510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K140446

B. Purpose for Submission:
The purpose of this submission is to migrate the BD ProbeTec™ *Chlamydia trachomatis* (CT) Q^x Amplified DNA Assay ("BD ProbeTec CTQ Assay") from the previously cleared (K081824) BD Viper System operating in extracted mode ("BD Viper") to the new BD Viper LT System.

C. Measurand:
Chlamydia trachomatis (CT) DNA

D. Type of Test:
Strand displacement nucleic acid amplification (SDA) assay

E. Applicant:
Becton, Dickinson and Company

F. Proprietary and Established Names:
BD ProbeTec™ *Chlamydia trachomatis* (CT) Q^x Amplified DNA Assay
BD ProbeTec CTQ Assay

G. Regulatory Information:
1. Regulation section:

   21 CFR 866.3120, *Chlamydia* serological reagents

2. Classification:

   Class I

3. Product code:

   MKZ

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

The BD ProbeTec *Chlamydia trachomatis* (CT) Q^x Amplified DNA Assay, when tested with either the BD Viper™ System in Extracted Mode or the BD Viper™ LT System, uses Strand Displacement Amplification technology for the direct, qualitative detection of *Chlamydia trachomatis* DNA in clinician-collected female endocervical and male urethral swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and male and female urine specimens (both UPT and Neat). The assay is also intended for use with gynecological specimens collected in BD SurePath™ Preservative Fluid or PreservCyt™ Solution using an aliquot that is removed prior to processing for either the BD SurePath or ThinPrep™ Pap test. The assay is indicated for use with asymptomatic and symptomatic individuals to aid in the diagnosis of chlamydial urogenital disease.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

Prescription only

4. Special instrument requirements:

The BD ProbeTec CTQ Assay uses SDA technology on the BD Viper System in Extracted Mode or the BD Viper LT System, which extracts, amplifies, and detects the target DNA.

I. Device Description:

The BD ProbeTec CTQ Assay uses SDA technology for the detection of CT DNA. The BD Viper LT System is a table-top instrument that is designed to be fully contained on a standard laboratory bench-top. The system performs automated extraction of nucleic acids from multiple specimen types in addition to amplification and detection of target nucleic acid sequences when utilized with legally marketed *in vitro* diagnostic assays.

J. Substantial Equivalence Information:

1. Predicate device name(s):

BD ProbeTec™ *Chlamydia trachomatis* (CT) Q^x Amplified DNA Assay on the BD Viper System

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

K081824

3. Comparison with predicate:

Item	**Predicate Device:** BD ProbeTec CTQ Assay on the BD Viper System – K081824	**Subject Device:** BD ProbeTec CTQ Assay on the BD Viper LT System – K140446
Intended Use	The BD ProbeTec CT 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 *Chlamydia trachomatis* DNA in clinician-collected female endocervical and male urethral swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and male and female urine specimens. The assay is indicated for use with asymptomatic and symptomatic individuals to aid in the diagnosis of chlamydial urogenital disease.	The BD ProbeTec *Chlamydia trachomatis* (CT) Q^x Amplified DNA Assay, when tested with either the BD Viper™ System in Extracted Mode or the BD Viper™ LT System, uses Strand Displacement Amplification technology for the direct, qualitative detection of *Chlamydia trachomatis* DNA in clinician-collected female endocervical and male urethral swab specimens, patient-collected vaginal swab specimens (in a clinical setting), and male and female urine specimens (both UPT and Neat). The assay is also intended for use with gynecological specimens collected in BD SurePath™ Preservative Fluid or PreservCyt™ Solution using an aliquot that is removed prior to processing for either the BD SurePath or ThinPrep™ Pap test. The assay is indicated for use with asymptomatic and symptomatic individuals to aid in the diagnosis of chlamydial urogenital disease.
Assay Results	Qualitative	Same as predicate
Technology	Strand displacement amplification (SDA)	Same as predicate
Instrument	BD Viper	BD Viper LT
Specimen Types	Key: liquid based cytology (LBC) urine preservative transport media (UPT)	Key: liquid based cytology (LBC) urine preservative transport media (UPT)

