 24, 48, 72, and 73 hours. The resulting data showed that the sample eluted into the Lysis buffer was stable at both room temperature and 2-8°C for up to 72 hours.

Controls:
Controls were run on the AmpliVue® Trichomonas Assay each day of testing. Quidel Molecular Trichomonas Control Set #M119 contains positive and negative controls and serves as an external processing and extraction control. These controls are described as follows:

a. The internal control is used to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and cassette detection. The internal control is included in the reaction tube.

b. The External assay positive control listed in "Materials Required But Not Provided" in the Package Insert contains intact non-viable trophozoites and has titers near the limit of detection for the assay. The external assay positive control is intended for monitoring substantial reagent and cassette failure.

c. The External assay negative control listed in "Materials Required But Not Provided" is the same matrix as the positive control, but is trophozoite-free. The external assay negative control is intended for detection of reagent or environment contamination or carry-over by either *T. vaginalis* DNA or amplicons.

d. Detection limit:

The limit of detection (LoD) of the AmpliVue® Trichomonas Assay was determined using quantified (trophozoite/mL) stocks of two *T. vaginalis* strains, one metronidazole-susceptible G3 and one metronidazole-resistant CDC888 serially diluted in negative vaginal matrix. The LoD is defined as the lowest concentration at which 95% of all replicates tested positive.

The strains were freshly grown and quantified using a hemocytometer. The cells were serially diluted in Liquid Stuart medium with negative vaginal matrix at five concentrations in the preliminary LoD determination study. Each dilution was tested as 10 replicates on three reagent lots.

LoD was confirmed by testing each reference strain with 20 replicates on three reagent lots in the negative vaginal matrix.

The assay LoD for *T. vaginalis* strain G3 is 307 trophozoites/mL and for strain CDC888 is 921 trophozoites/mL.

e. Reactivity:

A study was performed to verify the *in silico* inclusivity results with functional testing of the AmpliVue® Trichomonas Assay using 20 additional strains of *Trichomonas vaginalis* tested in triplicate at concentrations near the LoD.

Bacterial Strain	Strain Detected (Yes/No)
CDC899	Yes
CDC938	Yes
CDC963	Yes
CDC1031	Yes
CDC1256	Yes
PMGH25	Yes
BUSH20	Yes
CDC911	Yes
MOR31	Yes
CDC1080	Yes
B7708/1839	Yes
F1623	Yes
CDC1095	Yes
SD1	Yes
SA-384	Yes
CDC948	Yes
SD10	Yes
SA-A53	Yes
CDC1230	Yes
SA-A19	Yes

The inclusivity study demonstrated that all *T. vaginalis* strains listed above were detected by AmpliVue® Trichomonas Assay.

f. Analytical specificity:

Cross-reactivity:

A study was performed to evaluate the cross-reactivity of the AmpliVue® Trichomonas Assay with 45 microorganisms (36 bacteria, 4 yeasts, 4 viruses, 1 parasite) potentially found in specimens collected for testing *T. vaginalis* infection. Cross-reactive microorganisms (or DNA/RNA) were tested at clinically relevant levels of viruses ($\geq 10^5$pfu/mL or genome copies/mL), bacteria, yeast, and parasite ($\geq 10^6$cfu/mL or genome copies/mL). All organisms were diluted in Liquid Stuart medium and tested in negative vaginal matrix in triplicate using the AmpliVue® Trichomonas assay. The organisms included in the cross-reactivity study and their tested concentrations are shown in the table below.

