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PreservCyt Transport Medium Used for the ThinPrep Pap Test Is a Suitable Medium for Detection of *Chlamydia trachomatis* by the COBAS Amplicor CT/NG Test: Results of a Preliminary Study and Future Implications

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The commercial COBAS Amplicor CT/NG test (Roche Diagnostic Systems, Meylan, France) is a sensitive and specific method for detection of *Chlamydia trachomatis* infections. This test currently consists of using a nucleic acid amplification method to detect *C. trachomatis* in first-void urine specimens and in endocervical swabs collected in 2-sucrose-phosphate (2SP) transport medium. We conducted a prospective study to determine whether the automated COBAS Amplicor CT/NG test can detect *C. trachomatis* in cervical specimens collected in PreservCyt transport medium (ThinPrep Pap Test; Cytyc Corporation, Boxborough, Mass.). PreservCyt medium is used to preserve cervical samples before the preparation of ThinPrep slides. We collected 1,000 cervical specimens from young women (age range, 15 to 25 years) during routine Pap smear tests. Only specimens with normal cytology and in which the gynecologist found no clinical evidence of urogenital infections were selected. The samples were stored in PreservCyt transport medium at 15 to 20°C. *C. trachomatis* was detected in 22 of the 1,000 cervical specimens that had been stored in PreservCyt. To confirm the positive samples, the test was repeated on new endocervical swab specimens collected in 2SP transport medium. Only 9 of the 22 positive patients agreed to undergo this control, but all 9 retested positive. To evaluate the influence of storage conditions on the sensitivity of the *C. trachomatis* PCR test, all of the positive samples were stored at 15 to 20°C in PreservCyt transport medium and were retested every 2 weeks for 6 weeks. *C. trachomatis* was successfully amplified from all 22 specimens for the whole 6-week period. The prevalence of *C. trachomatis* infection was 2.2% in our study population. These results demonstrate that PreservCyt transport medium is a suitable transport medium for detection of *C. trachomatis* by the COBAS Amplicor CT/NG test. The ThinPrep Pap Test may enable gynecologists to monitor for both cervical lesions and *C. trachomatis* infections with a single endocervical specimen.

Chlamydia trachomatis infection is one of the most common sexually transmitted diseases and represents a major public health problem (48). In women, untreated *C. trachomatis* infections cause cervicitis and pelvic inflammatory disease, which may lead to infertility and ectopic pregnancy, and expose newborn infants to *C. trachomatis* (37). In the early 1990s, molecular diagnostic methods based on nucleic acid amplification tests such as PCR, ligase chain reaction, and transcription-mediated amplification were developed. These techniques have gradually replaced traditional techniques for detection of *C. trachomatis* (5, 32, 49). Commercial DNA amplification tests are now routinely used for diagnosis of *C. trachomatis* infections. Although these commercial tests are based on different nucleic acid amplification technologies, many studies have shown that nucleic acid amplification-based tests are more sensitive than culture-based tests (17, 28, 33, 35, 39, 40) and that they offer the opportunity to use noninvasive clinical samples (2, 3, 8, 12, 30, 31, 36). Therefore, nucleic acid amplification tests are considered to be a more suitable method for screening and diagnosing chlamydial infections (6, 14, 20, 30, 35, 44, 45, 46). A large proportion of infected individuals are asymptomatic (50% of infected men and 70 to 80% of infected women), and these individuals serve as a reservoir for the transmission of *C. trachomatis* (5). Thus, it is important that *C. trachomatis* be diagnosed at an early stage and that infected persons be treated to prevent the spread of this disease. Various *C. trachomatis* screening programs have shown that the prevalence of *Chlamydia* infections has decreased in recent years (1, 15, 41).

