

Feasibility of Using TriPath Imaging,[®] Inc. SurePath[™] Preservative Fluid with the BD ProbeTec[™] ET CT/GC Amplified DNA Assays^{*}

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REVISED ABSTRACT

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) cause two of the most prevalent sexually transmitted diseases worldwide. To prevent the spread of these infections and their debilitating sequelae, the Centers for Disease Control & Prevention recommends annual screening for all sexually active adolescent and young adult women, as well as older women with one or more risk factors. Similarly, the incidence of cervical cancer caused by the sexually transmitted human papillomavirus (HPV) has been reduced by widespread screening with cervicovaginal Papanicolaou (Pap) smears. TriPath Imaging,[®] Inc. SurePath[™] preservative fluid serves as a transport, preservative, and antibacterial medium for gynecologic specimens. The purpose of the present study with seeded samples was to demonstrate the feasibility of using specimens preserved in SurePath medium with the BD ProbeTec[™] ET CT/GC Amplified DNA Assays. In brief, a portion of the SurePath fluid was centrifuged at 2000 x g for 30 minutes and the supernatant discarded. The pellet was resuspended in ≥ 750 mL of BD ProbeTec ET CT/GC Sample Diluent before being assayed according to the manufacturer's instructions. The BD ProbeTec[™] ET CT/GC Amplified DNA Assays utilized an Amplification Control (AC) to monitor for inhibition of amplification and verify negative results. The analytical sensitivities for CT and GC were shown to be < 250 elementary bodies (EB)/mL and 690 cells/mL of SurePath fluid, respectively. Additional studies demonstrated that CT and GC organisms stored in SurePath medium were stable for at least 1 month at 2-30°C. These preliminary data demonstrate the feasibility of using Pap specimens preserved in SurePath medium for the detection of CT and GC DNA on the BD ProbeTec ET System. In turn, this offers the potential convenience of testing for three important STDs from a single gynecologic specimen.

INTRODUCTION

About half a million cases of cervical cancer are diagnosed worldwide each year with close to 300,000 deaths.¹ Sexually transmitted human papillomaviruses (HPVs) are the major cause of cervical cancer, with 5.5 million people infected annually in the United States alone.^{2,3} HPVs are separated into two groups—"low-risk" and "high-risk," each causing abnormal cell growth, but typically only the "high-risk" HPVs lead to cancer. High-risk HPVs include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, as well as others.⁴ Risk factors for cervical cancer include initiation of sexual activity at an early age, multiple sexual partners, and infection with a high risk strain of HPV. Widespread cervical cancer screening with the Papanicolaou (Pap) smear allows for the detection of abnormalities that may lead to invasive disease.⁵ Annual screening is recommended for all women aged 18 or older and those who are younger than 18 and sexually active.⁶ The TriPath Imaging,[®] Inc. SurePath[™] liquid-based Pap test is an FDA-approved thin-layer cell preparation process intended for use in the screening and detection of cervical cancer, pre-cancerous lesions, atypical cells and other cytologic categories as defined by The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses.⁷

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) infections are two of the most prevalent sexually transmitted diseases worldwide.⁸ In the United States alone in 2002, almost 850,000 cases of CT and more than 700,000 new cases of GC were reported to the Centers for Disease Control & Prevention (CDC).⁹ Among women, the rates of asymptomatic infection are 70-75% and 80% for CT and GC, respectively. If left untreated, the complications from these diseases include, pelvic inflammatory disease (PID), tubal infertility, and ectopic pregnancy.¹⁰ Routine screening of young women for CT and GC plays an important role in limiting the spread of these sexually transmitted diseases and their debilitating sequelae.¹⁰ The CDC's recommendations include annual screening for CT and GC of all sexually active women age 25 years and younger, as well as older women with risk factors (a new partner or multiple sex partners).⁸ The detection of CT and GC using nucleic acid amplification technologies has gained popularity over conventional methods due to increased sensitivity and specificity, and reduced time-to-result. The BD ProbeTec ET CT and GC Amplified DNA Assays utilize Strand Displacement Amplification (SDA) technology, coupled with fluorescent energy transfer (ET) to test for the presence of CT and GC DNA in clinical samples.

The purpose of this study was to determine the feasibility of using Pap specimens preserved in SurePath medium to detect CT and GC DNA in seeded samples using the BD ProbeTec ET System. The ability to test for two of the world's leading STDs (CT and GC) from a single specimen, in addition to routine screening for cytological abnormalities, would have important consequences in terms of patient care as well as management of resources within the testing laboratory.

