510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

k130268

B. Purpose for Submission:

To obtain clearance for a new device, BD ProbeTec™ *Trichomonas vaginalis* (TV) Q^x Amplified DNA Assay

C. Measurand:

138 base-pair region of *Trichomonas vaginalis* adhesion protein gene (AP65-1)

D. Type of Test:

Nucleic acid amplification assay

E. Applicant:

Becton, Dickinson and Company

F. Proprietary and Established Names:

BD ProbeTec™ *Trichomonas vaginalis* (TV) Q^x Amplified DNA Assay

TV Q^x 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:

   The BD ProbeTec™ *Trichomonas vaginalis* (TV) Q^X Amplified DNA Assay, when tested with the BD Viper™ System in Extracted Mode, uses Strand Displacement Amplification technology for the direct, qualitative detection of *Trichomonas vaginalis* DNA in clinician-collected female endocervical swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and female urine specimens. The assay is indicated for use with asymptomatic and symptomatic females to aid in the diagnosis of trichomoniasis.

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   BD Viper System

I. Device Description:

The BD ProbeTec *Trichomonas vaginalis* (TV) Q^x Amplified DNA Assay (TV Q^x Assay) is based on the simultaneous amplification and detection of target DNA using amplification primers and fluorescently-labeled detector probes. The assay employs strand displacement amplification (SDA) technology in similar fashion to the previously cleared assays k081824, k081825, and k103798. The reagents for SDA are dried in two separate disposable microwells: the Priming Microwell contains the amplification primers, fluorescently-labeled detector probe, nucleotides and other reagents necessary for amplification, while the Amplification Microwell contains the two enzymes (a DNA polymerase and a restriction endonuclease) that are required for SDA. The assay is designed for use with the BD ProbeTec Q^X specimen collection and transport devices, applicable reagents, the BD Viper System, and BD FOX™ Extraction Tubes. The BD Viper System pipettes a portion of the purified DNA solution from each Extraction Tube into a Priming Microwell to rehydrate the contents. After a brief incubation, the reaction mixture is transferred to a corresponding, pre-warmed Amplification Microwell which is sealed to prevent contamination and then incubated in one of the two thermally-controlled fluorescent readers. The presence or absence of *T. vaginalis* DNA is determined by calculating the peak fluorescence (Maximum Relative Fluorescent Units (MaxRFU)) over the course of the amplification process and by comparing this measurement to a predetermined threshold value.

The TV Q^x Assay utilizes two specimen collection and transport kits. These collection kits were cleared for use with BD Viper System in extracted mode (k081824, and k081825)

- BD ProbeTec Q^x Collection Kit for Endocervical or Lesion Specimens
- Vaginal Specimen Transport for the BD ProbeTec Q^x Amplified DNA Assays

J. Substantial Equivalence Information:

1. Predicate device name:

   APTIMA Trichomonas vaginalis Assay on the TIGRIS DTS System

2. Predicate 510(k) number:

   K102911

3. Comparison with predicate:

	APTIMA TV Assay on the TIGRIS DTS System (K102911)	**TV Q^x Assay on the BD Viper System**
Intended Use	The APTIMA Trichomonas vaginalis Assay is an *in vitro* qualitative nucleic acid amplification test (NAAT) for the detection of ribosomal RNA (rRNA) from *Trichomonas vaginalis* to aid in the diagnosis of trichomoniasis using the TIGRIS DTS System. The assay may be used to test the following specimens from symptomatic or asymptomatic women: clinician-collected endocervical swabs, clinician-collected vaginal swabs, female urine specimens, and specimens collected in PreservCyt solution.	The BD ProbeTec™ *Trichomonas vaginalis* (TV) Q^x Amplified DNA Assay, when tested with the BD Viper™ System in Extracted Mode, uses Strand Displacement Amplification technology for the direct, qualitative detection of *Trichomonas vaginalis* DNA in clinician-collected female endocervical swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and female urine specimens. The assay is indicated for use with asymptomatic and symptomatic females to aid in the diagnosis of trichomoniasis.
Assay Results	Qualitative	Qualitative
Technology	Molecular NAAT	Molecular NAAT

Instrument	TIGRIS DTS System	BD Viper System
Specimen Types	Female Urine (UTT[2]) Vaginal Swab Endocervical Swab PreservCyt solution	Female Urine (Neat) Vaginal Swab Endocervical Swab

K. Standard/Guidance Document Referenced:

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

MM03-A2, 2006 – Molecular Diagnostic Methods for Infectious Diseases, CLSI Approved Guideline

MM13-A, 2005 – Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods, CLSI Approved Guideline

EP17-A2, 2012 – Evaluation of Detection Capability for Clinical laboratory Measurement Procedures; CLSI Approved Guideline

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

L. Test Principle:

The BD ProbeTec *Trichomonas vaginalis* (TV) Q^x Amplified DNA Assay is based on strand displacement amplification (SDA) technology. The TV Q^x assay is designed to target unique sequences within the *T. vaginalis* genome, a 138 base pair region of AP65-1 Adhesion Protein gene.

All specimens undergo a pre-warm step in the BD Viper Lysing Heater to dissolve mucus which may be present in certain specimens and to homogenize the specimen. After cooling, the specimens are loaded onto the BD Viper System which then performs all the steps involved in extraction and amplification of target DNA, without further user intervention. The specimen is transferred to an Extraction Tube that contains ferric oxide particles in a dissolvable film and dried Extraction Control. A high pH is used to lyse the cells and liberate the DNA into solution. Acid is then added to lower the pH and induce a positive charge on the ferric oxide, which in turn binds the negatively charged DNA. The particles and bound DNA are then pulled to the sides of the Extraction Tube by magnets and the treated specimen is aspirated to waste. The particles are washed and a high pH Elution Buffer is added to recover the purified DNA. Finally, a Neutralization Buffer is used to bring the pH of the extracted solution to the optimum for amplification of the target. The BD Viper System pipettes a portion of the purified DNA solution from each Extraction Tube into a Priming

Microwell to rehydrate the contents. The reaction mixture is transferred to a corresponding, pre-warmed Amplification Microwell that contains the enzymes (DNA polymerase and restriction endonuclease) for Strand Displacement Amplification. The amplification well is then sealed to prevent contamination and incubated in one of the two thermally-controlled fluorescent readers. The presence or absence of *T. vaginalis* DNA is determined by calculating the peak fluorescence (MaxRFU) over the course of the amplification process and by comparing this measurement to a predetermined threshold value. The magnitude of the MaxRFU score is not indicative of the level of *T. vaginalis* DNA in the specimen.