High-risk populations, such as sexually active young women, should be screened for *C. trachomatis* and monitored for precancerous lesions of the cervix; however, the major problem is the technical feasibility of this kind of screening program. The ideal situation would be use of a single specimen for both cytological monitoring and detection of a number of pathogens so that the patient does not have to be examined repeatedly. Papanicolaou (Pap) smear tests are recommended for adolescent and young women for detection of abnormal cervical cytology (34). In 1996, the U.S. Food and Drug Administration approved a liquid method for collecting and preparing cervical

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samples for cytological screening (ThinPrep Pap Test; Cytyc Corporation, Boxborough, Mass.). The ThinPrep Pap Test preserves endocervical cells in a liquid alcohol (PreservCyt transport medium; Cytyc Corporation) to ensure optimal morphological preservation. These samples are subsequently used to prepare Pap slides, which are stained for microscopic examination. The ThinPrep Pap Test is significantly more effective than the conventional Pap smear test for the detection of precancerous lesions (16, 18, 22, 24, 26, 27, 29). We carried out a prospective study to evaluate the ability of the automated PCR COBAS Amplicor CT/NG test (9, 21) to detect *C. trachomatis* in the PreservCyt transport medium used for the ThinPrep Pap Test.

MATERIALS AND METHODS

Preliminary assays. Firstly, to assess the presence of endogenous amplification inhibitors and to determine the technical feasibility of using PreservCyt as a storage medium, 13 endocervical PreservCyt samples were selected. Seven of these specimens contained blood and were thus treated with 0.5 to 1 ml of an acetic acid solution to lyse red blood cells. These samples were processed as described below ("Pretreatment of PreservCyt specimens"). The COBAS Amplicor CT/NG test was performed in duplicate on each lysate. Secondly, we compared the in vitro sensitivities of the PreservCyt transport medium and the classical 2SP transport medium (0.2 M sucrose–0.02 M phosphate; QUELAB Laboratories Inc., Montréal, Canada) for detection of *C. trachomatis*. Four separate batches of the *C. trachomatis* serotype D reference strain (ATCC VR 885; stored at −80°C) were serially diluted with 2SP and with PreservCyt transport medium such that there were 10^3 to 10^{-2} inclusion-forming units (IFU)/ml. This reference strain stock was previously used to compare the sensitivities of all *C. trachomatis* diagnostic tests commercially available in France (4). Negative controls, consisting of the 2SP medium or the PreservCyt transport medium alone, were included. Each dilution was tested in duplicate by the COBAS Amplicor CT/NG test.

Patient population. Specimens were obtained between May 2000 and November 2000 from consenting patients registered with different general practitioners, dermatologists, gynecologists, and family planning centers. We collected 1,000 PreservCyt specimens from young women (age range, 15 to 25 years) during Pap screening (ThinPrep Pap Test). Samples were selected according to two criteria: (i) absence of clinical signs of urogenital infection, including genital or pelvic pain, dysuria, and urethritis, and (ii) absence of atypical cells such as inflammatory cells and cervical cancer cells or precursor lesions (ASCUS [atypical squamous cells of undetermined significance], low-grade squamous intraepithelial lesions, or high-grade squamous intraepithelial lesions).

Preparation of ThinPrep slides from PreservCyt specimens. PreservCyt specimens were collected by inserting a cytobrush into the endocervical canal. The cytobrush was immediately placed in a vial of PreservCyt transport medium. These specimens were stored at 15 to 20°C and transported to the laboratory within 24 h of collection. In the cytology laboratory (Laboratoire Claude Lévy, Paris, France), a ThinPrep 2000 processor (Cytyc Corporation) was used to prepare slides (one slide per patient). The ThinPrep 2000 processor automatically prepares cytological slides from cells suspended in PreservCyt transport medium. It disperses the samples and transfers cells onto a microscopic glass slide in a 20-mm-diameter circle. The slide is then automatically deposited into a 95% ethanol bath before being stained with Papanicolaou stain. ThinPrep slides were observed by two independent cytopathologists.

Cytological reports were formulated according to the Bethesda System, including evaluation of correct sampling of the transformation zone (presence of metaplastic and/or columnar cells). PreservCyt specimens were stored at 15 to 20°C for as long as 6 weeks, in case the sample had to be retested.

