Comparison of Methods for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae Using Commercially Available Nucleic Acid Amplification Tests and a Liquid Pap Smear Medium

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Annual screening for Chlamydia trachomatis infection is currently recommended for sexually active women 15 to 25 years old and for women older than 25 if they have a new or multiple sex partners and have not used condoms during the previous 3 months. Annual screening for cervical abnormalities using the Pap smear has achieved a substantial reduction in morbidity and mortality from cervical cancer. Screening for Neisseria gonorrhoeae infection has likely contributed significantly to the reduction in the rates of gonococcal infection. The introduction of liquid Pap smear methods using exfoliated cervical cells presents an opportunity to screen for these three conditions using one specimen. We evaluated the preservation of C. trachomatis and Neisseria gonorrhoeae DNAs from ThinPrep liquid media (PreservCyt; Cytyc Corp., Boxborough, Mass.); tested the feasibility of using a clinical specimen of this medium for the detection of cytologic abnormalities, C. trachomatis, and N. gonorrhoeae; evaluated the agreement between ligase chain reaction (LCR) performed on PreservCyt and LCR performed on a cervical specimen; and compared the performance of LCR performed on PreservCyt to those of LCR performed on a cervical specimen, culture, PCR performed on a cervical specimen, on urine, and on a vaginal specimen (a multiple-site infection status standard), and transcription-mediated amplification (for C. trachomatis only) from 255 sexually active adolescent women. The agreement between LCR performed on PreservCyt and LCR from a cervical swab in LCx transport medium was high (for C. trachomatis, agreement = 0.97 and kappa = 0.92; for N. gonorrhoeae, agreement = 0.99 and kappa = 0.96). Test performances were similar for LCR-urine, LCR-cervix, and LCR-ThinPrep, with sensitivities from 93 to 99% for C. trachomatis and 81 to 83% for N. gonorrhoeae and specificities from 95.5 to 99% for C. trachomatis and 99.1 to 99.6% for N. gonorrhoeae using a PCR-based multiple-site infection status standard. This is the first study to examine the agreement between liquid cytologic media and multiple nucleic acid amplification tests for the detection of C. trachomatis and N. gonorrhoeae from patient samples. Cytologic fluid shows promise for simultaneous screening for cytologic abnormalities and sexually transmitted infections.

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simultaneously. There would be no need for multiple specimen collection kits or different procedures for specimen processing. This single specimen would have the added advantage of being preserved and available for additional DNA detection tests (such as for HPV) if necessary.

The objectives of this study were (i) to determine the in vitro stability of chlamydial and gonococcal DNA in PreservCyt, (ii) to determine the feasibility of detecting C. trachomatis and N. gonorrhoeae from PreservCyt using a NAAT, (iii) to compare the agreement between ligase chain reaction (LCR) (LCx; Abbott Laboratories, Chicago, Ill.) performed on PreservCyt and LCR performed on a cervical swab, and (iv) to compare the test performances of LCR (on PreservCyt, cervical swab, and urine) using other NAAT and cervical culture reference standards and a patient reference standard based on PCR from multiple sites (any site positive).

During the time that this report was under internal review, licensure from the U.S. FDA was completed for the use of PreservCyt for the detection of C. trachomatis and N. gonorrhoeae using the COBAS AMPLICOR (Roche Diagnostic Systems, Branchburg, N.J.) C. trachomatis-N. gonorrhoeae test.

