Evaluation of a Modified Sanitary Napkin as a Sample Self-Collection Device for the Detection of Genital Chlamydial Infection in Women

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Received 20 November 2000/Returned for modification 22 February 2001/Accepted 2 May 2001

A modified sanitary napkin was compared with endocervical swab and urine specimens for the detection of urogenital Chlamydia trachomatis infection. Endocervical swabs and/or first-catch urine were collected from 510 women at medical or community settings in Quebec City. Participants were also asked to wear a modified sanitary napkin (Ezy-Detek) during 4 consecutive hours and to bring it back to the clinic or mail it to the laboratory. Endocervical and urine specimens were tested using the Cobas Amplicor CT/NG assay (Roche Diagnostic Systems) according to the manufacturer’s instructions, as were specimens collected with the napkin after adequate preparation. If the PCR test result was positive on the endocervical sample or on any two samples, a woman was considered to be infected. PCR testing results on paired samples were identical for 493 (96.6%) of 510 women. According to the definition given above, 58 (11.3%; 95% confidence interval [CI], 8.7 to 14.5%) women were infected with C. trachomatis. The sensitivity and specificity of PCR testing on modified sanitary napkin specimens were, respectively, 93.1% (54 of 58; 95% CI, 83.3 to 98.1%) and 98.9% (447 of 452; 95% CI, 97.4 to 99.6%) compared to 81.0% (47 of 58; 95% CI, 68.6 to 90.1%) and 100% (451 of 451; 95% CI, 99.2 to 100%) for urine specimens. The positive and negative predictive values were, respectively, 91.5% (54 of 59) and 99.1% (447 of 451) for the sanitary napkin specimens compared to 100% (47 of 47) and 97.6% (451 of 462) for urine samples. These results suggest that a modified sanitary napkin represents an effective noninvasive device for self-collection of specimens to detect urogenital C. trachomatis infection.

Chlamydia trachomatis infection is the most frequently reported sexually transmitted disease in the industrialized countries. The association of this infection with transmission of human immunodeficiency virus infection, pelvic inflammatory disease, infertility, ectopic pregnancy, and chronic pelvic pain (10, 20, 30) constitutes a major public health concern. Because most infected women have no specific symptoms or are asymptomatic, screening programs represent one of the main approaches to control this infection (20, 21).

The current literature provides very good information on the use of self-collection techniques, such as PCR or ligase chain reaction, for the detection of C. trachomatis in the lower genital tracts of women. Both technologies have been evaluated in depth using endocervical swab or urine samples. With a specificity higher than 98%, the sensitivity of PCR on either cervical specimens or urine samples varies from 80.0 to 100% (4, 12, 18, 19, 27, 28).

More recently, detection of chlamydial infection by these techniques has also been assessed on secretions recovered from vaginal introitus collected by clinicians or patients themselves (2, 5, 9, 15, 16, 22, 26, 31, 33) or from vaginal tampons (13, 23–25). Urine and self-collected samples represent easy and noninvasive techniques compared to the usual cervical swabbing, which necessitates vaginal speculum insertion and a gynecologic examination. In a study, as many as 60% of chlamydia-infected adolescents would have been missed if the urine screening program had not been in place, because they refused the gynecologic examination (14). In addition, specimens may be collected in nontraditional medical settings, such as community centers and schools, and eventually at home (6, 11, 15, 17). Although these methods have been shown to be relatively easy to use and sensitive for the detection of C. trachomatis, some women failed to collect adequate specimens or did not feel at ease with the technique (2, 16, 31). The objective of the present study was to evaluate a modified sanitary napkin (Ezy-Detek [EDI Inc., Sillery, Canada]) as a specimen self-collection device for detection of chlamydial infection in women. Results of PCR tests on sanitary napkin specimens were compared with those for endocervical and urine specimens obtained from the same women.

MATERIALS AND METHODS

Study population and specimen collection. The study population consisted of women at different medical or community settings in Quebec City. Women were recruited through family-planning clinics, teen clinics, and a public juvenile facility health service. Each of these women had a gynecologic examination, and an endocervical swab specimen was taken for C. trachomatis detection as part of the medical visit. In order to include a sufficient number of infected women, physicians from private medical clinics who received positive chlamydia test results for their patients were also invited to collaborate by asking infected women to participate in the study before treatment.

