DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

Quidel Corporation
Ronald H. Lollar
Senior Director, Clinical and Regulatory Affairs
2005 East State Street, Suite 100
Athens OH 45701

March 17, 2015

Re: K143329
Trade/Device Name: Amplivue® Trichomonas Assay
Regulation Number: 21 CFR 866.3860
Regulation Name: *Trichomonas vaginalis* nucleic acid amplification test system
Regulatory Class: II
Product Code: OUY
Dated: February 18, 2015
Received: February 19, 2015

Dear Mr. Lollar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Sally A. Hojvat -S

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K143329

Device Name
AmpliVue® Trichomonas Assay

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Applicant:

Quidel Corporation
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San Diego, California 92130
Telephone: 858-552-7910
Fax: 858-646-8045

Contact Information:

Ronald H. Lollar, Senior Director Clinical and Regulatory Affairs
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Athens, Ohio 45701
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Ron.Lollar@quidel.com

Date of preparation of 510(k) summary:

November 17, 2014

A. 510(k) Number:

K143329

B. Purpose for Submission:

To obtain substantial equivalence for the AmpliVue® Trichomonas Assay

C. Measurand:

Repeated DNA fragment located in *T. vaginalis* genome

D. Type of Test:

Helicase-dependent amplification (HDA)

510(k) Summary

E. Applicant:

Quidel Corporation

F. Proprietary and Established Names:

AmpliVue® Trichomonas Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OUY – *Trichomonas vaginalis* nucleic acid amplification test system	Class II	21 CFR 866.3860	Microbiology (83)

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 *in vitro* diagnostic use only
   - For prescription use only

4. Special instrument requirements:

   None

510(k) Summary

I. Device Description:

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

The vaginal swab is eluted in a lysis tube, and the cells are lysed by simple heat treatment. After heat treatment, an aliquot of the lysed specimen is transferred into a dilution tube. An aliquot of the diluted sample is 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 also includes an internal control to confirm the integrity of the assay reagents and cassette detection as well as to control for (or determine whether) HDA-inhibitors that may be present within the clinical specimens. The HDA reaction is asymmetric so that an excess of single stranded DNA (amplicon) is formed. 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 with the test result displayed as test and/or control lines in the window of the cassette. The dual-labeled probe-amplicon hybrid is then detected by the lateral flow strip within the cassette. The bottom line captures the test 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 colored lines visible to the naked eye.

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 opens 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 that contains 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 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.

510(k) Summary

Materials Provided:

AmpliVue® Trichomonas Assay Kit: M211
16 Tests per kit

Component	Quantity	Storage
Detection Cassettes	16/kit	2° to 30°C
Lysis Buffer Tubes (1.0 mL solution/tube)	16 tubes/kit	2° to 8°C
Dilution Buffer Tubes (1.5 mL solution/tube)	16 tubes/kit,	2° to 8°C
Reaction Tubes	16 tubes/kit	2° to 8°C
Amplicon Cartridge	16/kit	2° to 30°C

Materials required but not provided:

- External controls for *Trichomonas vaginalis* (e.g. Quidel Molecular Trichomonas Assay Control Kit: M119, which contains positive and negative controls. This positive control contains intact non-viable, trophozoites and has been titered to be near the limit of detection for the assay. This negative control is the same matrix as the positive control, but is trophozoite-free. These controls serve as an external processing and extraction control
- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Scissors or a blade
- Micro tube tray
- Heat block capable of 95° C ± 2° C temperature
- Heat block with heated lid capable of 64° ± 1° C temperature
- Thermometer

J. Substantial Equivalence Information:

1. Predicate device name(s):

   APTIMA Trichomonas vaginalis Assay (PANTHER® System)

2. Predicate 510(k) number(s):
   K122062

510(k) Summary

3. Comparison with predicate:

Similarities		
Item	**AmpliVue® Trichomonas Assay**	**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.

