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Effect of Endocervical Specimen Adequacy on Ligase Chain Reaction Detection of *Chlamydia trachomatis*

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Several studies have demonstrated that the sensitivity of a commercially available PCR test for the detection of *Chlamydia trachomatis* (Roche Diagnostics) is affected by the cellular quality of the endocervical swab specimens. The cellular adequacies of 1,633 female endocervical swab specimens were assessed and compared with the results of *C. trachomatis* detection obtained by ligase chain reaction (LCR; Abbott Laboratories). Specimen adequacy studies and LCR were performed with samples from the same swab, after demonstration of the stability of human epithelial cells in LCR transport medium. Prior to heat treatment of the swab specimen, an aliquot was removed and cytocentrifuged onto a slide. Cell spots were stained and examined at ×400 magnification for endocervical (columnar epithelial or metaplastic) cells and erythrocytes. The overall rate of positivity of the LCR was 6.5% (106 of 1,633 specimens) with pooled specimens (pools of 4 specimens each; reduced cutoff). Of the 1,633 specimens examined, 655 (40.1%) were found to contain one or more endocervical cells. The rate of positivity for *C. trachomatis* was 10.8% (71 of 655 specimens) among specimens containing endocervical cells, whereas it was 3.6% (35 of 978 specimens) among specimens lacking endocervical cells ($P < 0.0001$). There was no linear trend between the rate of positivity for *C. trachomatis* and the number of endocervical cells ($P = 0.24$). The rate of positivity for *C. trachomatis* was 5.4% (8 of 147 specimens) among specimens containing large numbers of erythrocytes (≥100 per high-power field), whereas it was 6.6% (98 of 1,486 specimens) among specimens containing less than 100 erythrocytes per high-power field ($P = 0.59$). These results show that the sensitivity of the Abbott *C. trachomatis* LCR test is affected by the presence of endocervical cells. Additionally, they indicate that the presence of a single endocervical cell is as good an indicator of specimen adequacy as the presence of many endocervical cells. The presence of a large number of erythrocytes was not associated with an increased rate of sensitivity of the LCR.

Chlamydia trachomatis is the most common bacterial sexually transmitted organism in the United States, with an estimated annual incidence of infections with this organism of 3 million to 4 million (3, 4). A variety of commercially available test methods exist for the detection of *C. trachomatis* infections, including cell culture, antigen detection enzyme immunoassay, direct fluorescent-antibody assay, nonamplified nucleic acid hybridization tests, and nucleic acid amplification tests. The analytical and clinical sensitivities of these methods vary greatly (2). In addition, the sensitivities of direct fluorescent-antibody assay, enzyme immunoassay, and nucleic acid hybridization tests have been shown to be affected by the cellular adequacy (presence of columnar epithelial or metaplastic [endocervical] cells or large numbers of erythrocytes [more than 100 per high-power microscopic field]) of female endocervical swab specimens (1, 8, 9, 15). More recently, the sensitivity of a PCR nucleic acid amplification test (Roche Diagnostics, Indianapolis, Ind.) has also been shown to be influenced by endocervical swab specimen adequacy. In three separate studies, rates of positivity for *C. trachomatis* by PCR ranged from 9.1 to 12.3% among specimens containing endocervical cells or large numbers of erythrocytes, whereas the rate of positivity was 0.9 to 3.5% for inadequate specimens (10, 11, 15). For those studies, the presence of one or more endocervical cells was the criterion used to represent an adequate specimen. Endocervical cells were not assessed quantitatively to determine if a linear relationship existed between cellular adequacy and *C. trachomatis* positivity. Beebe et al. (1) reported that the rate of positivity for *C. trachomatis* by a nonamplified nucleic acid test (PACE 2; GenProbe, San Diego, Calif.) increased with increasing numbers of endocervical cells. They applied a semiquantitative approach to assess specimen adequacy. To our knowledge, no studies that have described the use of nucleic acid amplification tests other than PCR, including the ligase chain reaction (LCR; Abbott Laboratories, Abbott Park, Ill.), have been published. In the present study, our first objective was to evaluate the suitability of the Abbott swab specimen transport medium for specimen adequacy testing. This would avoid the need to collect two swabs, which can potentially introduce sampling variability. Our second objective was to determine if the presence of endocervical cells or large numbers of erythrocytes influenced rates of positivity for *C. trachomatis* by LCR. Our final objective was to determine if a linear relationship existed between the number of endocervical cells and the rate of positivity for *C. trachomatis* by LCR.

