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AmpliVue®

Trichomonas ASSAY

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.

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

Prescription Use only

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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.

SUMMARY AND EXPLANATION OF THE TEST

Trichomonas vaginalis infection (trichomoniasis) is the most common curable, non-viral sexually transmitted disease (STD), with an estimated 7.4 million new cases occurring annually in the U.S.[1] Trichomoniasis may lead to preterm birth, low birth weight, and pelvic inflammatory disease when left untreated.[2] Effective diagnosis and treatment of *T. vaginalis* infections in women are important to prevent disease acquisition, transmission, and associated complications. Conventional identification methods for *T. vaginalis* infection from vaginal swabs include wet mount microscopy and culture. Wet mount microscopy is the most common method of *T. vaginalis* detection. Although this technique is rapid and inexpensive, it is only about 36 to 75% sensitive compared to culture even in the hands of trained operators.[3] Culture is the present gold standard for the diagnosis of *T. vaginalis* infection. However, the culture method is technically challenging and time consuming, requiring up to 7 days for getting the final result. The AmpliVue Trichomonas Assay is a nucleic acid amplification test based on Helicase-Dependent Amplification (HDA) technology and a disposable lateral-flow detection device.[4-8] The assay detects *T. vaginalis* DNA directly from vaginal swab. The assay has a turn-around time of approximately 50 minutes and it only uses an inexpensive heat block for performing the assay.

PRINCIPLE OF THE PROCEDURE

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.

Materials Provided

Cat. #M211
16 Tests per kit

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

MATERIALS REQUIRED BUT NOT PROVIDED

- External controls for *Trichomonas vaginalis* (e.g. Quidel Molecular Trichomonas Assay Control Kit, Cat. #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 micropipette tips
- Micropipette (accurate range between 20 to 200 µL)
- Stopwatch or timer
- Heat block capable of 95°C ± 2°C temperature
- Heat block with heated lid capable of 64°C ± 2°C temperature
- Vortex mixer
- Thermometer

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots. Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with *T. vaginalis* amplicons, do not open the reaction tubes post-amplification. DO NOT open the AmpliVue Detection Chamber after use. Opening the cartridge after use may result in amplicon contamination of the test area.
- Avoid microbial and deoxyribonuclease (DNAse) contamination of reagents when removing aliquots from tubes. The use of sterile DNAse-free disposable filter-blocked or positive displacement pipette tips is recommended.
- Use a new pipette tip for each specimen or reagents. Performing the assay outside of the listed time ranges for each step of the procedure can produce erroneous results. Assays not completed within specified time ranges should be repeated.
- Additional controls may be tested according to guidelines or requirements of Local, State, Provincial and/or Federal regulations or accrediting organizations. Separated or segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
- Wash hands thoroughly after performing the test. Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled. Dispose of unused reagents and waste in accordance with county, federal, provincial, state and local regulations.
- Wear suitable protective clothing, gloves, eye and face protection when using this kit. For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water. Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.

STORAGE AND HANDLING OF KIT REAGENTS

Store assay reagents and detection cassettes as indicated on their individual labels and as described in the MATERIALS PROVIDED table until the expiration date.

SPECIMEN COLLECTION AND TRANSPORT

Collect vaginal specimens using an appropriate collection and transport system.
Note: The collection and transport system used in the clinical evaluation was the BD BBL™ CultureSwab™.

Specimen Collection

1. Using the sterile swab, carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus.
2. Gently rotate the swab for 10 to 30 seconds against the vaginal wall ensuring the entire circumference of the swab has touched the vaginal wall.
3. Swab the lateral vaginal wall while removing the swab.
4. After collection, transport and store the swab at 2°C to 8°C for 7 days or room temperature (25 ± 2°C) for up to 2 days prior to testing.

Specimen Storage

Specimens may be stored between 2°C to 8°C for 7 days prior to testing. Specimens may be stored at up to 2 days at room temperature (25 ± 2°C) prior to testing.

ASSAY PROCEDURE

Heat Lysis

1. Warm the heat block to 95°C, 25 minutes prior to Heat Lysis Step 3.
2. Place the swab in a labeled Lysis Buffer tube and release the specimen by swirling the swab tip rapidly in the buffer for **at least 10 seconds**; remove the swab and discard as appropriate for your laboratory.
   Note: Processed clinical samples are stable in Lysis Buffer for up to 72 hours at room temperature and 2°C to 8°C.
3. Place the Lysis Buffer tubes in the 95°C ± 2°C heat block for 10 minutes ± 2 minutes.
   Note: Begin the lysis procedure after placing tubes in the heat block and waiting until the heat block returns to 95°C.