Pretreatment of PreservCyt specimens. PreservCyt specimens were tested by PCR within 1 week of collection. The PreservCyt transport medium containing residual endocervical cells was vortexed vigorously. For each specimen, two 1-ml samples were transferred into two 2-ml polypropylene tubes, each containing 1 ml of PreservCyt transport medium. The tubes were centrifuged at 12,500 × *g* for 15 min at 20°C. Supernatants were discarded, each cellular pellet was resuspended in 500 μl of 2SP transport medium, and cellular suspensions were pooled in a single tube (final volume, 1 ml). One hundred microliters of this suspension was added to 100 μl of CT/NG Lysis Buffer. After a 10-min incubation at room temperature, 200 μl of CT/NG Specimen Diluent was added to the lysate. The contents were mixed well by vortexing, and tubes were incubated at 90°C for 10 min. Lysates were centrifuged at 12,500 × *g* for 10 min. A COBAS Amplicor CT/NG test was performed with 50 μl of lysate, according to the manufacturer's instructions.

Control endocervical swab specimens in 2SP transport medium. To confirm the positive results obtained with the PreservCyt transport medium, patients with positive results (*C. trachomatis* A_{660}, >0.8) were asked by their gynecologists to give a new endocervical swab (alginate swabs) specimen collected by standard procedures into 2SP medium. The swabs were stored at 2 to 8°C and tested within 3 days of collection. One hundred microliters of CT/NG Lysis Buffer was mixed with 100 μl of the sample in 2SP transport medium and incubated at room temperature for 10 min. Two hundred microliters of CT/NG Specimen Diluent was added to each tube, and tubes were incubated at room temperature for 10 min. The COBAS Amplicor CT/NG test was performed with 50 μl of lysate, according to the manufacturer's instructions.

Amplification and detection of *C. trachomatis* by the COBAS Amplicor CT/NG PCR test. We transferred 50 μl of lysate to A-tubes containing 50 μl of Working Master Mix. The A-tubes were placed in a COBAS Amplicor thermocycler. An internal control (IC) was included with each amplification reaction to monitor inhibition and was coamplified with the *C. trachomatis* target DNA. AmpErase was incorporated to prevent carryover contamination by uracil-*N*-glycosylase. The COBAS Amplicor system automatically denatures the amplified DNA target, hybridizes the amplicons with the target-specific probes bound to magnetic microparticles, and detects the amplicon/probe complexes by a colorimetric reaction. The *C. trachomatis* target and the IC were detected in separate reactions with specific oligonucleotide capture probes.

Interpretation of results. Specimens yielding *C. trachomatis* signals above the positive cutoff (*C. trachomatis* A_{660}, >0.8) were interpreted as positive. Specimens in which the *C. trachomatis* signal was below the negative cutoff were interpreted as negative if the IC signal was above the assigned cutoff (*C. trachomatis* A_{660}, <0.2; IC A_{660}, >0.2). Specimens for which the *C. trachomatis* results were between the negative and positive cutoffs ($0.2 < C.\ trachomatis\ A_{660} < 0.8$) were considered to be in a gray zone if the IC A_{660} was >0.2. Specimens yielding signals below the cutoff values ($A_{660} < 0.2$) for both *C. trachomatis* and the IC were considered to contain inhibitory substances. For specimens containing inhibitory substances or in the gray zone, another aliquot of the original specimen was retested in duplicate. These specimens were considered to be positive if at least one of the duplicates yielded a *C. trachomatis* A_{660} of >0.8. They were interpreted as negative if the two repeat tests yielded a *C. trachomatis* A_{660} of <0.2 and the IC signals were above the assigned cutoff (IC A_{660}, >0.2). If the two repeat tests yielded a *C. trachomatis* A_{660} of <0.2 and the IC signal was below the assigned cutoff (IC A_{660}, <0.2), the specimens were considered to contain inhibitory substances and were excluded.

Evaluation of storage conditions in PreservCyt vials. PreservCyt specimens in which the *C. trachomatis* signal was above the positive cutoff (*C. trachomatis* A_{660} of >0.8) were stored at 15 to 20°C for 6 weeks. For each positive PreservCyt specimen, the PCR pretreatment was repeated as described above and the lysates were retested by the COBAS Amplicor CT/NG test 2, 4, and 6 weeks after collection (days 15, 30, and 45).

