

# Performance of the BD ProbeTec™ CT Q<sup>x</sup> and GC Q<sup>x</sup> Amplified DNA Assays with PreservCyt® Specimens on the BD Viper™ System in Extracted Mode

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## ABSTRACT

We have developed two novel assays for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) for use with the BD Viper™ System in extracted mode.\* Here we characterize the performance of the new BD ProbeTec™ CT Q<sup>x</sup> and GC Q<sup>x</sup> Amplified DNA Assays\* with PreservCyt® liquid-based cytology specimens (Cytoc, Corp.). A 0.5mL volume of PreservCyt specimen (removed prior to Pap processing is added to a pre-filled BD ProbeTec Liquid-Based Cytology Specimen Dilution Tube. Specimens require no further processing by the user, and are loaded directly onto the BD Viper System for DNA extraction and amplification.

The analytical limits of detection (95% proportion positive) for the CT Q<sup>x</sup> and GC Q<sup>x</sup> assays with clean PreservCyt Solution were estimated to be 8 Elementary Bodies (EB) and 4 GC cells/mL of diluted PreservCyt, respectively. With pooled PreservCyt clinical specimens, the analytical sensitivities were 10 EB and 27 GC cells/mL of diluted specimen. We also demonstrated the ability of the CT Q<sup>x</sup> and GC Q<sup>x</sup> assays to detect a multitude of clinically relevant serovars and strains when spiked into PreservCyt Solution at levels close to the analytical limits of detection. In addition, the two assays were shown to be tolerant to common exogenous and endogenous substances that may be present in PreservCyt clinical specimens, including but not limited to blood, mucous, semen, leukocytes and various prescription and over-the-counter medications. When CT and GC organisms were spiked into PreservCyt clinical matrix at levels near the analytical limits of detection, CT and GC DNA were successfully detected after storage in the PreservCyt vials for up to 30 days at 2-30°C.

We conclude that the CT Q<sup>x</sup> and GC Q<sup>x</sup> Amplified DNA Assays on the BD Viper System in extracted mode have the potential to offer competitive analytical sensitivity when used with PreservCyt liquid-based cytology specimens. This alternative specimen type offers physicians the convenience and flexibility of testing for two important sexually transmitted diseases from a single patient specimen, in addition to routine cervical cancer screening.

## INTRODUCTION

The World Health Organization estimates that there are 92 million new cases of infection due to *Chlamydia trachomatis* (CT) and 62 million due to *Neisseria gonorrhoeae* (GC) each year (1). In the United States, 76% of CT infections occur in women, of which 70-90% are asymptomatic. As a result, long-term health problems can develop before a woman even knows she is at risk (2, 3). If left untreated, CT and GC infections in women may cause long-term sequelae such as pelvic inflammatory disease and infertility.

The use of liquid-based cytology (LBC) specimens to diagnose CT and GC infections is an attractive alternative to that of traditional swab and urine sample types, offering convenience and cost savings to patients, physicians and laboratories by allowing multiple test results to be reported from a single specimen. However, special care must be taken in handling cytology specimens to avoid the potential for cross contamination and possible false-positive nucleic acid amplification test (NAAT) results. Cytoc Corporation obtained FDA clearance to allow for removal of up to 4mL of a given PreservCyt® cytology specimen prior to Pap processing. This provides the laboratory flexibility and helps mitigate the potential for cross contamination of specimens. With this development, removal of an aliquot of cytology medium for NAAT screening ("pre-quot") prior to preparation of the Pap smear is gradually gaining acceptance as the preferred methodology.