After giving written informed consent, participants were asked to complete an anonymous short questionnaire on risk behavior and medical history and to
provide a urine specimen. First-catch urine (15 to 30 ml) was collected in a screw-cap plastic jar, placed in plastic bags, and refrigerated until transport to the laboratory (within 4 days of collection). The modified sanitary napkin comprises a conventional sanitary napkin which was modified by adding a removable sampling filter between the upper porous and lower impermeable sheets. This device was provided to women with a postage-paid, preaddressed envelope. Women were asked to wear the modified sanitary napkin for a period of 4 consecutive hours during their usual activities (determined by a pretest study among 15 women with recently diagnosed chlamydial infections and 4 uninfected women) and then to put it in the plastic bag provided, writing the date and the times at which they put on and took off the napkin on the label. They could bring the envelope back to the recruitment site or mail it to the laboratory. Women who were enrolled at a medical visit at which they received a drug prescription were asked to provide a urine specimen and to wear the sanitary napkin before taking their treatment.

In order to assess the specimen self-collection device in a nonmedical context, a community-based organization providing services to street youth and a high-school-based clinic were asked to collaborate in the study. In these settings, C. trachomatis screening was performed on urine specimens. In the context of the present study, these young women were also asked to wear the sanitary napkin as described above. In these groups, women with positive PCR test results were invited to visit their physicians for adequate follow-up and treatment. A gynecological examination was performed and a cervical swab specimen was taken before treatment. Exclusion criteria were being younger than 14 years, under antiotherapy, or menstruating. Those with active severe vulvitis or known intolerance of sanitary napkins were also excluded.

Questionnaires and specimens were coded. The code number was written on the consent form in order to identify the subject. Only the medical team had access to name information. Medical examination and screening were available at recruitment sites or at local community health clinics. The study protocol was approved by the Ethics Committee of the Hôpital du St-Sacrement du Centre Hospitalier Affilié Universitaire and the Centre Hospitalier Universitaire de Québec-ChUL.

Laboratory procedures. The Amplicor PCR assay (Roche Diagnostic Systems, Inc., Branchburg, N.J.) was performed on both cervical and urine specimens within 5 days of collection. The sanitary napkins were kept at room temperature until their preparation. At the time of testing, the filter of the sanitary napkin was transferred into a 50-ml polypropylene tube containing 2 ml of Amplicor collection transport medium and vigorously agitated for 15 min. This preparation was done in a separate room from that used for PCR analysis. All samples were then processed by the Cobas Amplicor system according to the manufacturer’s instructions.

Chlamydia DNA detection with the Amplicor diagnostic kit is based on three different steps: (i) PCR amplification, (ii) hybridization with a C. trachomatis-specific probe, and (iii) detection of amplified material by a colorimetric reaction secondary to enzymatic reaction. The internal control DNA included in the Cobas Amplicor system was used to monitor the inhibition of amplification. Optical density (OD) was read on a spectrophotometer at 450 nm. OD values below 0.2 meant a negative result if the internal control was positive. Values equal to or greater than 0.8 meant a positive result, regardless of the internal control result. In order to avoid ambiguity, specimens interpreted as positive and those with OD values between 0.2 and 0.8 interpreted as equivocal results were reprocessed and reanalyzed, and their results were established according to the above definition. When the internal control was negative, indicating the presence of DNA inhibitors, aliquots of the original specimens were retested after 1:10 dilution or heating at 100°C for 15 min. Paired specimens with discordant results were restested by processing another aliquot of each original specimen.

Statistical analysis. With the advent of DNA amplification tests which have better sensitivity than culture, different combinations of alternative tests were used as an expanded reference standard for the calculation of the performance characteristics of a new technique, because no “gold standard” has been defined yet (1). Different methods used to resolve discrepant results have also been critically assessed (20, 21, 22, 28). In the present study, the performance characteristics of PCR in different specimens was calculated in three ways. First, only women with a positive PCR test result on the endocervical swab specimen were considered truly infected. This definition was based on the fact that, from a clinical point of view, PCR testing on endocervical swab specimens is currently used for the diagnosis of C. trachomatis infection in Quebec City. Because PCR testing on urine samples is available for specific groups of women, such as street youth and prostitutes, who are often reluctant to attend traditional medical settings, women with positive PCR test results on both urine and sanitary napkin specimens were also considered truly infected in the second analysis. Finally, PCR testing on the three types of specimens was assessed using a latent class model which did not assume any gold standard (29). According to each definition, relative sensitivity and specificity, 95% confidence intervals (95% CI), and the positive and negative predictive values for PCR tests on sanitary napkin, urine, and endocervical swab specimens were calculated. The sensitivity and specificity of PCR on each type of specimen were also calculated according to the latent class model.