RESULTS

Preliminary assays: preparation of PreservCyt specimens and in vitro sensitivity assay. Some preliminary tests were necessary to evaluate the technical feasibility of conducting a prospective study to detect *C. trachomatis* by the ThinPrep Pap test. One major problem with nucleic acid amplification tests is the presence of potential inhibitors in the biological samples, which can lead to false-negative results. Firstly, PreservCyt transport medium is an alcohol-based solution that may inhibit PCR. Secondly, for an optimal cytological examination, any bloody PreservCyt specimens must be pretreated with 0.5 to 1 ml of a pure acetic acid solution to lyse the red blood cells (hemoglobin is known to be a potential inhibitor of PCR). We evaluated the efficiency of the pretreatment procedure at removing potential PCR inhibitors from PreservCyt specimens. The IC was detected in all 13 PreservCyt specimens tested by the COBAS Amplicor CT/NG test (IC A_{660}, >0.8). These encouraging results led us to compare the detection sensitivity

TABLE 1. Comparison of the sensitivities of 2SP and PreservCyt (ThinPrep Pap test) for detection of *C. trachomatis* by the COBAS Amplicor CT/NG test

Batch and medium	A_{660} of the following dilution (IFU/ml)[a]:											
	10^3		10^2		10		1		0.1		0.01	
	C. trachomatis	IC	*C. trachomatis*	IC	*C. trachomatis*	IC	*C. trachomatis*	IC	*C. trachomatis*	IC	*C. trachomatis*	IC
Batch 1												
2SP	**3.24**	**3.84**	**3.54**	**3.99**	**3.99**	**3.99**	**3.99**	**3.99**	**2.03**	**3.99**	0.003	3.99
	3.21	**3.99**	**3.62**	**3.99**	**3.85**	**3.99**	**3.99**	**3.99**	0.007	3.99	0.003	3.99
PreserCyt	**3.17**	**3.84**	**3.72**	**3.99**	**3.99**	**3.99**	**3.42**	**3.99**	0.001	3.99	0.001	3.99
	3.28	**3.48**	**3.84**	**3.84**	**3.99**	**1.00**	**3.99**	0.002	3.99	0.002	3.99	
Batch 2												
2SP	**3.30**	**3.99**	**3.78**	**3.99**	**3.99**	**3.99**	0.002	3.99	0.002	3.99	0.002	3.76
	3.23	**3.99**	**3.60**	**3.77**	**3.99**	**3.77**	**3.12**	**3.77**	0.002	3.77	0.002	3.77
PreserCyt	**3.42**	**3.99**	**3.99**	**3.99**	**3.42**	**3.99**	0.002	3.99	0.002	3.99	0.003	3.99
	3.62	**3.99**	**3.84**	**3.99**	**3.99**	**3.99**	0.003	3.99	0.002	3.99	0.002	3.93
Batch 3												
2SP	**3.19**	**3.99**	**3.63**	**3.99**	**3.63**	**3.93**	**3.93**	**3.93**	**1.99**	**3.76**	0.002	3.76
	3.19	**3.93**	**3.63**	**3.63**	**3.93**	**3.76**	**2.36**	**3.93**	0.001	3.76	0.001	3.76
PreserCyt	**3.62**	**3.99**	**3.99**	**3.99**	**3.80**	**3.99**	0.002	3.99	0.001	3.99	0.001	3.99
	3.62	**3.99**	**3.80**	**3.99**	**2.19**	**3.79**	0.002	3.77	0.001	3.77	0.003	3.77
Batch 4												
2SP	**3.33**	**3.93**	**3.63**	**3.63**	**3.75**	**3.93**	**2.25**	**3.76**	**3.45**	**3.76**	0.002	3.76
	3.33	**3.93**	**3.39**	**3.93**	**3.75**	**3.76**	**3.99**	**3.63**	0.002	3.76	0.002	3.76
PreserCyt	**3.62**	**3.99**	**3.79**	**3.79**	**3.99**	**3.99**	0.003	3.99	0.002	3.79	0.002	3.79
	3.49	**3.99**	**3.99**	**3.79**	**3.79**	**3.79**	0.003	3.99	0.002	3.79	0.003	3.79

[a] Results for duplicate assays are shown. Positive results are boldfaced.

of the PreservCyt transport medium with that of the 2SP transport medium in a dilution assay using a titrated solution of purified infectious elementary bodies of *C. trachomatis* (serovar D, VR 885).

