

Performance of the BD ProbeTec™ CT Q^x and GC Q^x Amplified DNA Assays* with Urine and Vaginal Swab Specimens in the Presence of Potential Interfering Substances

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ABSTRACT

BACKGROUND: We have developed two novel assays for detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) for use with the BD Viper™ System in extracted mode. The purpose of this study was to investigate the performance of the assays with urine and vaginal swabs in the presence of endogenous and exogenous substances likely to be present in these specimen matrices.

METHODS: Pools of urine or expressed vaginal swabs were spiked with CT and GC at a low level near the analytical limits of detection of the assays, or left unspiked. Potential endogenous or exogenous interfering substances were added to each pool. Compounds tested included blood, leukocytes, mucus, semen, albumin, hormones, antibiotics and analgesics. Parallel controls lacking the interferents were also prepared. DNA was extracted on the BD Viper System and tested using the BD ProbeTec™ CT/GC Q^x Amplified DNA Assays. A non-amplified Extraction Control was included in each assay to monitor for reagent failure. Results were evaluated against pre-determined thresholds to determine the proportion positive/negative/non-reportable.

RESULTS: There was ≥95% agreement with expected results for both CT/GC positive and negative specimens. No failures of the Extraction Control were reported.

CONCLUSION: The performance of the BD ProbeTec CT/GC Q^x Amplified DNA Assays and Extraction Control with urine and vaginal swabs was robust to the levels of endogenous and exogenous substances tested in this study.

INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) infections are the most commonly reported notifiable diseases in the United States. As of 2006, reported CT infections in the US exceeded 1 million for the first time, while the Centers for Disease Control and Prevention estimates that more than 700,000 individuals in the United States contract GC each year.^{1,2} CT or GC infections may increase the risk of HIV infection and, if left untreated, may result in serious complications such as epididymitis in men and pelvic inflammatory disease or infertility in women.^{2,3} There is, therefore, a significant clinical need for sensitive and specific laboratory tests for the diagnosis of CT and GC infections. To this end, we have developed the BD ProbeTec™ CT/GC Q^x Amplified DNA Assays for use on the BD Viper™ System in extracted mode to provide high throughput capability for the detection of CT and GC in clinical specimens. Efficient walk away automation is accomplished on the BD Viper System through DNA extraction with proprietary FOX technology followed by amplification and real-time fluorescent detection. To confirm the validity of the process, a non-amplified extraction control (EC) is added to each sample and carried through extraction process. The system is designed for compatibility with a range of clinical specimens from either symptomatic or asymptomatic patients including male urethral swab specimens, female vaginal and endocervical swab specimens, and male and female urine specimens. The purpose of this study was to verify the performance of the CT/GC Q^x Amplified DNA Assays on the BD Viper System in extracted mode in the presence of a variety of endogenous and exogenous substances that are likely to be present in urogenital swab and urine specimens.

Figure 1. Specimen Spike Levels

Analyte / Specimen	Spike Level
CT (UPT Urine)	0 or 45 EB/mL*
GC (UPT Urine)	0 or 150 cells/mL
CT (Vaginal Swab)	0 or 90 EB/mL*
GC (Vaginal Swab)	0 or 300 cells/mL