510(k) Summary

Differences		
Item	**AmpliVue® Trichomonas Assay**	**APTIMA Trichomonas vaginalis Assay (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*
Performance Characteristics	Clinician-collected Vaginal Specimens: <u>Asymptomatic</u> Sensitivity 100% (95% CI 94.1 – 100) Specificity 98.3% (95% CI 96.9 – 99.1) <u>Symptomatic</u> Sensitivity 100% (95% CI 93.9 – 100) Specificity 97.9% (95% CI 95.5 – 99.0)	Clinician-collected Vaginal Specimens: <u>Asymptomatic</u> Sensitivity 100% (95% CI 75.8 – 100) Specificity 97.3% (95% CI 94.6 – 98.7) <u>Symptomatic</u> Sensitivity 100% (95% CI 93.7 – 100) Specificity 98.8% (95% CI 97.0 – 99.5)

510(k) Summary

K. Test Principle:

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

The vaginal swab is eluted in a lysis tube, and the cells are lysed by simple heat treatment. After heat treatment, an aliquot of the lysed specimen is transferred into a dilution tube. An aliquot of the diluted sample is 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 also includes an internal control to confirm the integrity of the assay reagents and cassette detection as well as to control for (or determine whether) HDA-inhibitors that may be present within the clinical specimens. The HDA reaction is asymmetric so that an excess of single stranded DNA (amplicon) is formed. 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 with the test result displayed as test and/or control lines in the window of the cassette. The dual-labeled probe-amplicon hybrid is then detected by the lateral flow strip within the cassette. The bottom line captures the test 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 colored lines visible to the naked eye.

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 opens 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 that contains 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 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.

510(k) Summary

L. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility

In order to confirm the reproducibility of the AmpliVue Trichomonas Assay a blinded and randomized study four-member panel containing *Trichomonas vaginalis* positive samples (3× LoD, 1× LoD, 1/9× LoD) and a negative sample were tested at three (3) test sites (one in-house laboratory and two (2) clinical sites). Each site tested a reproducibility panel and Assay Controls for five (5) days in triplicate. Testing was done by two operators at each site. Each operator ran the panel once a day using one lot of AmpliVue Trichomonas Assay. The AmpliVue Trichomonas Assay generated reproducible results in this study.

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 /mL)	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%
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	30/30	100%	88.6% to 100%	30/30	100%	88.6% to 100%	90/ 90	100 %	95.9% to 100%

510(k) Summary

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			
			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 High Negative sample has a detection target range of 20 to 80%. Data generated by all sites fall into this range.

The results suggest that there are no significant differences between different users and different sites on different days. Reproducibility studies are acceptable.

b. Linearity/assay reportable range:

Not applicable – This assay is qualitative.

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

Traceability:

Not applicable. This assay is qualitative.

Specimen Stability:

Not applicable

Controls:

Controls (Quidel Molecular Trichomonas Control Set #M119, which contains positive and negative controls, serves as an external processing and extraction control) were run on the AmpliVue® Trichomonas Assay each day of testing. 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. External assay positive control serves as the assay positive control. The positive control listed in "Materials Required But Not Provided" contains intact non-viable, trophozoites and has been titered to be near the limit of detection for the assay. Transfer 50 µL of positive control into a labeled dilution buffer tube and proceed with processing as described above in Step 1 of Amplification. The external assay positive control is intended to monitor substantial reagent and cassette failure

c. External assay negative control serves as the assay negative control. The negative control listed in "Materials Required But Not Provided" is the same matrix as the positive control, but is trophozoite-free. Transfer 50 µL of negative control into a labeled dilution buffer tube and proceed with processing as described above in Step 1 of Amplification. The external assay negative control is intended to detect reagent or environment contamination or carry-over by either *T. vaginalis* DNA or amplicons.

d. Detection limit:

The analytical sensitivity (limit of detection or LoD) of the AmpliVue® Trichomonas Assay was determined using quantified (trophozoite/mL) stocks of two (2) *T. vaginalis* stocks, one metronidazole-susceptible G3 and one metronidazole-resistant CDC888 serially diluted in negative 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 to five (5) concentrations in the preliminary LoD determination study. Each test dilution was run as 10 replicates in the presence of negative matrix on three reagent lots.

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

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

e. Analytical specificity:

Cross Reactivity:

A study was performed to evaluate the cross-reactivity of the AmpliVue® Trichomonas Assay with forty-five (45) microorganisms (36 bacteria, 4 yeasts, 4 viruses, 1 parasite) potentially found in specimens collected to test for *Trichomonas vaginalis* infection. Cross-reactive microorganisms were tested at clinically relevant levels of viruses (≥10^5pfu/mL or genomic copies/mL) and bacteria, yeast and parasite (≥10^6cfu/mL or genomic copies/mL) in the device. All organisms were diluted in Liquid Stuart medium and tested in negative matrix in triplicate in 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 genomic copies/mL
HIV-1 Subtype B RNA	1.14×10^6 genomic copies/mL
Herpes simplex virus I	7.96×10^6 TCID50/mL
Herpes simplex virus II	2.27×10^5 TCID50/mL

Microorganism	Concentration Tested
HPV 16 (SiHa)	4.3×10^6 genomic 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 genomic 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
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 genomic copies/mL

No cross-reactivity was seen with the AmpliVue® Trichomonas Assay with any of forty-five (45) microorganisms (36 bacteria, 4 yeasts, 4 viruses, 1 parasite) tested.