M. Performance Characteristics:

1. Analytical performance:

a. Reproducibility

Reproducibility of TV Q^x Assay was evaluated at two external and one internal site on one BD Viper System per site. Panels were comprised of four levels of *T. vaginalis* (ATCC strain 30001) seeded into urine specimen matrix or vaginal matrix in Q^x diluent. Each panel member was either left unspiked (negative), or was spiked with known amounts of *T. vaginalis* organism at 0.3X (high negative), 1X (low positive), and 3X LOD (moderate positive) for each respective specimen matrix. Samples in each panel were randomized and blinded to the user prior to shipment to the testing sites. Three replicates of each panel member were tested twice a day for five days on each BD Viper System in Extracted Mode.

The following table presents, for each panel member, MaxRFU data in terms of mean, standard deviation (SD), and coefficient of variation (CV) between sites, , between runs, within runs, and between days. Percent agreement with expected results is also shown. Samples with valid results were included in the analyses. The results indicate reproducible performance within and across all three sites for all panel members tested when compared to the expected outcome.

Summary of Reproducibility Data for TV Q^x Assay on the BD Viper System

					Within Run		Between Run Within Day		Between Day Within Site		Between Sites	
Specimen type	Panel	Agreement	95% CI	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Vaginal	Zero	100.0% (89/89)	(95.9%, 100.0%)	3.89	4.02	103.53	2.01	51.64	0.92	23.57	4.69	120.60
	High Negative (0.3X LOD)	43.3% (39/90)	(33.6%, 53.6%)	795.66	814.68	102.39	136.97	17.21	287.34	36.11	190.15	23.90
	Low Positive (1X LOD)	96.7% (87/90)	(90.7%, 98.9%)	1632.07	457.83	28.05	0.00	0.00	0.00	0.00	110.13	6.75
	High Positive (3X LOD)	100.0% (90/90)	(95.9%, 100.0%)	1756.68	297.78	16.95	0.00	0.00	104.51	5.95	260.45	14.83
Urine Matrix	Zero	100.0% (90/90)	(95.9%, 100.0%)	8.70	11.33	130.18	0.00	0.00	0.00	0.00	8.29	95.33
	High Negative (0.3X LOD)	39.3% (35/89)*	(29.8%, 49.7%)	976.96	913.87	93.54	0.00	0.00	0.00	0.00	0.00	0.00

	Low Positive (1X LOD)	92.1% (82/89)*	(84.6%, 96.1%)	1574.67	681.14	43.26	0.00	0.00	0.00	0.00	0.00	0.00
	High Positive (3X LOD)	100.0% (90/90)	(95.9%, 100.0%)	1822.52	364.46	20.00	0.00	0.00	0.00	0.00	172.88	9.49

* Four non-reportable results were due to extraction control failures which caused a reduction in the full number of replicates at one test site.

b. Precision

Precision of the TV Q^x assay was evaluated at one site over multiple days, by four users testing three instruments across calibration cycles, and multiple organism spike levels. Testing occurred in four phases on each of the three BD Viper Systems. Each phase consisted of three days of testing with two runs per day on three BD Viper Systems. The study was designed to capture the potential variation across calibration cycles with each instrument. Usually, the BD Viper System is calibrated every 30 days. In this study, the calibration cycles were artificially condensed to allow a shorter real-time interval between test phases.

Accuracy and Precision Testing Sequence

Duration	Part 1	Calibration	Part 2		Part 3	Calibration	Part 4
Duration	3 days		3 days		3 days		3 days

Twelve different panel members were tested in each run. Panel members consisted of specimen matrix unspiked or spiked with 0.3X, 1X, and 3X LoD. Testing was performed for 12 days in four phases with 360 observations per panel member.

Panel	Matrix	Spike Level	Total Tested	Number Correct	%Agreement
1	Q^x Vaginal	Neg	360	359	99.72%
2	Q^x Vaginal	0.3XLOD	360	236	65.56%
3	Q^x Vaginal	1XLOD	360	353	98.06%
4	Q^x Vaginal	3XLOD	360	360	100.00%
5	Urine matrix	Neg	360	359	99.72%
6	Urine matrix	0.3XLOD	360	260	72.22%
7	Urine matrix	1XLOD	360	351	97.50%
8	Urine matrix	3XLOD	360	360	100.00%

c. Linearity/assay reportable range:

Not applicable

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

Specimen Stability Studies:

Urine Specimen Stability:

Data to support the recommended storage and transport conditions for neat urine was generated with negative female urine specimens spiked with *T. vaginalis* strain 30001 at 3X LOD. Twenty-four assay replicates were tested for each condition (sample type/temperature/duration). The study results demonstrated the urine stability for the following conditions:
2–8°C for up to 7 days; 30°C for 18 and 24 hours; -20°C for up to 180 days.

Vaginal Dry and Expressed Swab Stability:

Pools of *T. vaginalis* negative vaginal swab matrix were used in analytical experiments to support the storage and transport stability claims for dry vaginal swabs and vaginal swabs expressed in Q^x swab diluent. Pools were spiked with *T. vaginalis* strain ATCC 30001 at 3X LOD. Twenty four assay replicates were tested for each condition (sample type/temperature/duration). The data supported the stability for dry vaginal swab specimens at 2 – 8°C for up to 14 days, at 30°C for up to 3 days, or at -20°C for up to 180 days and the stability of the expressed vaginal swab specimens at 2–8°C or 30°C for up to 30 days or at -20°C for up to 180 days.