Microorganism	**Concentration Tested**
Acinetobacter lwoffi	4.55×10^6 CFU/mL
Actinomyces israelii	6.63×10^6 CFU/mL
Atopobium vaginae	3.60×10^6 CFU/mL
Bacteroides fragilis	4.2×10^6 CFU/mL
Bifidobacterium adolescentis	1.00×10^6 CFU/mL
Campylobacter jejuni	1.72×10^6 CFU/mL
Candida albicans	2.00×10^6 CFU/mL
Candida glabrata	7.87×10^6 CFU/mL
Candida parapsilosis	2.87×10^6 CFU/mL
Candida tropicalis	2.15×10^6 CFU/mL
Chlamydia trachomatis	7.83×10^6 CFU/mL
Clostridium difficile	6.77×10^6 CFU/mL
Clostridium perfringens	1.06×10^6 CFU/mL
Corynebacterium genitalium	3.61×10^6 CFU/mL
Cryptococcus neoformans	1.92×10^6 CFU/mL
Enterobacter aerogenes	1.18×10^6 CFU/mL
Enterococcus faecalis	2.20×10^6 CFU/mL
Escherichia coli	1.13×10^6 CFU/mL
Fusobacterium nucleatum	8.05×10^6 CFU/mL
Gardnerella vaginalis	1.20×10^6 CFU/mL
Haemophilus ducreyi	2.97×10^6 genome copies/mL
HIV-1 Subtype B RNA	1.14×10^6 genome copies/mL
Herpes simplex virus I	7.96×10^6 TCID50/mL
Herpes simplex virus II	2.27×10^5 TCID50/mL
HPV 16 (SiHa)	4.3×10^6 genome copies/mL
Klebsiella oxytoca	1.63×10^6 CFU/mL
Lactobacillus acidophilus	2.00×10^6 CFU/mL
Lactobacillus jensenii	4.06×10^6 CFU/mL
Lactobacillus vaginalis	1.11×10^6 CFU/mL
Listeria monocytogenes	6.13×10^6 CFU/mL
Mobiluncus curtisii	3.2×10^6 CFU/mL
Mycoplasma hominis	1.30×10^6 CFU/mL
Neisseria gonorrhoeae	3.20×10^6 CFU/mL
Pentatrichomonas hominis	4.5×10^6 CFU/mL
Peptostreptococcus anaerobius	8.1×10^6 genome copies/mL
Prevotella bivia	3.01×10^6 CFU/mL
Propionibacterium acnes	6.63×10^6 CFU/mL
Proteus mirabilis	1.19×10^6 CFU/mL
Pseudomonas aeruginosa	1.32×10^6 CFU/mL
Staphylococcus aureus MRSA	7.52×10^6 CFU/mL

Microorganism	Concentration Tested
Staphylococcus epidermidis MRSE	1.75×10^6 CFU/mL
Streptococcus pyogenes	6.38×10^6 CFU/mL
Streptococcus agalactiae	2.20×10^6 CFU/mL
Trichomonas tenax	6.3×10^6 CFU/mL
Ureaplasma urealyticum	1.23×10^6 genome copies/mL

No cross-reactivity was observed with the AmpliVue® Trichomonas Assay when testing any of the 45 microorganisms.

Microbial Interference:

The 45 microorganisms tested in cross-reactivity study were evaluated in the presence of each of the two *T. vaginalis* strains (G3 and CDC888) at 2x LoD level. Each microorganism was diluted in Liquid Stuart medium to the desired concentration (10^6 or higher CFU/mL or genome copies/mL for bacteria, yeast, parasite or DNA/RNA and 10^5 or higher pfu/mL or TCID50/mL or genome copies/mL for viruses), and tested in triplicate in negative vaginal matrix. No interference was observed with the detection of each of the two *T. vaginalis* strains in the AmpliVue® Trichomonas Assay.

g. Interference:

A study was conducted to determine whether the AmpliVue® Trichomonas Assay is inhibited in the presence of 13 substances potentially present in specimens collected for *T. vaginalis* infection testing. Each of the potential interfering substances was tested in three replicates in the presence and absence of *T. vaginalis* strains G3 and CDC888 at 2x LoD in a negative vaginal matrix. Substances were tested at medically relevant concentrations. No interference was observed by the substances tested and listed in the table below.