Item	**Predicate Device:** BD ProbeTec CTQ Assay on the BD Viper System – K081824	**Subject Device:** BD ProbeTec CTQ Assay on the BD Viper LT System – K140446
	<u>Female specimens:</u> Endocervical swab Patient-collected vaginal swab Neat urine UPT urine <u>Male Specimens:</u> Urethral swab Neat urine UPT urine	<u>Female specimens:</u> Endocervical swab Patient-collected vaginal swab Neat urine UPT urine LBC specimens collected in SurePath preservative fluid LBC specimens collected in PreservCyt Solution <u>Male Specimens:</u> Urethral swab Neat urine UPT urine
Priming Microwell		
Primers	CT cryptic plasmid	Same as predicate
Detector	<u>Linear Detector</u> Flourescein (fluorophore) Dabcyl (quencher)	Same as predicate
Nucleotides	4 of 4 nucleotides required for SDA	Same as predicate
Non-specific reagents and cofactors	Buffering components, magnesium ions, salt, and stabilizing reagents	Same as predicate
Amplification Microwell		
Restriction enzyme	*Bso*BI restriction enzyme	Same as predicate
Polymerase	*Bst* DNA polymerase	Same as predicate
Nucleotides	0 of 4 nucleotides required for SDA	Same as predicate
Non-specific reagents and cofactors	Buffering components, magnesium ions, salt, and stabilizing reagents	Same as predicate

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

1. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.* Sections 5 and 7 – Protocols for Evaluation of the Limit of Blank & Limit of Detection and Verification of Detection

Capability Claims. CLSI document EP 17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
2. CLSI. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.* Sections 5, 6, and 8 – Specimen Collection, Specimen Transport and Storage, & Sample Storage. CLSI document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
3. CLSI. *Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline – Second Edition*. Sections 10 and 13 – Establishment and Evaluation of Performance Characteristics of Molecular Diagnostic Tests & Controlling False-Positive Nucleic Acid Target Amplification Reactions. CLSI document MM3-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2006.
4. FDA. *Assay Migration Studies for in vitro Diagnostic Devices.* Guidance for Industry and FDA Staff; 2013.

L. Test Principle:

The BD ProbeTec CTQ Assay is based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescently-labeled detector probe. 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 BD Viper LT 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 CT DNA is determined by calculating the peak fluorescence (Maximum Relative Fluorescence Units; Max RFU) over the course of the amplification process and by comparing this measurement to a predetermined threshold value.

In addition to the fluorescent probe used to detect amplified CT target DNA, a second fluorescently-labeled oligonucleotide is incorporated into each reaction. The Extraction Control (EC) oligonucleotide is labeled with a different dye than that used for detection of the CT-specific target and is used to confirm the validity of the extraction process. The EC is dried in the Extraction Tubes and is re-hydrated upon addition of the specimen and extraction reagents. At the end of the extraction process, the EC fluorescence is monitored by the BD Viper LT System and an automated algorithm is applied to both the EC and CT-specific signals to report specimen results as positive, negative, or EC failure.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

    a. Precision/Reproducibility:

Reproducibility

To create the panel for the reproducibility study, CT (serovar H) and *Neisseria gonorrhoeae*

(GC; ATCC strain 19424) were spiked into clinical matrix in their respective diluents: vaginal swab matrix in Swab Diluent for the BD ProbeTec CT/GC Amplified DNA Assays ("Q^x Swab Diluent"), female urine in Urine Preservative Transport media for the BD ProbeTec Q^x Amplified DNA Assays ("UPT"), or liquid based cytology specimens (LBC) in PreservCyt LBC media diluted into BD LBC Specimen Dilution Tubes.

Four levels were created for each matrix/diluent type: negative, high negative (C_{20}-C_{80}), low positive (1.5X LoD), and moderate positive (3X LoD). Reproducibility for GC can be found in K140448. The reproducibility panel was comprised of twelve panel members (3 clinical matrices x 4 levels = 12 panel members). Testing was conducted at 2 external sites (each had one BD Viper LT) and one internal site (one BD Viper LT and three BD Viper Systems). One lot of extraction and amplification reagents and one lot of the Control Set for the BD ProbeTec CT/GC Q^x Amplified DNA Assays ("BD CT/GC Q^x Control Set") were included in this study.

For the BD Viper LT System, there were two operators per site testing one run per day on one instrument over a total of 8 days. Each panel member was tested in duplicate on three BD Viper LT Systems over eight days by two operators (2 x 3 x 8 x 2 = 96 tests per level). The reproducibility the BD ProbeTec CTQ Assay on the BD Viper is shown in Table 1 below.