Endocervical Swab Specimen Stability:

Pools of negative endocervical swab matrix were spiked with *T. vaginalis* strain ATCC 30001 at 3X LOD in this study that demonstrated the endocervical swab specimens were stable at 2–8°C or 30°C for up to 30 days or at -20°C for up to 180 days. Twenty four assay replicates were tested for each condition (sample type/temperature/duration).

Post Pre-Warm Specimen Stability

Pools of *T. vaginalis* negative neat urine matrix were used to support the storage claims for pre-warmed neat urine specimens. Pools of neat urine were spiked with *T. vaginalis* ATCC strain 30001 at 3X LoD. The neat urine pools were dispensed in 2 mL volumes into BD Viper specimen tubes. The specimen tubes were pre-warmed at 114°C for 15 minutes and cooled for 15 minutes. After the pre-warm process, the specimen tubes were stored at 2–8°C for up to 7 days, at 30°C for up to 3 days, and at -20°C for up to 180 days. At each time point, samples were removed from storage and tested with the TV Q^x Assay on the BD Viper System in extracted mode. Twenty-four assay replicates were tested for each condition (sample type/temperature/duration) demonstrating that the urine specimens are stable at the above conditions after the pre-warm process.

Pools of *T. vaginalis* negative vaginal and endocervical swab specimen matrices in Q^x Swab Diluent were used to support the storage stability claims for pre-warmed expressed vaginal and endocervical swab specimens. For both types of matrices, pools were spiked with *T. vaginalis* strain ATCC 30001 at 3X LOD and aliquoted into 2 mL volumes in BD Viper specimen tubes. The tubes were pre-warmed at 114°C for 15 minutes and cooled for 15 minutes. After the pre-warm process, the specimen tubes were stored either at 2–8°C or 30°C for up to 30 days, or at -20°C for

up to 180 days. At each time point, samples were removed from storage and evaluated with the TV Q^x Assay on the BD Viper System in extracted mode. Twenty four assay replicates were tested for each condition (sample type/temperature/duration). The results supported the post pre-warm stability for expressed vaginal and endocervical swab specimens under above mentioned conditions.

Quality Control Results and Acceptability:

External Controls:

The control set for the assay is provided separately. One Positive and one Negative Control must be included in each assay run and for each new reagent kit lot number. Controls must be positioned according to the BD Viper Instrument User's Manual. The CT/GC/TV Q^x Positive Control will monitor for substantial reagent failure only. The CT/GC/TV Q^x Negative Control monitors for reagent and/or environmental contamination.

The CT/GC/TV Q^x Positive Control is comprised of recombinant plasmids that contain the SDA target regions for the CT Q^x, GC Q^x and TV Q^x Assays. The plasmids are not necessarily representative of native target DNA detected by the assay (e.g., their overall length is shorter than that of the complete gene or genomic sequence), nor are the controls representative of the specimen matrices indicated for use with the assays on the BD Viper System in extracted mode. The TV Q^x Negative Control is comprised of the same milieu as the Positive Control but without the plasmid DNA. The Positive and Negative Control formulations are dried in separate 4.5 mL specimen tubes. A QC pair (Positive Control and Negative Control) must be logged in for each plate to be tested and for each reagent kit lot number.

The location of the microwells is shown in a color-coded plate layout screen on the LCD Monitor. The plus symbol (+) within the microwell indicates the positive QC sample. The minus symbol (-) within the microwell indicates the negative QC sample.

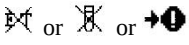
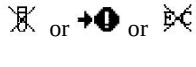
The CT/GC/TV Q^x Positive Control and the CT/GC/TV Q^x Negative Control must test as positive and negative, respectively. If controls do not perform as expected, the run is considered invalid and results will not be reported by the instrument. If either of the controls does not provide the expected results, the entire run should be repeated using a new set of controls, new extraction tubes, new extraction trough, new lysis trough, and new microwells.

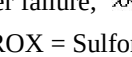
The Extraction Control (EC):

Each extraction tube contains a dried fluorescently labeled oligonucleotide that is incorporated into each tube. EC is labeled with a dye different that that used for detection of *T. vaginalis* specific target and is used to confirm the validity of the extraction process. The EC is dried in the Extraction Tubes and is rehydrated by the BD Viper System upon addition of the specimen and extraction reagents. At the end of the extraction process, the EC fluorescence is monitored by the instrument and an

automated algorithm is applied to both the EC and TV Q^x Assay specific signals to report results as positive, negative, or EC failure.

Interpretation of Quality Control Results

Control Type	Tube Result Report Symbol	TV Q^x MaxRFU	QC Disposition
CT/GC/TV Q^x Positive Control	OK	≥125	QC Pass
CT/GC/TV Q^x Positive Control		<125	QC Failure
CT/GC/TV Q^x Positive Control		Any value	QC Failure
CT/GC/TV Q^x Negative Control	OK	<125	QC Pass
CT/GC/TV Q^x Negative Control		≥125	QC Failure
CT/GC/TV Q^x Negative Control		Any value	QC Failure
CT/GC/TV Q^x Negative Control		<125	QC Failure

 = Fail,  = Extraction Transfer failure,  = Liquid Level failure, = Extraction Control failure, = Error, = ROX failure ROX = Sulforhodamine dye used to monitor EC performance.

e. Detection limit:

The limit of detection (LOD) for the TV Q^x Assay on BD Viper System was determined in specimen matrices (vaginal swab, endocervical swab, and urine) and in Q^x swab diluent. Two strains of *T. vaginalis*, one Metronidazole-sensitive and one Metronidazole-resistant, were used at varying concentrations to create an LOD panel consisting of six target levels. For each target level 20 replicates were tested across three lots of assay and extraction reagents on three BD Viper Systems to yield a total of 60 replicates at each target level. The resulting MaxRFU values were analyzed to determine proportion positive at each level that was used to generate positivity curves from which each 95% LOD was calculated.