ACKNOWLEDGEMENTS

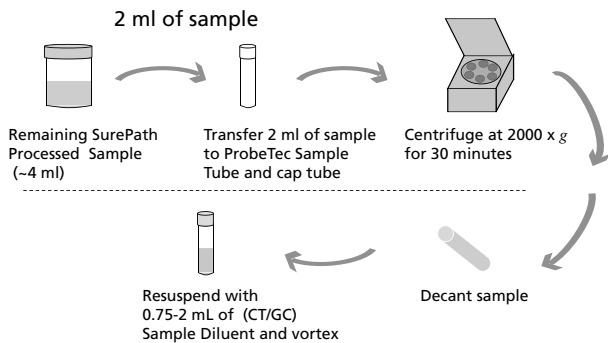
Special thanks to Paula Johnson for statistical support, Gerry Durmowicz for ProbeTec reagents, Craig Howell for TriPath reagents, and Kristina Carnaggio and David Holman for laboratory assistance.

MATERIALS AND METHODS

Background Information on the TriPath SurePath Gynecologic Samples

TriPath SurePath gynecologic specimens are collected using a broom-type sampling device (e.g. Cervex Brush®) or an endocervical brush/plastic spatula combination (e.g. Cytobrush® Plus GT or Pap Perfect® spatula), with detachable head(s). The tip of the collection device is separated from the stem and placed into a SurePath preservative fluid collection vial. The sample is capped and sent to the laboratory where it is processed using the PrepStain™ System. After a sample is removed for cytology, approximately 4 mL of fluid remains in the SurePath collection vial for use in CT/GC testing on the BD ProbeTec system (Figure 1).

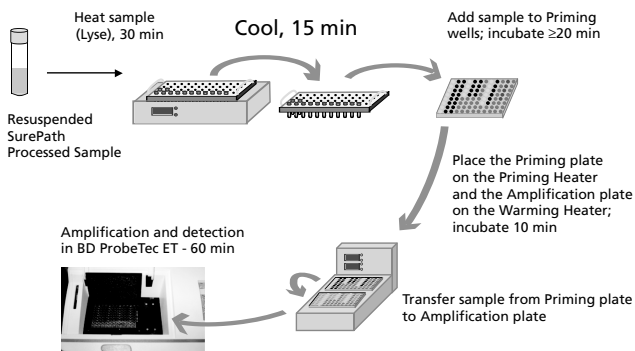
Figure 1. Sample Processing for the BD ProbeTec™ ET System from SurePath™ Preservative Fluid*



Background Information on the BD ProbeTec ET CT and GC Amplified DNA Assays¹¹

The BD ProbeTec ET CT and GC Amplified DNA Assays are based on the simultaneous amplification and detection of target nucleic acid. For each analyte, the SDA reagents are dried in two separate disposable microwells. The processed sample is added to the Priming Microwell containing the amplification primers, fluorescently-labeled detector probe and other reagents necessary for amplification. After a brief incubation, the reaction mixture is transferred to the Amplification Microwell that contains the two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The Amplification Microwells are sealed and then incubated in a thermally controlled fluorescent reader that monitors each reaction for the generation of amplified products (Figure 2). The presence of CT or GC is determined by relating the BD ProbeTec ET readings for the sample to pre-determined cutoff values. Each sample may be tested in three discrete microwells: CT, GC, and the Amplification Control (AC). The purpose of the AC is to verify negative results by identifying samples that may inhibit the SDA reaction.

Figure 2. Assay Workflow for CT/GC using the BD ProbeTec ET System



RESULTS

Limit of Detection

To determine analytical sensitivity, 2 mL of SurePath medium were aliquoted into a 4 mL ProbeTec sample tube and inoculated with a quantified suspension of CT elementary bodies (EB) or GC cells. Sixteen tubes for each condition were inoculated at the following levels: 0, 250, 500, 750, 1000, and 2000 EB/mL and 0, 80, 160, 320, 640, 1280, and 2000 GC cells/mL. All tubes were centrifuged for 30 minutes at 2000 x g. Supernatants were discarded and each cell pellet was resuspended in 2 mL of BD ProbeTec ET CT/GC Sample Diluent. Samples were then lysed and assayed in triplicate according to the BD ProbeTec ET package insert (Figures 3 and 4). A total of 48 data points were obtained at each level of inoculum.

Seeded Organism Stability

An analytical stability study was performed using SurePath medium that was seeded with both CT and GC organisms. Seeded samples were held at either 2-8°C or 30°C for up to one month.

