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Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in North American Women by Testing SurePath Liquid-Based Pap Specimens in APTIMA Assays[▽]

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The APTIMA COMBO 2 assay, which detects and amplifies rRNA from *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*, is approved for use on ThinPrep liquid-based Pap test specimens. The objective was to determine the clinical utility of the APTIMA assays (APTIMA COMBO 2 assay, APTIMA CT assay for *Chlamydia trachomatis*, and APTIMA GC assay for *Neisseria gonorrhoeae*) for screening women during their annual Pap exam, using SurePath liquid-based Pap test specimens. Two cervical samples were collected from 1,615 females attending six clinical sites in North America. A cervical broom sample was processed for cytology, with the residuum aliquoted into an APTIMA specimen transfer kit tube. The second cervical swab sample was put into APTIMA specimen transport medium, and both samples were tested with each APTIMA assay on a direct sampling system. Using a subject-infected status that utilized cervical-swab specimen results from two APTIMA assays, the prevalence was 7.9% for *Chlamydia trachomatis* and 2.5% for *N. gonorrhoeae*. For the liquid-based Pap samples, the sensitivities, specificities, positive predictive values, and negative predictive values for *Chlamydia trachomatis* detection were 85.2%, 99.5%, 93.2%, and 98.7%, respectively, for the APTIMA COMBO 2 assay and 89.1%, 98.7%, 85.7%, and 99.1%, respectively, for the APTIMA CT assay. For *N. gonorrhoeae* detection, the values were 92.5%, 100%, 100%, and 99.8%, respectively, for the APTIMA COMBO 2 assay and 92.5%, 99.9%, 97.4%, and 99.8%, respectively, for the APTIMA GC assay. The high predictive values support the use of the assays with SurePath liquid-based Pap specimens processed with the APTIMA specimen transfer kit.

Annual screening for cervical abnormalities using a Pap test has resulted in a substantial reduction in morbidity and mortality from cervical cancer (11). There are three liquid-based Pap tests (LPTs) approved by the United States Food and Drug Administration (FDA): PreservCyt ThinPrep (Cytyc, Boxborough, MA), SurePath (TriPath Care Technologies, Burlington, NC), and Cytotek MonoPrep (Monogen, Inc., Vernon Hills, IL). Liquid-based cytology has provided greater Pap testing accuracy, and the sample may serve as a specimen to test for infectious agents, such as human papillomavirus (2, 11), *Chlamydia trachomatis* (5, 14), and *Neisseria gonorrhoeae* (6, 9). The APTIMA COMBO 2 assay, APTIMA CT assay for *Chlamydia trachomatis*, and APTIMA GC assay for *Neisseria gonorrhoeae* are transcription-mediated amplification tests that utilize target capture specimen processing for the in vitro qualitative detection of rRNA from *C. trachomatis* and/or *N. gonorrhoeae*. The assays are approved for use on cervical swab (CS) samples, vaginal swab samples, urethral swab samples, and first-catch urine samples. The APTIMA COMBO 2 assay is approved for use with ThinPrep LPT specimens. A limited number of studies have been published using both amplified and nonamplified tests for *C. trachomatis* or *N. gonorrhoeae* in liquid-based specimens and have concentrated on the use of ThinPrep samples (1, 5–10; D. Fuller, T. Davis, and J. Talbott, unpublished data). Our objective was to determine the clinical utility of the APTIMA assays (Gen-Probe Incorporated) for screening women for *C. trachomatis* and *N. gonorrhoeae* during their annual Pap examination by using SurePath LPT specimens.

MATERIALS AND METHODS

A total of 1,615 female subjects signed consent forms while being enrolled during 2004 at six clinical sites in North America: University of North Carolina (UNC), Chapel Hill, NC; University of Alabama (UAB), Birmingham, AL; St. Joseph's Healthcare (SJH), Hamilton, ON, Canada; University of Oklahoma—Tulsa (UOT), Tulsa, OK; University of Illinois—Urbana (UIU), Urbana, IL; and Planned Parenthood of Minnesota/South Dakota (MNSD), St. Paul, MN. Each patient had a cervical broom collection into a SurePath preservative-fluid tube, which was processed for cytology. The residual specimen was transferred to an APTIMA specimen transfer tube and tested with each APTIMA assay. A CS sample was also collected by using the APTIMA collection kit. For each assay, the results on the SurePath specimen were compared to a subject-infected status where the patient's CS sample was positive by two APTIMA assays (APTIMA COMBO 2 and APTIMA CT and APTIMA COMBO 2 and APTIMA GC). Sensitivity, specificity, and predictive values were computed based on binomial distribution. Fisher's exact test was used to calculate *P* values, and 95% confidence intervals were calculated. The patient's age, ethnic origin, and reason for clinic visit were recorded. To determine the performance of the APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays on LPT specimens, positives, negatives, false positives, and false negatives were calculated.