The results obtained were highly reproducible for duplicate assays performed with each of the four batches (same assay run) for the 10^3- to 10-IFU/ml dilutions in both types of transport medium. At these dilutions, all assays performed with 2SP and with PreservCyt gave positive signals for *C. trachomatis* (Table 1). At the 1-IFU/ml dilution, one batch gave positive signals for *C. trachomatis* with both types of transport medium. The other three batches all gave negative signals for *C. trachomatis* with the PreservCyt medium, and all but one gave positive signals with the 2SP medium (Table 1).

None of the assays performed at the 0.01-IFU/ml dilution gave positive signals for *C. trachomatis*. The IC was detected in all samples, and all of the negative controls gave negative results. These conditions allowed us to determine the threshold of sensitivity (the highest dilution tested that gave positive duplicate results with the four batches of dilutions) to be the 10-IFU/ml dilution in PreservCyt medium.

Results of the COBAS Amplicor CT/NG test on PreservCyt specimens. *C. trachomatis* was detected in 22 of the 1,000 specimens (2.2%) (Table 2). No inhibition was observed in any of the 978 specimens with negative results as assessed by the IC of the COBAS Amplicor CT/NG test.

Storage conditions. For each positive PreservCyt specimen, a COBAS Amplicor CT/NG test was performed 15, 30, and 45 days after collection. All of the 22 positive specimens yielded positive results at each of these time points (*C. trachomatis* A_{660}, >0.8) (Table 3). Most of the PreservCyt specimens retested gave very similar optical density values at each time point. Optical density values decreased by one A_{660} unit for just five PreservCyt specimens (samples 4, 5, 19, 20, and 22) (Table 3). The storage conditions (45 days at 15 to 20°C) could be responsible for a slight degradation in DNA, which would affect the sensitivity of PCR.

Confirmation of infection by testing of independent endocervical swabs and prevalence of urogenital chlamydial infection. Only 9 of the 22 positive patients agreed to be retested. All nine endocervical swab specimens were positive (*C. trachomatis* A_{660}, >0.8). Because PreservCyt transport medium is conventionally used to monitor endocervical specimens for cervical cancer and because all specimens with abnormal cytology or in which there was evidence of urogenital infection were excluded from this study, we can consider our population asymptomatic. The prevalence of *C. trachomatis* in this popu-

TABLE 2. Performance of COBAS Amplicor CT/NG test for detection of *C. trachomatis* in ThinPrep Pap test samples[a]

Age (yr) of female population (no. of persons)[b]	No. of PCR results		Specimens with inhibition
	Positive	Negative	
15 (n = 5)	1	4	None
16 (n = 13)		13	None
17 (n = 31)	1	30	None
18 (n = 67)		67	None
19 (n = 76)		76	None
20 (n = 130)	5	125	None
21 (n = 132)	4	128	None
22 (n = 117)	4	113	None
23 (n = 150)	2	148	None
24 (n = 124)	3	121	None
25 (n = 155)	2	153	None
Total (n = 1,000)	22	978	None

[a] None of the women tested showed clinical signs of urogenital infection, and cytological results were normal for all specimens.
[b] *C. trachomatis* PCR results were positive for 2 of the 116 15- to 18-year-olds tested (prevalence, 1.7%) and for 20 of the 884 19- to 25-year-olds tested (prevalence, 2.3%).

lation was 2.2% (Table 2), with the highest prevalence in women aged 19 to 25 years (2.3%; 20 out of 884).