MATERIALS AND METHODS

In vitro stability of C. trachomatis and N. gonorrhoeae in PreservCyt. The stability of bacterial DNA for subsequent detection in PreservCyt medium was assessed by inoculating known numbers of bacteria into sterile media and incubating samples at a variety of temperatures and times. A suspension of C. trachomatis (D/13028/Cx) was prepared in phosphate-buffered saline and used to inoculate PreservCyt to yield 1,000, 100, or 10 inclusion-forming units per ml of PreservCyt. N. gonorrhoeae (ATCC 27629), suspended in phosphate-buffered saline, was inoculated into PreservCyt to achieve a final concentration of 1,000, 100, or 10 CFU per ml. For each inoculation, 200 ml of bacterial suspension was added to 20 ml of PreservCyt. Three samples of each dilution of the organisms were prepared in order to test the effects of storage temperature and time on DNA stability. One sample was stored at 4°C and tested after 24, 48, and 1 week of storage. The second and third samples were tested at similar time intervals, but the second sample was stored at 22°C and the third at 37°C (a total of 27 samples for each organism). All samples were tested with LCR as described later for clinical PreservCyt specimens. For comparison, Dacron swabs were allowed to absorb to saturation liquid from the various concentrations of bacterial suspensions prepared for the direct ThinPrep inoculation. These swabs were then immersed in PreservCyt to simulate clinical specimens. The 27 swabs in PreservCyt were stored under the same conditions as for direct inoculations and tested by LCx as described below.

Clinical study enrollment. Enrollment in this study took place within a larger longitudinal sexually transmitted disease study with multiple diagnostic and epidemiologic objectives among adolescents attending an adolescent clinic at a public pediatric hospital in Atlanta, Ga. Nonpregnant, human immunodeficiency virus-negative, sexually active adolescent females 12 to 19 years old were recruited to participate at a clinic visit if a pelvic exam was indicated. Young women who had received antibiotics in the previous month were excluded. After informing the adolescent and a parent or guardian (if the adolescent was <18 years old) about the study and obtaining the assent and consent of the adolescent and a parent or guardian, respectively, or the consent of adolescents aged 18 or 19 years, a structured face-to-face interview was performed by trained interviewers. The study visit concluded with a physical examination and specimen collection.

The study was reviewed and approved by the Institutional Review Boards at the Centers for Disease Control and Prevention and Emory University.

Specimen collection and handling. During the pelvic examination, the first specimen collected was an endocervical swab specimen for culture of N. gonorrhoeae. Next, a broom (Papette; Wallach Surgical Devices, Inc., Milford, Conn.) supplied with the ThinPrep kit (for the Pap smear) and study swabs were used to collect cervical samples in random order. Four swabs were obtained from the cervix for C. trachomatis culture or a different swab for C. trachomatis by amplification (TMA) (Amp CT; Gen-Probe, Inc., San Diego, Calif.), for LCR (LCx; Abbott Laboratories, Chicago, Ill.), and for PCR (COBAS AMPLICOR; Roche). The last two samples were also used for detection of N. gonorrhoeae. A vaginal swab collected by a clinician was tested for C. trachomatis and N. gonorrhoeae by PCR. The non-culture study swabs were supplied, collected, and transported in accordance with the manufacturer's instructions. Urine specimens were collected and tested for C. trachomatis and N. gonorrhoeae using LCR and PCR according to the manufacturer's instructions.

The PreservCyt specimens were sent to the hospital cytology laboratory. After the cytology slide had been prepared and read satisfactorily, the remaining liquid was transferred to the Centers for Disease Control and Prevention, 1.0 ml was tested for HPV (T. A. Tarkowski, unpublished data), and 1.0 ml was tested for C. trachomatis and N. gonorrhoeae using LCR.

Detection of C. trachomatis and N. gonorrhoeae. Endocervical swabs for C. trachomatis isolation were inoculated into M-4 transport medium (Micro Test Inc., Snellville, Ga.), held at 4°C, and transported to the laboratory within 12 h. The specimens were frozen at ~70°C until they were cultured. BGMK cells, grown on coverslips in 1-dram shell vials, were inoculated with 200 μl of inoculum and centrifuged at 1,000 × g (Sorvall T1000D table top centrifuge; Kendro Laboratory Products, Newtown, Conn.) for 2 h. The inoculated cells were cultured in Eagle’s minimal essential medium supplemented with 10% fetal calf serum and 1 μg of cycloheximide/ml. C. trachomatis inclusions were detected following 48 h of incubation at 37°C under 5% CO2 by staining methanol-fixed cells with a fluorescein-labeled anti-major outer membrane protein monoclonal antibody (Wampole Laboratories, Cranbury, N.J.).