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TABLE 1. Ethnic origin, symptom status, and reason for clinic visit of women from the six study sites collecting liquid-based Pap samples

Clinic site	Mean age (yr)	No. (%) of patients who were:					
		Black, non-Hispanic	White, Hispanic	White, non-Hispanic	Receiving annual exam	Receiving STD screening	Asymptomatic
UIU	37.8	13 (3.9)	4 (1.2)	311 (93.1)	283 (84.7)	3 (0.9)	325 (97.3)
MNSD	27.4	7 (3.6)	5 (2.6)	169 (87.1)	165 (85.1)	25 (12.9)	180 (92.8)
SJH	19.9	46 (23.6)	6 (3.1)	117 (60.0)	146 (74.9)	40 (20.5)	157 (80.5)
UOT	30.8	64 (13.8)	181 (38.9)	186 (40.0)	319 (68.6)	6 (1.3)	361 (77.6)
UAB	27.1	374 (91.9)	0 (0)	28 (6.9)	5 (1.2)	349 (85.7)	117 (28.7)
UNC	28.1	15 (75)	0 (0)	5 (25.0)	0 (0)	18 (90)	8 (40.0)
Total	29.6	519 (32.1)	196 (12.1)	816 (50.5)	918 (56.8)	441 (27.3)	1,148 (71.1)

RESULTS

Table 1 is a summation of the information gathered on the questionnaire and shows that the overall mean age of the women enrolled in the study was 29.6 years. Patients from the SJH site were younger (19.9 years) and from the UIU site older (37.8 years). Ethnicity or race varied among sites. The UIU and MNSD patients were predominantly white non-Hispanic women (93.1% and 87.1%, respectively), whereas those from UAB were predominantly black non-Hispanic women (91.9%). Women enrolled at the UNC site were 75% black non-Hispanic and 25% white non-Hispanic. At SJH, these percentages were 23.6% and 60%, respectively. The UOT enrollment had a more even distribution of the three predominant combinations. The UAB and UNC women were attending for sexually transmitted disease (STD) screening, whereas the other four study sites reported that most were attending the clinic for an annual examination. Overall, 71.1% (1,148/1,615) of the women were without symptoms. The majority of patients attending for their annual examination were asymptomatic compared to those attending for STD screening.

Table 2 shows the numbers of patients enrolled at each study site, varying from 20 from UNC to 465 at UOT. Of the 1,615 women enrolled, 113 were infected only with *C. trachomatis*, 15 with both *C. trachomatis* and *N. gonorrhoeae*, and 25 with only *N. gonorrhoeae*. A total of 20 patients were positive in the APTIMA CT assay but negative by APTIMA COMBO 2, and 3 were positive by APTIMA COMBO 2 but negative in the APTIMA CT test. One woman's CS sample was positive in the APTIMA GC test but negative in the APTIMA COMBO 2 assay. Overall, the prevalence of infection was 7.9% (128/1,615) for *C. trachomatis* (confidence interval [CI], 6.7 to 9.45) and 2.5% (40/1,615) for *N. gonorrhoeae* (CI, 1.8 to 3.45). The prevalence of *C. trachomatis* infections ranged from 0.9% at UIU to 17.0% at SJH. *N. gonorrhoeae* infections were most prevalent at UAB (7.4%) and not prevalent at UIU and UNC.