Table 1 - Reproducibility of the BD ProbeTec CTQ Assay on the BD Viper LT System

					Within Run		Between Run within Day		Between Day within Site		Between Site		Total	
Specimen Type	Panel	% Expected Results[#]	95% CI	Mean RFU	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
PreservCyt LBC	Negative	99.0% (95/96)	(94.3 - 99.8%)	14.6	104.7	718.8	0.0	0.0	7.2	49.1	6.9	47.4	105.2	722.0
	High Negative	41.7% (40/96)	(32.3 - 51.7%)	565.0	533.2	94.4	226.5	40.1	0.0	0.0	0.0	0.0	579.3	102.5
	Low Positive	100.0% (96/96)	(96.2 - 100.0%)	1435.3	372.3	25.9	0.0	0.0	85.2	5.9	86.4	6.0	391.6	27.3
	Moderate Positive	98.9% (93/94)[*]	(94.2 - 99.8%)	1752.4	241.0	13.8	0.0	0.0	36.1	2.1	0.0	0.0	243.7	13.9
Vaginal Swab	Negative	100.0% (96/96)	(96.2 - 100.0%)	22.1	28.6	129.3	8.7	39.5	0.0	0.0	5.0	22.5	30.3	137.0
	High Negative	24.0% (23/96)	(16.5 - 33.4%)	974.0	716.7	73.6	0.0	0.0	0.0	0.0	105.1	10.8	724.4	74.4
	Low Positive	95.8% (91/95)[*]	(89.7 - 98.4%)	1603.8	535.8	33.4	115.0	7.2	0.0	0.0	0.0	0.0	548.0	34.2
	Moderate Positive	100.0% (96/96)	(96.2 - 100.0%)	1780.6	320.3	18.0	0.0	0.0	59.6	3.3	52.2	2.9	330.0	18.5
Female UPT	Negative	99.0% (95/96)	(94.3 - 99.8%)	21.5	171.4	797.4	0.0	0.0	0.0	0.0	5.8	27.0	171.5	797.9
	High Negative	32.3% (31/96)	(23.8 - 42.2%)	678.3	578.6	85.3	276.0	40.7	0.0	0.0	0.0	0.0	641.1	94.5
	Low Positive	99.0% (95/96)	(94.3 - 99.8%)	1549.4	432.5	27.9	0.0	0.0	85.9	5.5	0.0	0.0	441.0	28.5
	Moderate Positive	100.0% (96/96)	(96.2 - 100.0%)	1747.0	223.9	12.8	133.5	7.6	0.0	0.0	22.5	1.3	261.6	15.0

[*]There were two moderate positive LBC samples and one low positive swab sample which resulted in an extraction transfer error and therefore no valid results were available for analysis.

[#] The results for negative panel members were calculated according to an expected result of 'negative for CT.' All other panel members were calculated according to an expected result of 'positive for CT.'

For the BD Viper System, there were two operators testing three runs per day on each of the 3 instruments (6 runs total per day) over a total of 4 days. Each panel member was tested in duplicate on three BD Viper Systems over four days by two operators (2 x 6 x 4 x 2 = 96 tests

per level).The reproducibility the BD ProbeTec CTQ Assay on the BD Viper is shown in Table 2 below.