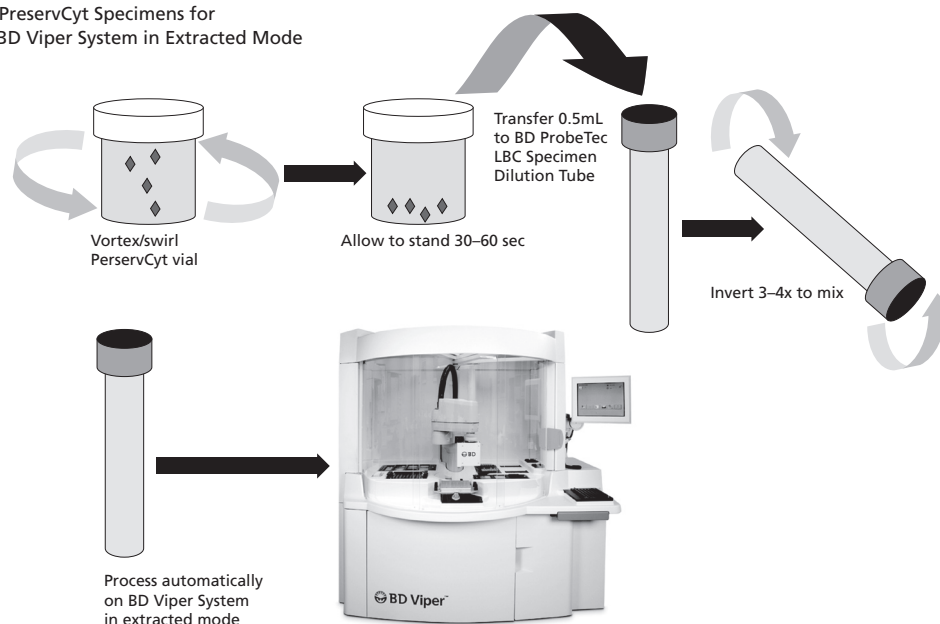
Here we describe the analytical performance of the new BD ProbeTec™ CT/GC Q<sup>x</sup> Amplified DNA Assays on the BD Viper™ System in extracted mode with PreservCyt® LBC specimens. This fully automated assay system involves the chemical lysis of cells, followed by non-specific binding of DNA to positively-charged iron particles, washing of the bound nucleic acid and elution in an amplification-compatible buffer. Amplification and detection of target DNA are performed on-board the BD Viper System using homogeneous real-time Strand Displacement Amplification (SDA) and fluorescent detection. Included in each reaction is a fluorescently labeled Extraction Control (EC) oligonucleotide. The non-amplified EC is labeled with a different dye than that used for detection of the CT and GC target DNA. Recovery of the EC confirms the validity of the extraction process for CT and/or GC negative results.

## REFERENCES

1. World Health Organization. 2001. Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates. WHO.
2. Centers for Disease Control and Prevention. STD surveillance 2005: national profile. <http://www.cdc.gov/std/stats/chlamydia.htm>.
3. US Preventive Services Task Force. 2001. Screening for chlamydial infection: recommendations and rationale. *Am J Prev Med* 20 (3S): 90-94.

## METHODS AND RESULTS

Figure 1. Preparation of PreservCyt Specimens for Testing on the BD Viper System in Extracted Mode



**PREPARATION OF PRESERVCYT SPECIMENS (Figure 1):** PreservCyt specimens are prepared for testing on the BD Viper System in extracted mode prior to processing on the ThinPrep™ 2000 (Cytoc, Corp.) instrument.

**RESULTS ALGORITHM ASSAY** results were determined by calculating the peak fluorescence (Maximum Normalized Relative Fluorescent Units [MaxRFU]) over the course of the amplification process and by analysis of EC values:

CT Q <sup>x</sup> or GC Q <sup>x</sup> MaxRFU	EC Value	Interpretation
≥125	Any	Positive for CT or GC DNA
<125	Positive	Negative for CT or GC DNA
<125	Negative	EC failure

**ANALYTICAL LIMITS OF DETECTION (Table 1):** Clean PreservCyt Solution and pooled clinical specimens were diluted and seeded with CT and GC organisms at six different concentrations. A total of 72 assay replicates was generated at each target level across three different BD Viper Systems and reagent lots.

Table 1. CT/GC Q<sup>x</sup> Assays: Analytical Limits of Detection (LOD) in PreservCyt Solution

Target	LOD Point Estimate	Lower 95% Confidence Interval	Upper 95% Confidence Interval
CT Q <sup>x</sup> – Clean System	8 EB/mL	4 EB/mL	11 EB/mL
CT Q <sup>x</sup> – Clinical Matrix	10 EB/mL	6 EB/mL	13 EB/mL
GC Q <sup>x</sup> – Clean System	4 cells/mL	0 cells/mL	8 cells/mL
GC Q <sup>x</sup> – Clinical Matrix	27 cells/mL	12 cells/mL	43 cells/mL

**SEROVARS AND STRAINS (Table 2):** Clean PreservCyt Solution was diluted and seeded separately with each of 16 CT serovars (15 EB/mL) and 17 GC strains (50 cells/mL). Twenty-four assay replicates were generated for each serovar and strain.