RESULTS

From September 1999 to May 2000, 510 women participated in the study: 246 (48.2%) were recruited through medical clinics, among whom 24 were enrolled after laboratory-diagnosed but untreated chlamydial infection; 157 (30.8%) were high school students; and 107 (20.9%) were incarcerated adolescents or street youth. Another 54 women (39 from medical clinics, 10 from high school, and 5 street youth or incarcerated adolescents) who provided endocervical and/or urine specimens were excluded because they did not mail or bring back the sanitary napkin. Most (90.6%) participants were less than 25 years old. A history of sexually transmitted disease (STD) was reported by 23.9% (122 of 510) of participants, while 4.3% (22 of 510) reported having had a sexual partner infected with an STD in the previous 6 months. In addition, 12.6% (64 of 510) of women reported genital symptoms at their enrollment, such as vaginal discharge, abdominal pain, and a variety of other complaints.

A total of 294 women provided matched endocervical, urine, and sanitary napkin specimens, and 215 participants provided only urine and sanitary napkin specimens, whereas cervical swab and sanitary napkin specimens were collected from 1 woman. Three hundred forty-two women (67.1%) sent in their sanitary napkins by mail, while 168 (32.9%) brought them back to the recruitment site. According to the information on the label, 96.1% women wore the sanitary napkin for at least 4 h. While cervical and urine specimens were tested within 5 days of collection, sanitary napkin specimens were processed from 1 to 82 days of collection (median, 9 days; 98.7% within 30 days).

Overall, 64 (5.0%) of 1,286 PCR specimens were found to be inhibitory at initial testing (this information was missing for 20 endocervical, 5 urine, and 3 sanitary napkin samples). The inhibition rates were 4.4% (12 of 275), 4.4% (22 of 504) and 5.9% (30 of 507) for endocervical, urine, and sanitary napkin specimens, respectively. PCR inhibitors were removed after dilution in 61 of 64 initially inhibitory samples, while the three other samples (one urine and two sanitary napkin samples) required heating at 100°C after dilution to eliminate the inhibition. Retested samples confirmed positive results for eight endocervical and seven sanitary napkin specimens and yielded additional positive results for two endocervical, one urine, and five sanitary napkin specimens. Altogether, PCR test results on paired samples were identical for 96.6% (493 of 510) of participants (94.9 and 99.0% of those who provided three and two samples, respectively) (Table 1). This proportion was similar for women who mailed their sanitary napkins in and those who brought them back to the recruitment site (96.5 versus 97.0%) and for the sanitary napkin specimens analyzed within 10 days of collection versus the others (97% in each instance).

The PCR test results were positive on two or three paired samples (accordingly) from 46 women (Table 1). In addition, 12 of 17 women with discrepant PCR test results were considered infected because the endocervical swab specimen or at least two specimen types tested positive by PCR. Overall, 39
TABLE 1. Results of C. trachomatis testing by PCR on endocervical swab, urine, and modified sanitary napkin specimens collected from 510 women in Quebec City, Canada

<table>
<thead>
<tr>
<th>No. of women with profile</th>
<th>Result* by PCR test on specimen type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endocervical</td>
</tr>
<tr>
<td>239</td>
<td>–</td>
</tr>
<tr>
<td>207</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>2†</td>
<td>–</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>7†,d</td>
<td>+</td>
</tr>
<tr>
<td>4†,d</td>
<td>+</td>
</tr>
<tr>
<td>1†</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>295</td>
</tr>
</tbody>
</table>

*–, negative; +, positive; NA, not applicable.
†These samples produced discrepant results on one or two specimen types.
‡These women were classified as not infected with C. trachomatis because they had a negative PCR test result on the endocervical swab specimen or they had a positive PCR test result on only one specimen.
§These women were classified as infected with C. trachomatis because they had a positive PCR test result on the endocervical swab specimen or on at least two specimens.

(15.9%) of 246 women recruited through medical clinics were infected with C. trachomatis (of whom 24 were enrolled after a recent positive test result), as were 2 (1.3%) of 157 high school students and 17 (15.9%) of 107 incarcerated adolescents or street youth. A minority (18 of 58) of infected women reported symptoms such as vaginal discharge (10 women), abdominal pain (3 women), and different other complaints (5 women).