Dilution

1. Vortex the Lysis Buffer tube for 5 seconds to mix the solution.
2. Transfer 50 µL of the specimen to a labeled Dilution Buffer tube (Blue Cap) and vortex for 5 seconds.

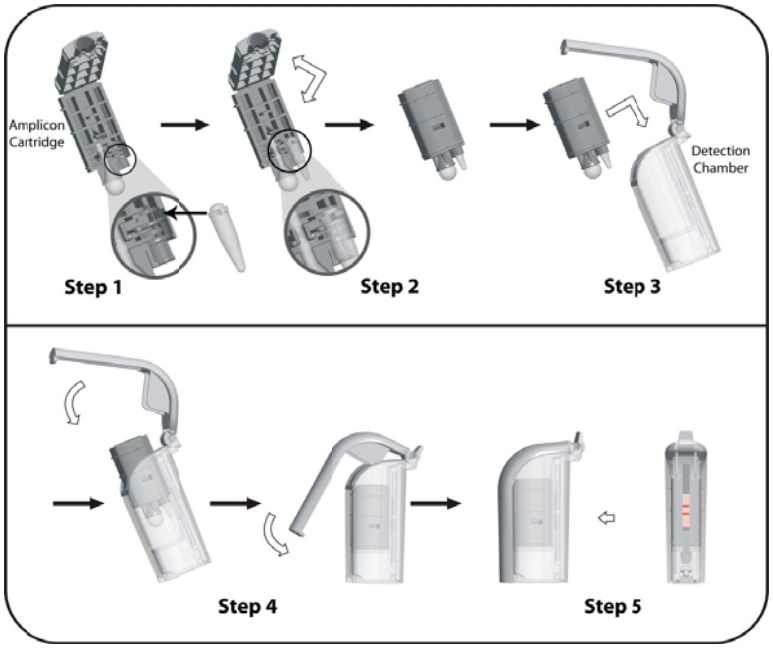
Amplification

1. Transfer 50 µL of diluted sample to a labeled Reaction Tube containing lyophilized reagents and rehydrate the dry reagents by pipetting up and down 3-5 times to mix. Close the lid tightly and proceed to the next step **immediately**.
   a. **Note:** Remove the required number of reaction tubes from the protective pouch, remove the excess air, and reseal the bag.
2. Incubate the Reaction Tube at 64°C ± 2°C for 25 minutes in a heat block with a heated lid.

Detection

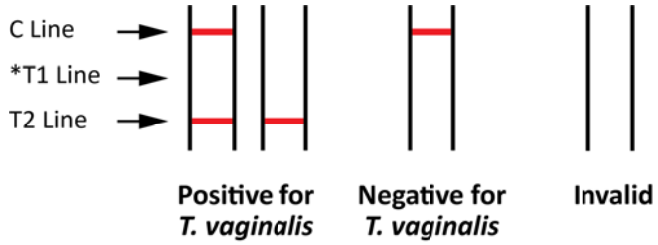
1. Tear open a new Detection Cassette package. Label the Cassette appropriately.
   a. Make sure the buffer bulb is attached in the correct position of the Amplicon Cartridge (see drawing Figure 1).
2. Place the Reaction Tube into the Amplicon Cartridge (Figure 1, step 1).
   NOTE: Be sure to place the HINGE of the Reaction Tube cap into the largest slot adjacent to the buffer bulb.
3. Close the Amplicon Cartridge (Figure 1, step 2) ensuring that it snaps shut.
   a. If the cartridge does not snap shut, reposition the Tube within the cartridge.
   b. Insert the closed Amplicon Cartridge into the Detection Chamber (Figure 1, step 3).
   c. Make sure the arrow faces the detection strip (Reaction Tube should be aligned with the razor blade and the plastic bulb containing the running buffer should be aligned with the pin). Mark or label the Cassette on the top and/or side of the outer casing.
4. Keep the device upright and press the handle of the outer casing to close the device (Figure 1, step 4). The handle will lock (click) into place when closed completely (Figure 1, step 5).
5. Read results at 10 minutes through the front window and record the results.
6. Discard the used Detection Chambers in sealed bags and as appropriate for your laboratory.

