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Comparison of Three Assays for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in SurePath Pap Samples and the Role of Pre- and Postcytology Testing

M. Chernesky,[a] D. Jang,[a] E. Portillo,[a] M. Smieja,[a] J. Kapala,[b] C. Doucette,[b] J. Sumner,[b] R. Ewert,[c] C. MacRitchie,[d] and J. Gilchrist[a]

St. Joseph's Healthcare/McMaster University, Hamilton, ON, Canada[a]; Gamma-Dynacare Medical Laboratories, Brampton, ON, Canada[b]; Evergreen Health Centre, Toronto, ON, Canada[c]; and Hamilton Community Health Centre, Hamilton, ON, Canada[d]

***Chlamydia trachomatis* and *Neisseria gonorrhoeae* are common causes of sexually transmitted infections, and there is interest in screening SurePath liquid-based Pap (L-Pap) samples with Aptima Combo 2 (AC2), Amplicor (AMP), and ProbeTec ET (PT) assays. SurePath L-Pap samples and a cervical swab (CS) were collected from 394 women attending health clinics in Hamilton and Toronto, ON, Canada. L-Pap samples were tested with the three assays prior to being processed for cytology, and the CS sample was tested with AC2. The prevalence of *C. trachomatis* was 8.9%, and that of *N. gonorrhoeae* was 1.5%. By using the positives from CS testing, as well as CS negatives corresponding to L-Pap samples that tested positive in 2 of 3 assays, the sensitivities of AC2, AMP, and PT for *C. trachomatis* in precytology samples were calculated to be 97.1% (34 of 35 positive samples were detected), 91.4% (32 of 35 were detected), and 77.1% (27 of 35 were detected), respectively. Six women were infected with *N. gonorrhoeae*. After cytology processing, the results of testing the remaining liquid in the L-Pap vial and the cell-enriched fraction for *C. trachomatis* by AC2 showed positive agreements of 98.9% (kappa [*k*], 0.93) and 98.7% (*k*, 0.92), respectively, with the results of testing precytology L-Pap samples. Although all testing showed high specificity, testing for *C. trachomatis* by AC2 was significantly more sensitive than testing by PT for SurePath samples ($P = 0.02$). Newer versions of AMP (Cobas 4800) and PT (Q^x with XTR technology) need published evaluations for detecting *C. trachomatis* and *N. gonorrhoeae* in L-Pap samples. *C. trachomatis* testing can be performed with similar results on pre- and postcytology SurePath samples.**

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections are the most frequently reported bacterial sexually transmitted diseases in North America (1). Because of the high rates of asymptomatic infection, which may lead to upper genital tract complications such as pelvic pain, ectopic pregnancy, and infertility, control requires some form of screening to identify and treat infected patients and their partners (9). Specimens from cervical cancer screening programs are being considered for *C. trachomatis* and *N. gonorrhoeae* screening by testing of PreservCyt ThinPrep (Hologic) or SurePath (BD Diagnostics-TriPath) samples. Nucleic acid amplification tests such as the Aptima Combo 2 (AC2), Amplicor (AMP), and ProbeTec ET (PT) assays are cleared by the U.S. FDA for the detection of *C. trachomatis* and *N. gonorrhoeae* using swabs and urine samples. AC2 and AMP are cleared for use on ThinPrep liquid-based Pap (L-Pap) samples; AC2 has been validated using SurePath samples, and AMP and PT protocols for SurePath testing have been presented at scientific meetings. The objective of this study was to compare the performances of the three assays for precytology SurePath L-Pap samples collected from patients attending clinics for routine Pap testing. A secondary objective was to compare L-Pap samples before and after processing for cytology.

MATERIALS AND METHODS

Study design. This was a cross-sectional study which enrolled 394 women between 15 and 71 years of age attending health clinics for routine care in Hamilton and Toronto, Ontario, Canada. Patients signed informed-consent forms prior to participation. Patients with antibiotic use in the past 3 weeks and those pregnant past the first trimester were excluded from the study. Each collection package included information about the study, an informed-consent form, and collection kits for each assay. Each item was prelabeled with a unique identifier. The clinician collected three samples in the following order: (i) a SurePath L-Pap sample obtained using an established procedure with a Cervex-Brush, (ii) a cervical swab (CS) sample obtained using an Aptima unisex swab (Gen-Probe Inc.) and placed into specimen transport medium, and (iii) a second L-Pap SurePath sample. Samples were shipped on the day of collection to Gamma-Dynacare Medical Laboratories in Brampton, Ontario, Canada.