DISCUSSION

The aim of this study was to evaluate the technical feasibility of using a routine PCR test to detect *C. trachomatis* in cervical specimens collected in the PreservCyt transport medium, a collection medium for the Pap smear (ThinPrep Pap Test). We evaluated the technical feasibility (DNA isolation procedure) and the in vitro sensitivity of *C. trachomatis* detection in PreservCyt transport medium in preliminary assays. Because PreservCyt transport medium is an alcohol-based solution and because endocervical cells are collected with cytobrushes, which induce bleeding, we adapted the treatment of PreserCyt specimens in an attempt to remove potential PCR inhibitors (COBAS Amplicor CT/NG test). The COBAS Amplicor IC was detected in all PreservCyt specimens tested (bloody and nonbloody samples), indicating that there were no inhibitors after the sample preparation procedure.

TABLE 3. Impact of storage conditions on the performance of the COBAS Amplicor CT/NG test

Sample no.	*C. trachomatis* A_{660} at:			
	Day 0	Day 15	Day 30	Day 45
1[a]	3.04	3.05	3.05	2.73
2[a]	3.08	2.95	3.01	2.77
3[a]	3.16	3.37	2.95	3.48
4	>4	>4	>4	2.76
5	2.97	2.95	2.47	1.95
6	1.97	3.13	3.37	3.10
7[a]	3.29	3.29	3.42	3.43
8[a]	3.03	3.33	2.26	2.98
9	3.37	3.72	3.73	3.73
10[a]	3.72	3.55	3.33	3.21
11	3.85	3.73	3.43	3.74
12	3.28	3.10	2.64	2.77
13	3.21	3.33	3.13	3.13
14[a]	>4	3.26	ND[b]	3.56
15	3.18	3.11	3.64	3.16
16	2.78	3.86	ND	3.39
17[a]	3.08	3.64	3.11	3.19
18[a]	2.78	3.51	3.26	2.83
19	3.09	3.29	ND	1.94
20	3.44	3.03	ND	2.15
21	2.69	ND	ND	2.69
22	3.06	ND	ND	1.72

[a] For this sample, a second independent PCR test was performed on an endocervical swab specimen collected in 2SP.
[b] ND, not determined.

We used four separate batches of the *C. trachomatis* serotype D reference strain that had been serially diluted in 2SP and in PreservCyt transport medium to compare the in vitro sensitivity and reproducibility of the PCR COBAS Amplicor CT/NG test for detection of *C. trachomatis*. The threshold of sensitivity obtained with titrated dilutions in PreservCyt medium was 10 IFU/ml. This means that exactly 0.125 IFU of *C. trachomatis* was tested in the assay. In France, a group of experts used the same *C. trachomatis* strain and protocol to compare the sensitivities of all of the commercially available *C. trachomatis* diagnostic tests in 1997. They concluded that the sensitivity threshold does not have to be higher than 100 IFU/ml (4). It is difficult to explain the difference in sensitivity observed between the two types of medium.

It is possible that the pretreatment step (centrifugation and resuspension in 2SP) carried out before the dilutions in PreserCyt influenced the sensitivity. This pretreatment step was carried out because we wanted to treat the PreservCyt dilutions in the same way as the PreservCyt specimens. The pretreatment may reduce the amount of material present before the nucleic acid extraction step, which would in turn reduce the number of DNA copies in the amplification mixture. This might explain the loss of reproducibility observed with the higher dilutions. The dilutions prepared in 2SP medium are not subjected to this pretreatment step (they are lysed immediately). We are currently studying the pretreatment step involved in the preparation of PreservCyt specimens using both quantitative real-time PCR and the COBAS Amplicor CT/NG test for detection of *C. trachomatis*.

Based on the results of our preliminary assays, we decided to screen 1,000 PreservCyt specimens with the COBAS Amplicor CT/NG test. PreservCyt specimens were collected from a population of women (age range, 15 to 25 years) during routine Pap screening. PreservCyt specimens were selected according to two criteria: normal cytology and the absence of clinical symptoms of infection mentioned by the practitioners. Thus, this population could be considered homogeneous and asymptomatic. Of the 1,000 endocervical specimens collected in PreservCyt transport medium, 22 were positive (prevalence, 2.2%). The specificity of diagnosis of chlamydial infection could be confirmed for only nine of these women, who agreed to give another endocervical sample under classical conditions (alginate swabs, 2SP transport medium). The COBAS Amplicor test confirmed that all nine of these patients were positive for *Chlamydia*. For all 1,000 PreservCyt specimens tested, no inhibition was observed, as assessed by the IC of the COBAS Amplicor test.