MATERIALS AND METHODS

Study population and specimen collection. Endocervical swab specimens were collected from females attending 28 Iowa clinics participating in the Centers for Disease Control and Prevention-funded Infertility Prevention Program. Clinic types included sexually transmitted disease (STD) clinics, family planning clinics, and correctional facility clinics. The proportions of specimens by clinic type were

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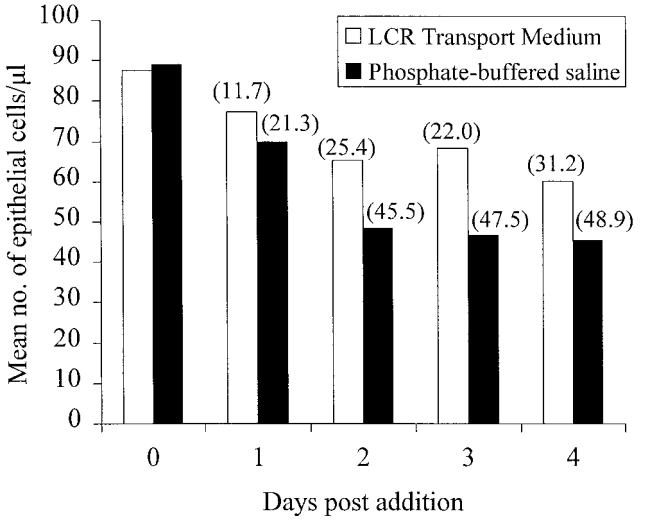

FIG. 1. Epithelial cell counts in swab specimen transport medium (Abbott Laboratories) and phosphate-buffered saline. Numbers in parentheses represent the percent decrease in cell counts versus the counts at day zero (immediately after addition of cells to one of the media).

as follows: STD clinics, 22.4%; family planning clinics, 61.6%; and correctional facility clinics, 16.0%. Specimens were chosen from clinics with high rates of positivity for *C. trachomatis* so that an adequate number of positive specimens could be obtained. The prevalences of *C. trachomatis* infection by clinic type were as follows: STD clinics, 14.7%; family planning clinics, 5.4%; and correctional facility clinics, 8.5%. Specimens were collected with the STD Swab Specimen Collection and Transport kit according to the instructions provided by the manufacturer (Abbott Laboratories). Specimens were transported to the State Hygienic Laboratory at ambient temperature. Transit times ranged from approximately 6 h to 3 days. This study was performed between September 2000 and January 2001.

Epithelial cell stability in LCR transport medium. To assess the stability of human epithelial cells in the Abbott specimen transport medium, freshly suspended cells of human lung origin (A549; American Type Culture Collection, Manassas, Va.) were counted in a hemocytometer and were added to swab transport medium tubes ($n = 5$) and to controls tubes containing phosphate-buffered saline (pH 7.2) ($n = 5$) to a final concentration of approximately 90 cells/μl. Tubes were allowed to sit at room temperature (specimen shipping temperature). At time zero and at 1, 2, 3, and 4 days, an aliquot was removed and the cells were counted in a hemocytometer.

Specimen adequacy testing. Upon arrival of the specimen in the laboratory and prior to the heating step, an aliquot was removed and applied to a glass slide by cytocentrifugation. To avoid the risk of specimen-to-specimen contamination, an extended-length, plugged pipette tip was used to remove the aliquot for specimen adequacy testing. Briefly, 100 μl was cytocentrifuged (Cytospin 3; Shandon, Inc., Pittsburgh, Pa.) for 6 min at 400 rpm. After the slides were air dried, they were stained by the Diff-Quik method. The slides were dipped five times each in Diff-Quik fixative solution, solution I, and solution II. The slides were rinsed by dipping them in distilled water and were allowed to air dry. Mounting medium was applied, and the spot was covered with a coverslip. Stained slides were examined initially at ×100 magnification for detection of columnar epithelial and metaplastic cells and erythrocytes. Confirmation of endocervical cells and counting of erythrocytes were performed at a magnification of ×400.

***C. trachomatis* LCR.** The *C. trachomatis* LCR (LCX; Abbott Laboratories) was performed according to the manufacturer's instructions, except that pooled specimens were tested. Briefly, 25 μl from each of four specimens was added to one amplification vial containing the LCR master mixture. Following detection, pools with sample value-to-cutoff value (S/CO) ratios <0.2 were considered negative. All four specimens were reported as negative. Pools with S/CO ratios ≥0.2 were considered positive. The four specimens from positive pools were retested individually (100 μl per amplification). Individual specimens with an S/CO ratio ≥1.00 were considered positive for *C. trachomatis*, and the results were reported as such.