Testing. The cytology technologist removed the two L-Pap vials from the study package, carefully subjected the samples to a vortex, and then combined and mixed the samples to ensure homogeneity. The mixed samples were then divided back into the original vials. One vial was used for Pap cytology, and the other (referred to herein as a precytology L-Pap sample) was placed back into the study collection package containing the CS and study forms and shipped overnight to St. Joseph's Healthcare Infections Research Laboratory (IRL) in Hamilton, Ontario, Canada, where upon receipt, aliquots were transferred into respective assay transport tubes and tested by the three assays within 48 h. The CS was also tested by AC2. Approximately 1 week later, following the performance of L-Pap cytology analysis, paired processed samples (the remaining liquid in the L-Pap vial and the cell-enriched fraction) were shipped to the IRL for comparative testing by the AC2 test.

Aptima Combo 2 (Gen-Probe Inc.) testing of the CS was performed as indicated in the test package insert. One milliliter of the L-Pap sample was transferred into a Gen-Probe Aptima specimen transfer tube containing 2.9 ml of Aptima transport medium, and 400 μl was tested using the manual direct detection system (5).

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Address correspondence to M. Chernesky, chernesk@mcmaster.ca.

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TABLE 1 Testing profiles for patients infected with *C. trachomatis* and/or *N. gonorrhoeae*

Infecting agent	AC2 result for CS sample	Result for precytology SurePath L-Pap sample by:			No. of patients:		
		AC2	AMP	PT	≤25 yr	>25 yr	Total
C. trachomatis	+	+	+	+	23	2	
	+	+	+	−	6	0	
	+	+	−	−	1	0	
	+	−	−	−	1	0	
	+	+	−	+	0	1	
	−	+	+	+	1	0	
Total no. of positive samples	34	34	32	27			35
N. gonorrhoeae	+	+	+	+	4	0	
	+	+	−	−	1	0	
	+	+	+	−	1	0	
Total no. of positive samples	6	6	5	4			6

TABLE 2 Sensitivities, specificities, and positive and negative predictive values for detecting *C. trachomatis* infection[a]

Test	No. of positive samples detected	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
AC2	34	97.1 (85.1–99.9)	100 (99.0–100)	100	99.7
AMP	32	91.4 (76.9–98.2)	99.7 (98.6–100)	97.0	99.2
PT	27	77.1 (60.0–89.6)	100 (99.0–100)	100	97.8

[a] Values are for testing of SurePath L-Pap samples from 35 infected and 359 uninfected women before processing of the samples for cytology. CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value. *P* values: AC2 versus PT, $P = 0.02$; AC2 versus AMP, $P = 0.48$; AMP versus PT, $P = 0.07$.

The Amplicor test (Roche Diagnostics) was performed using a protocol published by the Cytyc Corporation in 2003 as a package insert for the PreservCyt solution kit (2). A 500-μl aliquot was subjected to a vortex for 5 s and then transferred into a 1.5-ml screw-cap tube. The sample was then centrifuged at 12,500 × *g* for 10 min before the supernatant fluid was discarded. Kit lysis buffer (100 μl) was added to the pellet before it was subjected to a vortex for 10 s. After incubation of the sample at room temperature for 15 min, 100 μl of specimen diluent was added, and the sample was mixed and incubated for an additional 10 min at room temperature. A 100-μl aliquot was transferred into a tube containing a working master mix for processing according to the instructions in the package insert.

ProbeTec ET (BD Diagnostics) testing was performed using an experimental protocol (C. Martinaitis, T. Poth, K. Williams, C. Welborn, D. Shank, and T. Hellyer, presented at the Clinical Virology Symposium, Clearwater, FL, 8 to 11 May 2005). Two milliliters of the L-Pap sample was transferred into a 4-ml tube for centrifugation at 2,000 × *g* for 30 min. Pellets were resuspended in 1 ml BD *C. trachomatis*/*N. gonorrhoeae* sample diluent buffer, subjected to a vortex, and heated, and then 150 μl was tested according to the instructions in the test package insert.

Data analysis. Calculations of sensitivity, specificity, and predictive values were made by using two-by-two tables based on a reference standard of the CS being positive or, if the CS was negative, L-Pap samples being positive in 2 of 3 tests. Confidence Interval Analysis software version 2.2 (T. Bryant, 2004) was used. A *P* value of <0.05 was deemed statistically significant.

