

# Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from Vaginal Swabs\* Using Second Generation Assays\* on the BD ProbeTec™ ET System

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## ABSTRACT

Here we describe the performance characteristics of second-generation BD ProbeTec™ ET assays for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) with the new BD ProbeTec Vaginal Specimen Transport (VST). The BD ProbeTec CT/GC - Q<sup>x</sup> Amplified DNA Assays target a different region of the genome than the existing BD ProbeTec ET CT/GC Amplified DNA Assays. The CT/GC - Q<sup>x</sup> Assays also incorporate an Internal Amplification Control (IAC) to monitor sample inhibition and verify that proper amplification conditions exist for each specimen. To determine the analytical sensitivities of these assays with a vaginal swab matrix, CT/GC negative self-collected vaginal swabs from volunteer donors were expressed in 2 mL Sample Diluent and pooled. Sub-aliquots of the pooled swab matrix were then seeded with CT and GC at 0, 20, 100, 250, 500 and 1000 organisms/mL, prior to testing. The Limits of Detection (LODs) were determined to be 43 Elementary Bodies (EB)/mL and 75 particles/mL for CT and GC, respectively. The stability of CT and GC on vaginal swabs was evaluated by spiking a concentrated pool of expressed vaginal swabs with CT and GC organisms at approximately 2X their respective LODs, then seeding polyester swabs with the spiked vaginal matrix. Both organisms were found to be stable on the seeded swabs for 30 days at 2-8°C and -20°C, and for 14 days when stored at 30°C. The clinical performance of the vaginal swab was evaluated by testing paired endocervical (Reference Method) and vaginal swab (assayed with CT/GC - Q<sup>x</sup> Assays) specimens from 795 patients from 3 clinical sites. Results are summarized in the table below:

	CT - Q <sup>x</sup> Assay	GC - Q <sup>x</sup> Assay
Positive Percent Agreement	98.7% (75/76)	100% (29/29)
Negative Percent Agreement	98.9% (698/706)	99.3% (761/766)
Indeterminate Rate	0.1% (1/698)	0% (0/761)

We have demonstrated that the BD ProbeTec VST, in conjunction with the new, second generation CT/GC - Q<sup>x</sup> Assays, provides a viable alternative to conventional endocervical and urine specimens for the detection of CT and GC on the BD ProbeTec ET System.

## INTRODUCTION

Infections caused by *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) are the most common sexually transmitted bacterial diseases in the United States and worldwide. In 2003, 877,438 CT infections were reported to the Centers for Disease Control and Prevention from the 50 states and the District of Columbia.<sup>1</sup> From 1987 to 2003, the reported rate of CT infection in women increased from 78.5 cases to 466.9 cases per 100,000 population.<sup>1</sup> With 335,104 cases reported in 2003, gonorrhea is the second most frequently reported communicable disease in the United States.<sup>1</sup>

While endocervical swabs and urine specimens are routinely used for the diagnosis of CT and GC infections in women, both suffer from disadvantages. Endocervical swabs typically carry a high bacterial load but require collection by a trained physician and, while urine is readily obtained, inherent variability in specimen quality may compromise clinical sensitivity. Physician or self-collected vaginal swabs are less invasive than conventional endocervical specimens and studies have shown self-collection is an acceptable approach to patients. If adequate clinical performance can be demonstrated, they offer an attractive alternative to traditional specimen types when a pelvic examination is not otherwise indicated, especially for disease screening programs.