Figure 1



INTERPRETATION OF RESULTS

- Any pink to red colored visible T2 line should be recorded as positive (+) and no T2 line should be recorded as negative (-); for example, "T2+" = Visible T line and "T2-" = No T line (See diagram below).
- The T2 line detects *T. vaginalis* DNA.
- The C line detects the internal control DNA in the absence or presence of the target *T. vaginalis* DNA. In the presence of the target *T. vaginalis* DNA, the control line intensity may vary with each test. Any pink to red colored visible line in the control signifies a valid test.



The interpretation of the assay results is done according to the following criteria:

Test line (T) Reading	Control line (C) Reading	Interpretation of result
T2+	C+	*T. vaginalis* DNA detected (Positive)
T2+	C-	*T. vaginalis* DNA detected (Positive)
T2 -	C+	No *T. vaginalis* DNA detected (Negative)
T2 -	C-	Invalid: failure due to inhibitory specimen, or reagent failure, or device failure. Repeat test with original specimen dilution.

Note: The T1 line is not used on this assay. The presence of a T1 line should be considered invalid for this assay. Repeat test with lysed specimen.

QUALITY CONTROL

The AmpliVue Trichomonas Assay incorporates several controls to monitor assay performance.

1. The internal control is used to control for HDA inhibitory specimens and to confirm the integrity of assay reagents and Cassette detection. The internal control is included in the Reaction Tube.
2. External assay positive control serves as the assay positive control. The positive control listed in MATERIALS REQUIRED BUT NOT PROVIDED section 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.
3. External assay negative control serves as the assay negative control. The negative control listed in MATERIALS REQUIRED BUT NOT PROVIDED section 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.

LIMITATIONS

- Although there is no need for reagent preparation, the main laboratory technique required is pipetting; good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- This assay has been tested using only the clinician collected vaginal swabs. Performance with other specimen types and patient collected vaginal swabs has not been evaluated.
- This test does not replace cervical exams and endocervical specimens for diagnosis of female urogenital infections. Patients may have cervicitis, urethritis, urinary tract infections, vaginal infections due to other causes or concurrent infections with other agents.
- Similar to other diagnostic test, results from AmpliVue Trichomonas Assay should be interpreted in conjunction with other clinical data available to the clinician.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown *T. vaginalis* variants and may result in a false negative result with the AmpliVue Trichomonas Assay.
- A positive test result does not necessarily indicate the presence of viable organisms.
- Negative test result does not preclude a possible infection and may occur from improper specimen collection, handling or storage, presence of inhibitors, technical error, sample mix-up or because the number or organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the instructions given in this insert is necessary to avoid erroneous results. Use of this assay should be limited to personnel trained on the procedure.
- Assay performance has not been evaluated in presence of *Dientamoeba fragilis.*
- Therapeutic success or failure cannot be determined with the AmpliVue Trichomonas Assay since nucleic acid may persist following appropriate antimicrobial therapy.
- Performance of AmpliVue Trichomonas Assay has not been evaluated in pregnant women or in patients with less than 16 years of age.
- Performance of AmpliVue Trichomonas Assay has not been evaluated at temperatures less than 15°C or greater than 30°C.

EXPECTED VALUE

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

CLINICAL PERFORMANCE

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. A 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 this document. 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		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 an FDA-cleared *Trichomonas vaginalis* molecular device.

ANALYTICAL PERFORMANCE

Limit of Detection

The Limit of Detection (LOD) of the AmpliVue Trichomonas Assay was determined using limiting dilutions of two (2) *Trichomonas vaginalis* reference strains, one metronidazole-susceptible strain G3 and one metronidazole-resistant strain CDC888.

The assay LOD for Trichomonas vaginalis strain G3 is 307 trophozoites/mL and for strain CDC888 the LOD is 921 trophozoites/mL. These concentrations were demonstrated on three Validation Lots.

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
CDC1230	Yes
SA-A19	Yes

Analytical Specificity – Microbial Interference

A study was performed to evaluate the performance of the AmpliVue Trichomonas Assay 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. Each microorganism was diluted in liquid Stuart medium to the desired concentration (10^6 or higher CFU/mL or copies/mL for bacteria, yeast or DNA/RNA and 10^5 or higher pfu/mL or TCID50/mL for viruses), and tested in triplicate in the presence of each of the two (2) *T. vaginalis* (G3 and CDC888) strains at 2x LOD level. No interference was observed with the detection of each of the two (2) *T. vaginalis* strains in the AmpliVue Trichomonas Assay. The organisms and their concentrations included in the interference study are shown in the table below.