Endocervical swabs for N. gonorrhoeae isolation were placed in a transport vial and transported within 15 min to the hospital laboratory. There, the swabs were planted on Martin-Lewis agar plates and placed in a 5% carbon dioxide incubator at 35°C. An oxidase test and Gram staining were performed on suspected N. gonorrhoeae colonies after 24 and 48 h. If the oxidase test was positive and the Gram staining identified gram-negative diplococci, further testing using either the NIH card or the rapid NH strip (bio-Merieux Vitek, Hazelwood, Mo.) was used to confirm the presence of N. gonorrhoeae.

The cervical swab and urine specimens collected for each of the nonculture tests were processed according to the directions provided by the manufacturer in the package inserts. Vaginal swabs were processed in the same manner as cervical swabs. PreservCyt specimens were processed for C. trachomatis and N. gonorrhoeae by aspirating 1.0 ml of medium into a 1.5-ml tube and centrifuging the tube at 14,000 × g (Eppendorf centrifuge model 5415C) for 15 min. The supernatant was discarded, the pellet was resuspended in 1.0 ml of LCx urine resuspending buffer (the assay mixture), and the tubes were placed in a heating block at 95 to 100°C for 15 min for amplification. The samples were then tested by LCR according to the manufacturer's protocol. The interpretation of positive and negative results was in accordance with the manufacturer’s guidelines. Lab- roratorians were unaware of any clinical information or the results of other tests performed on each patient.

Sample size and statistics. We calculated sample sizes for a one-sided confidence interval for a kappa statistic assuming that the agreement between LCRs on PreservCyt and on a cervical specimen would be 0.88. We calculated agreement and the kappa statistic because our objective was to ascertain agreement between two specimen collection methods using the same assay. The kappa statistic takes into consideration chance agreement.

Since the prevalence of N. gonorrhoeae was expected to be lower than that of C. trachomatis, we used the more conservative sample size for N. gonorrhoeae as the sample size for both comparisons. Given a conservative estimate of 0.10 for the prevalence of N. gonorrhoeae, 275 patient samples would be needed to achieve a 95% confidence interval with a lower limit of 0.80, assuming a kappa of 0.88. During the study period, the actual prevalence of N. gonorrhoeae was 11%, and sample collection was discontinued after analyses were complete for 255 adolescent women.

To compare the test performances for LCR on cervical specimens in PreservCyt and on LCx medium and for LCR on urine, we used four different reference standards for C. trachomatis (based on culture, TMA, and PCR) and three reference standards for N. gonorrhoeae (based on culture and PCR). The culture, TMA, and PCR reference standards were based on cervical samples, and the fourth (for C. trachomatis) and third (for N. gonorrhoeae) PCR patient reference standards were based on cervical, vaginal, and urine samples. Because samples were taken from all three genital sites (urine, vagina, and cervix), the PCR patient reference standard may better approximate the infection status of the patient than a single type of specimen. If any sample from a given person was found to be positive, the test was considered positive. We used the software package StatXact version 4.0.1 (Cytel, Cambridge, Mass.) to calculate 95% confidence intervals of sensitivity and specificity.


RESULTS

In vitro detection of C. trachomatis and N. gonorrhoeae in PreservCyt. In the stability experiments, C. trachomatis and N. gonorrhoeae were detected by LCR in all 27 spiked samples and all swabs kept at each temperature (4, 22, and 37°C) and tested at each time point, including those inoculated with the fewest organisms and maintained for 1 week at 37°C. No observable differences were detected between the directly inoculated ThinPrep samples (n = 27) and those that were inoculated with a spiked swab (n = 27).