Here, we describe the analytical and clinical performance of two second-generation BD ProbeTec™ assays for the detection of CT and GC using the new BD ProbeTec Vaginal Specimen Transport (VST). Evolving CLIA regulations are increasing the requirements for quality assurance at all stages of nucleic acid testing within the clinical laboratory in order to verify both assay and instrument performance.<sup>2</sup> As a result, we have developed the BD ProbeTec CT/GC Q<sup>x</sup> Assays to amplify and detect both the target analyte (if present) and an internal amplification control (IAC) in a single microwell. For each assay, the IAC is dried in the priming microwell to monitor for inhibitory specimens and verify that proper conditions exist for amplification. The CT/GC Q<sup>x</sup> assays employ the same workflow as the current CT/GC Assays, including ready-to-use, dried, unit dose reagents and a closed microwell format that minimizes potential for contamination. In the present study, we used seeded vaginal swab specimens from healthy volunteers to determine the analytical sensitivity of the Q<sup>x</sup> assays for CT and GC and assess the stability of these organisms in a vaginal swab matrix. In addition, a comparison was made between the performance of the Q<sup>x</sup> assays with the VST and that obtained with the current BD ProbeTec ET CT/GC Amplified DNA Assays using paired endocervical swabs.

## METHODS

**Data Analysis:** CT/GC Q<sup>x</sup> data were analyzed using a novel Passes After Threshold (PAT) algorithm (Figure 1).

### Limit of Detection (LOD) in Vaginal Swab Matrix.

- Self-collected vaginal swabs were obtained from healthy volunteers using the VST, expressed in Sample Diluent and pooled.
- Aliquots of expressed swab matrix were spiked with CT elementary bodies (EB) and GC particles at 0, 20, 100, 250, 500, or 1000/mL.
- Samples were processed as indicated in Figure 2.
- 32 replicates were tested at each of the 6 target levels.
- Q<sup>x</sup> assay results for CT and GC are summarized in Figures 3 and 4, respectively.

### Organism Stability in a Vaginal Swab Matrix

- Self-collected vaginal swabs were obtained from healthy volunteers using the VST.
- Vaginal swabs were pooled by expressing and concentrating the swabs in Phosphate Buffered Saline/Bovine Serum Albumin.
- Negative swabs were created by seeding sterile VST swabs with 100 µL of unspiked concentrated vaginal matrix.
- Positive swabs were created by spiking the concentrated vaginal pool with CT and GC at 160 EB/mL and 220 particles/mL, respectively. These levels represent 2X the upper confidence interval of the respective Q<sup>x</sup> assay LODs, as indicated in Figures 3 and 4.
- Swabs were then placed at either -20°C, 2-8°C or 30°C.
- Negative Swabs were tested at baseline and 7 days (n=24 swabs/time point/temperature). Positive swabs were tested at Baseline, 7, 14 and 30 days (n=24 swabs/time point/temperature). Results are summarized in Figure 5.

### Performance Characteristics of Vaginal Specimen Transport:

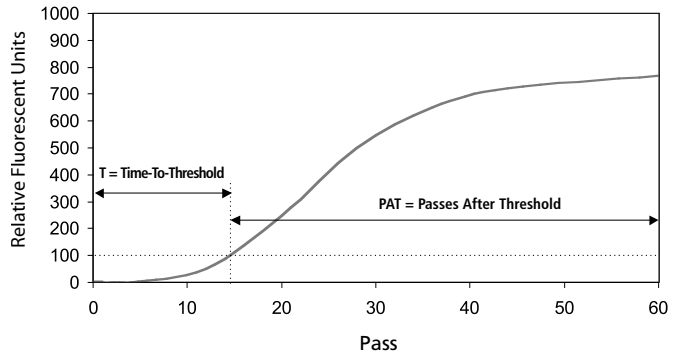
- Paired endocervical (Culturette™ Direct) and VST swab specimens, were provided by the following sites:

Table 1. Collection sites for comparison of VST and endocervical swab performance.