TABLE 2. Numbers of single and dual infections with *C. trachomatis* and/or *N. gonorrhoeae* determined by positive results using APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays performed on CS samples of 1,615 women at six study sites[a]

Site	No. of patients	No. (%) with infection status of:			Total no. (%) infected with:	
		CT^+ GC^-	CT^+ GC^+	CT^- GC^+	CT	GC
UIU	334	3 (0.9)	0 (0)	0 (0)	3 (0.9)	0 (0.0)
MNSD	194	7 (3.6)	0 (0)	1 (0.5)	7 (3.6)	1 (0.5)
SJH	195	31 (15.9)	2 (1.0)	4 (2.1)	33 (17.0)	6 (3.1)
UOT	465	22 (4.7)	2 (0.4)	1 (0.2)	24 (5.2)	3 (0.6)
UAB	407	49 (12.0)	11 (2.7)	19 (3.4)	60 (14.7)	30 (7.4)
UNC	20	1 (5.0)	0 (0)	0 (0)	1 (5.0)	0 (0.0)
Total	1,615	113 (6.9)	15 (0.9)	25 (1.5)	128 (7.9)	40 (2.5)

[a] CT, *C. trachomatis*; GC, *N. gonorrhoeae*.

Table 3 summarizes the results of testing the CS samples for *C. trachomatis* and *N. gonorrhoeae* using the APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays, according to the presence or absence of symptoms. Infection with *C. trachomatis* was diagnosed in 52 (11.1%) of 467 symptomatic patients, compared to 76 (6.6%) of 1,148 patients without STD symptoms ($P = 0.003$); *N. gonorrhoeae* was diagnosed in 17 (3.6%) and 23 (2.0%) of symptomatic and asymptomatic patients, respectively ($P = 0.075$).

The calculation of the agreement of results of testing CS and LPT samples using the APTIMA COMBO 2 assay was performed for *C. trachomatis* detection and showed positive agreement (PA) of 78.2% (65.0 to 88.2%), negative agreement (NA) of 99.5% (98.3 to 99.9%), and overall agreement (OA) of 97.0% (95.0 to 98.4%) in symptomatic patients, compared to PA of 86.8% (77.1 to 93.5%), NA of 99.5% (98.9 to 99.8%), and OA of 98.6% (97.7 to 99.2%) in asymptomatic patients. The respective results for *N. gonorrhoeae* detection for the two specimen types with the APTIMA COMBO 2 assay were PA of 88.2% (63.6 to 98.5%), NA of 100% (99.2 to 100%), and OA of 99.6% (98.5 to 99.9%) in patients with symptoms, compared to PA of 95.7% (78.1 to 99.9%), NA of 100% (99.7 to 100%), and OA of 99.9% (99.5 to 100%) in those that were asymptomatic.

The prevalences, sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) for *C. trachomatis* and *N. gonorrhoeae* determined by testing the LPT samples in the assays are summarized in Table 4. The APTIMA COMBO 2 assay performed on the LPT samples detected 109 of the 128 CS sample-positive patients (85.2%). The APTIMA CT assay performed on the LPT sample detected 114 of the positives (89.1%). The APTIMA CT assay recorded 19 (1.1%) extra positives and 14 (0.8%) extra negatives. The APTIMA COMBO 2 test detected 8 (0.5%) extra positives and 19 (1.2%) extra negatives. The percent sensitivities of the APTIMA COMBO 2 and APTIMA CT assays for the detection of *C. trachomatis*-infected women by testing the

TABLE 3. Numbers of *C. trachomatis* and *N. gonorrhoeae* infections according to the presence or absence of symptoms from 1,615 patients whose CS samples were tested using the APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays[a]

Infection status	Result for CS sample tested using:			No. of patients				% Prevalence (CI) of:	
	APTIMA COMBO 2	APTIMA CT	APTIMA GC	Symptomatic for:		Asymptomatic for:		CT	GC
				CT	GC	CT	GC		
Infected[b]									
CT	+	+	−	52		76		7.9 (6.7–9.45)	
GC	+	−	+		17		23		2.5 (1.8–3.45)
Noninfected									
CT	−	−	−	406		1,058			
GC	−	−	−		449		1,125		
Noninfected									
CT	−	+	−	6		14			
GC	−		+		1		0		
Noninfected									
CT	+	−	−	3		0			
GC	+	−	−		0		0		
Total				467	467	1,148	1,148		

[a] CT, *C. trachomatis*; GC, *N. gonorrhoeae*.
[b] CS sample positive for *C. trachomatis* or *N. gonorrhoeae* in two tests.