Study population, cytology, and prevalence of C. trachomatis and N. gonorrhoeae. Of the 291 participants eligible for analysis, 36 had PreservCyt specimens with insufficient liquid, after cytologic and HPV testing were completed, for C. trachomatis or N. gonorrhoeae testing, leaving 255 participants for analysis. The mean age was 16.6 years; 64% had normal cytology, 22% had atypical cells of undetermined significance, 14% had low-grade squamous intraepithelial lesions, and 0.5% had high-grade squamous intraepithelial lesions. HPV results will be reported separately (the data are not included). Seventy (27%) adolescents had chlamydial infections, and 27 (11%) had gonococcal infections by cervical PCR.

Agreement of C. trachomatis and N. gonorrhoeae detection from cervical samples using LCR-cervix and LCR-ThinPrep. Two-hundred and fifty-five women were tested for C. trachomatis and N. gonorrhoeae by LCR on cervical specimens in PreservCyt (LCR-ThinPrep), LCR transport medium (LCR-cervix), and urine (LCR-urine) and by PCR on cervical (PCR-cervix), vaginal (PCR-vagina), and urine (PCR-urine) specimens. N. gonorrhoeae culture was also performed on the 255 women. Because of missing reagents or kits, only 212 of the 255 were tested by TMA on a cervical specimen (TMA-cervix) and C. trachomatis culture.

C. trachomatis DNA was detected by LCR in both LCR transport media and PreservCyt from cervical samples from 71 patients. C. trachomatis was not detected by either LCR-cervix or LCR-PreservCyt from 176 patient samples. Five patients had C. trachomatis detected by LCR-cervix but not by LCR-PreservCyt, and three had C. trachomatis detected by LCR-PreservCyt but not by LCR-cervix. The agreement between LCR-cervix and LCR-PreservCyt was 0.97, and the kappa was 0.92 (Table 1). Of five patients positive by LCR-cervix but negative by LCR-PreservCyt, two were positive by PCR-urine, PCR-vagina, PCR-cervix, TMA-cervix, culture-cervix, and LCR-urine; one was positive by all six other tests except urine LCR, one was positive by all six other tests except PCR-cervix, PCR vagina, and culture, and one was positive only by TMA-cervix. Of the three samples negative by LCR-cervix and positive by LCR-PreservCyt, all were negative by the six other tests.

N. gonorrhoeae DNA was detected by LCR in both LCx transport media and PreservCyt from cervical samples from 25 patients. N. gonorrhoeae was not detected by either LCR-cervix or LCR-PreservCyt from 228 samples. The agreement between LCR-cervix and LCR-PreservCyt was 0.99, and the kappa was 0.96 (Table 1). The one patient positive by LCR-cervix but negative by LCR-PreservCyt was positive by all other tests except PCR-urine, and the one patient negative by LCR-cervix but positive by LCR-PreservCyt was positive only by PCR-vagina.

Test performance for C. trachomatis and N. gonorrhoeae detection from PreservCyt using culture, TMA, and PCR reference standards. The sensitivities, specificities, and 95% confidence intervals of LCR-PreservCyt, LCR-cervix, and LCR urine compared to the culture, TMA, and PCR standards for the detection of C. trachomatis are shown in Table 2, and those for the detection of N. gonorrhoeae are shown in Table 3. Because of the small sample size for these comparisons, the confidence intervals, particularly for N. gonorrhoeae, are wide. There were no statistically significant differences in sensitivity

<table>
<thead>
<tr>
<th>Organism detected</th>
<th>Results (LCR-cervix/LCR PreservCyt)</th>
<th>Agreement</th>
<th>Kappa</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. trachomatis</td>
<td>+/+</td>
<td>71</td>
<td>0.97</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>+/–</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>–/+</td>
<td>176</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>–/–</td>
<td>179</td>
<td></td>
<td></td>
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</table>