Table 2. Detection of *C. trachomatis* Serovars and *N. gonorrhoeae* Strains in PreservCyt Solution

CT Serovar	CT Q <sup>x</sup> Assay: Percent Positive @15 EB/mL	GC Strain	GC Q <sup>x</sup> Assay: Percent Positive @50 cells/mL
A	100 (24/24)	19424	100 (24/24)
B	100 (24/24)	27628	100 (24/24)
Ba	100 (24/24)	27629	100 (24/24)
C	100 (24/24)	27630	100 (24/24)
D	100 (24/24)	27632	100 (24/24)
E	100 (24/24)	27633	100 (24/24)
F	100 (24/24)	27631	100 (24/24)
G	100 (24/24)	21823	100 (24/24)
H	100 (24/24)	51803	100 (24/24)
I	100 (24/24)	23051	100 (24/24)
J	100 (24/24)	31407	100 (24/24)
K	100 (24/24)	31953	100 (24/24)
LGV1	100 (24/24)	35201	100 (24/24)
LGV2	100 (24/24)	31397	100 (24/24)
LGV3	100 (24/24)	31151	100 (24/24)
Variant (E)*	100 (24/24)	43785	100 (24/24)
		51804	100 (24/24)

\* Variant strain of serovar E from Northern Europe with a 377 base pair deletion in the ORF1 of the cryptic plasmid.

**METHODS AND RESULTS (CONTINUED)**

**INTERFERING SUBSTANCES (Tables 3&4):** Potential interfering substances were tested by addition to pooled clinical specimen matrix diluted and seeded with 90 CT EB/mL and 300 GC cells/mL. Parallel controls without organisms were also tested. Thirty-two assay replicates were generated for each potential interfering substance.

Table 3. Summary of Potential Interfering Substances Spiked into PreservCyt Clinical Specimen Matrix

Potential Interfering Substance	Level Tested Per 20mL PreservCyt Vial*
<b>Contraceptive Pool:</b> contraceptive film, foam and gel	200µL
<b>Anti-Fungal / Anti-Vaginosis Pool:</b> clotrimazole, miconazole nitrate, tioconazole, clindamycin	200µL
<b>Over The Counter Medication Pool:</b> anti-itch gel, liquid vaginal lubricant, hemorrhoid cream	200µL
<b>Prescription Pool:</b> Acyclovir, Metronidazole	200µL
<b>Bovine Cervical Mucous</b>	200mL (1% v/v)
<b>Whole Blood (Heparin)</b>	200mL (1% v/v)
<b>Semen</b>	200mL (1% v/v)
<b>Isolated Human Leukocytes</b>	2 x 10 <sup>7</sup> (1x 10 <sup>6</sup> /mL)
<b>Glacial Acetic Acid + Blood</b>	Acetic Acid = 1mL (5% v/v) Blood = 200µL (1% v/v)
<b>1x10<sup>6</sup> GC Cells/mL in CT Q<sup>x</sup> Assay:</b>	2 x 10 <sup>7</sup>
<b>1x10<sup>6</sup> CT EB/mL in GC Q<sup>x</sup> Assay:</b>	2 x 10 <sup>7</sup>

\* Levels represent those which a typical cytology collection device (brush/spatula or broom) would bring into a 20mL vial of PreservCyt specimen

Table 4. Effect of Potentially Interfering Substances in PreservCyt Clinical Specimen Matrix

