

Evaluation of Swab Interfering Substances on the BDProbeTec™ ET* *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay

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ABSTRACT

■ The BDProbeTec™ ET system allows the detection of *C. trachomatis* and *N. gonorrhoeae* from female endocervical swabs, male urethral swabs, and urine using homogeneous Strand Displacement Amplification. The system includes an Amplification Control to identify inhibitory samples (indeterminate results), and an Equivocal Zone where results cannot be reported as positive or negative (equivocal results). Potentially interfering substances which may be associated with swab specimens were delivered onto CULTURETTE™ DIRECT female swabs that were previously co-inoculated with 200 *C. trachomatis* elementary bodies/reaction and 200 *N. gonorrhoeae* cells/reaction. Negative swabs were not inoculated with organisms. Interfering substances investigated included over-the-counter (OTC) spermicidal products containing Nonoxinol-9, several OTC and prescription topical treatments for vaginosis, acyclovir cream, other common vaginal creams and lubricants, mucus, leukocytes, and seminal fluid at vast excess concentrations. Blood was examined at 1%, 2%, 5%, 10% and 15% (v/v) of the seeded swab volume. Data indicated that blood $\geq 5\%$ (v/v) and leukocytes at 250,000 cells/ μL may cause equivocal results and blood $\geq 15\%$ (v/v) and leukocytes at 250,000 cells/ μL may cause indeterminate results. No interference was detected with any other compounds tested or blood at $\leq 2\%$ (v/v). The BDProbeTec™ ET *C. trachomatis* and *N. gonorrhoeae* Amplified DNA Assay gave indeterminate or equivocal results from highly bloody or purulent swab specimens, but no interference was seen with mucus, ointments and other exogenous substances which may be present in swab specimens.

BD ProbeTec™ ET



INTRODUCTION

The BDProbeTec™ ET System *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) amplified DNA assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescent labeled detector probe. The SDA reagents are dried in two separate disposable microwell strips. The processed sample is added to the Priming Microwell which contains the amplification primers, fluorescent labeled detector probe, and other reagents necessary for amplification. After incubation, the reaction mixture is transferred to the Amplification Microwell, which contains two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The Amplification Microwells are sealed to prevent contamination and then incubated in a thermally controlled fluorescent reader which monitors each reaction for the generation of amplified products.

Each sample and control is tested in three discreet microwells: *C. trachomatis*, *N. gonorrhoeae* and the amplification control. The purpose of the amplification control is to identify samples that contain compounds which inhibit the SDA reaction. Results are reported through an algorithm as positive, negative, indeterminate or equivocal.

BDProbeTec™ Swab Sample Processing

Collect endocervical or male urethral swab specimen
(For endocervical swabs, remove excess mucus
from the cervical os with the cleaning swab)

Transport swab (dry) to testing lab at room temperature or 2-8°C.

Place swab into 2mL pre-filled Sample Diluent Tube.

Swirl swab in diluent for 5-10 seconds.

Express swab against side of tube, then discard swab.

Vortex expressed material.

Heat in lyse block for 30 min at 110°C.

INTERFERING SUBSTANCES: METHODS

1. Interfering substances tested in this study included endogenous substances likely to be present in patients' specimens (blood, mucus, etc.) and possible exogenous substances present in specimens or used in the specimen collection process (medications, spermicides, etc.). See Table 1.
2. CULTURETTE™ DIRECT female swabs were co-inoculated with each organism at a single level: 4000 CT EBs/swab and 4000 GC cells/swab (200/reaction). These levels were chosen to provide a suitable level of positivity to determine significant changes in sample results due to interfering substances. Negative swabs were not inoculated with organisms. Potential interfering compounds were dispensed onto swabs. In most cases, the level of interfering substance represented vast excess concentrations.
3. Three (3) replicates from each processed sample were assayed from each of six (6) positive swabs, and three (3) replicates from each processed sample were assayed from each of three (3) negative swabs for interfering substances except blood. Three (3) replicates from each processed sample were assayed from each of six (6) negative swabs for each concentration of blood.
4. Data were analyzed through at least one of the following procedures: testing for a statistical difference between two proportions, and/or logistic regression analysis, and /or linear regression analysis, as appropriate. See Table 2.