Forty-seven (92.2%) of 51 women who were C. trachomatis positive by PCR on the endocervical swab specimen also had a positive PCR test on the sanitary napkin specimen. In symptomatic and asymptomatic women, these rates were, respectively, 94.4% (17 of 18) and 90.9% (30 of 33). For seven participants, both urine and sanitary napkin specimens were C. trachomatis positive; one of these had a negative PCR test on the endocervical swab specimen. Unfortunately, the other six women did not provide endocervical swab specimens (Table 1). Four women had a positive PCR test on the endocervical swab specimen and a negative PCR test on urine and sanitary napkin specimens, while three others had a positive PCR test on the sanitary napkin sample and a negative PCR test on urine and endocervical swab specimens (Table 1).

The performance characteristics of PCR for each type of specimen, calculated by using either a positive endocervical swab specimen exclusively or at least two positive specimens as a standard, are presented in Table 2, as well as the sensitivity and specificity obtained with the latent class model. The sensitivity of PCR on sanitary napkin specimens was higher than that on urine samples (92 to 100% versus 78 to 85%), whereas the relative specificities were comparable (98 to 99% and 99 to 100%, respectively). These results did not change significantly when symptomatic and asymptomatic infected women were compared (data not shown).

TABLE 2. Performance characteristics for detection of C. trachomatis by PCR in each type of specimen, according to the definition of infection used as a reference standard, among women from Quebec City, Canada

<table>
<thead>
<tr>
<th>Reference standard and specimen</th>
<th>Sensitivity* (95% CI)</th>
<th>Specificity* (95% CI)</th>
<th>PPV†</th>
<th>NPV‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive PCR on endocervical swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitary napkin (n = 295)</td>
<td>92.1; 47/51 (81.1–97.8)</td>
<td>98.4; 240/244 (95.9–99.6)</td>
<td>92.1 (47/51)</td>
<td>98.4 (240/244)</td>
</tr>
<tr>
<td>Urine (n = 294)</td>
<td>78.4; 40/51 (64.7–88.7)</td>
<td>99.6; 242/243 (97.7–100)</td>
<td>97.6 (40/41)</td>
<td>95.7 (242/253)</td>
</tr>
<tr>
<td>Endocervical swab</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Positive PCR on endocervical swab or on at least 2 specimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitary napkin (n = 510)</td>
<td>93.1; 54/58 (83.3–98.1)</td>
<td>98.9; 447/452 (97.4–99.6)</td>
<td>91.5 (54/59)</td>
<td>99.1 (447/451)</td>
</tr>
<tr>
<td>Urine (n = 509)</td>
<td>81.0; 47/58 (68.6–90.1)</td>
<td>100; 451/451 (99.2–100)</td>
<td>100 (47/47)</td>
<td>97.6 (451/462)</td>
</tr>
<tr>
<td>Endocervical swab (n = 295)</td>
<td>97.9; 47/48 (88.9–99.9)</td>
<td>100; 247/247 (98.5–100)</td>
<td>100 (47/47)</td>
<td>99.6 (247/248)</td>
</tr>
<tr>
<td>No reference standard (latent class, model) (n = 294 for all specimens)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitary napkin</td>
<td>100</td>
<td>98.8 (97.4–99.8)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Urine</td>
<td>85.2 (75.0–95.4)</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Endocervical swab</td>
<td>97.6 (92.8–97.7)</td>
<td>98.4 (96.8–100)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Values are percentages. Where a second value is listed, it is the number of specimens that were true positive/(number true positive plus the number true negative).
†PPV, positive predictive value. Values are percentages, with the number of specimens that were true positive/(the number true positive plus the number false positive) given in parentheses.
‡NPV, negative predictive value. Values are percentages, with the number of specimens that were true negative/(the number true negative plus the number false negative) given in parentheses.
NA, not applicable.
infection was more likely to be detected in sanitary napkin specimens than in urine specimens. As previously reported, the sensitivity of DNA techniques on female urine samples may be suboptimal because *C. trachomatis* infection is localized in the uterine cervix in most women (21). Conversely, some infected women may harbor *C. trachomatis* only in the urethra, and this infection may be missed by testing only endocervical or vaginal swab specimens (4, 6, 15, 18, 19, 27, 28). Thus, testing on single specimens (either urogenital or urine samples) may fail to identify all infected women (4, 15, 18, 19, 32).