Substances	Final Conc.
K-Y Personal Lubricant Jelly	1%
Ortho Options Gynol II Extra Strength Vaginal Contraceptive Jelly	1%
Summer's Eve Ultra Extra Strength Feminine Deodorant Spray	1%
Vagisil Creme Maximum Strength	1%
Estradiol	1%
Mucin from Porcine Stomach	1%
Glacial acetic acid	1%

Substances	Final Conc.
CVS Vinegar & Water Extra Cleansing Disposable Douche	1%
Seminal fluid	1%
Whole blood with EDTA	10%
Summer's Eve Douche, Medicated	1%
Acyclovir (Acycloguanosine)	5% (w/v), active concentration in Zovirax cream
	1% of active ingredient of Zovirax cream
Metronidazole	0.75% (w/v), active concentration in Vandazole gel
	1% of active ingredient of Vandazole gel

h. Assay cut-off:

Not applicable

i. Carryover-Cross Contamination:

In the Carryover-Cross Contamination study High positive samples (containing approximately 2.5x 10^6 trophozoites/mL) were tested in series alternating with negative samples, and a minimum of five runs were performed between two operators. For each run, five replicates of *T. vaginalis* high positive sample in Liquid Stuart medium alternating with five replicates of negative Liquid Stuart medium were tested in the AmpliVue® Trichomonas Assay along with an external positive control and an external negative control. In total, five runs consisting of five samples positive for *T. vaginalis* and five negative samples were tested by two operators for a total of 50 positive and 50 negative samples.

Consecutive testing of alternating *T. vaginalis* high positive samples and *T. vaginalis* negative samples resulted in no carry over or cross contamination.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

A multi-center study was conducted to evaluate the AmpliVue® Trichomonas Assay from April to November 2014. A total of 992 clinician-collected vaginal swab specimens were obtained from symptomatic (n=342) or asymptomatic (n=650) patients at four locations in the United States and one location in Canada. Specimens were obtained from each subject after informed consent was obtained.

For each subject, three vaginal specimens were collected using polyester or rayon Swabs w/ Liquid Stuart's, and one vaginal specimen collected with a collection swab from a FDA-cleared molecular device. The four clinician collected vaginal swabs were used for reference testing and AmpliVue® Trichomonas Assay. The first two polyester/rayon swabs were randomized, one swab was tested for the Wet Mount (reference method) and the other swab was used for the InPouch TV Culture (reference method). The third swab was used for testing the AmpliVue® Trichomonas Assay**.** The FDA-cleared molecular device collection swab was used for discordant testing.

All sensitivity and specificity calculations were based on a composite reference method of Wet Mount and InPouch TV culture. The specimen was considered as positive for *T. vaginalis* if either the wet mount or the *T. vaginalis* culture was positive. The specimen was considered negative for *T. vaginalis* if both of the reference methods were negative.

One specimen was removed from the study due to a delay in the culture inoculation. Eight specimens yielded invalid results upon initial testing with the AmpliVue® Trichomonas Assay (0.8%). Upon re-testing six of the specimens yielded valid results (5 negative and 1 positive results). Two specimens yielded a second invalid result (0.2%). The table below shows the sensitivity, specificity, PPV, and NPV of the AmpliVue® Trichomonas Assay and the prevalence of *T. vaginalis* (by asymptomatic, symptomatic status and combined) in the study population.

Performance Characteristics of the AmpliVue® Trichomonas Assay by Symptom Status compared to the Composite Reference Method

Site Number	Symptom Status	N	TP	FP	TN	FN	Prev%	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Combined	Asymptomatic	647	61	10	576	0	9.4	100 (94.1 to 100)	98.3 (96.9 to 99.1)	85.9 (76.0 to 92.2)	100 (99.3 to 100)
	Symptomatic	342	59	6	277	0	17.3	100 (93.9 to 100)	97.9 (95.5 to 99.0)	90.8 (81.3 to 95.7)	100 (98.6 to 100)
	All	989	120	16*	853	0	12.1	100 (96.9 to 100)	98.2 (97.0 to 98.9)	88.2 (81.7 to 92.6)	100 (99.6 to 100)