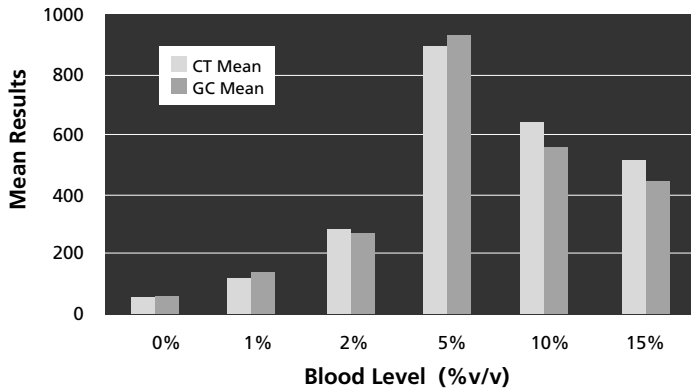
Table 1. Interfering Substances List

Interfering Substances	Swab Concentration
Contraceptive Pool:	15% v/v
Gynol II® contraceptive Jelly	5%
Koromex contraceptive Gel	5%
Delfin® contraceptive Foam	5%
OTC Pool #1:	15% v/v
Vagistat®-1 (6% Tioconazole)	3.8%
Gyne-Lotrimin® (1% Clotrimazole)	3.8%
Miconazole 7 (2% Miconazole nitrate)	3.8%
Femstat®-3 (2% Butoconazole nitrate)	3.8%
OTC Pool #2:	15% v/v
K-Y® Jelly	5%
Vagisil® anti-itch cream	5%
Preparation H® hemorrhoidal cream	5%
Prescription Pool:	15% v/v
Zovirax® (5% acyclovir)	7.5%
MetroGel-Vaginal® (0.75% metronidazole)	7.5%
Mucus, bovine cervical	15% v/v
Whole Blood	15%, 10%, 5%, 2%, 1% v/v
Seminal Fluid specimen pool	15% v/v
Human leukocytes	250,000/μL

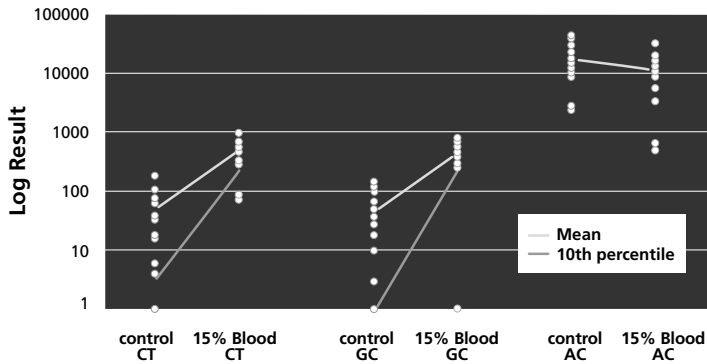
Table 2. Result Assignment Algorithm

Result Assignment	<i>C. trachomatis</i> (CT)	Amplification Control (AC)
CT Positive	CT result ≥ 2000	Not applicable
CT Negative	CT result < 1000	AC result ≥ 1000
Indeterminate	CT result < 1000	AC result < 1000
Equivocal	1000 ≤ CT result < 2000	Not applicable
Result Assignment	<i>N. gonorrhoeae</i> (GC)	Amplification Control (AC)
GC Positive	GC result ≥ 2000	Not applicable
GC Negative	GC result < 1000	AC result ≥ 1000
Indeterminate	GC result < 1000	AC result < 1000
Equivocal	1000 ≤ GC result < 2000	Not applicable

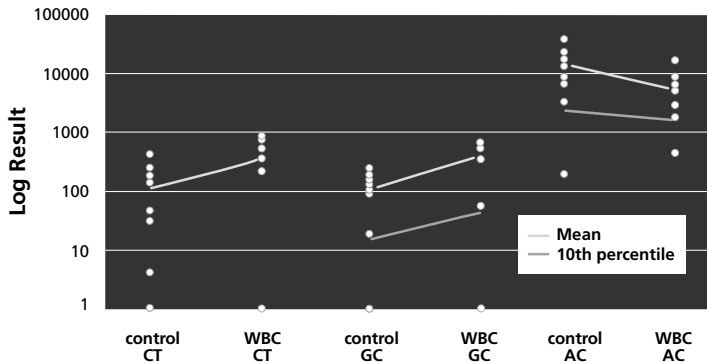
Effect of Blood on Negative Results
 Mean Result as a Function of Blood Level
 (Negative Cutoff = 1000)



Grossly bloody swabs
 Swab Interfering Substances: 15% Blood
 Negative Sample (Negative Cutoff = 1000)



Human leukocytes on swabs
 Swab Interfering Substances: leukocytes
 Negative Sample (Negative Cutoff = 1000)



RESULTS

Interpretation	Substance
Does not interfere.	Blood, 1%, 2% Mucus Seminal fluid Vagistat®-1 (6% Tioconazole) Gyne-Lotrimin® (1% Clotrimazole) Miconazole 7 (2% Miconazole nitrate) Femstat®-3 (2% Butoconazole nitrate) K-Y® Jelly Vagisil® anti-itch cream Preparation H® hemorrhoidal cream Zovirax (5% Acyclovir) MetroGel-Vaginal® (0.75% metronidazol) Nonoxinol-9 containing products Gynol II® contraceptive jelly Koromex contraceptive gel Delfin® contraceptive foam
May cause equivocal results.	Blood, 5%, 10%, 15% Leukocytes
May cause indeterminate results.	Blood, 15% Leukocytes

CONCLUSION

- Swab specimens containing 5% to 15% (v/v) blood or 250,000 WBC/μL may cause indeterminate or equivocal results.
- Swab specimens containing 1% to 2% blood do not interfere.
- Mucus, ointments or other exogenous substances at vast excess concentrations do not interfere.

