

Effect of Potentially Interfering Substances on the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine Samples Using the BDProbeTec™ ET System

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ABSTRACT

■ The BDProbeTec™ ET system is an amplified DNA probe assay for the direct qualitative detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) in endocervical swabs, male urethral swabs, and urine using homogeneous strand displacement amplification. The assay includes an amplification control (AC) that can help identify inhibitory samples, which are reported as Indeterminate. Without the AC, it would be impossible to distinguish between true negative results and inhibited reactions. A negative result is only reportable if the corresponding AC result is positive. Clinical urine samples may contain a variety of compounds that could potentially interfere with the ability of the BDProbeTec™ ET system to detect CT and GC. To examine these effects, different compounds were added to simulated urine samples that contained both CT and GC at levels of 200 EB per test and 200 cells per test, respectively. Compounds (n=23) tested included antibiotics, leukocytes, analgesics, bilirubin, blood, albumin, glucose, mucus, serum and talc. In addition, simulated urine samples with a low pH (pH=4) or a high pH (pH=8) were tested for the effect of pH on the assay. Urine sample processing included incubation of the urine sample with a urine preservative pouch for 2 hours followed by centrifugation, resuspension in diluent, and assay. Analysis of the data indicated that blood (5% v/v), phenazopyridine (10 µg/ml), and bilirubin (10 µg/ml) had a minor effect on the assays, and had the potential to generate false negative or indeterminate results in some samples. However, these effects were minimal, and none of the compounds tested showed any significant effect on the CT or GC assays. In conclusion, the majority of the interfering substances tested here had little or no effect on the ability of the BDProbeTec™ ET system to detect CT and GC in urine samples. Furthermore, the AC proved effective in discriminating false negative results due to inhibition from true negatives.

BD ProbeTec™ ET



INTRODUCTION

The BDProbeTec™ ET system can detect *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in male and female urine samples. A simple urine processing method was developed which included incubation with a urine processing pouch (UPP) for removal of potential interfering substance, followed by centrifugation to collect the CT and/or GC cells.

In order to evaluate the sample processing method, urine specimens containing a variety of substances had to be tested in the system. These substances included compounds that were taken internally and excreted in the urine, as well as substance that were applied to the genital area and could be present in a urine sample.

Substances were added to simulated urine specimens; both spiked (with CT and GC) or unspiked specimens. The compounds were evaluated for several effects on the assay including false positives, false negatives, and inhibition of the system's internal control (amplification control).

The results of this study will help identify compounds that could potentially affect the performance of the BDProbeTec™ system.



Table 1.

Interfering Substances List	
Interferant	Concentration
<u>Powder Pool:</u> Feminine Spray/talcum powder	5% v/v
Mucus, bovine cervical	5% v/v
Whole Blood	5% v/v
Human serum	5% v/v
Seminal Fluid specimen pool	5% v/v
Human leukocytes	250,000/ μ L
<u>Oral Contraceptives:</u> (Norethindrone + 17 α Ethinylestradiol)	1 μ g/mL each
Phenazopyridine Hydrochloride (Pyridium)	10 μ g/mL
Bilirubin	10 μ g/mL
4-Acetamidophenol + Acetylsalicylic Acid	1 mg/mL + 10 μ g/mL
β -Naphthaleneacetic Acid	0.1 mg/mL
Human Serum Albumin + Glucose	1 mg/mL + 15 mg/mL
<u>Antibiotic Pool #1:</u> Amoxicillin trihydrate Metronidazole Tetracycline Hydrochloride Sodium Cefotaxime	1 mg/mL 1 mg/mL 1 mg/mL 1 mg/mL
<u>Antibiotic Pool #2:</u> Sulfamethoxazole Trimethoprim Erythromycin	1 mg/mL 10 μ g/mL 1 mg/mL
High pH	pH = 8.0
Low pH	pH = 4.0

DEFINITIONS

Urine Preservative Pouch (UPP) — Pouch containing resin that removes potential interfering substances from the urine samples.