LOD was confirmed using 20 replicates at the estimated LOD for each matrix type with one lot of reagents and one BD Viper System for each strain of *T. vaginalis*.

The Limit of Detection (LOD) for the TV Q^x Assay with *T. vaginalis* ATCC strains 30001 and 50143 in BD Q^x Swab Diluent when extracted on the BD Viper System was 54.5 and 55.5 trichomonads/mL, respectively. The LODs for neat urine, vaginal and endocervical specimen matrices are presented in the Table below.

LOD Estimates for TV Q^x Assay

Specimen type	ATCC Strain	LOD (TV/mL)
Neat Urine	30001	109.7
	50143	108.2
Vaginal	30001	74.4
	50143	88.4
	30184*	152.8
Endocervical	30001	64.8
	50143	76.2

* LoD for an additional ATCC *T. vaginalis* strain was determined in vaginal swab specimen matrix. Please see the Inclusivity section below for additional information.

f. Inclusivity:

Four ATCC *Trichomonas vaginalis* strains 30185, 30237, 50144, and 30184 were evaluated in the inclusivity study for TV Q^x assay on BD Viper System in urine and vaginal swab specimen matrix. A total of 20 assay replicates were run for each strain and specimen matrix. One ATCC strain 30184 could not meet the 95% positivity criteria for vaginal matrix LoD confirmation; therefore, a full LoD testing was performed with this strain in vaginal swab matrix. The same panel that was used in the LoD study with six target levels was tested with 60 assay replicates for each level, using two lots of assay reagents, three lots of extractions reagents, and three BD Viper Systems. The resulting MaxRFU values were analyzed to determine proportion positive at each level that was used to generate positivity curves from which each 95% LOD was calculated. The TVQ LOD for strain 30184 in vaginal matrix was determined to be 152.8 TV/mL (95% CI: 122.9, 182.7).

g. Analytical specificity:

DNA from 54 organisms was extracted on the BD Viper System and tested with the TV Q^x Assay. All potentially cross-reactive species were tested at approximately $1x10^6$ CFU/mL for bacteria and yeast, and for viruses/pathogens at $1x10^6$ viral particles/mL or genomic RNA/DNA equivalents per mL, or organisms/mL (or at the highest concentration possible). One organism, *Trichomonas tenax was identified as* a cross-reactant at concentration above $1.0x10^4$ organisms/mL. This limitation is included in the labeling. No other organisms cross-reacted in the TV Q^x Assay when tested on the BD Viper System.

Microorganisms tested for Potential Cross Reactivity

Organism	Final Concentration	Organism	Final Concentration
Acinetobacter baumannii	1.03 x 10^9 CFU/mL	HPV-18	6.13 x 10^8 cells/mL
Actinomyces israelii	6.23 x 10^7 CFU/mL	Human Immunodeficiency Virus (HIV-1)	1.00 x 10^8 vp/mL
Atopobium vaginae	5.70 x 10^7 CFU/mL	*Klebsiella oxytoca*	6.27 x 10^8 CFU/mL
Bacteroides fragilis	1.04 x 10^9 CFU/mL	*Lactobacillus acidophilus*	1.37 x 10^7 CFU/mL
Bifidobacterium bifidum	7.57 x 10^7 CFU/mL	*Lactobacillus jensenii*	4.00 x 10^7 CFU/mL
Campylobacter jejuni	2.87 x 10^7 CFU/mL	*Lactobacillus vaginalis*	6.10 x 10^7 CFU/mL
Candida albicans	8.50 x 10^6 CFU/mL	*Listeria monocytogenes*	8.50 x 10^8 CFU/ml
Candida glabrata	2.05 x 10^7 CFU/mL	*Mobiluncus curtisii*	6.47 x 10^8 CFU/mL
Candida parapsilosis	2.20 x 10^7 CFU/mL	*Moraxella catarrhalis* (*Branhamella* sp.)	1.93 x 10^8 CFU/mL
Candida tropicalis	7.00 x 10^6 CFU/mL	*Mycobacterium smegmatis*	1.23 x 10^7 CFU/mL
Chlamydia trachomatis	3.50 x 10^6 EB/mL	*Mycoplasma genitalium*	1.00 x 10^7 CFU/mL
Clostridium difficile	6.10 x 10^7 CFU/mL	*Mycoplasma hominis*	1.00 x 10^6 CFU/mL
Clostridium perfringens	5.63 x 10^7 CFU/mL	*Neisseria gonorrhoeae*	4.63 X 10^8 CFU/mL
Corynebacterium genitalium biovar 1	1.16 x 10^8 CFU/mL	*Pentatrichomonas hominis*	3.39 x 10^5 Organisms/mL
Cryptococcus neoformans	6.33 x 10^6 CFU/mL	*Peptostreptococcus anaerobius*	2.37 x10^7 CFU/mL
Enterobacter aerogenes	8.23 x 10^8 CFU/mL	*Prevotella bivia*	7.03 x 10^8 CFU/mL
Enterobacter cloaceae	1 x 10^6 CFU/mL	*Proteus mirabilis*	7.97 x 10^8 CFU/mL
Enterococcus faecalis	5.13 x 10^8 CFU/mL	*Pseudomonas aeruginosa*	1.20 x 10^9 CFU/mL
Escherichia coli	6.17 x 10^8 CFU/mL	*Staphylococcus aureus*, non-protein A	4.14 x 10^9 CFU/mL
Fusobacterium nucleatum	3.00 x 10^6 CFU/mL	*Staphylococcus aureus*, protein-A producing	4.80 x 10^9 CFU/mL
Gardnerella vaginalis	4.07 x 10^8 CFU/mL	*Staphylococcus epidermidis*	1.80 x 10^8 CFU/mL
Haemophilus ducreyi	9.93 x 10^8 CFU/mL	*Staphylococcus saprophyticus*	3.47 x 10^8 CFU/mL
Herpes Simplex Virus Type 1	1.00 x 10^7 vp/mL	*Streptococcus pyogenes* (Group A)	3.73 x 10^8 CFU/mL
Herpes Simplex Virus Type 2	1.00 x 10^7 vp/mL	*Streptococcus agalactiae* (Group B)	5.67 x 10^8 CFU/mL
HPV-6	1.57 x 10^9 cells/mL	*Trichomonas tenax*	1.50 x 10^6 Organisms/mL
HPV-11	4.87 x 10^8 cells/mL	*Ureaplasma urealyticum*	1.00 x 10^6 CFU/mL
HPV-16	8.43 x 10^8 cells/mL	*Veillonella parvula*	7.47 x 10^8 CFU/mL