Statistical analyses. All statistical analyses were performed with Statistical Analysis software (version 8; SAS Institute, Inc., Cary, N.C.). Differences in the rates of positivity for *C. trachomatis* were analyzed by use of the *z* statistic. The two-sample *t* test for equal means was used to compare the mean numbers of endocervical cells or erythrocytes in specimens positive and negative for *C. trachomatis*. Logistic regression analysis was used to evaluate the association between the number of endocervical cells and positivity for *C. trachomatis*. The level of significance was maintained at a P value <0.05.

RESULTS

The number of human epithelial cells in swab specimen transport medium (Abbott Laboratories) decreased 31% after 4 days of storage at room temperature, whereas the decrease in cell number was almost 49% in phosphate-buffered saline (Fig. 1). Cell counts in transport medium and in phosphate-buffered saline remained fairly stable between 2 and 4 days. On the basis of these results we felt that the Abbott transport medium provided an adequate degree of stability (structural integrity) for human epithelial cells, and we elected to assess the numbers of endocervical cells and erythrocytes directly from the swab specimen transport medium.

Initially, an aliquot of the swab specimen transport medium was pipetted onto a glass slide, smeared over an area approximately 15 by 30 cm, air dried, and then stained by the Diff-Quik method. However, these slides were difficult to read, as there was significant background staining and poor contrast among different cell types. Cytocentrifugation produced a spot (diameter, 5 mm) with minimal background staining (data not shown) that could be read in a much shorter time than was possible with a smear. All of the data presented in this report were obtained with cell spots prepared by cytocentrifugation.

Of the 1,633 cervical swab specimens analyzed, 106 (6.5%) were positive for *C. trachomatis* by LCR. Our laboratory had implemented specimen pooling prior to beginning the specimen adequacy study. Consistent with results from other studies (6, 7, 13), we had previously determined that the sensitivity of LCR for *C. trachomatis* with pooled specimens by use of a reduced cutoff value was comparable to that from the testing of individual specimens (S. J. Jirsa, R. Teske, and M. J. Loeffelholz, Abstr. Natl. STD Prevention Conf., abstr. P58, 2000).

Of the 1,633 specimens examined, 655 (40.1%) were found to contain one or more columnar epithelial or metaplastic (endocervical) cells (Table 1). The rate of positivity for *C. trachomatis* was 10.8% (71 of 655) among specimens that contained at least one endocervical cell, whereas it was 3.6% (35

TABLE 1. Results for specimens positive and negative for *C. trachomatis* by LCR according to presence of endocervical cells

C. trachomatis LCR result	No. (%) of specimens		Mean no. of endocervical cells[a]
	Without endocervical cells	With endocervical cells	
Negative	943	584	6.8
Positive	35[b]	71[c]	14.6
Total	978 (59.9)	655 (40.1)	

[a] Number of endocervical cells per cell spot.
[b] Rate of positivity for *C. trachomatis* among specimens without endocervical cells, 35 of 978 (3.6%) specimens.
[c] Rate of positivity for *C. trachomatis* among specimens with one or more endocervical cell, 71 of 655 (10.8%) specimens.

TABLE 2. Rate of positivity for *C. trachomatis* by LCR by number of endocervical cells

No. of endocervical cells[a]	No. of specimens tested	No. (%) of specimens positive
1–10	379	40 (10.6)
11–30	172	17 (9.9)
31–50	47	6 (12.8)
51–99	27	4 (14.8)
≥100	30	4 (13.3)

[a] Number of endocervical cells per cell spot.

of 978) among specimens that lacked endocervical cells ($P < 0.0001$). The mean number of endocervical cells in specimens positive for *C. trachomatis* was 14.6, whereas the mean number was 6.8 cells in specimens negative for *C. trachomatis* ($P = 0.0088$). The distribution of specimens and the rates of positivity for the specimens by number of endocervical cells are shown in Table 2. There was no linear trend between the rate of positivity for *C. trachomatis* and the number of endocervical cells ($P = 0.24$).

The rate of positivity for *C. trachomatis* was 5.4% (8 of 147) among specimens containing large numbers of erythrocytes (≥100 per high-power field), whereas it was 6.6% (98 of 1,486) among specimens containing less than 100 erythrocytes per high-power field ($P = 0.59$) (Table 3). The mean number of erythrocytes for specimens positive for *C. trachomatis* was 15.6, whereas the mean number of erythrocytes for specimens negative for *C. trachomatis* was 16.9.