Performance Characteristics of the AmpliVue® Trichomonas Assay by Symptom Status compared to the Composite Reference Method											
Site Number	Symptom Status	N	TP	FP	TN	FN	Prev%	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Site 1	Asymptomatic	133	26	3	104	0	19.5	100 (87.1 to 100)	97.2 (92.1 to 99.0)	89.7 (73.6 to 96.4)	100 (96.4 to 100)
	Symptomatic	163	27	2	134	0	16.6	100 (87.5 to 100)	98.5 (94.8 to 99.6)	93.1 (78.0 to 98.1)	100 (97.2 to 100)
	All	296	53	5	238	0	17.9	100 (93.2 to 100)	97.9 (95.3 to 99.1)	91.4 (81.4 to 96.3)	100 (98.4 to 100)
Site 2	Asymptomatic	46	5	1	40	0	10.9	100 (56.6 to 100)	97.6 (87.4 to 99.6)	83.3 (43.6 to 97.0)	100 (91.2 to 100)
	Symptomatic	69	17	1	51	0	24.6	100 (81.6 to 100)	98.1 (89.9 to 99.7)	94.4 (74.2 to 99.0)	100 (93.0 to 100)
	All	115	22	2	91	0	19.1	100 (85.1 to 100)	97.8 (92.5 to 99.4)	91.7 (74.2 to 97.7)	100 (95.9 to 100)
Site 3	Asymptomatic	206	20	3	183	0	9.7	100 (83.9 to 100)	98.4 (95.4 to 99.4)	87.0 (67.9 to 95.5)	100 (97.9 to 100)
	Symptomatic	41	7	2	32	0	17.1	100 (64.6 to 100)	94.1 (80.9 to 98.4)	77.8 (45.3 to 93.7)	100 (89.3 to 100)
	All	247	27	5	215	0	10.9	100 (87.5 to 100)	97.7 (94.8 to 99.0)	84.4 (68.2 to 93.1)	100 (98.2 to 100)
Site 4	Asymptomatic	260	10	3	247	0	3.8	100 (72.2 to 100)	98.8 (96.5 to 99.6)	76.9 (49.7 to 91.8)	100 (98.5 to 100)
	Symptomatic	35	3	1	31	0	8.6	100 (43.8 to 100)	96.9 (84.3 to 99.4)	75.0 (30.1 to 95.4)	100 (89.0 to 100)
	All	295	13	4	278	0	4.4	100 (77.2 to 100)	98.6 (96.4 to 99.4)	76.5 (52.7 to 90.4)	100 (98.6 to 100)
Site 5	Asymptomatic	2	0	0	2	0	0	N/A	100 (34.2 to 100)	N/A	100 (34.2 to 100)
	Symptomatic	34	5	0	29	0	14.7	100 (56.6 to 100)	100 (88.3 to 100)	100 (56.6 to 100)	100 (88.3 to 100)
	All	37	5	0	31	0	13.5	100 (56.6 to 100)	100 (89.0 to 100)	100 (56.6 to 100)	100 (89.0 to 100)

* Eight (8) of sixteen (16) Composite reference negative/AmpliVue positive specimens were positive by a FDA-cleared *Trichomonas vaginalis* molecular device.

*b. Clinical specificity**:***

See section M 3a.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The prevalence of *T. vaginalis* (by asymptomatic, symptomatic status, and combined) detected by the AmpliVue® Trichomonas Assay in the multi-center study was calculated and is provided in the table below.

Symptom Status	All Sites Combined	Site 1	Site 2	Site 3	Site 4	Site 5
Asymptomatic	11.0%	21.8%	12.8%	11.2%	5.0%	0.0%
Symptomatic	19.0%	17.8%	26.1%	22.0%	11.4%	14.7%
Combined	13.7%	19.6%	20.7%	13.0%	5.8%	13.5%

N. Proposed Labeling:

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

O. Conclusion:

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