h. Interference

The effect of endogenous and exogenous substances that may be present in urine and vaginal swab specimens on the sensitivity and specificity of the TV Q^x Assay on the BD Viper System was evaluated in an interference study.

The table below describes the substances that were individually spiked into urine and vaginal sample matrices. Testing included 20 replicates containing each potentially interfering substance alone as well as 20 replicates containing each substance spiked with *T. vaginalis* at 3X LoD in each matrix. ATCC strain 50143 or 30001 was used for spiking the urine or vaginal specimen matrix respectively. The study also included sample matrices unspiked or spiked with *Trichomonas vaginalis* without interfering substances as a control. The results are summarized in the table below.

Interfering Substances Tested

Interpretation	Swab	Urine Matrix
No interference observed at levels listed	Whole Blood (≤ 60%) Seminal Fluid Mucus Over the counter vaginal products and contraceptives Hemorrhoidal cream Prescription vaginal treatments Leukocytes ($1x10^6$ cells/mL) Intravaginal hormones	Phenazopyridine Hydrochloride Whole Blood (≤ 1% v/v) Acidic urine (pH 5.0) Alkaline urine (pH 9.0) Horomone pool Analgesic pool Antibiotics Bilirubin Mucus Albumin (≤ 1mg/mL) Glucose Semen (5% v/v) Over the counter deodorant spray and powder Leukocytes ($2.5x10^6$ cells/mL)
May cause Extraction Control (EC) failures	Blood (> 60%)	Not applicable
May cause false negative results	Not applicable	Not applicable

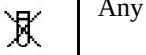
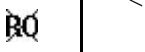
V/V = volume/volume

i. Assay cut-off:

The TV Q^x Assay is designed for and validated on the BD Viper System in Extracted Mode. The assay test results are automatically interpreted by the BD Viper System software. The presence or absence of *T. vaginalis* DNA is determined by calculating the peak fluorescence (MaxRFU) over the course of the amplification process and by comparing this measurement to a predetermined threshold value. If the *T. vaginalis*-specific signal is greater than or equal to a threshold of 125 MaxRFU, the EC fluorescence is ignored by the algorithm. If the *T. vaginalis*-specific signal is less than a threshold of 125 MaxRFU, the EC fluorescence is utilized by the algorithm in the interpretation of the result.

Interpretation of Test Results for the TV Q^x Assay

Tube Report Result	TV Q^x MaxRFU	Report	Interpretation	Result
⊕	≥125	*T. vaginalis* DNA detected by SDA	Positive for *T. vaginalis* DNA. *T. vaginalis* organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.	Positive
⊖	<125	*T. vaginalis* DNA not detected by SDA	Presumed negative for *T. vaginalis* DNA. A negative result does not preclude	Negative

			T. vaginalis infection because results are dependent on adequate specimen collection, absence of inhibitors, and the presence of sufficient DNA to be detected.	
	<125	Extraction Control Failure. Repeat test from initial specimen tube or obtain another specimen for testing.	*T. vaginalis*, if present, is not detectable.	Extraction Control Failure
	Any value	Extraction Transfer Failure. Repeat test from initial specimen tube or obtain another specimen for testing	*T. vaginalis*, if present, is not detectable.	Extraction Transfer Failure
	Any value	Liquid Level Failure. Repeat test from initial specimen tube or obtain another specimen for testing.	*T. vaginalis*, if present, is not detectable.	Liquid Level Failure
	Any value	Error. Repeat test from initial specimen tube or obtain another specimen for testing.	*T. vaginalis*, if present, is not detectable.	Error
	<125	ROX Channel Failure. Repeat test from initial specimen tube or obtain another specimen for testing.	*T. vaginalis*, if present, is not detectable.	ROX Failure

The performance of the TV Q^x Assay with the preliminary assay cutoff (125 MaxRFU) was evaluated in three phases at external clinical sites: a cutoff analysis (to verify the preliminary assay cutoff prior to the main clinical trial), a clinical trial (to validate the performance of the assays in different clinical settings), and a reproducibility study. These three phases confirmed that the preliminary cutoff values for assay was acceptable.

j. Contamination and Carryover Study

An internal study was conducted to evaluate the risk of producing a false positive result with the TV Q^x Assay protocol in either the same run on the BD Viper System in Extracted Mode (within run cross-contamination) or in a subsequent run (between run carryover). Testing was conducted using negative and positive samples on three BD Viper Systems utilizing CTQ/GCQ/TVQ Panel Mode (login mode in which a single sample tube may be tested with the CT Q^x, GC Q^x, and TV Q^x Assays on the BD Viper System) as the worst-case for carryover and crossover contamination rates. CTQ/GCQ/TVQ Panel Mode utilizes the most complex series of robotic arm

movements and incorporates the longest instrument run time. This study utilized the most sensitive BD ProbeTec Q^X Amplified DNA Assay (CTQ Assay based on analytical LOD, K081824). Positive samples were seeded with a representative analyte (10^5 CT EBs/mL). The experimental series was comprised of 6 consecutive runs using alternating positive and negative samples arranged in a checkerboard pattern (at a positivity rate of 50%) on each BD Viper System. The overall rate of contamination was ≤0.49% (3/612) in TVQ mode and ≤0.39% (3/807) in CTQ/GCQ/TVQ panel mode.

k. Fresh vs. Frozen samples:

This study was done to verify that specimens which have been frozen prior to processing on the BD Viper System perform equivalently to those that are not frozen prior to processing. Urine and vaginal swab specimens expressed in Q^x diluent, unspiked or spiked with *T. vaginalis* at 3X LoD were tested either fresh or after storing at -20˚C for 7 days. Each fresh/frozen pair was considered as one set of sample and if the frozen result did not match the fresh then it was a "disagreement". 44 such sets of fresh/frozen were tested at each *T. vaginalis* concentration for each specimen matrix.