liquid-based Pap samples ranged from 75% at UOT to 100% at three of the six study sites (data not shown). Table 4 also shows that the specificities of the APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays were very high (98.7 to 100%). The specificities did not vary significantly from site to site ($P = 0.003$). In testing LPT specimens, the percent sensitivities and specificities, respectively, by site for the detection of *C. trachomatis* were 100 and 100 at UIU, 100 and 100 at MNSD, 82.3 and 96.9 at SJH, 75.0 and 99.8 at UOT, 86.7 and 99.7 at UAB, and 100 and 100 at UNC using the APTIMA COMBO 2 test. The percent values with the APTIMA CT test were 100 and 99.7, 100 and 99.5, 87.9 and 93.8, 83.3 and 99.5, 90.0 and 98.6, and 100 and 100 at each respective site. Overall, the PPV and NPV were 93.2% and 98.7% using the APTIMA COMBO 2 test and 85.7% and 99.1% when the APTIMA CT assay was used to diagnose *C. trachomatis* infections. From site to site, the NPV for both tests showed a tight range between 96.9 and 100%, with three of the sites at 100% (data not shown). The three sites with the NPV at 100% also demonstrated their PPV at 100%. UAB and UOT demonstrated strong PPV and NPV, in the mid- to high 90% range. The SJH site recorded the lowest PPV, at 84.8% with the APTIMA COMBO 2 test and 76.3% for the APTIMA CT assay.

Calculations of the sensitivities, specificities, and predictive values for *N. gonorrhoeae* are also summarized in Table 4. By APTIMA COMBO 2 testing of the LPT sample, the overall prevalence of *N. gonorrhoeae* infections was 2.3%, ranging from 0% at UIU and UNC to 3.1% at SJH (data not shown), and 92.5% of the CS samples positive for *N. gonorrhoeae* were detected by both the APTIMA COMBO 2 and APTIMA GC assays. PPV and specificity were 100% overall and at each of the study sites. Sensitivity was 100% at three of the four sites where *N. gonorrhoeae* was found, with only three extra negatives by APTIMA COMBO 2 and APTIMA GC reported at UAB (sensitivity, 90%). The values reported for the APTIMA GC assay were equally impressive for sensitivity, specificity, PPV, and NPV, with consistent reporting from site to site.

DISCUSSION

To our knowledge, this is the first publication on the use of the APTIMA assays (APTIMA COMBO 2, APTIMA CT, and

TABLE 4. Performance of APTIMA COMBO 2, APTIMA CT, and APTIMA GC assays on 1,615 processed SurePath liquid-based Pap specimens

Organism	Assay	No. of positives (%)	No. of false positives	No. of negatives	No. of false negatives	% Sensitivity (CI)	% Specificity (CI)	PPV (%)	NPV (%)
C. trachomatis	APTIMA COMBO 2	109 (6.7)	8	1,479	19	85.2 (77.8–90.8)	99.5 (99.0–99.8)	93.2	98.7
	APTIMA CT	114 (7.1)	19	1,468	14	89.1 (82.3–93.9)	98.7 (981–99.3)	85.7	99.1
N. gonorrhoeae	APTIMA COMBO 2	37 (2.3)	0	1,575	3	92.5 (79.6–98.4)	100 (99.8–100)	100	99.8
	APTIMA GC	37 (2.3)	1	1,574	3	92.5 (79.6–98.4)	99.9 (99.6–100)	97.4	99.8

APTIMA GC) to detect women infected with *C. trachomatis* and/or *N. gonorrhoeae* by testing the residuum from a SurePath liquid-based Pap collection. Our study involved a substantial number (n = 1,615) of women from six different geographical regions in North America, with a range of races and ethnicities, seeking either an annual pelvic examination or screening for a sexually transmitted infection. Although the majority of women screened were asymptomatic, 41.1% (69/168) of the infected women had symptoms (Table 3). Performance of the APTIMA assays for *C. trachomatis* demonstrated very high specificities and NPV (Table 4). The PPV were 93.2% for the APTIMA COMBO 2 assay and 85.7% for the APTIMA CT assay. The NPV for *C. trachomatis* were 98.7% for the APTIMA COMBO 2 assay and 99.1% for the APTIMA CT assay. One of the study sites accounted for seven of the false positives, suggesting that the results may have been influenced by technical difficulties in processing the samples or collection of the LPT sample. Examination of these hypotheses is under way. These observations may also be a reflection of the exquisite analytical sensitivity of the APTIMA assays (3, 4), which allows for the detection in samples of small amounts of the target nucleic acid, near the assay cutoffs (12). This observation with *C. trachomatis* has not been observed for *N. gonorrhoeae* and may be a reflection of the APTIMA COMBO 2 and APTIMA GC assays having lower analytical sensitivities for the gonococcus than for *C. trachomatis* or of a consistently higher level of *N. gonorrhoeae* than *C. trachomatis* in clinical specimens.