Table 2 – Reproducibility of the BD ProbeTec CTQ Assay on the BD Viper System

					Within Run		Between Run within Day		Between Day within Instrument		Between Instrument		Total	
Specimen Type	Panel	% Expected Results[#]	95% CI	Mean RFU	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
PreservCyt LBC	Negative	99.0% (95/96)	(94.3 - 99.8%)	14.1	135.6	962.7	0.0	0.0	1.1	7.8	4.2	29.9	135.6	963.2
	High Negative	44.8% (43/96)	(35.2 - 54.7%)	723.3	771.3	106.6	0.0	0.0	211.8	29.3	0.0	0.0	799.8	110.6
	Low Positive	95.8% (92/96)	(89.8 - 98.4%)	1542.5	530.9	34.4	0.0	0.0	0.0	0.0	0.0	0.0	530.9	34.4
	Moderate Positive	99.0% (95/96)	(94.3 - 99.8%)	1951.3	269.2	13.8	0.0	0.0	0.0	0.0	112.7	5.8	291.8	15.0
Vaginal Swab	Negative	100.0% (96/96)	(96.2 - 100.0%)	1.3	5.6	437.0	2.9	224.2	0.0	0.0	1.7	133.2	6.5	508.9
	High Negative	30.2% (29/96)	(21.9 - 40.0%)	832.6	759.7	91.2	0.0	0.0	0.0	0.0	0.0	0.0	759.7	91.2
	Low Positive	97.9% (94/96)	(92.7 - 99.4%)	1660.9	445.5	26.8	0.0	0.0	0.0	0.0	54.6	3.3	448.8	27.0
	Moderate Positive	99.0% (95/96)	(94.3 - 99.8%)	1677.3	413.3	24.6	0.0	0.0	105.1	6.3	71.1	4.2	432.3	25.8
Female UPT	Negative	100.0% (96/96)	(96.2 - 100.0%)	0.6	2.5	407.6	0.0	0.0	0.0	0.0	0.6	92.3	2.6	417.9
	High Negative	29.2% (28/96)	(21.0 - 38.9%)	973.5	795.9	81.8	201.9	20.7	0.0	0.0	0.0	0.0	821.1	84.3
	Low Positive	100.0% (96/96)	(96.2 - 100.0%)	1780.6	394.4	22.1	0.0	0.0	0.0	0.0	0.0	0.0	394.4	22.1
	Moderate Positive	100.0% (96/96)	(96.2 - 100.0%)	1925.5	225.7	11.7	0.0	0.0	49.3	2.6	34.7	1.8	233.6	12.1

[#] The results for negative panel members were calculated according to an expected result of 'negative for CT.' All other panel members were calculated according to an expected result of 'positive for CT.'

The data presented in Tables 1 and 2 demonstrate good reproducibility for the BD ProbeTec CTQ Assay on the BD Viper LT and the BD Viper System. On both systems, the assay shows ≥ 95% detection of CT at low positive/close to the detection limit and moderate positive levels. For the high negative samples, detection was >5% and <95% positive on both systems. Taken together, this data indicates that the reproducibility for the BD Viper LT and the BD Viper System is similar.

b. Linearity/assay reportable range:
N/A

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

Post Pre-Warm Specimen Stability

The existing workflow previously cleared for the BD ProbeTec CTQ Assay on the BD Viper includes a pre-warm step for swab and urine samples. The workflow proposed in K140446 for the BD Viper LT System includes a pre-warm step for LBC specimens in SurePath or PreservCyt media so all sample types (urine, swabs, and LBC specimens) can be tested on the same instrument run. For this reason, a post pre-warm specimen stability study was conducted to support the storage and transport stability claims for LBC specimens in SurePath or PreservCyt LBC media.

Mini-pools were created by diluting CT and GC negative LBC specimens in either SurePath or PreservCyt LBC media at a ratio of 1:3.4 mL (12 mini-pools were created for each LBC media). Each mini-pool was split equally. Half were spiked with CT serovar H and GC strain ATCC 19424 (at 90 EB/mL and 300 cells/mL, respectively) and half were not spiked (negative samples). Results for GC can be found in K140448. From these split mini-pools, 2.2 mL aliquots were removed and dispensed into BD LBC Specimen Dilution Tubes (15 samples per group; 30 samples total/mini-pool). Fourteen of these tubes were pre-warmed in the BD Viper LT heat block at 114°C for 15 min, one tube was read at "baseline" (no warming).

After the pre-warm step, the seven tubes were stored at the following conditions: two tubes at 2-8°C for 7 days, two tubes at 30°C for 7 days, and two tubes at -20°C for 90 days (one tube was available for testing and one was available for retesting, if required). At each storage condition/time point, one negative and one positive tube from each of the 12 mini-pools was removed and tested in a dual dispensing mode, which generated two replicate test results (1 tube x 2 replicates x 12 mini-pools = 24 measurements/storage condition/time point for each LBC media type). Four lots of extraction reagents, 2-3 lots of amplification reagents, three lots of the BD CT/GC Q^x Control Set, and three BD Viper LT Systems were included in this study. Results are summarized in Tables 2 and 3 below.

Table 3 - PreservCyt Specimens Post Pre-Warm Stability Results

Temperature	Timepoint	Positives	Negatives
N/A	Baseline	24/24 (Pass)	0/24 (Pass)
2-8 °C	Day 3	24/24 (Pass)	0/24 (Pass)
30 °C	Day 3	24/24 (Pass)	0/24 (Pass)
2-8 °C	Day 7	24/24 (Pass)	0/24 (Pass)
30 °C	Day 7	24/24 (Pass)	0/24 (Pass)
-20 °C	Day 90	24/24 (Pass)	0/24 (Pass)
-20 °C	Day 90	24/24 (Pass)	0/24 (Pass)*

*Data generated by Out of Specification (OOS) testing after contaminated environment was deamed clean.