It has been suggested that vaginal swabs may collect biologic material from both the cervix and the urethra (31). However, when the performance of different self-collected samples had been assessed, the sensitivity of PCR on combined urine and vaginal swab specimens was 96%, compared to 83% when PCR was performed only on vaginal swab specimens and 74% when it was performed only on first-catch urine samples (15). In the same way, the sensitivity of a *C. trachomatis* PCR test on a combined urine and cervical swab specimen was higher compared to testing on separate samples (32). It would be interesting to assess the performance of the sanitary napkin specimen compared to that of the corresponding self-collected vaginal swab specimen in detecting *C. trachomatis* infection in women. Unfortunately, self-taken vaginal introitus swab specimens were not collected in the present study because the requirement for too many types of samples to be provided by the women could have led to low participation rates. Further studies would be needed to assess women’s attitudes to these different sampling methods and to evaluate whether sanitary napkin specimens represent an efficient alternative to sampling of multiple sites and may thus improve the cost-effectiveness of urogenital *C. trachomatis* infection diagnosis in women. Sanitary napkins represent additional important advantages. They are a common product previously used by almost all women, and they have less-stringent transport criteria than urine or endocervical and vaginal swab specimens. Indeed, in the pretest study, sanitary napkin samples were kept at room temperature for up to 6 weeks before being tested without alteration in the PCR testing results (data not shown).

For four women the PCR test was positive on the endocervical swab specimen but negative on urine and sanitary napkin samples. The test results of these specimens were confirmed by retesting of the original samples, and the negative samples had no evidence of PCR inhibitors. Cross-contamination in these endocervical samples is unlikely because of the consistent results in separate analyses and the strict use of a contamination prevention protocol. These results may reflect the fact that these infections would be missed by testing only on modified sanitary napkin specimens. However, three of these four women were from the same recruitment site, and they presented multiple behavior problems according to the health care provider who recruited them. These results may indicate an improper use of the sanitary napkin that we could not identify as well as a lack of detection of the infection, possibly related to an insufficient number of copies of organisms present in the aliquot tested.

On the other hand, two out of three women with negative PCR test results on endocervical swab and urine samples but positive results on the sanitary napkin specimen, confirmed by retesting of the original samples, were treated because they presented symptoms clinically consistent with *C. trachomatis* infection. False-positive results with sanitary napkins are possible, but false-negative results from endocervical swab specimens related to inadequate specimen collection may also explain these discrepant results (1, 3). Sanitary napkin specimens from two women who did not provide endocervical swab specimens were positive, whereas urine samples was negative. These women were considered noninfected in the calculation of the performance characteristics of PCR on sanitary napkin specimens.

The results of the present study suggest that a modified sanitary napkin represents an easy, noninvasive, and efficient specimen self-collection device for *C. trachomatis* testing by PCR. It may be particularly useful for screening programs in nonclinical settings, allowing for the provision of STD services to high-risk groups, such as incarcerated adolescents, street youth, and persons involved in prostitution who are not inclined to attend traditional medical settings. Since this device allows very easy self-collection of specimens, it could also be useful to increase the accessibility of *C. trachomatis* screening for women in general. Further studies are required to evaluate whether this specimen self-collection device may represent an accurate and cost-effective approach to the detection not only of *C. trachomatis* but also of other STDs.

ACKNOWLEDGMENTS

We thank the collaborating physicians as well as the directors and staff of collaborating organizations: Les Oeuvres de la Maison Dauphine Inc.; the Centre-Jeunesse de Québec, l’Escale; the Collège St-François-Xavier Garneau; the CEGEP Ste-Foy; the Clinique de Planification des Naissances of the Hôpital St-François d’Assise and of the Centre Hospitalier Universitaire Laval du Centre Hospitalier Universitaire de Québec; the CLSC Haute-Ville, Basse-Ville/Limoilou; and CLSC La Source, Quebec City, Canada. We also acknowledge Roche Diagnostic Systems, Inc. for providing AMPLICOR CT/NG test kits and EZY-Detek (EDI) Inc. for providing modified sanitary napkins. This study was supported by the Division of STD Prevention & Control, Bureau of HIV/AIDS, STD & TB, Laboratory Center for Disease Control, Health Canada. M. Alary is a research scholar of the Fonds de la Recherche en Santé du Québec (970097-103).

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