Potential Interfering Substance	Assay	CT/GC Seeded Specimens: Percent Positive	CT/GC Negative Specimens: Percent Positive	CT/GC Negative Specimens: Percent Positive for EC
Contraceptive Pool	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Anti-Fungal/Anti-Vaginosis Pool	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Over The Counter Medication Pool	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Prescription Pool	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Bovine Cervical Mucous	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Whole Blood (Heparin)	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Semen	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
Isolated Human Leukocytes	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	100 (32/32)	3.1 (1/32)	100 (32/32)
Glacial Acetic Acid + Blood	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>	96.9 (31/32)	0 (0/32)	100 (32/32)
1x10 <sup>6</sup> GC cells/mL (in CT Q <sup>x</sup> Assay)	CT Q <sup>x</sup>	100 (32/32)	0 (0/32)	100 (32/32)
	GC Q <sup>x</sup>			
1x10 <sup>6</sup> CT EB/mL (in GC Q <sup>x</sup> Assay)	CT Q <sup>x</sup>			
	GC Q <sup>x</sup>	100 (32/32)	3.1 (1/32)	100 (32/32)

**SPECIMEN STABILITY (Figures 2&3):** Twelve pools of CT/GC negative PreservCyt specimen matrix were seeded with 396 CT EB and 1320 GC cells/mL. Volumes of each pool were stored at either 2-8°C or 30°C for up to 30 days. On days 0, 7, 21 and 30, 0.5mL was removed from each pool, added to a BD ProbeTec LBC Specimen Dilution Tube and tested on the BD Viper System in extracted mode. Final organism levels in the diluted specimens were 90 CT EB/mL and 300 GC cells/mL.

Figure 2. Stability of *C. trachomatis* DNA in PreservCyt Clinical Specimen Matrix

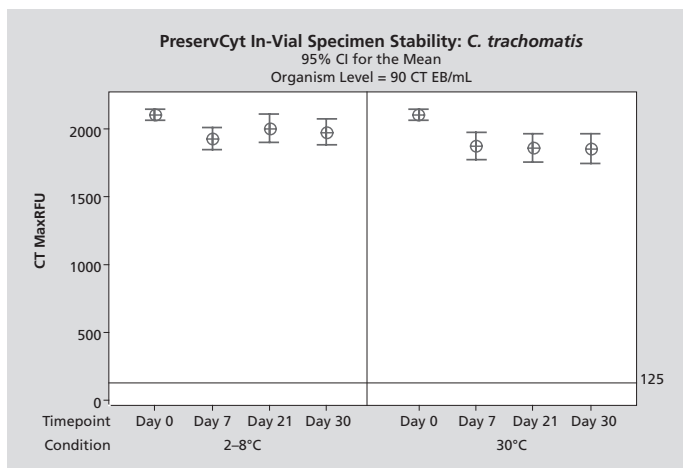
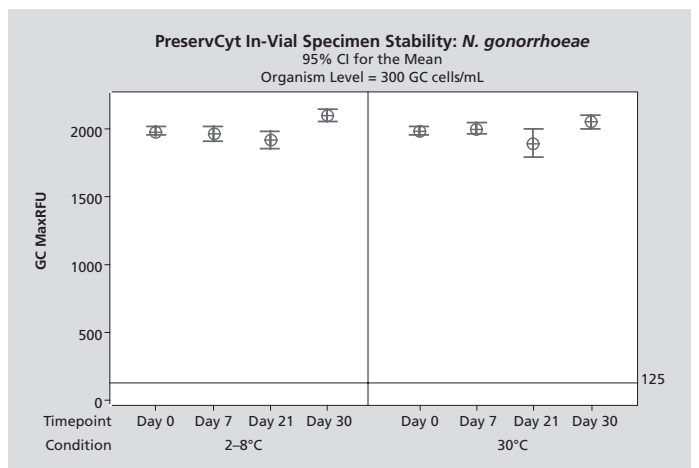


Figure 3. Stability of *N. gonorrhoeae* DNA in PreservCyt Clinical Specimen Matrix



## CONCLUSIONS

- The results presented here demonstrate the potential of the BD ProbeTec CT/GC Q<sup>x</sup> Amplified DNA Assays on the BD Viper System in extracted mode to provide competitive analytical performance with PreservCyt liquid-based cytology specimens. This alternative specimen type offers improved convenience to patients, physicians and laboratories with the prospect of making CT/GC screening more cost effective, and thereby facilitating increased detection of asymptomatic, but transmissible, infections.