Site	Endocervical Swab Reference Method
Planned Parenthood of Houston Houston, TX	BD ProbeTec ET
Dupage County Health Department DuPage, IL	BD ProbeTec ET
Baltimore City Health Department Baltimore, MD	Roche Amplicor PCR assay

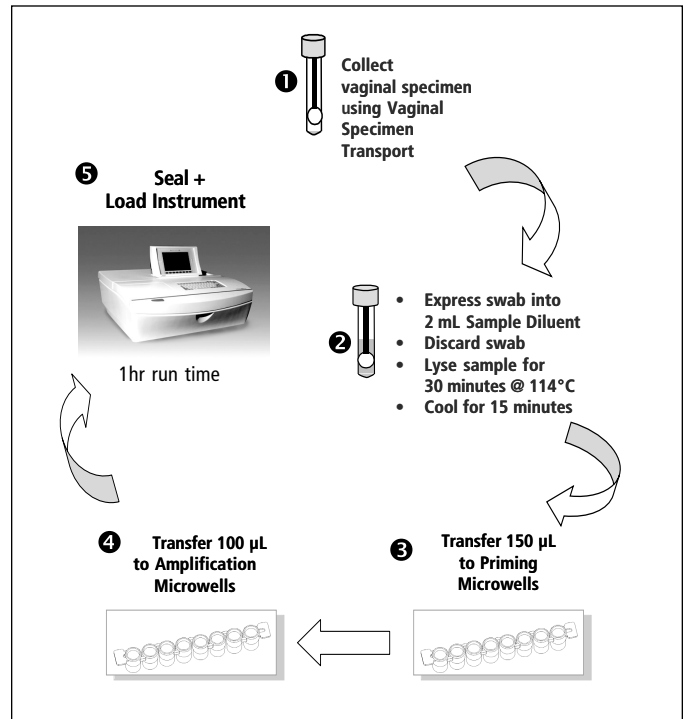
- All specimens were collected from symptomatic women by a trained physician and tested at the collection site using the reference methods listed in Table 1.
  - VST specimens were processed as described in Figure 2 and tested in the CT/GC Q<sup>x</sup> assays.
- Results are summarized in Figures 6 and 7.

Figure 1. BD ProbeTec™ ET System PAT Algorithm



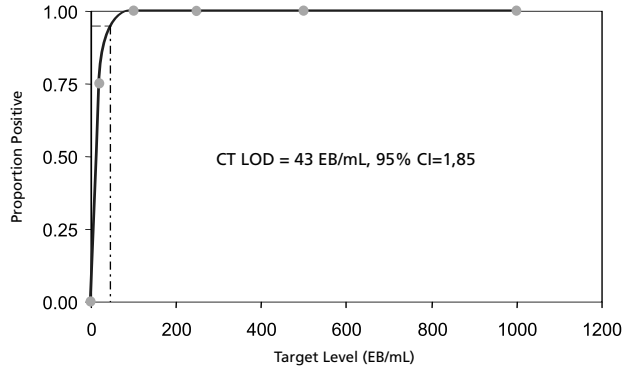
- T3 is the time at which the background corrected signal crosses a pre-determined threshold
  - T3 = Time-To-Threshold
- The same threshold is used for every sample
- **Passes After Threshold score = 60 – T3**
- Lower T3 scores and corresponding higher PAT values correlate with more efficient SDA
- Positive samples: PAT > 0
- Negative samples: Target PAT = 0 and IAC PAT > 0
- Indeterminate sample: Target PAT = 0 and IAC PAT = 0