Overall percent agreement between fresh and frozen specimens was calculated by sample type for the TV Q^x Assay using the MaxRFU algorithm and a cutoff threshold of 125 MaxRFU.

Overall Agreement Between Combined (Positive and Negative) Fresh and Frozen Samples

Specimen Type	Target	Total Tested	Number in Agreement	% Agreement
Vaginal Matrix	TV Negative	44	44	100%
	3x LOD (223.2 TV/mL)	44	44	100%
Neat Urine	TV Negative	44	44	100%
	3x LOD (329.1 TV/mL)	44	42	95.5%

2. Comparison studies:

a. Method comparison with predicate device:
Not Applicable

b. Matrix comparison:
Not applicable

3. Clinical studies:

Performance characteristics for the BD TV Q^x Assay on BD Viper System were established in multi-center clinical study using prospectively collected specimens from consenting subjects during 2012. First void urine specimens between 20 – 60 mL, self-collected vaginal swabs in a clinical setting, and clinician-collected endocervical swabs were obtained from 1222 symptomatic and asymptomatic female subjects attending family planning, OB/GYN, and sexually transmitted disease clinics at seven (7) geographically diverse clinical sites in North America. Subjects were classified as symptomatic if they presented to the clinic with abnormal vaginal discharge, itching, dysuria, or odor as determined by the inclusion/exclusion criteria. The final data analysis included 1197 evaluable subjects. Exclusions from the data analysis were made due to specimens not collected, enrollment issues, transport errors, collection errors, shipping errors, processing errors, or BD Viper System operating errors. The final data analysis included 735 compliant results for the neat urine specimen type, 838 compliant results for the vaginal swab specimen type, and 995 compliant results for the endocervical swab specimen type.

All sensitivity and specificity calculations were based on the total number of TV Q^x Assay results for the neat urines, vaginal, and endocervical specimens as compared to the composite reference of the wet mount and the commercially available *T. vaginalis* culture test method. The subject was considered to be positive for *T. vaginalis* if either the wet mount or *T. vaginalis* culture result was positive. Subjects were considered negative for *T. vaginalis* if both of the reference methods were negative.

The following tables show the sensitivity, and specificity of the TV Q^x Assay on the BD Viper System for each specimen type by symptom status as well as sensitivity, specificity, prevalence, PPV, and NPV of the TV Q^x Assay by specimen type and collection site. Subjects were classified as symptomatic if symptoms were reported by the subject. Subjects were classified as asymptomatic if the subject did not report symptoms.

TV Q^x Assay Performance Compared to Composite Reference by Symptomatic Status

			Performance Compared to Composite Reference			
Specimen Type	**Status**	**n**	**Sensitivity**	**95% C.I.**	**Specificity**	**95% C.I.**
Neat Urine	A	289	93.1% (27/29)	(78.0%, 98.1%)	99.6% (259/260)	(97.9%, 99.9%)
	S	446	96.4% (80/83)	(89.9%, 98.8%)	98.1% (356/363)	(96.1%, 99.1%)
	Total	735	95.5% (107/112)[A]	(90.0%, 98.1%)	98.7% (615/623)[B]	(97.5%, 99.3%)
Vaginal	A	343	93.5% (29/31)	(79.3%, 98.2%)	99.0% (309/312)	(97.2%, 99.7%)
	S	495	100.0% (85/85)	(95.7%, 100.0%)	99.0% (406/410)	(97.5%, 99.6%)
	Total	838	98.3% (114/116)	(93.9%, 99.5%)	99.0% (715/722)[C]	(98.0%, 99.5%)

Endocervical	A	505	92.2% (47/51)	(81.5%, 96.9%)	99.1% (450/454)	(97.8%, 99.7%)
	S	490	98.8% (82/83)	(93.5%, 99.8%)	99.8% (406/407)	(98.6%, 100.0%)
	Total	995	96.3% (129/134)	(91.6%, 98.4%)	99.4% (856/861)[D]	(98.6%, 99.8%)
All Specimen Types Combined	A	1137	92.8% (103/111)	(86.4%, 96.3%)	99.2% (1018/1026)	(98.5%, 99.6%)
	S	1431	98.4% (247/251)	(96.0%, 99.4%)	99.0% (1168/1180)	(98.2%, 99.4%)
	Overall	2568	96.7% (350/362)	(94.3%, 98.1%)	99.1% (2186/2206)	(98.6%, 99.4%)

A = asymptomatic, C.I. = confidence interval, n = number, S = symptomatic

[A] Of the five neat urine Viper negative, composite reference positive, one was also negative by alternate NAAT test.
[B] Of the eight neat Viper positive, composite reference negative, six were also positive by alternate NAAT test.
[C] Of the seven vaginal Viper positive, composite reference negative, four were also positive by alternate NAAT test.
[D] Of the two endocervical Viper positive, composite reference negative, both were also positive in at least one specimen tested by alternate NAAT test.

