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DIAGNOSIS OF C. TRACHOMATIS (CT) AND N. GONORRHOEAE (GC) IN SUREPATH® L-PAP SAMPLES USING APTIMA COMBO 2 AND PROBETEC (PT) ASSAYS

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REVISED ABSTRACT

Background: Liquid-based Pap (L-Pap) samples collected for cervical cancer screening are convenient samples for testing for CT and GC. We compared APTIMA® Combo 2 (AC2) and ProbeTec™ (PT) assays to detect CT and GC from SurePath (TriPath) L-Pap samples.

Methods: A total of 394 women consented to collection of a cervical swab (CS) and 2 SurePath L-Pap collection vials using Cervex-Brushes™. The 2 L-Pap samples were pooled before processing one half for Pap cytology (cyt). Aliquots of the other half of the L-Pap fluid (precyt remnant) were tested for CT and GC using published testing protocols as follows: [a] transferring within 48 hours 1ml into specimen transport media [STM] before testing 400µl in AC2 [Gen-Probe Inc.]; [b] placing 500µl into 2ml of PT diluent then testing 150µl in PT [Becton Dickinson]. The CS was tested by AC2. To compare the presence of analytes in the L-Pap post cytology (postcyt) remnant versus gradient sample left after cytological processing, both samples were tested by AC2.

Results: The prevalence of CT was 8.9% (35/394) and GC was 1.5% (6/394). Two patients were infected with both organisms. Twenty-seven (77.1%) of the 35 CS-positive women (CT-positive) were positive in the L-Pap sample in both CT assays. The percent sensitivity and specificity of AC2 was 97.1, 100 and 77.1, 100 for PT. For GC, AC2 identified all 6 (100%) infections while PT missed two (66.7% sensitivity). Specificity for GC was 100% for both tests. Thirty one of 34 CT and 6 of 6 GC- positive L-Pap samples were positive in both the remnant and gradient samples tested after cytology processing.

Conclusions: Using published testing protocols for the detection of CT and GC in SurePath L-Pap samples demonstrated very high sensitivity and specificity for the AC2 assay which may have been due to the transfer of the L-Pap sample into STM within 48 hours. The protocol used for PT testing of L-Pap samples in this study was less impressive, missing several positive patients. Remnant and gradient L-Pap samples detected equal numbers of CT- and GC-positives by AC2, but 3 less than the precyt samples.

INTRODUCTION

There are three liquid based cytology systems cleared by the US Food and Drug Administration for liquid-based Pap (L-Pap) cytology: PreservCyt® ThinPrep (Hologic), SurePath (BD Diagnostics-TriPath) and Cytotek MonoPrep (Monogen). All use broom, brush or spatula sampling of the cervix placed into their proprietary transport media. APTIMA Combo 2 (Gen-Probe) and AMPLICOR (ROCHE) have FDA clearance for the use of ThinPrep L-Pap for C. trachomatis (CT) and N. gonorrhoeae (GC) testing.

OBJECTIVES

1. To compare the performance of APTIMA Combo 2 (AC2) and ProbeTec (PT) on pre-cytology SurePath L-Pap samples for the diagnosis of CT and GC.
2. To compare AC2 performance on post cytology remnant and gradient samples.

METHODS

Patient recruitment: 394 women (>ages 15) from April 2008-February 2009 attending health centre/ OB/GYN clinic for routine care signed an IRB approved informed consent to participate. Study participants with antibiotic use in the past 3 weeks and women pregnant past the first trimester were excluded.

Physician collection: Each collection package included information/informed consent forms and collection kits, labeled with an unique study identifier. The physician collected three samples which were in a randomized order (Fig. 1): Two L-Pap samples using a Cervex-Brush® and immersed into the SurePath specimen vial; an APTIMA cervical swab (Gen-Probe Inc.) [see diagram]. Samples were shipped the same day to Gamma Dynacare Medical Laboratories (GDC), Brampton site.

Laboratory specimen handling/testing: The cytology technologist removed only the two L-Pap vials from the study package and carefully mixed both vials to ensure homogeneity, then divided the L-Pap samples evenly. One vial remained for Pap cytology at GDC and the other was placed into the study package with the rest of the study samples and shipped immediately to St. Joseph's Healthcare Infections Research Group (SJH), where the L-Pap sample was prepared for AC2 and PT within 48 hours (Fig. 2). After cytological processing, the gradient component from the processed sample and the postcyt remnant sample in the SurePath vial were tested by AC2.

APTIMA Combo 2 (Gen-Probe Inc.): As described previously, after vortexing the SurePath vial, 1mL of L-Pap was transferred into the APTIMA STM tube. Both the cervical swab STM and L-Pap processed STM were tested in the DTS 400/ Leader HC+ system.

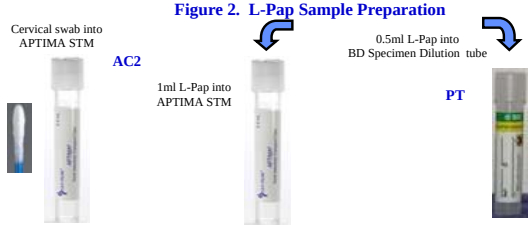
BD ProbeTec (Becton Dickinson): As described previously, 0.5mL of L-Pap was transferred into the BD specimen dilution tube, inverted 3-4x and tested according to the manufacturers' instructions.

Sensitivity and Specificity calculations: A patient was considered positive if the CS was positive or the L-Pap sample was positive by AC2 and PT. Confidence intervals were calculated using analysis software (version 2.1.2, 2004; T. Bryant, University of Southampton, UK).

Figure 1. Physician instructions for specimen collection

- Insertion of the swab into the endocervix and rotated twice.
- Insertion of the central bristles of the cervex-brush into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate in a clockwise direction five times.

Figure 2. L-Pap Sample Preparation



RESULTS

Table 1. Numbers of patients positive for CT and GC by specimen and tests

Infection	Specimen/Test			Totals
	CS	L-PAP		n
CT	AC2	AC2*	BD	
(n=35)	+	+	+	27
	+	+	-	7
	-	+	+	1
GC	+	+	+	4
(n=6)	+	+	-	2

- 3 extra CT-positive patients were identified using the precyt remnant sample compared to the postcyt remnant or gradient samples.

Table 2. Sensitivity and Specificity of AC2 and PT on SurePath L-Pap samples

Assay	CT		GC	
	%Sensitivity (95% C.I)	%Specificity (95% C.I)	%Sensitivity (95% C.I)	%Specificity (95% C.I)
AC2	97.1 (34/35) (84.2-99.5)	100 (359/359) (98.6-100)	100 (6/6) (61.0-100)	100 (388/388) (98.7-100)
PT	77.1 (27/35) (59.0-87.2)	100 (359/359) (98.6-100)	66.7 (4/6) (43.6-97.0)	100 (388/388) (98.7-100)

CONCLUSIONS

- The prevalence of infection was 8.9% (35/394) for CT and 1.5% (6/394) for GC.
- SurePath L-Pap testing demonstrated excellent sensitivity and specificity with the APTIMA Combo 2 assay with some advantage to testing a precyt remnant specimen.
- Although the specificity was 100%, 20% of the CT positives and 33.3% of GC positives were missed using this ProbeTec protocol for SurePath samples

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