MOTA units — Results generated by the BDProbeTec™ ET system. The values represent the normalized fluorescent units obtained after system software analysis of the raw data.

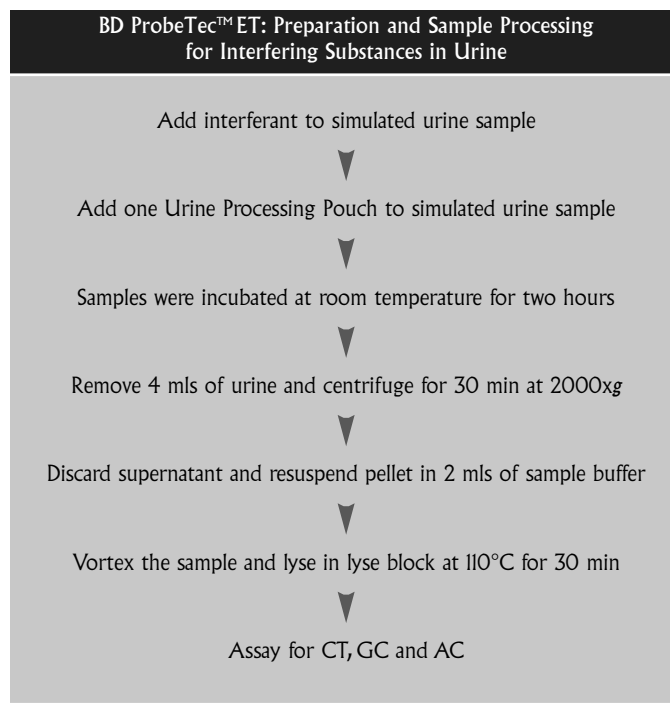
Positive results — Assays where the MOTA value is 2000 or greater

Negative results — Assays where the MOTA value is less than 2000.

Amplification Control (AC) — a separate assay run alongside the CT and GC assays using the same processed clinical sample. This assay includes the presence of target DNA at a level that allows the identification of inhibitory samples.

Indeterminate results — When the MOTA value of the AC well is less than 1000, the sample is considered inhibitory, and the result is indeterminate.

Figure 1.



METHODS AND MATERIALS

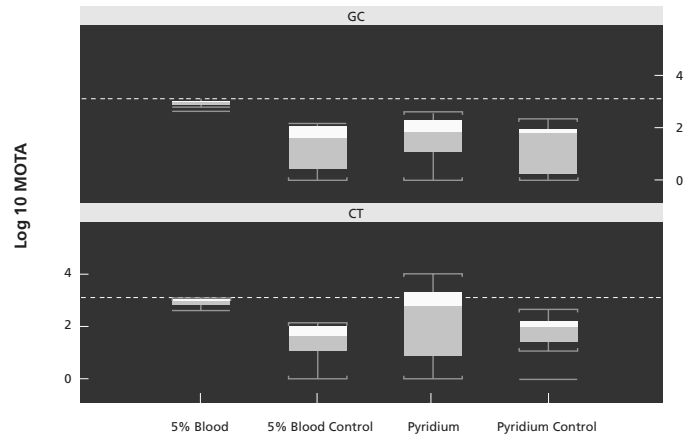
- Interfering substances tested in this study (Table 1) included endogenous substances likely to be present in patient's specimens (blood, mucus, etc.) and possible exogenous substances present in specimens or used in the specimen collection process (medication, spermicides, etc.)
- Positive simulated urine samples (35 ml) were co-inoculated with each organism at 200 CT EB's per test and 200 GC cells per test. These levels were chosen to provide a suitable level of positivity for identification of significant changes in results due to interfering substances. Negative urine samples (25 ml) were not inoculated with microorganisms.
- Potential interfering compounds were dispensed into the simulated urine. See Table 1 for concentration of each compound. One Urine Preservative Pouch was added to each urine sample and incubated at room temperature for minimum of two hours.
- Aliquots (4 ml) were removed from each sample and processed as described in Figure 1.
- Three (3) replicates from each processed sample were assayed from each of six (6) positive urine samples, and each of three (3) negative urine samples.
- Data were analyzed through at least one of the following procedures: testing for statistical difference between two proportions, and/or logistic regression analysis, and/or linear regression analysis as appropriate.

