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Evaluation of Copan eSwab® for the collection of Specimens for *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Herpes Simplex Virus* by Molecular Methods

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ABSTRACT

Introduction

eSwab is a FDA approved device for the collection and transport of clinical microbiology specimens. Each collection kit consists of a pre-labeled polypropylene screw-cap tube with conical shaped bottom filled with 1 mL of Liquid Amies transport medium and a specimen collection swab which has a tip flocked with soft nylon fiber. The liquid Amies transporting medium, is used as a supportive medium that can sustain the viability of a plurality of organisms that include clinically important aerobes, anaerobes and fastidious bacteria such as *Neisseria gonorrhoeae* during transit to the testing laboratory. In addition, to bacterial supportive medium, eSwab offers condition that will maintain intracellular microorganism's viability such as *Chlamydia trachomatis* (CT) and intracellular virus if stored at 4°C .

The purpose of this study is to validate eSwab for collection and transport of specimens for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and Herpes Simplex virus (HSV) testing using molecular methods.

Methods

A total of 100 specimens were used for this study; 60 specimens for CT/NG, and 40 specimens for HSV. Specimens were spiked with cell pellets collected from 1mL of previously tested positive ThinPrep® and 0.5mL of previously tested positive M4 specimens. In addition, to determine the lowest detection limits (LOD), five eSwabs were spiked with NG (bacterial control) ranging from 106 to 102 copies; five eSwabs were spiked with, CT (GenProbe® positive control containing 5fg) and HSV 1 and 2 plasmid DNA ranging from 1000 to 10 copies/reaction. For CT/NG testing, 0.9mL, 0.5mL, and 0.2mL of spiked eSwab specimen were then transferred to ThinPrep® transfer media (GenProbe) and run on the TIGRIS APTIMA assay. Results were compared using Relative Light Units (RLU). For HSV testing, 0.5mL and 0.2mL of the spiked eSwab specimen were extracted on the MagNA Pure and then tested using a real-time PCR HSV assay (Qiagen, Inc.). Crossing point (Cp) was used to compare the results.

Results

Of all tested specimens, 41 were positive for CT, 5 were positive for NG and 2 were positive for both CT and NG. The remaining 11 tests were negative and matched the results reported by the reference method. Two specimens that were discrepant, one negative and one positive for CT/NG were confirmed by repeat analysis giving the assay a sensitivity of 96% and specificity of 100%, with an overall correlation of 98.33%. Of 40 specimens tested for HSV, 25 were positive and 15 were negative, and all matched those reported by the reference method. Interestingly, there was no significant difference in RLUs or Cp between 0.9mL, 0.5mL and 0.2mL for CT/NG and between 0.5mL and 0.2mL for HSV. The results for analytical sensitivity and LOD, correlated with expected results.

Conclusions

The eSwab collection and transport system used for collection and transport of clinical microbiological specimens is an optimal and reliable device and meets or exceeds all required standards for collection and transport of clinical specimens for CT, NG and HSV testing by molecular methods.

INTRODUCTION

Microbial viability and stability depend on collection, storage and the medium used for collection. The many collection devices used for microbiology and molecular specimen collection are sometimes confusing for clinicians. The eSwab is a FDA approved collection and transport device. It consists of 1 mL of Liquid Amies transport medium and a specimen collection swab which has a tip flocked with soft nylon fiber. Liquid Amies is a supportive medium that can sustain the viability of clinically important aerobes, anaerobes and fastidious bacteria such as *Neisseria gonorrhoeae (NG)*. The eSwab offers conditions that will maintain an intracellular microorganism's viability, such as *Chlamydia trachomatis* (CT), and intracellular viruses if stored at 4°C.

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) infections are two of the most common sexually transmitted infections worldwide. *C. trachomatis* can cause nongonococcal urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and Pelvic Inflammatory Disease (PID). These infections are often asymptomatic in both males and females. Children born to infected mothers are at significantly higher risk for inclusion conjuctivitis and chlamydial pneumonia. *N. gonorrhoeae* is the causative agent of gonorrheal disease. The majority of gonorrheal infections are uncomplicated lower genital tract infections and may be asymptomatic. However, if left ntreated in women, infections can cause PID and can manifest as endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscesses.

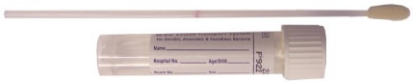
The classical presentation of primary HSV-1 infection is herpes gingivostomatitis, an infection of the oral mucosa.

Herpes genitalis is a classical presentation of HSV-2 infection. Approximately 85% of these cases are caused by HSV-2, the rest caused by HSV-1. The most serious consequence of genital HSV infection is neonatal herpes. This usually results from exposure of the infant to the virus secreted by the mother at time of vaginal delivery. The mortality rate for untreated infants who develop disseminated infection is greater than 70%.

Here we evaluated performance of each assay in our laboratory used for the detection of CT/NG and HSV with the eSwab collection device.

Results from this validation study demonstrated 100% for sensitivity and specificity and a correlation of 100% for the HSV tests. The CT/NG gave a sensitivity of 96% and specificity of 100% with an overall correlation of 98.33%.

MATERIALS AND METHODS



For the HSV portion of the study:

•Total of 40 specimens were spiked with cell pellet collected from 0.5mL of previously tested M4 specimens.