Table 4 - SurePath Specimens Post Pre-Warm Stability Results

Temperature	Timepoint	Positives	Negatives
N/A	Baseline	24/24 (Pass)	0/24 (Pass)
2-8 °C	Day 3	24/24 (Pass)	0/24 (Pass)
30 °C	Day 3	24/24 (Pass)	0/24 (Pass)
2-8 °C	Day 7	24/24 (Pass)	0/24 (Pass)
30 °C	Day 7	24/24 (Pass)	0/24 (Pass)
-20 °C	Day 90	24/24 (Pass)	0/24 (Pass)
-20 °C	Day 90	24/24 (Pass)	0/24 (Pass)

The data indicates that, for all conditions and time points tested, the percent positive rate for pre-warmed CT positive and percent negative rate for CT negative LBC samples was 100% for samples stored in either PreservCyt or SurePath LBC media.

Run Control Failure Rate

This analysis was conducted to determine the failure rate for the BD CT/GC Q^x Control Set across four different studies. One positive and negative control result was recorded for each run for each of the following:

Clinical agreement study = 56 runs

Carry-over study (in K140446, referred to as "contamination validation study") = 63 runs

LoD/LoB validation studies = 71 runs

Reproducibility study = 51 runs

A total of 241 results were collected for each control type. There were no false negatives found in the positive control samples assessed (0/241 x 100 = 0% failure rate). There were 2 false positives found in the negative control samples assessed (2/241 x 100 = 0.83% failure rate).

Environmental Study

The environmental study was conducted to assess the performance of the BD ProbeTec CTQ Assay on the BD Viper LT when the instrument is exposed to variations in environmental temperature and humidity. One BD Viper LT was placed in an environmental chamber and equilibrated to either 1) 18°C with 20% humidity (low temperature, low humidity) or 2) 27°C with 85% humidity (high temperature, high humidity). Three lots of positive and negative controls from the BD CT/GC Q^x Control Set were reconstituted with Viper QC Wash Buffer and run as samples. For the negative controls, 30 "samples" were prepared from each lot with one run per lot. An extraction control (EC) was included in each of the negative controls to show that the negative result was valid (30 negative samples/ECs x 3 lots = 90 measurements). For the positive controls, 90 "samples" were prepared from each lot with 3 runs/lot (90 positive samples/lot x 3 lots = 270 measurements).

Each rack was loaded with all positive or all negative controls (30/rack). These were logged in as "samples." On each rack, one pair of positive and negative controls were run normally (e.g., reconstituted, extracted, and amplified by the BD Viper LT). This study required 12 runs total with one lot of extraction and amplification reagents for testing with the assay. The results are summarized in Table 5 below.

Table 5 - Environmental System Testing Summary

Conditions/Parameter	BD ProbeTec CTQ Assay Percent Correct (N)			Pass/Fail ≥97%
	EC	Negative	Positive	
1: 18°C / 20% RH	100% (90/90)	100% (90/90)	100% (270/270)	Pass
2: 27°C / 85% RH	100% (90/90)	100% (90/90)	100% (270/270)	Pass

There were no false negatives or false positives detected in this study, indicating that the performance of the BD ProbeTec CTQ Assay on the BD Viper LT is not affected by the temperature and humidity tested in this study.

d. Detection limit:

Limit of Detection (LoD)

The following clinical matrices were tested in the LoD studies: vaginal swabs in Q^x Swab Diluent, urine in UPT, and SurePath or PreservCyt LBC specimens in LBC Specimen Dilution Tubes. For all LoD studies, CT serovar H (ATCC VR879; strain UW-43/Cx) and GC (ATCC strain 19424) were paired for testing and CT serovar D (ATCC VR885; strain UW-3Cx) and GC (ATCC strain 49226) were paired for testing. Results of the LoD studies for GC can be found in K140448.

i. LoD Detection – BD Viper and BD Viper LT

For each matrix, four mini-pools were prepared and then used to create LoD detection panels (one panel was created for each of the four mini-pools). Each LoD detection panel contained six analyte levels for CT and GC (CT concentrations in Table 6 below; GC results can be found in K140448). There were six replicates created for the six analyte levels (4 mini-pools x 6 analyte levels x 6 replicates for each of the analyte levels = 144 total tubes tested per matrix).