DISCUSSION

The data from the present study indicate that the presence of endocervical (columnar epithelial or metaplastic) cells significantly affects the sensitivity of the LCR test for *C. trachomatis*. Our findings are consistent with those reported by others, who evaluated a PCR test for *C. trachomatis* (10, 11, 15). This is not surprising since the sensitivities of these two nucleic acid amplification methodologies for detection of *C. trachomatis* are comparable (2, 5, 14). In contrast to the data presented by Beebe et al. (1), we found no correlation between the number of endocervical cells and rates of positivity for *C. trachomatis*. That is, the rate of positivity for *C. trachomatis* by LCR for specimens containing ≥100 endocervical cells per slide was not significantly different from the rate of positivity for specimens containing only 1 to 10 cells. However, Beebe et al. (1) evaluated a nonamplification nucleic acid test (PACE 2; GenProbe) which is less sensitive than amplification-based tests such as LCR for the detection of *C. trachomatis* (2, 12). One explanation for the different findings is that when highly sensitive nucleic acid amplification tests are used, a single endocervical cell (as assessed by the method that we describe) provides a sufficient target for amplification and detection. An additional explanation is the different methods used to prepare slides. Beebe et al. (1) applied the entire specimen collected from a swab onto a slide, whereas we sampled one-fifth of the swab contents (100 μl from a total transport medium volume of 500 μl). Unfortunately, it is not possible to directly compare our actual cell counts with the semiquantitative data from the study Beebe et al. (1), in which cell numbers were converted into scores.

Since the present study was conducted between September 2000 and January 2001 and specimens were transported at ambient temperature, we cannot exclude the possibility that some specimens were exposed to freezing temperatures. We did not assess epithelial cell stability (structural integrity) after freezing and thawing.

Of interest are our data that suggest that large numbers of erythrocytes do not affect rates of positivity for *C. trachomatis*. This is in contrast to data from other studies that indicate that the presence of a large number of erythrocytes (≥100 per high-power field) is indicative of an adequately collected specimen (15). Again, it is possible that this discrepancy could be due to different slide preparation methods. However, our data on the association between erythrocytes and rates of positivity for *C. trachomatis* did not approach statistical significance. We did not assess the stability of erythrocytes in specimen transport medium. While many specimens were found to contain erythrocytes on microscopic analysis, without a quantitative assessment of stability, we cannot rule out the possibility that inadequate preservation of erythrocytes influenced the data.

Of great concern from medical and public health standpoints is the specimen inadequacy rate of nearly 60% that was quite consistent among all clinics submitting specimens (data not shown). The rate of positivity for *C. trachomatis* among these inadequate specimens was only a third of that among adequate specimens (3.6 versus 10.8%). This, together with the fact that as many as 75% of *C. trachomatis* infections in females are asymptomatic, suggests that many infected females might not be receiving proper treatment due to the lack of positive laboratory results or clinical findings.

Our data and those from other studies indicate that essentially all tests for the detection of *C. trachomatis* in endocervical swab specimens from females, including nucleic acid amplification methods, are affected by specimen adequacy (1, 8–11, 15). Periodic cytological evaluation of specimens is warranted to assess collection technique and signal the need for initiation of collector training, when necessary. Unfortunately, cytological evaluation of all specimens is labor-intensive and cost prohibitive. Rejection of inadequate specimens or addition of disclaimers to negative test results should be done only if all specimens are tested for adequacy. Periodic cytological evaluation or evaluation of new clinics or new clinicians may be

TABLE 3. Results for specimens positive and negative for *C. trachomatis* by LCR according to presence of erythrocytes

C. trachomatis LCR result	No. (%) of specimens		Mean no. of erythrocytes[a]
	With <100 erythrocytes	With ≥100 erythrocytes	
Negative	1,388	139	16.9
Positive	98[b]	8[c]	15.6
Total	1,486 (91.0)	147 (9.0)	

[a] Number of erythrocytes per high-power field (×400 magnification).
[b] Rate of positivity for *C. trachomatis* among specimens with <100 erythrocytes, 98 of 1,486 (6.6%) specimens.
[c] Rate of positivity for *C. trachomatis* among specimens with ≥100 erythrocytes: 8 of 147 (5.4%) specimens.

sufficient to maintain acceptable overall adequacy rates. Studies evaluating the effectiveness of various specimen adequacy screening and training programs are under way in our laboratory and other laboratories.

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