Figure 2. Sample Preparation for Vaginal Swabs



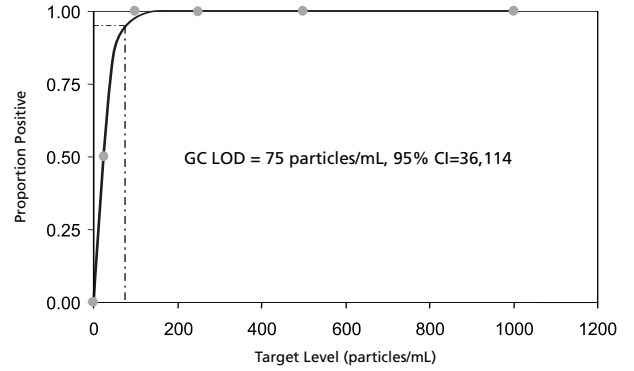
**RESULTS**

Figure 3. CT Limit of Detection in Vaginal Swab Matrix



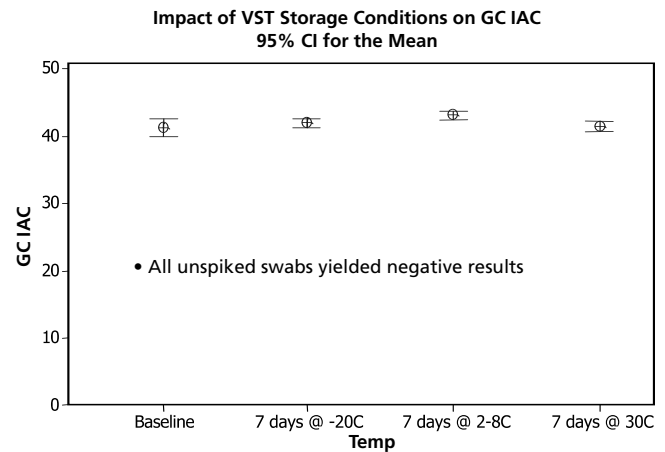
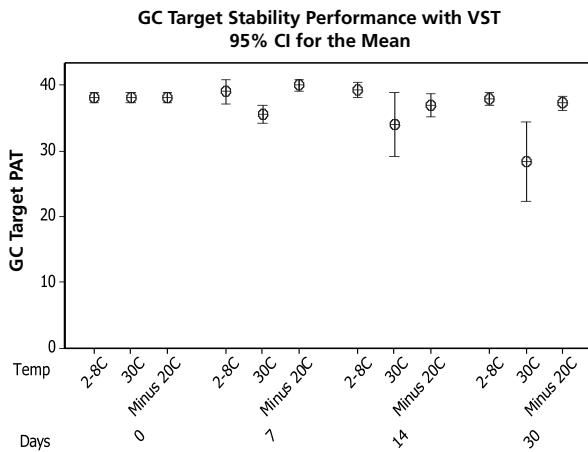
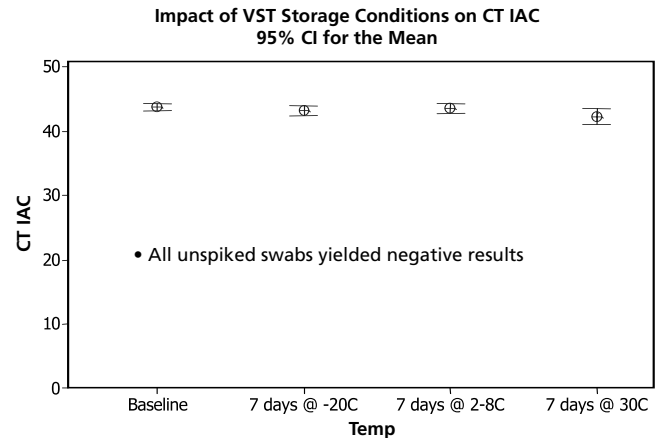
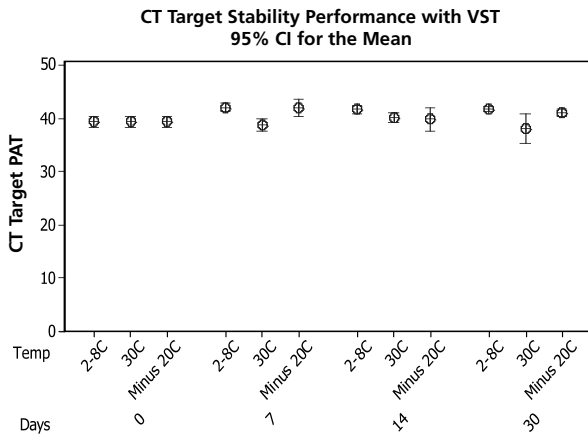
Levels tested included 0, 20, 100, 250, 500 and 1000 EB/mL.

Figure 4. GC Limit of Detection in Vaginal Swab Matrix



Levels tested included 0, 20, 100, 250, 500 and 1000 particles/mL.