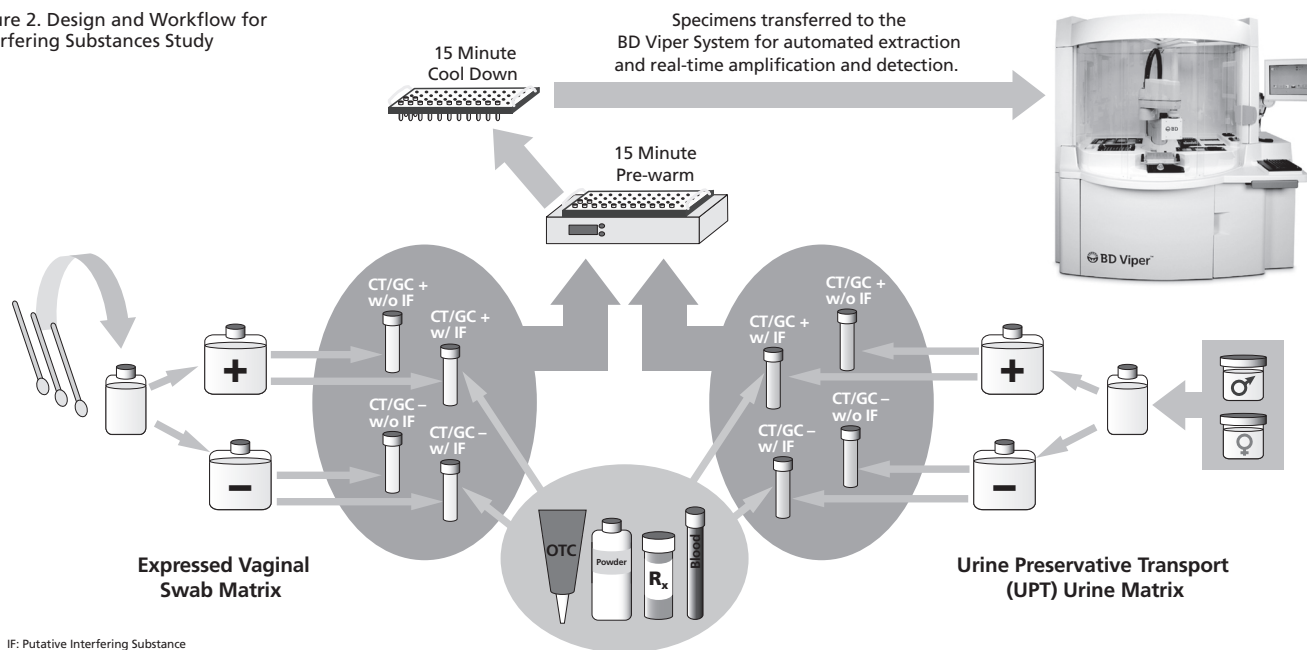
* EB: Elementary Bodies

REFERENCES

1. <http://www.cdc.gov/STD/stats/chlamydia.htm>
2. <http://www.cdc.gov/STD/Gonorrhea/STDFact-gonorrhea.htm>
3. <http://www.cdc.gov/STD/Chlamydia/STDFact-chlamydia.htm>

METHODS

Figure 2. Design and Workflow for Interfering Substances Study

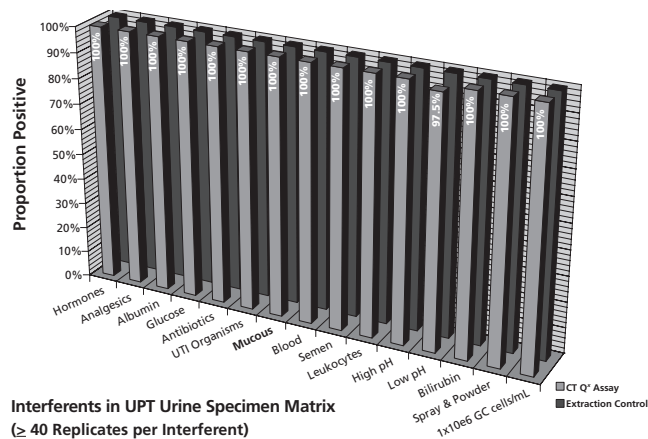
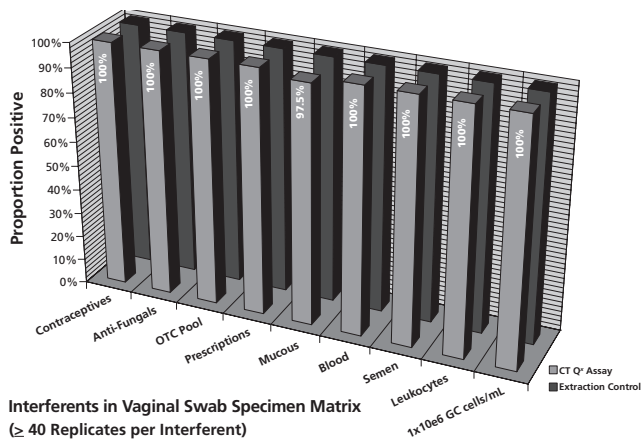