TABLE 1. Agreement of LCR-PreservCyt with LCR-cervix for detection of C. trachomatis and N. gonorrhoeae

<table>
<thead>
<tr>
<th>Test</th>
<th>Culture* (n = 212)</th>
<th>TMA* (n = 217)</th>
<th>PCR* (n = 255)</th>
<th>PCR+ (n = 255)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCR-PreservCyt</td>
<td>90 (79–97)</td>
<td>89.4 (84–94)</td>
<td>92 (82–97)</td>
<td>96 (88–99)</td>
</tr>
<tr>
<td>LCR-cervix</td>
<td>96 (86–99.5)</td>
<td>90 (84–94)</td>
<td>100 (94–100)</td>
<td>100 (95–100)</td>
</tr>
<tr>
<td>LCR-urine</td>
<td>92 (80–98)</td>
<td>91 (85–95)</td>
<td>93 (83–98.0)</td>
<td>97 (90–99.6)</td>
</tr>
</tbody>
</table>

*Performed on PreservCyt (LCR-PreservCyt), LCx transport media (LCR-cervix), and urine (LCR-urine) using culture (one specimen), TMA (one specimen), and PCR (one and three specimens) reference standards for detection of C. trachomatis.
or specificity by LCR using any specimen source. Depending on the reference standard used, the ranges of sensitivities and specificities for LCR-PreservCyt, LCR-cervix, and LCR-urine varied by 2 to 4%. There were larger differences between reference standards than between specimen types.

We also combined test results to create a multitest reference standard based on any one of the following tests being positive: culture, TMA-cervix (for C. trachomatis), PCR-urine, PCR-vagina, or PCR-cervix. Using this multitest standard, the sensitivities and specificities of LCR-PreservCyt, LCR-cervix, and LCR-urine for C. trachomatis were 91, 97, and 93% and 97, 99.4, and 98.3%, respectively, and for N. gonorrhoeae they were 74, 74, and 76% and 100, 100, and 99.5%, respectively (data not shown).

### DISCUSSION

Millions of women are screened for cytologic abnormalities by the ThinPrep method yearly (B. Lentrichia, personal communication). The liquid-based ThinPrep method for collecting, processing, and preparing the Pap smear provides accurate cytologic screening, and the remaining PreservCyt can be used for the detection of other sexually transmitted disease pathogens. The pathogens tested for in this analysis, C. trachomatis and N. gonorrhoeae, are the causes of two of the most commonly reported infections in the United States.

We have shown that PreservCyt is a very stable medium for the detection of these pathogens; that it is an acceptable specimen type; that its use can be combined with HPV testing; and that, when combined with LCR testing, it has performance characteristics equivalent to those of LCR, PCR, and TMA (for C. trachomatis). PreservCyt could be used as an alternative medium for the collection and transport of specimens for the detection of C. trachomatis and N. gonorrhoeae. The medium can be stored at room temperature for at least 7 days, which is an added advantage. Further research should evaluate the duration of storage, although for patient management, rapid diagnosis is of the utmost importance. Currently available testing methods can be used to detect C. trachomatis and N. gonorrhoeae using PreservCyt (1). Further comparisons of PreservCyt with other NAAT technologies that have also been approved for urine specimens would be useful.

Agreement between LCR testing of a cervical specimen in LCx transport medium and testing of a specimen in PreservCyt for detection of C. trachomatis and N. gonorrhoeae was very high. LCR on PreservCyt resulted in high sensitivity and specificity, which appeared to be similar to that for LCR-urine or LCR-cervix. The rate of false positives and false negatives may reflect the one added chance for laboratory contamination (during cytologic preparation), although the sensitivity and specificity we found are similar to those in other published studies of the performance of LCR (2, 3). The small sample size in this study is inadequate to determine whether the sensitivity and specificity of LCR on a PreservCyt sample differs from those of LCR on other samples.