TV Q^x Assay Performance Compared to Wet Mount and *T. vaginalis* Culture Composite Reference Result (by collection site)

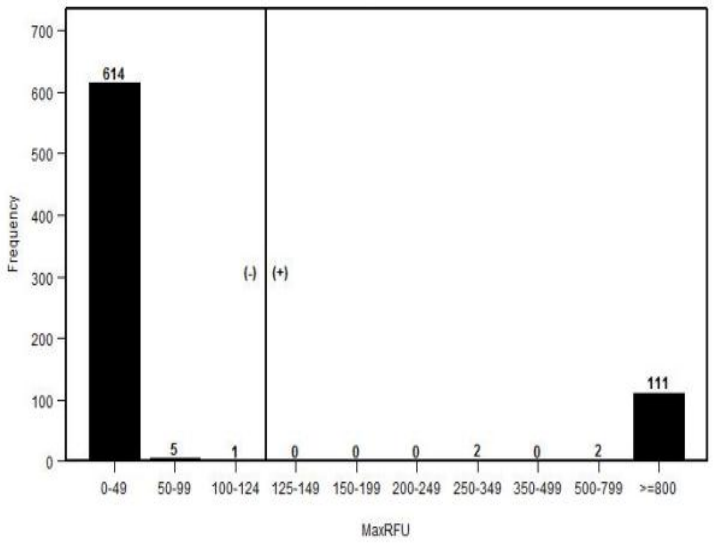
				Performance Compared to Composite Reference					
Specimen Type	**Clinical Site**	**Prev**	**n**	**Sensitivity**	**95% C.I.**	**Specificity**	**95% C.I.**	**PPV%**	**NPV%**
Neat Urine	1	29.1%	55	81.3% (13/16)	(57.0%, 93.4%)	100.0% (39/39)	(91.0%, 100.0%)	100.0%	92.9%
	2	27.4%	95	100.0% (26/26)	(87.1%, 100.0%)	98.6% (68/69)	(92.2%, 99.7%)	96.3%	100.0%
	3	14.4%	125	94.4% (17/18)	(74.2%, 99.0%)	97.2% (104/107)	(92.1%, 99.0%)	85.0%	99.0%
	4	2.8%	142	100.0% (4/4)	(51.0%, 100.0%)	99.3% (137/138)	(96.0%, 99.9%)	80.0%	100.0%
	5	9.9%	71	100.0% (7/7)	(64.6%, 100.0%)	98.4% (63/64)	(91.7%, 99.7%)	87.5%	100.0%
	6	7.4%	122	100.0% (9/9)	(70.1%, 100.0%)	100.0% (113/113)	(96.7%, 100.0%)	100.0%	100.0%
	7	25.6%	125	96.9% (31/32)	(84.3%, 99.4%)	97.8% (91/93)	(92.5%, 99.4%)	93.9%	98.9%
Vaginal	1	28.6%	56	100.0% (16/16)	(80.6%, 100.0%)	100.0% (40/40)	(91.2%, 100.0%)	100.0%	100.0%
	2	27.1%	96	100.0% (26/26)	(87.1%, 100.0%)	100.0% (70/70)	(94.8%, 100.0%)	100.0%	100.0%
	3	14.4%	125	94.4% (17/18)	(74.2%, 99.0%)	98.1% (105/107)	(93.4%, 99.5%)	89.5%	99.1%
	4	2.8%	142	100.0% (4/4)	(51.0%, 100.0%)	97.8% (135/138)	(93.8%, 99.3%)	57.1%	100.0%
	5	6.5%	169	90.9% (10/11)	(62.3%, 98.4%)	100.0% (158/158)	(97.6%, 100.0%)	100.0%	99.4%
	6	7.4%	122	100.0% (9/9)	(70.1%, 100.0%)	100.0% (113/113)	(96.7%, 100.0%)	100.0%	100.0%
	7	25.0%	128	100.0% (32/32)	(89.3%, 100.0%)	97.9% (94/96)	(92.7%, 99.4%)	94.1%	100.0%

Endocervical	1	24.7%	97	91.7% (22/24)	(74.2%, 97.7%)	100.0% (73/73)	(95.0%, 100.0%)	100.0%	97.3%
	2	23.9%	113	100.0% (27/27)	(87.5%, 100.0%)	100.0% (86/86)	(95.7%, 100.0%)	100.0%	100.0%
	3	15.6%	154	95.8% (23/24)	(79.8%, 99.3%)	100.0% (130/130)	(97.1%, 100.0%)	100.0%	99.2%
	4	3.8%	213	100.0% (8/8)	(67.6%, 100.0%)	98.0% (201/205)	(95.1%, 99.2%)	66.7%	100.0%
	5	6.5%	170	81.8% (9/11)	(52.3%, 94.9%)	100.0% (159/159)	(97.6%, 100.0%)	100.0%	98.8%
	6	7.4%	121	100.0% (9/9)	(70.1%, 100.0%)	100.0% (112/112)	(96.7%, 100.0%)	100.0%	100.0%
	7	24.4%	127	100.0% (31/31)	(89.0%, 100.0%)	99.0% (95/96)	(94.3%, 99.8%)	96.9%	100.0%

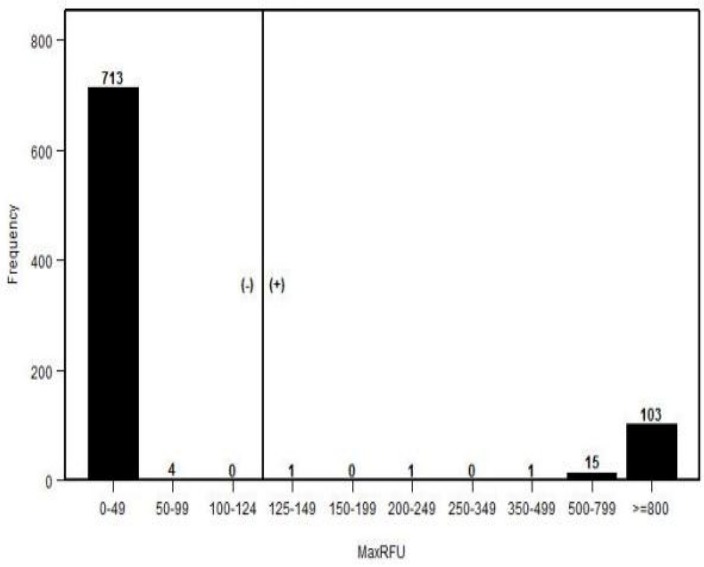
C.I. = confidence interval, n = number, NPV = negative predictive value, PPV = positive predictive value, Prev = prevalence, Spec = specimen

The results obtained with the TV Q^x Assay during the clinical performance testing were analyzed to generate frequency distributions of MaxRFU values. The data summarized in the following three tables indicate that positive and negative results obtained with the TV Q^x Assay were well separated from the cutoff of 125 MaxRFU.