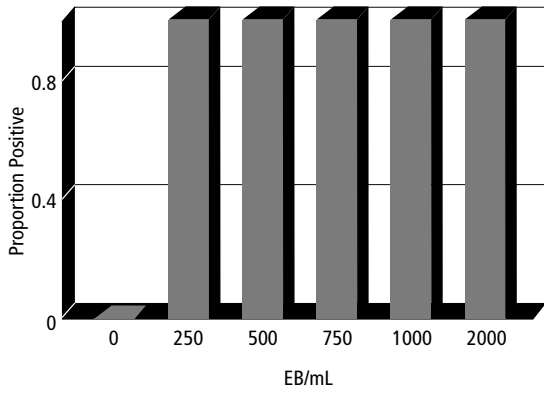
2-8°C Stability. Half of 50 tubes containing 2 mL of SurePath medium were seeded with 2000 CT EB and GC cells/mL and the other half with 4000 EB and GC cells/mL. Twenty-five tubes with 2 mL of SurePath medium were left uninoculated for use as negative controls. At baseline (day 0) and each subsequent time point, five tubes at each organism level were tested. Each tube was centrifuged for 30 minutes at 2000 x g, decanted, and resuspended in 2 mL of CT/GC Sample Diluent. The remaining samples were stored at 2-8°C until the appropriate time point for testing. All of the samples at each time point were tested according to the BD ProbeTec ET package insert as follows: all seeded samples were tested for CT and GC in duplicate (n = 20 assay results per analyte); all negative samples were tested with CT/GC/AC microwells (n = 15 assay results per analyte). See Figures 5, 6, & 7.

30°C Stability. The study design was similar to that performed with samples stored at 2-8°C with the following modifications. At baseline, 60 tubes containing 2 mL of SurePath medium were inoculated with 2000 CT EB and GC cells/mL. Another 60 tubes containing 2 mL of SurePath medium were inoculated with 4000 CT EB and GC cells/mL. Sixty tubes containing 2 mL of SurePath medium were left uninoculated for use as negative controls. Twelve tubes at each target level were processed and tested at baseline. The remaining samples were stored at 30°C +/- 3°C until the appropriate time point. All samples were assayed in duplicate (n = 24 assay results per analyte per time point). The AC was not run for specimens seeded with CT/GC. See Figures 8, 9, & 10.

REFERENCES

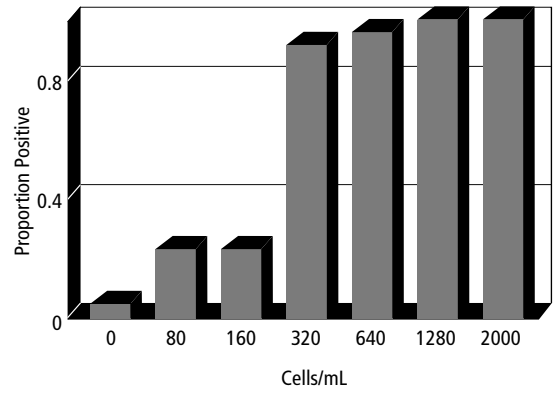
- 1 HRP biennial report 2000-2001, Chapter 3 Preventing reproductive tract infections
- 2 www.who.int/vaccine_research/documents/new_vaccines/en/index8.html
- 3 http://cis.ncis.nih.gov/fact/3_30.htm
- 4 <http://cancer.gov/cancerinfo/wyntk/cervix>
- 5 http://cis.ncis.nih.gov/fact5_16.htm
- 6 <http://intellihealth.com>
- 7 Package insert for the TriPath Imaging® Inc. PrepStain™ System Product Insert
- 8 www.who.int/docstore/hiv.GRSTI/003.htm
- 9 www.cdc.gov/std/facts.htm
- 10 www.cdc.gov/od/oc/media/pressrel/fs02509.htm
- 11 Package insert for the BD ProbeTec ET Chlamydia trachomatis and Neisseria gonorrhoeae Amplified DNA Assay

Figure 3. Proportion Positive for CT LOD in SurePath Preservative Fluid



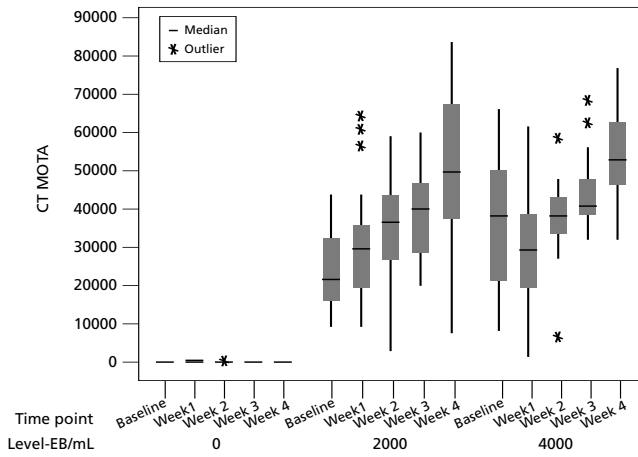
The LOD for CT is less than 250 EB/mL.