RESULTS

The 394 women ranged in age from 15 to 71 years (median age, 24 years). The prevalence of *C. trachomatis* was 8.9% (35 of 394 women were infected), and that of *N. gonorrhoeae* was 1.5% (6 of 394 women were infected). Two patients were infected with both organisms. Table 1 summarizes the testing profiles of the patients positive for *C. trachomatis* and/or *N. gonorrhoeae* with the 3 assays. Most of the *C. trachomatis*-infected patients (74.3%; 26 of 35) were positive by all 3 assays with the precytology L-Pap sample, and an additional 6 were positive by 2 of the 3 tests. One patient who was *C. trachomatis* negative by AC2 testing of the CS had positive L-Pap sample results in all three assays, providing a total of 35 *C. trachomatis* positives. The AC2 test identified 34 of 35 *C. trachomatis* positives, missing 1 by CS testing and another by L-Pap testing. The AMP assay detected 32 positives, and PT found 27 positives. The majority of *C. trachomatis* infections (91.4%; 32 of 35) were in women 25 years or younger; the prevalence among women in this age group was 13% (32 of 246 were infected), compared to 2% (3 of 148 were infected) among women older than 25 years. Of the patients infected with *N. gonorrhoeae*, 66.6% (4 of 6) were positive by all 3 tests and 83.3% (5 of 6) were positive by at least 2 of the 3 tests. AC2 detected all of the *N. gonorrhoeae* positives by testing of the CS or L-Pap sample. The AMP assay missed 1 *N. gonorrhoeae* positive, and PT missed 2. All 6 women infected with *N. gonorrhoeae* were less than 26 years old.

Table 2 summarizes the sensitivities, specificities, and predictive values of the 3 assays performed on precytology L-Pap samples. AC2 testing had a sensitivity of 97.1% (34 of 35 positives were detected) and a specificity of 100%. The AMP test identified 91.4% of the *C. trachomatis*-positive patients (32 of 35) and 1 false positive (specificity, 99.7%). The difference between the sensitivities of AC2 and AMP was not significant ($P = 0.48$). The PT test was 100% specific, but the sensitivity was 77.1% (27 of 35 positives were detected) (AC2 versus PT, $P = 0.02$; AMP versus PT, $P = 0.07$). Although the number of *N. gonorrhoeae* positives was low, specificities were high for all tests, but the ProbeTec missed 2 positives, Amplicor missed 1 positive, and Aptima identified all 6 positives by testing precytology L-Pap samples.

After processing of the L-Pap samples for cytology, the remaining liquid in the L-Pap vial and the cell-enriched fraction were tested by AC2 and the results were compared to the results from precytology testing. The outcomes for *C. trachomatis* testing are shown in Table 3. Agreement between results for the precytology samples and those for the remaining liquid in the L-Pap vial (98.9%; kappa [*k*], 0.93) and the cell-enriched fraction (98.7%; *k*, 0.92) after cytology processing was strong. Agreement between results for the 2 postcytology samples was also strong (99.2%; *k*, 0.95; data calculations not shown). For the 6 *N. gonorrhoeae*-positive patients, results for all 3 L-Pap samples were in 100% agreement (data not shown).

DISCUSSION

The findings in this study confirm and expand previous observations comparing the performances of the 3 amplification assays for identifying *C. trachomatis*- and/or *N. gonorrhoeae*-infected patients. We (2, 3, 6) and others (7) have shown previously that the

TABLE 3 Agreement between results for *C. trachomatis* testing of pre- and postcytology L-Pap samples from 394 women by the Aptima Combo 2

Postcytology L-Pap sample type and result	No. of precytology L-Pap samples testing:		Total no. of samples	% agreement between results for pre- and postcytology samples
	Positive	Negative		
Liquid remaining in L-Pap vial				98.9 (*k*, 0.93)
Positive	30	0	30	
Negative	4	360	364	
Cell-enriched fraction				98.7 (*k*, 0.92)
Positive	31	2	33	
Negative	3	358	361	

AC2 test may be more sensitive than the other 2 assays for clinical samples when head-to-head comparisons are performed and the outcomes of testing more than one specimen type are used to expand the reference standard (8). Previous studies have shown that differences in analytical sensitivity of nucleic acid amplification tests (6) and inhibitors of DNA amplification (2, 3) may account for lower sensitivities in the AMP and PT assays.

The sensitivity of AC2 for *C. trachomatis* in SurePath L-Pap samples in the present study was equal to that for testing of a CS (97.1%) and higher than those observed previously in a multicenter study (5) and two validation studies (4, 7), where AC2 sensitivities for SurePath L-Pap samples ranged from 80 to 89.3%, with high predictive values. The study by Khader et al. (7) demonstrated higher sensitivity for women under the age of 26 than for older women (93.1% versus 80.7%). The higher sensitivity values in the present study may be attributed to several reasons: more rapid introduction of the L-Pap specimens into the Aptima transfer tubes to stabilize RNA before testing, use of an expanded reference standard of CS positivity by AC2 or 2 positive L-Pap sample results, and a greater proportion of younger women infected (Table 1).