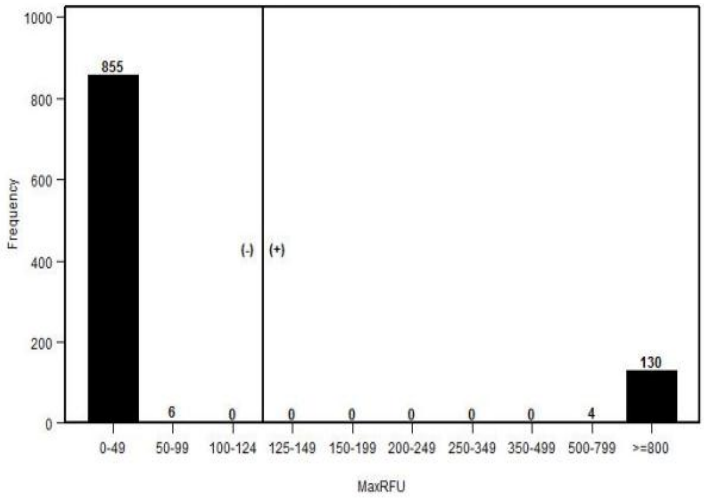
MaxRFU Distribution for Neat Urine



MaxRFU Distribution for Vaginal Swab Specimen



MaxRFU Distribution for Endocervical Swab Specimen



There were no CT/GC/TV Q^x Positive Control Failures from 235 TV Q^x runs. For the CT/GC/TV Q^x Negative Control, there was 1 CT/GC/TV Q^x Negative control failure from the 235 TV Q^x runs. The CT/GC/TV Q^x Positive and Negative Control MaxRFU values observed in the clinical trial are shown in Table below.

CT/GC/TV Q^x Control Information

Control	MaxRFU					
	n	Range	5th Percentile	Mean	Median	95th Percentile
Negative Control	234	0 – 56	0	11	8	32
Positive Control	235	724 – 2304	982	1419	1335	1960

n = number

4 Clinical cut-off**:**

A multi-site clinical trial was conducted to verify the pre-established cutoff value (threshold). The Cutoff Trial was designed to assess the performance of the TV Q^x Assay as defined by sensitivity and specificity relative to the reference methods (InPouch TV Culture and Wet Mount) when using the preliminary cutoff value. To evaluate the performance of the TV Q^x Assay, clinical specimens were collected from female participants at clinical centers across the United States, according to established inclusion/exclusion criteria. For specimen testing during the Cutoff Trial with the TV Q^x Assay, three BD Viper Systems were utilized at three external clinical sites. The MaxRFU values from the dataset were examined for distribution. Receiver operating curve (ROC) analysis was performed for the assay to verify that the current MaxRFU cutoff of 125 was appropriate for the TV Q^x Assay on the BD Viper System. ROC curves were created by specimen type (vaginal, neat urine, and endocervical) and overall. The data analysis showed that the current cutoff for positive results of > 125 is appropriate.

5. Expected values/Reference range:

A summary of the prevalence of *T. vaginalis*, by specimen type, as determined by the composite reference method during the clinical study (April 2012-August 2012) is shown in the following table.

TV Q^x Assay Prevalence by Specimen Type and Collection Site

	Prevalence (%) (# positive/# tested)							
Specimen Type	**All Sites**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**
Neat Urine	15.2 (112/735)	29.1 (16/55)	27.4 (26/95)	14.4 (18/125)	2.8 (4/142)	9.9 (7/71)	7.4 (9/122)	25.6 (32/125)
Vaginal	13.8 (116/838)	28.6 (16/56)	27.1 (26/96)	14.4 (18/125)	2.8 (4/142)	6.5 (11/169)	7.4 (9/122)	25.0 (32/128)
Endocervical	13.5 (134/995)	24.7 (24/97)	23.9 (27/113)	15.6 (24/154)	3.8 (8/213)	6.5 (11/170)	7.4 (9/121)	24.4 (31/127)

N. Instrument Name:

The BD Viper System

O. System Descriptions:

1. Modes of Operation:
Operation of the BD Viper System operating in extracted mode (BD Viper) remains the same as previously cleared (K081824/K081825/K103798).

2. Software:

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

   Yes ___X___ or No _____

3. Specimen Identification:

   Barcode or key entered accession numbers and patient sample location identification

4. Specimen Sampling and Handling:

   Automated

5. Calibration:

   Self-calibrated; the BD Viper instrument has 8 channels, one for each row of a 96-well plate. Each channel has slightly different optical characteristics. To minimize these differences, the reader has normalizers in each channel. After the normalizer is read, the readings are used by the Built-In-Test to verify that the detection system is in calibration. Any readings for a given normalizer that fall out of the expected range cause the system to report error conditions. The readers are recalibrated when a software upgrade is performed, when new Normalizers are logged in, and when System Parameters are cleared.

6. Quality Control:

   In addition to the extraction and external controls described in Section M.1.*d.*, the Specimen process controls which test the entire system may also be run in accordance with the requirements of appropriate accrediting organizations. For this purpose, known positive specimens can serve as controls by being processed and tested in conjunction with unknown specimens. Specimens used as processing controls must be stored, processed, and tested according to the package insert instructions. Specimen processing controls for *T. vaginalis* may also be prepared in the laboratory using commercially available Gibson Laboratories Tri-Valent™ Swab Positive Control (Cat. # TVS-01).

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

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

R. Conclusion:

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