Figure 4. Proportion Positive for GC LOD in SurePath Preservative Fluid



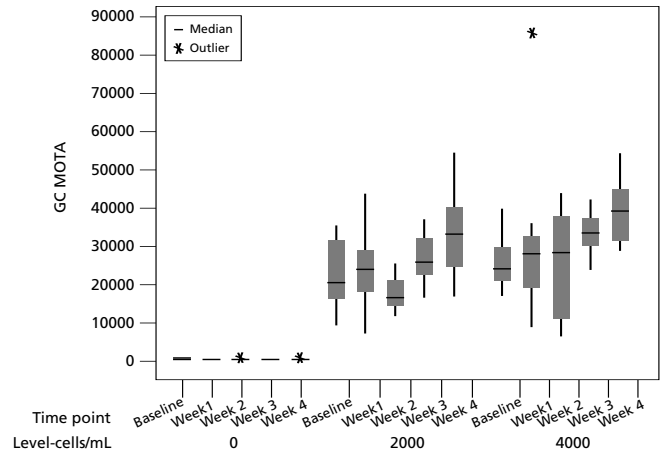
The LOD for GC is 690 cells/mL.

Figure 5. Stability at 2-8°C of CT EB Seeded into SurePath Preservative Fluid



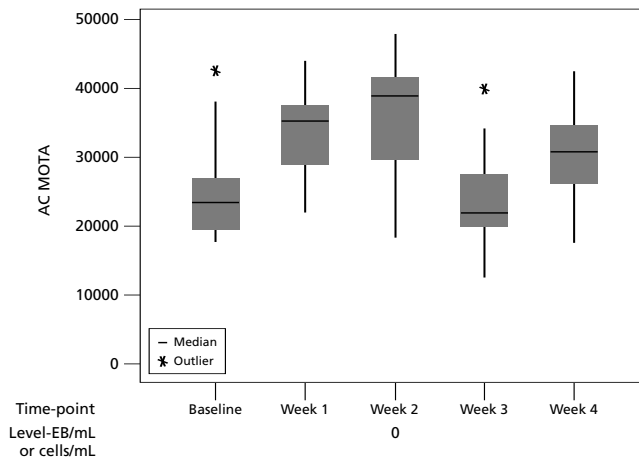
Note: At weeks 1 & 2, n = 10 for negative samples

