

Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine using the BD ProbeTec™ CT Q^x and GC Q^x Amplified DNA Assays on the BD Viper™ System in Extracted Mode

T.L. Schlitzer, J.T. Kendall, C.D. Welborn, C.A. Martinaitis, Y. J. Liu, and J.M. Harris

BD Diagnostics • Sparks, MD

ABSTRACT

We have developed two novel assays for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) on the BD Viper™ System in extracted mode.* Here we describe the analytical performance of the new BD ProbeTec™ CT Q^x and GC Q^x Amplified DNA Assays* with Neat urine specimens (without preservative) as well as urine specimens stored in the Urine Preservative Transport (UPT) for the BD ProbeTec (CT/GC) Q^x Amplified DNA Assays. Both the Neat and UPT urine samples utilized pierceable caps and were extracted on the BD Viper System.

The analytical sensitivities of the CT Q^x and GC Q^x assays with Neat and UPT-treated urine were determined by spiking pooled CT/GC negative specimens from healthy donors at the following target levels: 0, 1, 3, 7, 15, and 30 Elementary Bodies (EB)/mL for CT and 0, 5, 10, 20, 50, and 100 cells/mL for GC. The 95% limits of detection (LODs) for CT and GC with Neat urine were 14 EB/mL (95% confidence intervals: 8, 21 EB/mL) and 21 cells/mL (12, 30), respectively, while for UPT-treated urine the LODs were 6 EB/mL (4, 9) and 25 cells/mL (13, 38).

Specimen stability studies were conducted using multiple pools of Neat and UPT-treated urine that were spiked with CT and GC organisms at a low level, near the analytical LODs of the Q^x assays, and which were then stored under different conditions. CT and GC DNA was successfully detected in Neat urine stored at 30°C for up to 30 hours and at 2-8°C for up to 7 days. With UPT-treated urine, CT and GC DNA was stable at 2-30°C for up to 30 days. Stability of CT/GC DNA was also demonstrated when Neat urine specimens were held at 2-8°C for up to 24 hours and 30°C for up to 8 hours prior to transfer to the UPT.

The BD ProbeTec CT Q^x and GC Q^x assays, when used in conjunction with extraction on the BD Viper System, were able to detect low numbers of organisms in both Neat and UPT-treated urine. The stability of the CT/GC DNA in Neat and UPT-treated urine specimens in tubes with pierceable caps was also demonstrated over a broad range of temperatures that provide flexible alternatives for transport and storage prior to extraction on the BD Viper System.

INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) continue to be the two most commonly reported sexually transmitted organisms in the US. In 2006, there were >1 million new cases of CT infection reported, nearly three times the number reported for GC infection.¹ There is therefore a critical need for sensitive and specific methods for detection of these increasingly important infections.

To this end, we have developed two novel assays for the detection of CT and GC on the BD Viper™ System in extracted mode. The BD ProbeTec™ CT/GC Q^x Amplified DNA Assays use homogeneous real-time Strand Displacement Amplification (SDA) to detect CT and GC in a variety of sample types following DNA extraction using ferric oxide. Here we demonstrate the analytical sensitivity of the CT/GC Q^x assays with both neat urine specimens and urine specimens treated with the BD Urine Preservative Transport (UPT) tube. In addition, we evaluated the stability of CT and GC DNA in neat and UPT-treated urine specimen pools across a broad range of storage temperatures.