Table 6 – CT Levels (in EB/mL) Tested in LoD Detection Study for Each Matrix Type

Level	Vaginal Swab in Q^x Swab Diluent		UPT Urine	SurePath LBC Specimen in LBC Diluent	PreservCyt LBC Specimen in LBC Diluent
	Initial	Repeat*			
0	0	0	0	0	0
1	3	5	1	3	3
2	7	10	3	7	7
3	15	20	7	15	15
4	30	40	15	30	30
5	60	60	30	60	60

*Repeat of CT serovar H vaginal swab LoD with new CT target levels and increased replicates due to variable positive rates and poor reproducibility at low target levels thus impacting the model fit and the accuracy of the LoD estimates. Target levels chosen are representative of the claimed LoD per K091824.

The LoD detection study was conducted in one day for each matrix type by two operators running samples on 3 BD Viper and 3 BD Viper LT Systems. Table 7 demonstrates the setup of a rack on one instrument. Each rack was run twice on the same instrument (4 replicates x 6 levels x 2 runs/rack = 48 measurements/rack).

Table 7 – Sample Rack Set up for LoD Detection Study

		Level 5 MP 1	Level 4 MP 1	Level 3 MP 1	Level 2 MP 1	Level 1 MP 1	null MP 1
		Level 5 MP 2	Level 4 MP 2	Level 3 MP 2	Level 2 MP 2	Level 1 MP 2	null MP 2

		Level 5 MP 3	Level 4 MP 3	Level 3 MP 3	Level 2 MP 3	Level 1 MP 3	null MP 3
Pos Cont	Neg Cont	Level 5 MP 4	Level 4 MP 4	Level 3 MP 4	Level 2 MP 4	Level 1 MP 4	null MP 4

(Level = analyte level; MP = mini-pool)

Three different extraction and amplification reagent lots and three lots of BD CT/GC Q^x Control Set were used for the LoD detection study

A target titration plot was created by graphing the percent positive versus the concentration of CT EBs/mL in Log_{10}. Probit analysis was used to estimate the 95% LoD concentration for the BD Viper LT and BD Viper System. Results of the LoD detection study are shown in the Table 8 below.

Table 8 - LoD for BD Viper LT and BD Viper Systems

Specimen Matrices	Serovar	BD Viper LT LoD (95% CI)	BD Viper LoD (95% CI)	LoD Ratio (95% CI)
Vaginal Swabs in Q^x Swab Diluent	CT Serovar H	47 (32, 69)	45 (31, 67)	1.0 (0.9, 1.1)
	CT Serovar D	53 (33, 85)	48 (30, 78)	1.0 (0.9, 1.2)
UPT Urine	CT Serovar H	39 (28, 55)	27 (19, 37)	1.1 (1.0, 1.2)
	CT Serovar D	16 (11, 22)	12 (9, 17)	1.1 (1.0, 1.3)
SurePath LBC Sample in LBC Diluent	CT Serovar H	17 (12, 27)	26 (17, 40)	0.9 (0.7, 1.0)
	CT Serovar D	16 (13, 21)	14 (11, 18)	1.0 (0.9, 1.2)
PreservCyt LBC Sample in LBC Diluent	CT Serovar H	14 (11, 18)	15 (12, 20)	1.0 (0.9, 1.1)
	CT Serovar D	11 (8, 15)	15 (11, 20)	0.9 (0.7, 1.0)

The data shown above is acceptable as it meets the pre-determined acceptance criteria that the LoD ratio between the BD Viper LT and BD Viper Systems must be 0.8-1.2 (BD Viper LT/BD Viper).

ii. LoD Confirmation – BD Viper LT

For each matrix, one pool was created and then split into two aliquots of equal volume. One aliquot was spiked with CT serovar H and the other aliquot was spiked with CT serovar D. The LoD concentrations from the LoD determination study (see table 8 above) were used for spiking levels. Twenty replicates were generated from each aliquot. All samples were tested using one lot of reagents and one BD Viper LT. The results of the LoD confirmation are shown in Table 9 below.