Although the number of *N. gonorrhoeae* infections was lower, both the APTIMA COMBO 2 and APTIMA GC assays were highly sensitive and specific for it. The PPV and NPV ranged from 97.4 to 100% (Table 4). Previous studies to determine whether liquid-based Pap specimens might be suitable for the detection of *C. trachomatis* or *N. gonorrhoeae* have used direct fluorescent antibody staining, AMPLICOR PCR, or the ligase chain reaction (LCR) test on PreservCyt ThinPrep samples. A study by Inhorn et al. (8) compared the results of *C. trachomatis* direct fluorescent antibody staining of ThinPrep LPT samples to the results for CS smears from 636 women and reported PA in 43 (6.8%) and discrepant results in 11 (1.7%). Adjudication of the discrepant results by LCR and PACE 2 nucleic acid hybridization favored the ThinPrep sample (45%) and the CS smear (55%). The sensitivities at the three study sites varied from 62.5% to 100%, but the specificities were consistent across sites. This type of variation from site to site was also observed in our study and emphasizes the need to train both microbiology and cytology technologists in handling the dual-testing protocols. Hopwood et al. (7), testing CS and ThinPrep samples, showed 100% concordance of 19 LCR positives and 562 negatives, which were stable for 5 months. Koumans et al. (9) tested ThinPrep samples from 255 sexually active adolescent women, using LCR for *C. trachomatis* and *N. gonorrhoeae*, and also performed other forms of testing on urine and CS samples (LCR for *C. trachomatis* and *N. gonorrhoeae*, PCR for *C. trachomatis* and *N. gonorrhoeae*, transcription-mediated amplification for *C. trachomatis*, and culture), which broadened the reference standard for comparison of tests and samples to diagnose infected patients. They reported strong agreement (0.97) for *C. trachomatis* between LCR on ThinPrep and LCR on CS samples collected into the kit transport medium (kappa, 0.92, where kappa is a statistical measurement of interobserver agreement which compensates and corrects for the proportion of agreement that might be due to chance); for *N. gonorrhoeae* the agreement was 0.99 (kappa, 0.96). The sensitivity of LCR on the liquid-based Pap sample was higher for detection of *C. trachomatis* (93%) than for *N. gonorrhoeae* (81%). In contrast to the ThinPrep studies of Koumans, we found that with SurePath specimens, the *N. gonorrhoeae* assays were more sensitive (APTIMA COMBO 2, 92.5%, and APTIMA GC, 92.5%) than the *C. trachomatis* tests (APTIMA COMBO 2, 85.2%, and APTIMA CT, 89.1%). The lower values for *C. trachomatis* testing of the SurePath liquid-based Pap samples may have been related to low levels of *C. trachomatis* rRNA being present, which may lead to variable results using multiple tests (13), and to the volume of residuum available for testing. We did not record volumes routinely, but Hawthorne et al. (6) showed that the volume of liquid-based Pap material from a ThinPrep transport tube may impact the number of reactive samples reported. Using COBAS AMPLICOR PCR, they showed that 840 samples with a volume of >5 ml were positive for *C. trachomatis* in 33 (3.9%) and for *N. gonorrhoeae* in 8 (0.9%), compared to 80 samples with a volume of <5 ml which were negative for both *C. trachomatis* and *N. gonorrhoeae*. Some of these studies have led to Cytyc Corporation seeking FDA approval for AMPLICOR testing of ThinPrep samples, and Gen-Probe, Inc., recently received clearance from the FDA for APTIMA COMBO 2, APTIMA CT, and APTIMA GC testing of ThinPrep samples transferred to an LPT. In a multicenter trial using the APTIMA assays to test ThinPrep liquid-based Pap specimens (Fuller et al., unpublished data), sensitivities and specificities for *C. trachomatis* (96.7 and 99.3%) and *N. gonorrhoeae* (92.3 and 99.8%) which were not influenced by cervical symptoms in 1,626 women were found. The present study, using SurePath liquid-based Pap samples for 1,615 women from six centers in North America, showed some variations in sensitivities and specificities among study sites for *C. trachomatis* testing, but the high predictive values indicate that SurePath liquid-based Pap test residuum could be used to screen women for *C. trachomatis* and *N. gonorrhoeae* in the APTIMA assays.

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