REFERENCE: ¹ <http://www.cdc.gov/std/stats/trends2006.htm>

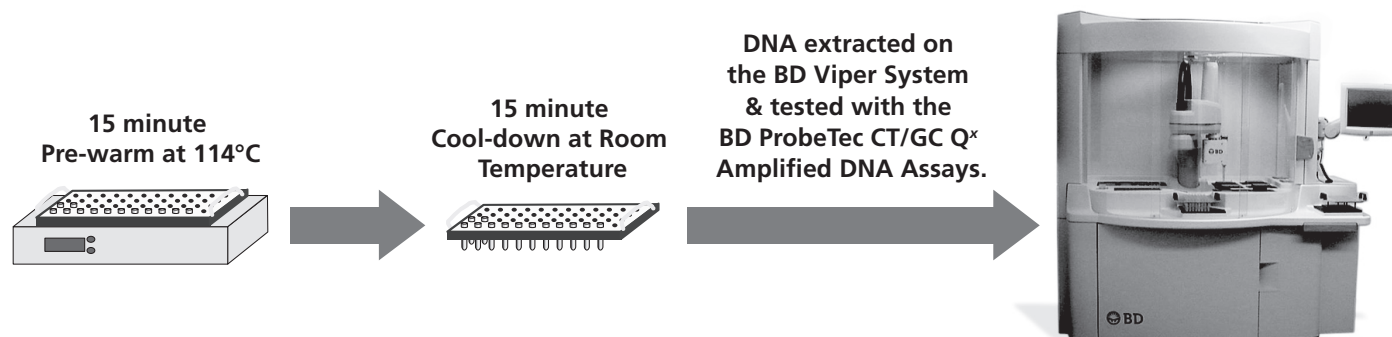
METHODS

Preparation of Urine Pools

- Male and female neat urine samples were obtained from healthy volunteers and pooled in equal volumes.
- Studies were conducted either directly with the pooled neat urine or after treatment of the pooled samples with the UPT.

Specimen Processing and Analysis

- After preparation according to the methods outlined below, all samples were dispensed into BD Viper Sample Tubes that were fitted with pierceable caps.
- Specimen processing and testing were conducted on the BD Viper System in extracted mode.



Analytical Sensitivity (Figures 1 & 2):

- Aliquots of neat and UPT-treated urine specimens were spiked with CT at 0, 1, 3, 7, 15, & 30 Elementary Bodies (EB)/mL, and 0, 5, 10, 20, 50, & 100 cells/mL for GC.
- 72 assay replicates were generated at each target level for each specimen type.

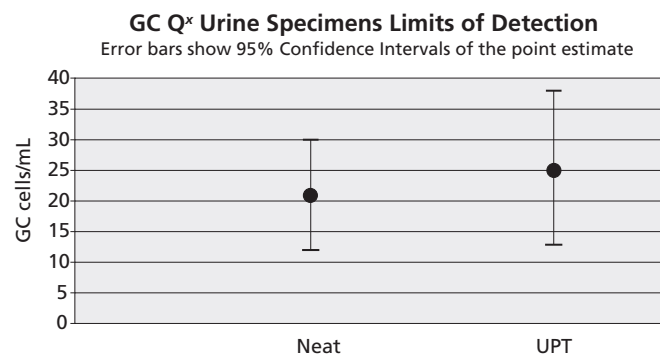
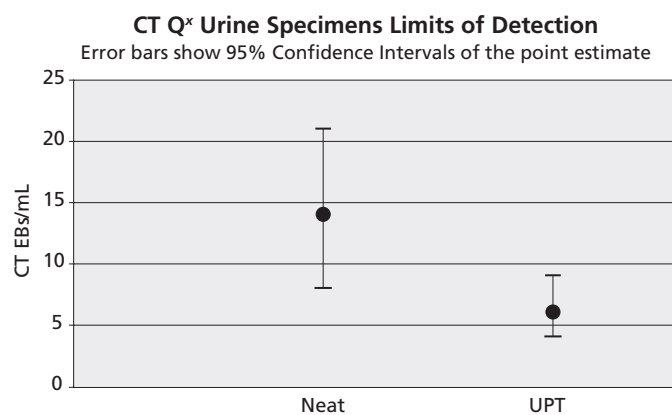
CT/GC DNA Stability in Neat and UPT Urine Specimens (Figures 3-5):

- CT/GC negative specimens were prepared from pooled neat urine specimens.
- CT/GC positive specimens were created by spiking pooled neat urine at 45 CT EB/mL and 150 GC cells/mL.
- 32 assay replicates were generated at each time-point and for each stability condition. (Table 1)

Table 1. CT/GC Q^x Urine Specimen Stability Study Design

Urine Specimen	Condition	Storage Temperature	Test Points
Neat		30°C	0, 8, 24, 30h
		2-8°C	0, 1, 3, 7 days
UPT	8h hold at 30°C prior to UPT	2-8°C	0, 14, 21, 30 days
		30°C	
	24h hold at 2-8°C prior to UPT	2-8°C	
		30°C	

Figures 1 & 2. Analytical Sensitivity Data



METHODS (continued)