Table 9 – Results of LoD Confirmation Study

Specimen Matrices	Serovar	Total # Tested	Total Positive	Total Negative	Percent Positive
Vaginal Swabs in Q^x Swab Diluent	CT Serovar H	20	19	1	95%
	CT Serovar D	20	20	0	100%
UPT Urine	CT Serovar H	20	19	1	95%

	CT Serovar D	20	19	1	95%
SurePath LBC Sample in LBC Diluent	CT Serovar H	20	20	0	100%
	CT Serovar D	20	19	1	95%
PreservCyt LBC Sample in LBC Diluent	CT Serovar H	20	20	0	100%
	CT Serovar D	20	20	0	100%

The results from the LoD confirmation study were found to be acceptable as they met the pre-determined acceptance criteria of 95% positive agreement with the LoD levels from the LoD determination study.

Limit of Blank (LoB)

Data from CT negative specimens (Level 0) from the analytical LoD studies described above were combined to calculate an overall LoB for the BD ProbeTec CTQ Assay on the BD Viper LT. Data from all specimen matrices were compared and the worst (or highest) LoB estimate among all the specimen matrices was selected as the overall LoB (68 MaxRFU). Because this value is below the assay threshold of 125 MaxRFU, this demonstrates that the assay has an acceptable separation between background fluorescence obtained from CT negative specimens and the assay cut-off for a positive result.

e. Analytical specificity:

Carry-Over/System Contamination

The carry-over study was conducted to look for the presence of contamination in negative specimens due to carry-over of DNA during nucleic acid extraction and PCR amplification in the BD Viper LT. Each run consisted of 15 high positive and 15 negative samples arranged in alternating positive or negative rows. To create high positive samples, 1 x 10^5 EB/mL of CT was spiked into either one of two diluents (listed below). These diluents were selected because they represent two most challenging settings that can cause sample contamination.

1. 3 mL of Q^x Swab Diluent: This volume was selected to represent a maximum fill volume, thereby maximizing the amount of fluid adhering to the outside of the pipette tip.
2. PreservCyt LBC media diluted in BD LBC Specimen Dilution tube diluent (1.7 mL LBC diluent + 0.5 mL PreservCyt = 2.2 mL; "LBC Specimen Matrix"): This LBC media was selected because it has a high alcohol content and is therefore more likely to drip from the pipette tips during sample transfer due to low surface tension.

Each run consisted of 30 samples. Seven runs were conducted per instrument with 3 BD Viper LT Systems for a total of (30 samples/run x 7 runs x 3 instruments) 630 measurements for each diluent. Runs were conducted by three operators over a period of three days. Two lots of extraction reagents, one lot of amplification reagents, and two lots of the BD CT/GC Q^x Control Set were used in this study.

Table 10 – Carry Over on Viper LT

BD Viper LT System	Q^x Sample Diluent			LBC Specimen Matrix		
	n	Positive Results	Percent Positive	n	Positive Results	Percent Positive
1	210	0	0.00%	210	0	0.00%
2	210	1	0.48%	210	0	0.00%
3	210	1	0.48%	210	0	0.00%
Overall	630	2	0.32%	630	0	0.00%

The overall rate of carry-over was 0.32% (2/630) for CT spiked into the Q^x Swab Diluent and 0.0% (0/630) for CT spiked into PreservCyt media diluted in BD LBC Specimen Dilution tube diluent. These results are acceptable as they are comparable to the levels of carry-over cleared for the BD ProbeTec CTQ Assay on the BD Viper in K081824.

f. Assay cut-off:

The assay cut-off was established previously in K081824.

2. Comparison studies:

a. Method comparison with predicate device:

Clinical Comparison Study

The results of the BD ProbeTec CTQ Assay run on the BD Viper LT were compared to the results obtained with the BD Viper System in a clinical comparison study. Clinical samples were collected between October 2012 and April 2013 from four geographically diverse clinical sites in North America (OB/GYN, sexually transmitted disease and family planning clinics). For each female subject, specimens were collected in the following order: (1) a first void urine specimen, (2) five patient-collected vaginal swab specimens, and (3) a SurePath liquid based cytology (LBC) specimen and a PreservCyt LBC specimen. The LBC specimen collection order was randomized throughout the study. For each male subject, a first void urine specimen was collected. Each urine specimen (male and female) was aliquoted into five UPTs (BD urine Preservative Transport for the Q^x Amplified DNA Assays).