Microorganism	Concentration Tested
Acinetobacter lwoffi	4.55E+06 CFU/mL
Actinomyces israelii	6.63E+06 CFU/mL
Atopobium vaginae	3.60E+06 CFU/mL
Bacteroides fragilis	4.2E+06 CFU/mL
Bifidobacterium adolescentis	1.00E+06 CFU/mL
Campylobacter jejuni	1.72E+06 CFU/mL
Candida albicans	2.00E+06 CFU/mL
Candida glabrata	7.87E+06 CFU/mL
Candida parapsilosis	2.87E+06 CFU/mL
Candida tropicalis	2.15E+06 CFU/mL
Chlamydia trachomatis	7.83E+06 CFU/mL
Clostridium difficile	6.77E+06 CFU/mL
Clostridium perfringens	1.06E+06 CFU/mL
Corynebacterium genitalium	3.61E+06 CFU/mL
Cryptococcus neoformans	1.92E+06 CFU/mL
Enterobacter aerogenes	1.18E+06 CFU/mL

Microorganism	Concentration Tested
Enterococcus faecalis	2.20E+06 CFU/mL
Escherichia coli	1.13E+06 CFU/mL
Fusobacterium nucleatum	8.05E+06 CFU/mL
Gardnerella vaginalis	1.20E+06 CFU/mL
Haemophilus ducreyi	2.97E+06 copies/mL
HIV-1 Subtype B RNA	1.14E+06 copies/mL
Herpes simplex virus I	7.96E+06 TCID50/mL
Herpes simplex virus II	2.27E+05 TCID50/mL
HPV 16 (SiHa)	4.3E+06 copies/mL
Klebsiella oxytoca	1.63E+06 CFU/mL
Lactobacillus acidophilus	2.00E+06 CFU/mL
Lactobacillus jensenii	4.06E+06 CFU/mL
Lactobacillus vaginalis	1.11E+06 CFU/mL
Listeria monocytogenes	6.13E+06 CFU/mL
Mobiluncus curtisii	3.2E+06 CFU/mL
Mycoplasma hominis	1.30E+06 CFU/mL
Neisseria gonorrhoeae	3.20E+06 CFU/mL
Pentatrichomonas hominis	4.5E+06 CFU/mL
Peptostreptococcus anaerobius	8.1E+06 copies/mL
Prevotella bivia	3.01E+06 CFU/mL
Propionibacterium acnes	6.63E+06 CFU/mL
Proteus mirabilis	1.19E+06 CFU/mL
Pseudomonas aeruginosa	1.32E+06 CFU/mL
Staphylococcus aureus MRSA	7.52E+06 CFU/mL
Staphylococcus epidermidis MRSE	1.75E+06 CFU/mL
Streptococcus pyogenes	6.38E+06 CFU/mL
Streptococcus agalactiae	2.20E+06 CFU/mL
Trichomonas tenax	6.3E+06 CFU/mL
Ureaplasma urealyticum	1.23E+06 copies/mL

None of the organisms used in the study demonstrated interference with the AmpliVue Trichomonas Assay.