Figure 3. Neat Urine Specimen Stability Data

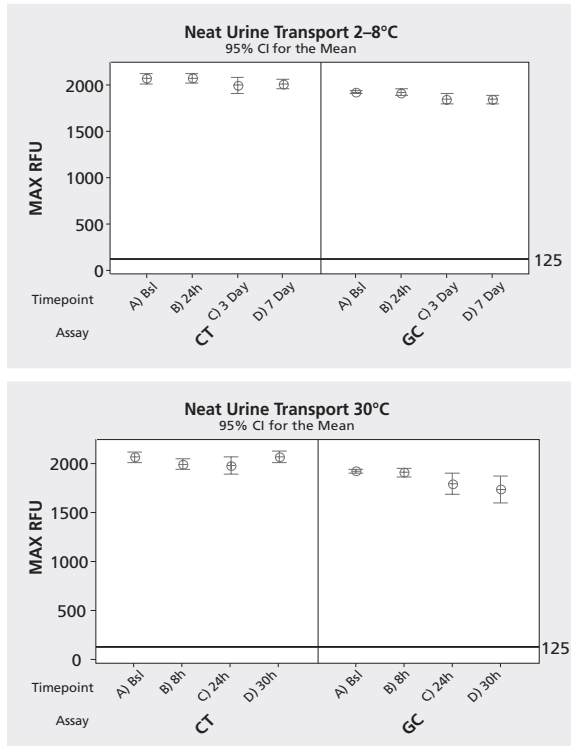


Figure 4. UPT Urine Specimen Stability 8h Hold

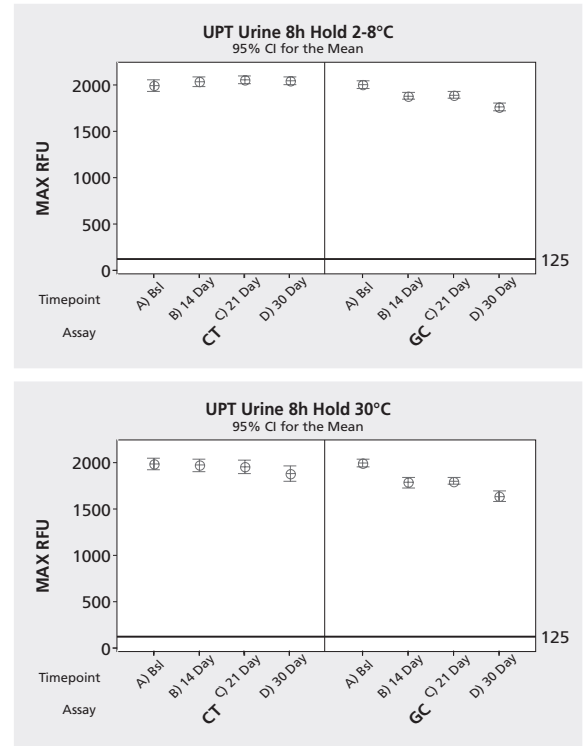
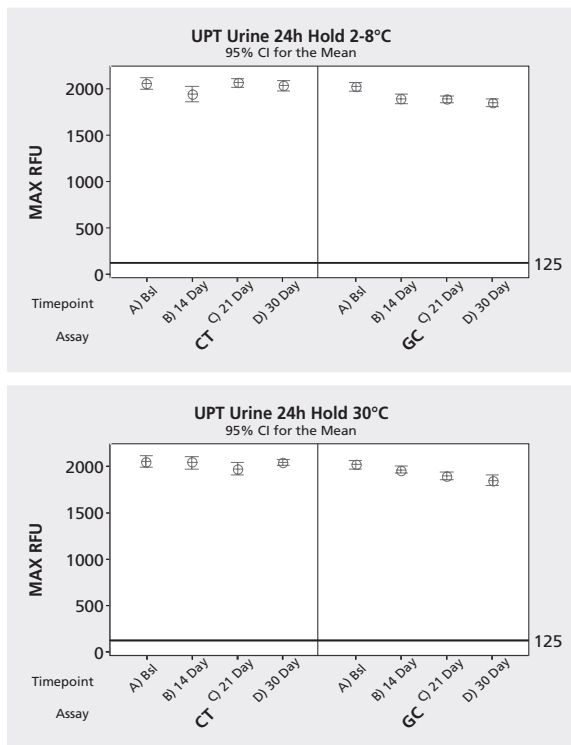


Figure 5. UPT Urine Specimen Stability 24h Hold



CONCLUSIONS

■ The analytical limits of detection for the BD ProbeTec CT/GC Q^x Amplified DNA Assays with neat and UPT urine were shown to be as follows:

Assay	Specimen Matrix	CT EB or GC cells/mL	
		LOD Point Estimate	95% Confidence Interval
CT Q ^x	Neat	14	8,21
	UPT	6	4,9
GC Q ^x	Neat	21	12,30
	UPT	25	13,38

- In neat urine specimen pools, CT and GC DNA was shown to be stable for 7 days at 2-8°C and 30 hours at 30°C.
- In UPT-treated urine specimen pools, CT and GC DNA was shown to be stable for up to 30 days at 2-30°C.