Interference: Microorganism

A study was conducted to determine if the AmpliVue® Trichomonas assay is inhibited in the presence of forty-five (45) microorganisms (36 bacteria, 4 yeasts, 4 viruses, 1 parasite) potentially found in specimens collected to test for *Trichomonas vaginalis* infection. Potentially interfering microorganisms were tested at clinically relevant levels of viruses ($\geq10^5$pfu/mL or genomic copies/mL) and bacteria, yeast and parasite ($\geq10^6$cfu/mL or genomic copies/mL) in the device. All organisms were diluted in Liquid Stuart medium and tested in negative matrix in triplicate in the AmpliVue® Trichomonas assay. The organisms included in the interference study and their tested concentrations are shown in the table below.

510(k) Summary

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 genomic copies/mL
HIV-1 Subtype B RNA	1.14×10^6 genomic 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 genomic 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 genomic copies/mL
Prevotella bivia	3.01×10^6 CFU/mL

Microorganism	Concentration Tested
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
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 genomic copies/mL

No interference was seen with the AmpliVue® Trichomonas Assay with any of forty-five (45) microorganisms (36 bacteria, 4 yeasts, 4 viruses, 1 parasite) tested.

Interference: Substances

A study was conducted to determine if the AmpliVue® Trichomonas assay is inhibited in the presence of a panel of thirteen (13) substances potentially present in specimens collected to test for *Trichomonas vaginalis* infection. Each of the potential interfering substances was tested in three replicates in the presence and absence of near LoD (2×) levels of two strains of *Trichomonas vaginalis* in the AmpliVue® Trichomonas Assay. Substances were introduced into the assay at concentrations which were medically relevant.

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%
CVS Vinegar & Water Extra Cleansing Disposable Douche	1%
Seminal fluid	1%

Substances	Final Conc.
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

There was no evidence of interference caused by the substances tested.

Analytical Reactivity (Inclusivity):

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

Bacterial Strain	Strain Detected (Yes/No)
CDC1230	Yes
SA-A19	Yes

f. Assay cut-off:

Not applicable.

2. Clinical studies:

a. Clinical Sensitivity:

A multi-center study was performed to evaluate the AmpliVue® Trichomonas Assay using nine hundred ninety-two (992) clinician-collected vaginal swab specimens obtained from symptomatic (n=342) or asymptomatic (n=650) patients. The clinician categorized the patients as symptomatic or asymptomatic at the time of specimen collection. The study was performed April to November 2014 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 (3) vaginal specimens were collected using polyester or rayon Swabs w/ Liquid Stuart's, and one (1) vaginal specimen collected with a collection swab from a FDA-cleared molecular device. The four (4) clinician collected vaginal swabs were used for reference and AmpliVue testing. 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. As specimen was considered positive if either test was positive.

One (1) specimen was removed from the study due to a delay in the culture inoculation. Eight (8) specimens yielded invalid results upon initial testing with the AmpliVue® Trichomonas Assay (0.8%). These specimens were re-tested according to the instruction provided in the Package Insert. Six (6) of the specimens yielded valid results when re-tested (5 negative and 1 positive result). Two (2) 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 clinician designations and combined).

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)
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 negative/AmpliVue positive specimens were positive by a FDA-cleared *Trichomonas vaginalis* molecular device.

b. Clinical specificity:

510(k) Summary

See Section 3a**.**

4. Clinical cut-off**:**

Not applicable

5. Expected values:

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

Symptom Status	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%

Positive and Negative Predictive Values

The estimated positive predictive value (PPV) and negative predictive value (NPV) of the AmpliVue Trichomonas Assay across different hypothetical prevalence rates are shown in the table below. These calculations are based on the overall estimated sensitivity and specificity for clinician-collected vaginal swab specimens in the AmpliVue Trichomonas Assay clinical study.

Hypothetical PPV and NPV of the AmpliVue Trichomonas Assay		
Prevalence %	PPV (%)	NPV (%)
1	33.3	100
2	50.0	100
5	72.5	100
10	85.5	100
15	89.8	100
20	93.0	100
25	94.4	100

Conclusion:

The analytical and clinical study results for the AmpliVue® Trichomonas Assay support the determination of substantial equivalence to the predicate device.