Analytical Specificity – Cross-reactivity

A study was performed to evaluate the cross-reactivity of the AmpliVue Trichomonas Assay with any 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 ($\geq 10^5$ pfu/mL) and bacteria ($\geq 10^6$ cfu/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	4.55E+06 CFU/mL
Actinomyces israelii	6.63E+06 CFU/mL
Atopobium vaginae	3.60E+06 CFU/mL
Bacteroides fragilis	4.2E+06 CFU/mL
Bifidobacterium adolescentis	1.00E+06 CFU/mL
Campylobacter jejuni	1.72E+06 CFU/mL
Candida albicans	2.00E+06 CFU/mL
Candida glabrata	7.87E+06 CFU/mL
Candida parapsilosis	2.87E+06 CFU/mL
Candida tropicalis	2.15E+06 CFU/mL
Chlamydia trachomatis	7.83E+06 CFU/mL
Clostridium difficile	6.77E+06 CFU/mL
Clostridium perfringens	1.06E+06 CFU/mL
Corynebacterium genitalium	3.61E+06 CFU/mL
Cryptococcus neoformans	1.92E+06 CFU/mL
Enterobacter aerogenes	1.18E+06 CFU/mL
Enterococcus faecalis	2.20E+06 CFU/mL
Escherichia coli	1.13E+06 CFU/mL
Fusobacterium nucleatum	8.05E+06 CFU/mL
Gardnerella vaginalis	1.20E+06 CFU/mL
Haemophilus ducreyi	2.97E+06 copies/mL
HIV-1 Subtype B RNA	1.14E+06 copies/mL
Herpes simplex virus I	7.96E+06 TCID50/mL
Herpes simplex virus II	2.27E+05 TCID50/mL
HPV 16 (SiHa)	4.3E+06 copies/mL
Klebsiella oxytoca	1.63E+06 CFU/mL
Lactobacillus acidophilus	2.00E+06 CFU/mL
Lactobacillus jensenii	4.06E+06 CFU/mL
Lactobacillus vaginalis	1.11E+06 CFU/mL
Listeria monocytogenes	6.13E+06 CFU/mL
Mobiluncus curtisii	3.2E+06 CFU/mL
Mycoplasma hominis	1.30E+06 CFU/mL
Neisseria gonorrhoeae	3.20E+06 CFU/mL
Pentatrichomonas hominis	4.5E+06 CFU/mL
Peptostreptococcus anaerobius	8.1E+06 copies/mL
Prevotella bivia	3.01E+06 CFU/mL
Propionibacterium acnes	6.63E+06 CFU/mL
Proteus mirabilis	1.19E+06 CFU/mL
Pseudomonas aeruginosa	1.32E+06 CFU/mL
Staphylococcus aureus MRSA	7.52E+06 CFU/mL
Staphylococcus epidermidis MRSE	1.75E+06 CFU/mL
Streptococcus pyogenes	6.38E+06 CFU/mL
Streptococcus agalactiae	2.20E+06 CFU/mL
Trichomonas tenax	6.3E+06 CFU/mL

Microorganism	Concentration Tested
Ureaplasma urealyticum	1.23E+06 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.

Analytical Specificity – Interfering 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 (2x) 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 Concentration
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%
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.

Precision – Repeatability

The Precision/Within Laboratory Repeatability was determined via a study, where a four-member panel (3x LOD, 1x LOD, 1/9x LOD and a negative sample) was tested by two (2) operators, three samples per concentration, twice a day (2X) for twelve (12) days.

The AmpliVue Trichomonas Assay produces results that are highly reproducible. This observation is based on the following findings:

- All negative samples generated negative results for *Trichomonas vaginalis*.
- The percentage of positive High Negative samples is 36%, this is within the target range of 20% to 80%.
- The percentage of positive of the Low Positive samples was 100%.
- The percentage of positive of the Moderate Positive samples was 100%.

Precision – 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 (3x LOD, 1x LOD, 1/9x 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.

Category	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	#expected results/# tested	*% Agreement*	*#expected results/# tested*	*% Agreement*	*#expected results/# tested*	*% Agreement*			
High Negative (34 trophozoites /mL)	9/30	30%	11/30	37%	19/30	63%	39/90	43%	33.6% to 53.6%
Low Positive (307 trophozoites /mL)	30/30	100%	30/30	100%	29/30	97%	89/90	99%	94.0% to 99.8%
Moderate Positive (921 trophozoites /mL)	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%
Negative Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95.9% to 100%

Carryover – Cross Contamination

A stock of high positive sample containing approximately (2.5x 10^6 trophozoites/mL) of *Trichomonas vaginalis* was prepared for testing in the assay workflow. In each run of testing, processing of high positive samples in Liquid Stuart medium was alternated with negative Liquid Stuart medium only samples to assess the risk of cross contamination. In total, five runs consisting of 5 samples positive for *T. vaginalis* and 5 negative samples were tested by two operators for a total of 25 positive and 25 negative samples.

Consecutive testing of alternating *T. vaginalis* high positive samples and *T. vaginalis* negative samples resulted in no carry over or cross contamination as 25/25 *T. vaginalis*-positive samples tested *T. vaginalis*-positive and 25/25 *T. vaginalis*-negative samples tested *T. vaginalis*-negative.

CUSTOMER AND TECHNICAL SUPPORT

To place an order or for Technical Support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact custserv@quidel.com or technicalsupport@quidel.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

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GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult e-labeling instructions for use
 WARNING: Harmful if swallowed (oral)	IVD For *In Vitro* diagnostic use
 Contains sufficient for 16 determinations	CONT Contents/Contains