Figure 6. Stability at 2-8°C of GC Cells Seeded into SurePath Preservative Fluid



Note: At weeks 1 & 2, n = 10 for negative samples

Figure 7. AC Results from Unseeded SurePath Preservative Fluid Stored at 2-8°C



Note: At weeks 1 & 2, n = 10 for AC

Figure 8. Stability at 30°C of CT EB Seeded into SurePath Preservative Fluid

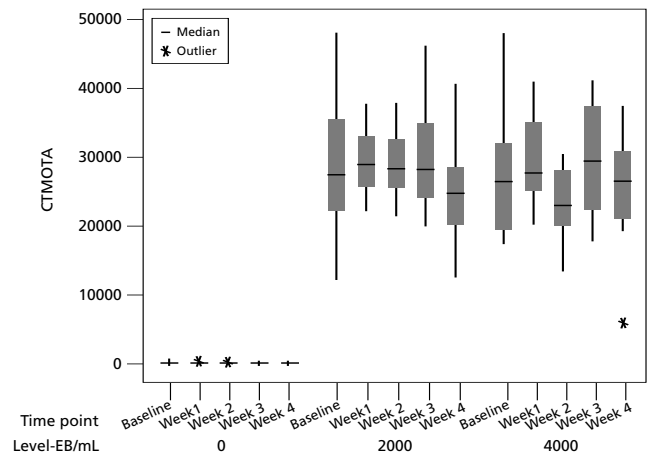


Figure 9. Stability at 30°C of GC Cells Seeded into SurePath Preservative Fluid

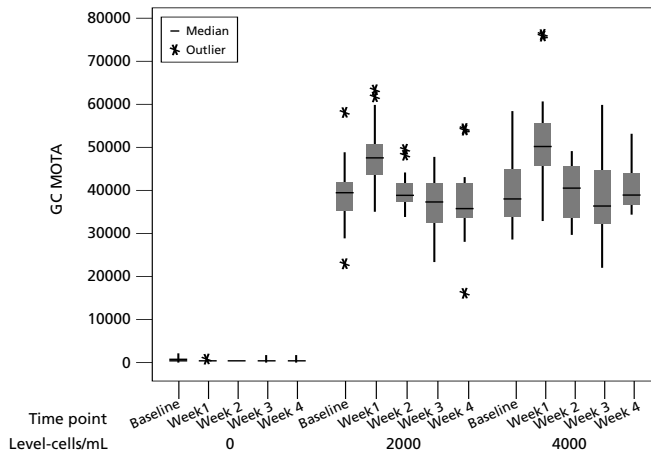
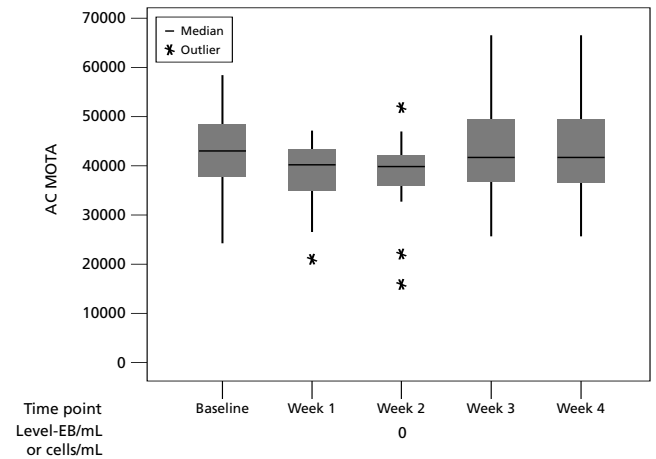


Figure 10. AC Results from Unseeded SurePath Preservative Fluid Stored at 30°C



Effect of Resuspension Volume Following Centrifugation

An experiment was performed to determine whether reducing the volume of Sample Diluent used to resuspend the SurePath specimens after centrifugation increased the risk of indeterminate results. A total of 28 tubes were each filled with 2 mL of SurePath medium and inoculated with 2000 EB and GC cells/mL. The samples were processed as described above using the modifications to the resuspension volume shown in Table 1. See Figure 11.

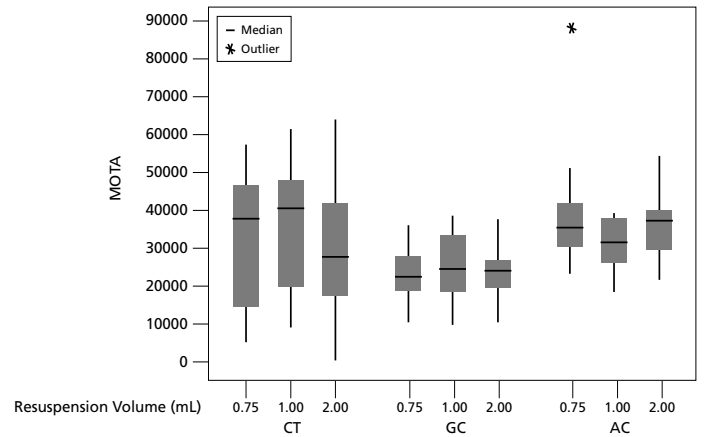
Table 1. Resuspension Volumes for Centrifuged SurePath Specimens

Number of Tubes Tested	Number of Assay Replicates	Volume of Sample Diluent (mL)	Theoretical Organism Concentration in Sample Diluent* (CT EB or GC cells/mL)	Concentration Factor
5	15	2	2000	1.0
8	16	1	4000***	2.0
15	15	0.75	5333	2.7

** Assumes 100% recovery following centrifugation.

*** Note: n = 8 for AC.

Figure 11. Effect of Resuspension Volume on CT/GC/AC Performance



Reducing the resuspension volume did not result in any indeterminate results.

CONCLUSIONS

- The analytical sensitivity of the BD ProbeTec ET System using organisms seeded into SurePath medium was shown to be <250 EB/mL for CT and 690 cells/mL for GC.
- CT and GC organisms spiked into SurePath medium were shown to be stable for 1 month at 2-30°C.
 - These results are consistent with TriPath's claim for SurePath preservative fluid with cytological samples of four weeks of stability at 15°C to 30°C.
- We demonstrated that decreasing the resuspension volume following centrifugation of SurePath specimens has the potential to enhance analytical sensitivity without detriment to the rate of indeterminate results.
- The preliminary data presented here demonstrate the feasibility of using SurePath preservative fluid as a sample type with the BD ProbeTec ET System for the detection of CT and GC DNA.
 - Nevertheless, further studies are required with clinical specimens in order to optimize the specimen processing procedure and determine clinical performance.