• Of these 25 were positive and 15 negative M4 specimens.

•All specimens were tested using, 0.5 and 0.2ml of spiked specimens.

•In addition, for analytical sensitivity, 6 eSwab specimens were spiked with HSV plasmid DNA (3 with HSV1 and 3 with HSV3) ranging from 10 through 1000 copies.

• Dilutions were run in duplicate between runs and days.

•To further investigate analytical specificity, five HSV positive specimens were spiked with CT/GC and GBS extracted DNA, two specimens were spiked with lysed blood and three specimens were spiked with feces.

•Sample extraction was performed using the MagNA Pure® automated extraction system (Roche, Inc.) and MagNA Pure® LC Total Nucleic Acid Isolation Kit according to the manufacturer's instructions.

• DNA was extracted from 200 and 500 µL of spiked specimens and eluted to 100 µL,.

•The real-time PCR was performed on the LightCycler® using 5 µL of eluted DNA and 15 µL of the LightCycler® Fast Start DNA Master Hybridization Probe reaction mix. Results were compared using crossing point.

To further investigate analytical sensitivity and specificity 6 eSwab specimens were spiked with HSV plasmid DNA (3 with HSV1 and 3 with HSV2) ranging from 10 copies through 1000 copies. Dilutions were run in duplicate between runs and days. The lowest detection for HSV 1 was 10 copies with the average Cp of 27.2 and the CV% of 8.7%, and for HSV 2 was 100 copies with the average Cp of 28.3 and the CV of 4.9%, demonstrating good analytical sensitivity and assay reproducibility. The internal control also demonstrated that did not interfere with the target amplification as it was in the expected range and constant for those with the higher or lower detection rate (Table 2).

To further investigate analytical specificity, five HSV positive specimens were spiked with CT/GC and GBS extracted DNA, two specimens were spiked with lysed blood and three specimens were spiked with feces and all reported positive as expected with the expected Cp and Tm.

DNA Conc. (copies)	1X10^{2}	1X10^{3}	1X10^{4}	0
HSV1 Cp	18.5	23.25	27.19	0
HSV2 Cp	27.14	28.32	0	0
Internal Control Cp	16.16	15.61	15.87	14.24
Internal Control Cp	14.78	15.04	15.15	14.24

For the CTNG portion of the study:

•Total of 60 specimens were used for this study.

• Of these 26 specimens were spiked with cell pellet collected from 1mL of previously positive ThinPrep specimens.

•Of these 13 were bacterial eSwab and 13 viral eSwab media. All specimens were tested using 0.9, 0.5 and 0.2ml of specimen. 34 additional specimens were prepared by spiking with pellet collected from 0.5mL of previously tested M4 specimens (from our reference laboratory).

•Of these 24 were positive and 10 negative M4 specimens. As M4 specimens are more concentrated, testing from these specimens was performed using 0.5 and 0.2 mL of specimen only.

•In addition, five eSwab specimens were spiked with GC (bacterial control) using McFarland dilutions ranging from 10^{6} to 10^{2} copies, and five eSwab specimens were also spiked with GenProbe positive control that contains 5fg of CT rRNA at different dilution ranging from 5fg/mL through 0.65fg/mL which equals one infectious forming unit (IFU). Dilutions were run in duplicate between runs and days.

•All specimens were transferred to ThinPrep transfer media (GenProbe) and run on the TIGRIS assay and compare to previously reported results.

• Correlation between 0.9, 0.5, and 0.2 specimens was performed using Relative Light Units (RLU). In addition, 26 specimens prepared by spiking with ThinPreps were also tested on Cobas Amplicor CT/GC assay.

RESULTS

CTNG on the GenProbe Tigris

eSwab Spiked	Total	Positive	Negative	Sensitivity	Specificity	PPV	NPV
ThinPreps	26	26/26	0/0	100%	100%	100%	100%
M4 Medium	34	23/24	12/11	100%	92%	100%	92%
0.9mL	13	13/13	0/0	100%	100%	100%	100%
0.5ml	60	58/59	12/11	96.60%	100%	95.10%	100%
0.2ml	60	58/59	12/11	96.60%	100%	95.10%	100%

HSV using RT PCR

Reproducibility

eSwab Spiked	Total	Positive	Negative	Sensitivity	Specificity	PPV	NPV
M4 Medium	40	25/25	15/15	100%	100%	100%	100%
0.5ml	40	25/25	15/15	100%	100%	100%	100%
0.2ml	25	25/25	0/0	100%		100%	

eSwab Spiked	Total	Positive	Negative	Sensitivity	Specificity	PPV	NPV
M4 Medium	40	25/25	15/15	100%	100%	100%	100%
0.5ml	40	25/25	15/15	100%	100%	100%	100%
0.2ml	25	25/25	0/0	100%		100%	

CONCLUSIONS

This validation study demonstrated that eSwab collection and transport system used for collection and transport of clinical specimens containing aerobes, anaerobes and fastidious bacteria from the collection site to our laboratory is an optimal and reliable device and meet or exceeds all required standards for collection and transport of clinical specimens for CT and CG testing in our lab using GenProbe TIGRIS assay.

It also demonstrated that eSwab system used for collection and transport of clinical specimens used for HSV testing using by real-time PCR assay in our laboratory is an optimal and reliable device and meet or exceeds all required standards.