The strengths of this evaluation include the fact that swabs used for testing were collected in random order. When multiple swabs are collected, the order of swab collection may play a role in the performance of the test in detecting the target. An additional strength of this analysis was the use of multiple specimen site reference standards using alternate NAATs to determine sensitivity and specificity of the test under evaluation. Much controversy has surrounded the evaluation of test performance and the choice of a "gold standard" (7, 6). Recent work has suggested that the inclusion of multiple tests with different targets to evaluate a new test may provide the most accurate information about test performance (3). The use of these standards could lead to lower estimates of sensitivity but higher estimates of specificity for the test under evaluation, because all possible infections are classified as true infections (for example, compare sensitivity and specificity results for culture and the PCR standard). Our findings did not consistently reflect this potential bias because of the small sample size.

Because the sample size for this study was based on the kappa statistic, there were wide estimates of sensitivity and specificity. However, the point estimates for the sensitivity and specificity of LCR-urine and LCR-cervix are well within other published estimates of performance for these tests (2, 3). The use of the ThinPrep broom alone for specimen collection may have reduced the cellularity of the PreservCyt sample. Most specimen collection for use with PreservCyt uses both a spatula and a cytobrush (11). We used the broom alone for the collection of cervical material for cytology because most of the women were nulliparous and the broom appeared to capture both endocervical and exocervical cells well (data not shown). However, the use of the broom alone could have reduced the likelihood of detecting C. trachomatis and N. gonorrhoeae. A potential disadvantage of an approach that includes testing for C. trachomatis and N. gonorrhoeae as well as cytologic screening and HPV testing is that more than one laboratory may need to share or divide a single specimen. Unresolved questions include which test should be the priority after cytology is

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**TABLE 3. Test performance of LCR for N. gonorrhoeae**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (Culture) (%)</th>
<th>Specificity (Culture) (%)</th>
<th>Sensitivity (PCR) (%)</th>
<th>Specificity (PCR) (%)</th>
<th>Sensitivity (PCR) (%)</th>
<th>Specificity (PCR) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCR-PreservCyt</td>
<td>86 (64–97)</td>
<td>97 (95–99)</td>
<td>89 (71–97)</td>
<td>99 (97–99)</td>
<td>81 (63–93)</td>
<td>99.9 (98–99)</td>
</tr>
<tr>
<td>LCR-cervix</td>
<td>86 (64–97)</td>
<td>97 (94–99)</td>
<td>89 (71–97)</td>
<td>99 (97–99)</td>
<td>81 (63–93)</td>
<td>99.9 (98–99)</td>
</tr>
<tr>
<td>LCR-urine</td>
<td>85 (62–97)</td>
<td>97 (94–99)</td>
<td>88 (70–97)</td>
<td>99 (96–99)</td>
<td>83 (64–94)</td>
<td>99.1 (97–99)</td>
</tr>
</tbody>
</table>

*Performed on PreservCyt (LCR-PreservCyt), LCx transport media (LCR-cervix), and urine (LCR-urine) using culture (one specimen) and PCR (three specimens) reference standards for detection of N. gonorrhoeae.

*Cervix.

*Cervix, vagina, and urine.

*Values in parentheses are 95% confidence intervals.
completed, whether reimbursement for a test will be provided if only one specimen is collected, and whether sequential testing will increase the chances of contamination.

This study provides additional evidence that a single cervical specimen can be used for cervical cytology and screening for C. trachomatis and N. gonorrhoeae using nucleic acid amplification (1), even among these adolescents, who theoretically may have had fewer cellular PreservCyt samples. The advantages of a single specimen include the need for only one collection device, the preservation of cellular material and nucleic acid in one liquid medium, and the ability to detect not only C. trachomatis and N. gonorrhoeae but other vaginal-cervical infections as well (5, 9). If these results are confirmed, providers and women could benefit from the simplicity and versatility of using a single specimen for many recommended routine screening activities.

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