The prevalence of *C. trachomatis* infection in this asymptomatic population of women was 2.2%, with a higher prevalence for women aged 19 to 25 years. Our laboratory routinely diag-

noses *C. trachomatis* from endocervical swab specimens collected in 2SP transport medium. The endocervical swab specimens tested routinely and the PreservCyt specimens tested in this study were collected from women living in the same geographic areas. Last year, 7,335 endocervical swab specimens were routinely analyzed by the COBAS Amplicor CT/NG test; 232 of the specimens tested were positive (3.01%). Between January and June 2001, 121 specimens out of 6,400 were positive (1.92%). These prevalences are similar to that observed in our study on PreservCyt specimens and are in agreement with those reported previously in France (7, 15, 47). Although the prevalence observed in our study is similar to that reported in different studies in the same geographic area, we are aware that in a low-risk population (prevalence of <5%), the positive predictive value decreases and a nonculture test must be confirmed by a second method (Clinical Practice Guidelines). We are currently examining more samples from the youngest patients (age range, 15 to 20 years) to see if the prevalence changes.

Because PreservCyt specimens are stored for 6 weeks at 15 to 20°C in the cytopathology laboratory in case the results are ambiguous and new slides need to be made, we evaluated the influence of storage conditions on PCR sensitivity. We found that PreservCyt specimens can be stored for 6 weeks under these conditions without affecting the sensitivity of DNA amplification. For all positive PreservCyt specimens, *C. trachomatis* target DNA could be successfully amplified even after 6 weeks of storage. These results confirm the findings of a previous study showing that PreservCyt transport medium preserves the integrity of nucleic acids (10). These authors used cord blood lymphocytes, cancer cell lines, or clinical cervical samples collected in PreservCyt transport medium to show that RNA integrity was still intact after storage at room temperature or at 4°C for 24 h. Even after 1 month at 4°C in PreservCyt transport medium, Northern blotting detected intact 28S and 18S RNAs.

Although our results prove that PreservCyt transport medium is a suitable collection medium for routine screening of *C. trachomatis* infections with the COBAS Amplicor CT/NG test, other recent studies have reported the technical feasibility of detecting *C. trachomatis* from the ThinPrep Pap Test by use of molecular methods (DNA probe or ligase chain reaction test) and by use of a direct fluorescence assay (19, 25). Moreover, many studies have evaluated the detection of human papillomavirus (HPV) DNA in the residual sample volume of liquid-based Pap tests (11, 42, 43). In clinical practice, HPV is currently detected by molecular methods (in situ hybridization, Hybrid Capture Test, or PCR) to identify women infected with high-risk HPVs or women with equivocal cervical lesions (50). Finally, a recent study has demonstrated that PCR methods can be used to detect herpes simplex virus in ThinPrep cervical cytologic specimens and that this test is useful when the microcopic diagnosis is equivocal (13).

This is the first evaluation of the COBAS Amplicor CT/NG test for detection of *C. trachomatis* by the fluid-based ThinPrep Pap method. Our preliminary data suggest that the PCR test for detection of *C. trachomatis* can be used to screen PreservCyt specimens from populations of women who undergo annual Pap smear tests. Cervical dysplasia can occur during adolescence, especially when women have multiple sexual partners. This population is exposed to sexually transmitted diseases such as *Chlamydia* and HPV infections, especially types 16 and 18, which are a high risk factor for invasive cancer in adults (23, 37, 38).

The ThinPrep Pap Test can be used both to monitor for cervical cancer and to screen for *C. trachomatis* with the same specimen, without repeatedly examining the patient. The combination of DNA amplification tests is a new and promising approach, which can be used to detect multiple pathogens (such as HPV, herpes simplex virus, *C. trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma* spp.) from a single PreservCyt specimen. This will lead to important cost savings and benefit to the patient.

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