RESULTS

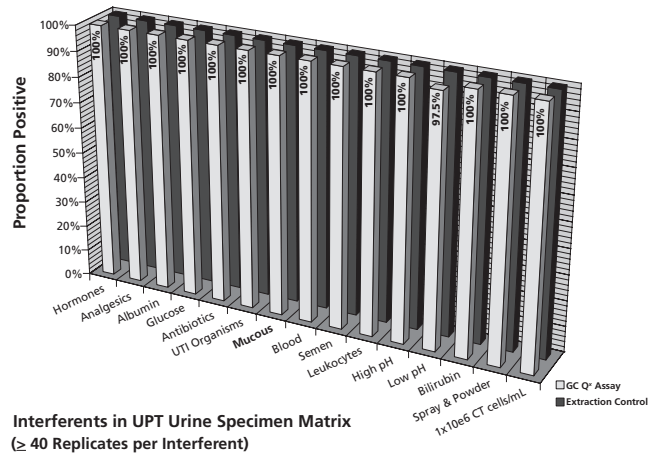
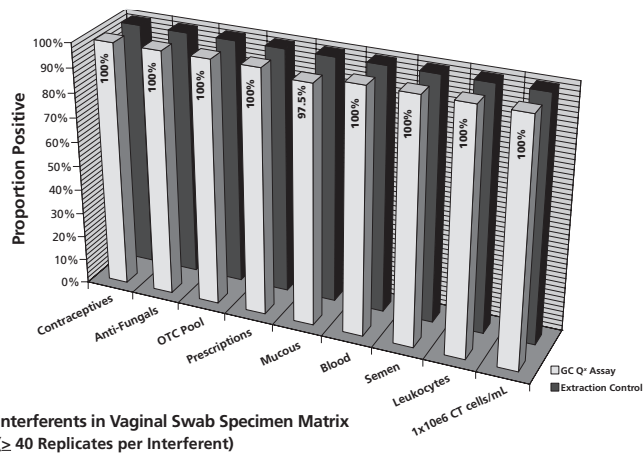
Figures 3-6 show the proportion of positive results obtained in the presence of potential interfering substances. EC Results depicted

are from CT/GC negative specimens only. $\leq 5\%$ of CT/GC negative specimens yielded positive Q^x assay results (data not shown).

Figures 3 & 4. CT Q^x Amplified DNA Assay



Figures 5 & 6. GC Q^x Amplified DNA Assay



RESULTS (continued)

Figure 7. Summary of Potential Interfering Substances Tested

Specimen Matrix	Category of Potential Interferents	Potential Interfering Substances Tested	Concentration Tested
Vaginal Swab	Contraceptives	3 pooled Nonoxynol-9 products	5% v/v
	Anti-Fungals	4 pooled products	5% v/v
	Over The Counter Pool	3 pooled non-prescription products	5% v/v
	Prescription Medications	2 pooled prescription products	5% v/v
	Mucous	n/a	5% v/v
	Blood	n/a	60%
	Semen	n/a	5% v/v
	Leukocytes	n/a	1x10 ⁶ cells/mL
	1x10 ⁶ GC cells/mL	n/a	1x10 ⁶ cells/mL
	1x10 ⁶ CT EBs/mL	n/a	1x10 ⁶ EBs/mL
UPT Urine	Hormones	2 pooled hormones; Norethindrone, 17- α -ethinylestradiol	≥ 1.2 ng/mL
	Analgesics	4 pooled analgesics; 4-acetamidophenol, Acetylsalicylic acid, Naproxen, Ibuprofen	≥ 25 μ g/mL Each
		Phenazopyridine	200 μ g/mL
	Spray & Powder	Feminine Deodorant Spray	1.7% v/v
		Talcum Powder	3.3% v/v
	Antibiotics	5 pooled antibiotics; Amoxicillin Metronidazole Tetracycline Azithromycin Ceftriaxone	75.2 μ g/mL
			120 μ g/mL
			15 μ g/mL
			12 μ g/mL
			733 μ g/mL
			≥ 60 μ g/mL
	Organisms Associated with Urinary Tract Infection	2 pooled organisms; <i>E. coli</i> , <i>G. vaginalis</i>	3.6x10 ⁵ /mL
			3.6x10 ⁵ /mL
		2 pooled organisms; <i>C. albicans</i> , <i>T. vaginalis</i>	1x10 ⁵ /mL
			1x10 ⁴ /mL
	Mucous	n/a	5% v/v
	Blood	n/a	1% v/v
	Semen	n/a	5% v/v
	Leukocytes	n/a	2.5 x10 ⁶ cells/mL
	High pH	n/a	pH 9.0
Low pH	n/a	pH 4.0	
Bilirubin	n/a	0.2 mg/mL	
Albumin	n/a	1 mg/mL	
Glucose	n/a	1.2 mg/mL	
1x10 ⁶ GC cells/mL	n/a	1x10 ⁶ cells/mL	
1x10 ⁶ CT EBs/mL	n/a	1x10 ⁶ EBs/mL	

CONCLUSIONS

- The BD ProbeTec CT/GC Q^x Amplified DNA Assays when tested on the BD Viper System in extracted mode are robust to the presence of a variety of endogenous and exogenous substances that may be found in urogenital swab and urine specimens.
- At the concentrations tested, none of the substances evaluated were observed to have a negative impact on the analytical assay performance or the recovery of the Extraction Control.