Figure 5. CT/GC Stability Performance with VST



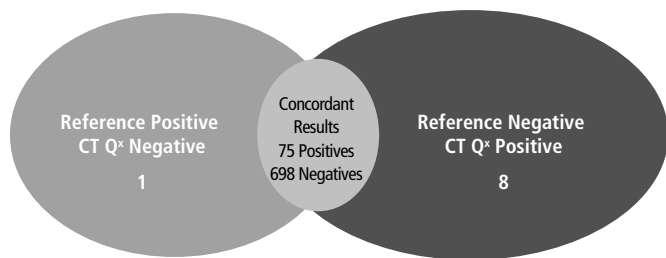
**ACKNOWLEDGEMENTS:**

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**REFERENCES:**

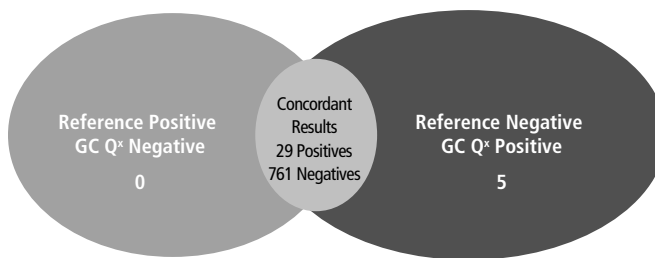
1. Division of STD Prevention. STD surveillance 2003- Centers for Disease Control and Prevention.
2. CLIA Regulations 42 CFR 493.1256, Standard:Control Procedures

Figure 6. Clinical Performance of CT Q<sup>x</sup> Assay with VST



- Repeat testing of all specimens with positive or discrepant results yielded the following:
  - 75/75 (100%) Q<sup>x</sup> +/Reference + samples repeated positive
  - 5/8 (63%) Q<sup>x</sup> +/Reference - samples repeated positive
  - 1/1 (100%) Q<sup>x</sup> -/Reference + sample tested positive
- Indeterminate Rate for the CT Q<sup>x</sup> assay with the VST = 0.1% (1/698)

Figure 7. Clinical Performance of GC Q<sup>x</sup> Assay with VST



- Repeat testing of all specimens with positive or discrepant results yielded the following:
  - 29/29 (100%) Q<sup>x</sup> +/Reference + samples repeated positive
  - 5/5 (100%) of the Q<sup>x</sup> +/Reference - results repeated positive
- Indeterminate Rate for the GC Q<sup>x</sup> assay with the VST = 0% (0/761)

### CONCLUSIONS

- We have developed second-generation, real-time homogeneous SDA-based assays for the detection of CT and GC in a closed system format on the BD ProbeTec ET System.
- Based on the data presented here:
  - The BD ProbeTec CT/GC Q<sup>x</sup> Amplified DNA assays had low indeterminate results for CT Q<sup>x</sup> (0.1%) and GC Q<sup>x</sup> (0.0%).
  - The CT/GC Q<sup>x</sup> assays are compatible with the new BD ProbeTec Vaginal Specimen Transport.
  - When compared with paired endocervical swabs tested using the existing BD ProbeTec ET CT/GC assays or the Roche Amplicor PCR assay, vaginal swabs tested with the Q<sup>x</sup> assays demonstrated the following clinical performance:

Positive Percent Agreement	CT	98.7% (75/76)
Positive Percent Agreement	GC	100% (29/29)
Negative Percent Agreement	CT	98.9 (698/706)
Negative Percent Agreement	GC	99.3% (761/766)

- The Q<sup>x</sup> assays incorporate the same workflow and Sample Diluent as the existing BD ProbeTec ET CT and GC Assays in addition to:
  - Amplification of alternative target sequences.
  - Improved throughput (integration of Internal Amplification Control).
  - Enhanced ability to comply with evolving quality control requirements.
- The new BD ProbeTec Vaginal Specimen Transport provides:
  - A dry swab transport system with stability of CT and GC over a range of temperatures and storage conditions.
  - A viable alternative to conventional endocervical and urine samples for the diagnosis of CT and GC infections in women.