RESULTS

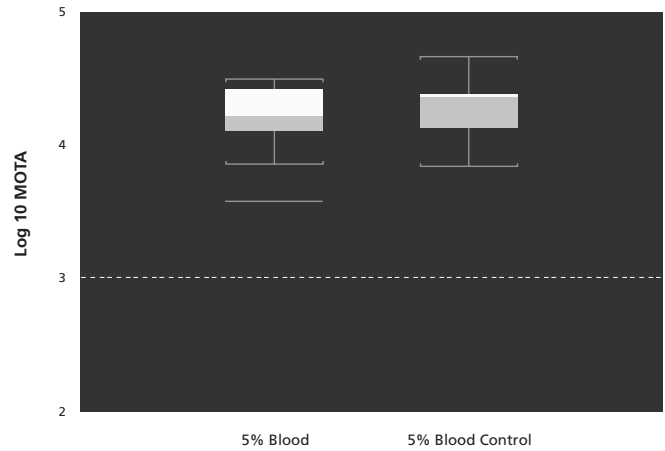
Table 2.

Interpretation	Substances
Does not interfere	Feminine Spray/talcum powder
	Mucus, bovine cervical
	Seminal Fluid specimen pool
	Oral Contraceptives (norethindrone + 17 α Ethinylestradiol)
	Phenazopyridine Hydrochloride
	4-Acetamidophenol + Acetylsalicylic Acid
	β -Naphthaleneacetic Acid
	Human Serum Albumin + Glucose
	Amoxicillin trihydrate
	Metronidazole
	Tetracycline Hydrochloride
	Sodium Cefotaxime
	Sulfamethoxazole
	Trimethoprim
	Erythromycin
	High pH
Low pH	
May cause indeterminate results	Whole Blood
	White blood cells
	Bilirubin

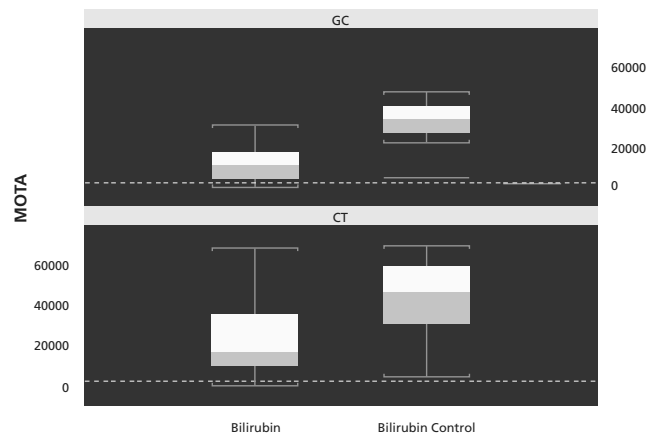
Negative (unspiked) Simulated Urine Samples



Amplification Control (AC) Results from Negative (unspiked) Simulated Urine Samples



Positive (spiked) Simulated Urine Samples



CONCLUSION

- Majority of the interfering substances had no effect on the ability of the BDProbeTec™ ET system to detect CT and GC in urine samples.
- Urine Specimen containing blood (5% v/v), phenazopyridine (10 μ g/ml) and bilirubin (10 μ g/ml) had a minor effect on the assays, and had the potential to generate false negative or indeterminate results in some samples.
- Other ointments, endogenous and exogenous substances at vast excess concentrations did not interfere.