A total of 823 subjects were enrolled (170 males and 653 females). Specimens were collected from subjects presenting with symptoms of CT and/or GC (symptomatic) and shipped to BD on cold packs for screening, aliquoting, and panel assembly. Following exclusion of non-compliant subjects and non-evaluable specimens, a total of 617 compliant female subjects and 167 compliant male subjects were available for the clinical comparison panel assembly.

To create the clinical comparison panel, for each subject, a UPT specimen was tested on the BD Viper. These results were used to confirm that the target number of CT positive panel members had been met. The clinical comparison panel consisted of randomly selected positive and negative specimens with four aliquots created from each specimen type. The number of

specimens selected for the clinical comparison panel was based on minimums suggested by FDA in previous correspondence (minimum of 40 CT positive and 40 CT negative). The positive and negative specimens were randomized within the panel and the testing sites were blinded to the results for each panel member. Panels were identical across all testing sites. Three external sites tested the panels on the BD Viper LT. An internal site tested the panels on the BD Viper System. Results from the two instruments were compared and expressed statistically as positive and negative percent agreement.

Table 11 - PPA and NPA for the BD ProbeTec CTQ Assay on the BD Viper LT System versus the BD Viper System

			Positive Percent Agreement		Negative Percent Agreement	
Gender	Specimen Type	Site	Percent	95% CI	Percent	95% CI
Female	Vaginal Swab	A	100.0% (46/46)	(92.3%, 100.0%)	95.0% (57/60)	(86.3%, 98.3%)
		B	100.0% (46/46)	(92.3%, 100.0%)	98.3% (59/60)	(91.1%, 99.7%)
		C	100.0% (46/46)	(92.3%, 100.0%)	91.7% (55/60)	(81.9%, 96.4%)
		Total	100.0% (138/138)	NA	95.0% (171/180)	(90.6%, 98.3%)
	Q^x UPT	A	97.9% (46/47)	(88.9%, 99.6%)	98.3% (58/59)	(91.0%, 99.7%)
		B	97.9% (46/47)	(88.9%, 99.6%)	100.0% (59/59)	(93.9%, 100.0%)
		C	95.7% (45/47)	(85.8%, 98.8%)	98.3% (58/59)	(91.0%, 99.7%)
		Total	97.2% (137/141)	(92.2%, 100.0%)	98.9% (175/177)	(96.6%, 100.0%)
	BD SurePath	A	100.0% (39/39)	(91.0%, 100.0%)	97.0% (65/67)	(89.8%, 99.2%)
		B	100.0% (39/39)	(91.0%, 100.0%)	97.0% (65/67)	(89.8%, 99.2%)
		C	100.0% (39/39)	(91.0%, 100.0%)	97.0% (65/67)	(89.8%, 99.2%)
		Total	100.0% (117/117)	NA	97.0% (195/201)	(93.0%, 100.0%)
	PreservCyt	A	97.5% (39/40)	(87.1%, 99.6%)	97.0% (64/66)	(89.6%, 99.2%)
		B	95.0% (38/40)	(83.5%, 98.6%)	98.5% (65/66)	(91.9%, 99.7%)
		C	95.0% (38/40)	(83.5%, 98.6%)	98.5% (65/66)	(91.9%, 99.7%)
		Total	95.8% (115/120)	(89.2%, 100.0%)	98.0% (194/198)	(96.0%, 99.5%)
Male	Q^x UPT	A	97.9% (47/48)	(89.1%, 99.6%)	100.0% (65/65)	(94.4%, 100.0%)
		B	95.8% (46/48)	(86.0%, 98.8%)	100.0% (65/65)	(94.4%, 100.0%)
		C	95.8% (46/48)	(86.0%, 98.8%)	98.5% (64/65)	(91.8%, 99.7%)
		Total	96.5% (139/144)	(92.4%, 100.0%)	99.5% (194/195)	(98.5%, 100.0%)
Total	All	Total	97.9% (646/660)	(96.0%, 99.4%)	97.7% (929/951)	(96.3%, 98.8%)

*Denominator based on the results from the BD Viper System and numerator based on the results from the BD Viper LT System. For reproducibility by site, the 95% CI was calculated using the score method. For the total reproducibility, the 95% CI was calculated using the bootstrap approach (except when there was 100% agreement at all sites, then the 95% CI cannot be calculated).

NA = Not Applicable

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity and Specificity

N/A

4. Clinical cut-off**:**

N/A

5. Expected values/Reference range:

N/A

N. Proposed Labeling:

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

O. Conclusion:

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