Experiments to compare the precytology samples with the remaining liquid in the L-Pap vials and the cell-enriched fraction after cytology processing showed strong agreement between pre- and postcytology testing results, indicating that any of the 3 types of L-Pap samples can be used for testing for the 2 infections by AC2.

We used an experimental AMP protocol which had been created for ThinPrep and a PT protocol designed for SurePath, and we showed the AMP test to have a high sensitivity of 91.4% for *C. trachomatis* while PT had a lower sensitivity of 77.1% ($P = 0.07$). The *C. trachomatis* results for PT are similar to those seen previously in a head-to-head ThinPrep comparison (2), where the sensitivity was 72.4%. The differences between AC2 and PT calculated in the present study are statistically significant ($P = 0.02$). The sensitivities for *N. gonorrhoeae* were 100% for AC2, 83.3% for AMP, and 66.6% for PT compared to 100%, 85.7%, and 85.7% in the previously published ThinPrep study (2). The total number of *N. gonorrhoeae* positives in the present study was too low to calculate significant differences.

While this study was being conducted, next-generation assays from Roche (Cobas 4800 CT/NG) and Becton Dickinson (ProbeTec CT/GC Q^x for use on the BD Viper system with XTR technology) appeared. A review of the literature and Internet searching failed to identify any peer-reviewed publications on either assay performed with SurePath samples. We were unable to find data presented for the Cobas 4800 assay or Amplicor for testing of SurePath L-Pap samples. However, several meeting presentations reporting performance data for BD assays with L-Pap samples are available. Using the first-generation ProbeTec CT/GC assays on SurePath preservative fluid, Martinaitis et al. spiked *C. trachomatis* and *N. gonorrhoeae* into SurePath media and reported a protocol for detection in the ProbeTec assay (C. Martinaitis, T. Poth, K. Williams, C. Welborn, and T. Hellyer, presented at the Clinical Virology Symposium, Clearwater, FL, 8 to 11 May 2005). Keller et al. collected 2 cervical swabs and SurePath ($n = 91$) or PreservCyt ($n = 125$) L-Pap samples. With data for an infected patient (whose CS was positive by AC2 and *C. trachomatis* Q^x and *N. gonorrhoeae* Q^x assays) as the standard, the sensitivity and specificity of the *N. gonorrhoeae* Q^x assay were 100% and 95.7% for SurePath samples. Similarly high values were recorded for the *C. trachomatis* Q^x assay with PreservCyt L-Pap samples (L. Keller, K. Eckert, P. Fine, D. Fuller, W. LeBar, M. Larsen, and J. Lebed, presented at the Clinical Virology Symposium, Daytona Beach, FL, 27 to 30 April 2008). A third presentation described results from a multicenter study of 1,715 patients attending family planning clinics, obstetric-gynecological offices, and sexually transmitted disease clinics (J. Lebed, P. Fine, D. Fuller, S. Ginde, E. W. Hook, W. LeBar, M. Martens, W. McCormack, L. Mena, S. Taylor, B. Van Der Pol, and C. Meyers, presented at the Clinical Virology Symposium, Daytona Beach, FL, 19 to 22 April 2009). Three cervical swabs and a SurePath broom or brush/spatula sample were collected. An infected patient (with 2 of 3 CS samples testing positive by the AC2, ProbeTec, and ProbeTec Q^x assays) was used as the reference standard to determine the sensitivities and specificities of Q^x assays for *C. trachomatis* and *N. gonorrhoeae* detection in SurePath samples. The researchers reported high rates in symptomatic and asymptomatic patients for both analytes and no differences between collection by the cervix broom and collection by the brush with spatula. This similarity of results from different collection devices confirms data previously reported (4). The 3 unpublished studies discussed above do not provide sufficient data on the samples with discordant findings used in setting the reference standard to assess the validity of the results and do not indicate whether the L-Pap samples were tested pre- or postcytology. Examination of the FDA clearance statement for the Q^x assay on SurePath samples shows clearance for an aliquot that is removed prior to processing for the BD SurePath L-Pap test.

The present study showed strong agreement between AC2 results for pre- and postcytology L-Pap testing, so either pre- or postcytology samples could be tested by the Aptima Combo 2 assay, which displayed high sensitivity, specificity, and predictive values. The AMP assay performed very well for *C. trachomatis*, but the PT assay had lower sensitivity than we expected. With newer-generation tests available, further studies should be performed to assess their performance on L-Pap samples.

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