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Influence of Endocervical Specimen Adequacy on PCR and Direct Fluorescent-Antibody Staining for Detection of *Chlamydia trachomatis* Infections

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The cellular quality of the endocervical swab specimen used for the detection of *Chlamydia trachomatis* may dramatically impact the sensitivity of the diagnostic assay used. An evaluation of the adequacy of 319 endocervical swab specimens from women attending two inner-city sexually transmitted disease and family planning clinics, as well as five high school-based family planning clinics, was performed, and the resulting data were compared with the diagnostic results obtained by both Amplicor PCR and Microtrak direct fluorescent-antibody (DFA) staining. The swab from each patient was rolled across the open circular area of a DFA slide and then used to inoculate a transport tube for PCR (Roche), after which the swab was discarded. The slides were stained and examined by epifluorescence microscopy for the presence of *C. trachomatis* elementary bodies and for the presence and number of cell types to determine specimen adequacy. Cellular adequacy for a cervical swab specimen was defined as the presence of one or more columnar epithelial or metaplastic epithelial cells or the presence of more than 100 erythrocytes per high-power microscopic field. Of the 319 specimens read by DFA, 204 (63.9%) were determined to be adequate. There were 34 (10.7%) positive specimens by DFA and/or PCR. Twenty-nine (9.1%) specimens were positive by PCR, 20 (6.3%) specimens were DFA positive, and 15 (4.7%) were concordantly positive by both tests. The prevalence of chlamydia among adequate specimens was 14.2% (29/204), compared to 4.3% (5/115) for inadequate specimens ($P < 0.0001$). Variations in specimen quality and the sensitivity of the diagnostic assay used have a significant impact on determining the prevalence of *C. trachomatis* in a population.

More than 4 million *Chlamydia trachomatis* infections occur annually in the United States, leading to significant and costly complications and sequelae, such as pelvic inflammatory disease, ectopic pregnancy, and infertility (8, 9, 15, 25, 28). Because symptoms among most infected women and men are often mild or absent, widespread screening has been recommended by the Centers for Disease Control and Prevention and in a recent Institute of Medicine report as a means to control this epidemic (9, 14). Until recently, screening has been limited by the availability of sensitive and specific assays. The advent of the newer DNA amplification techniques has resulted in assays which have sensitivities of greater than 90% and specificities approaching 100% (2–7, 10–13, 16, 17, 22, 23, 26, 27, 29, 30). Previously, a significant concern with regard to the performance of culture as well as nonculture assays has been the cellular adequacy of the cervical specimen, since chlamydiae are intracellular organisms which infect the columnar epithelial cells. The presence or absence of these cells has been shown to significantly affect the sensitivity and the specificity of the detection of chlamydia by the Chlamydiazyme assay (Abbott Diagnostics, Abbott Park, Ill.) as well as by direct fluorescent-antibody (DFA) staining (18, 21, 24). Additionally, the quality of the cervical specimen has been shown to affect the positivity rate of the commercial PCR assay (Roche Molecular Systems) (20). We wanted to ascertain whether specimen adequacy could be determined by performing DFA staining of a single cervical swab specimen, which would then also be used to determine the presence of *C. trachomatis* by the PCR assay (Roche), and whether the adequacy of the specimen affected the positivity rates of the two assays.

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MATERIALS AND METHODS

Study population. As part of the ongoing Centers for Disease Control and Prevention-funded Infertility and Chlamydia Initiative for Public Health Region III, all females attending sexually transmitted disease (STD), family planning, and school-based clinics are screened for *Chlamydia* infections. For a subsample of these patients, clinicians were directed to use the endocervical swab sample to make a slide for DFA staining and then to inoculate a PCR assay transport tube. The slide samples were collected on three separate occasions for 1 week over a 1-year period during 1995 and 1996. Clinicians were not told that the slides were being evaluated for specimen cellular adequacy. There were 129 specimens collected from two STD clinics, 106 obtained from two family planning clinics, and 84 received from five city high school clinics.

Specimen collection. Clinicians were told by clinic supervisors that a comparison study of DFA and PCR was being conducted and were instructed to roll the swab specimen for approximately 180° or more on a Microtrak slide (Behring Diagnostics, San Jose, Calif.) before inoculating the PCR transport tube (Roche Diagnostic Systems, Branchberg, N.J.) according to the manufacturer's instructions. All specimens and slides were sent by courier the same day to the Chlamydia Research Laboratory at Johns Hopkins University for testing. Specimens were processed upon receipt by the laboratory and refrigerated at 4°C overnight, or for up to 3 days for specimens received on a Friday, prior to testing.

Assays and statistical analyses. (i) DFA. Slides were fixed in methanol for 5 min and then stained for 15 min at room temperature with a fluorescein-conjugated monoclonal antibody (Behring Diagnostics). They were washed in phosphate-buffered saline, air dried, and read by epifluorescence microscopy at a magnification of ×630 or ×1,000. The slides were evaluated for the presence of elementary bodies, columnar epithelial or metaplastic cells, polymorphonuclear cells, vaginal squamous epithelial cells, and erythrocytes. According to criteria set forth by Kellogg et al., a specimen was considered to be adequate on a cellular-component basis if it contained any columnar epithelial or metaplastic cells, with or without the other cells, or if it contained more than 100 erythrocytes per high-power field (18, 20, 21). Specimens containing this many erythrocytes could not be judged for the presence of columnar cells because the erythrocytes mask the other cells present in a sample (18).

TABLE 1. PCR and DFA results for adequate and inadequate endocervical specimens from 319 females

Specimen category	No. (%) of specimens tested	Chlamydia test results			Total no. of positives (%)
		No. PCR positive and DFA positive	No. PCR positive and DFA negative	No. PCR negative and DFA positive	
Adequate[a]	204 (63.9)	15	10	4	29 (85.3)
Inadequate[b]	115 (36.1)	0	4	1	5 (14.7)
Total	319	15	14	5	34 (10.7)

[a] Prevalence of positives in adequate specimens: 29 of 204 (14.2%).
[b] Prevalence of positives in inadequate specimens: 5 of 115 (4.3%).

(ii) PCR. PCR (Amplicor; Roche Diagnostic Systems) was performed according to the manufacturer's instructions. Briefly, upon arrival of the specimen in the laboratory, 1 ml of transport-tube specimen was treated with 1 ml of specimen diluent, incubated for 10 min at room temperature, and then refrigerated. After the treated specimen had been refrigerated overnight or for up to 4 days, the assay was performed by addition of 50 μl of the processed sample to a master mix containing the manufacturer's PCR test components and amplification in the Perkin-Elmer (Norwalk, Conn.) 9600 thermocycler. Contamination was controlled by the use of separate rooms for the processing of specimens, the mixing of PCR reagents, the addition of specimens to the prepared PCR plate, and the performance of amplification steps. Additionally, area-dedicated pipettors and lab coats as well as aerosol barrier pipette tips were used throughout the procedures, and gloves were frequently changed. After the amplified samples were denatured in the 96-microwell plate, they were hybridized in a probe-coated microwell plate, and the colorimetric assay was completed by using an avidin-enzyme conjugate and a substrate. The plate was read spectrophotometrically at 450 nm, and a positive result was reported when a specimen had an optical density of greater than 0.5.

Statistical analyses. Tests of comparison were performed by Student's *t* test.

RESULTS

There were 319 cervical specimens, 204 (63.9%) of which were graded as adequate by using the criteria of Kellogg et al. (18, 20, 21). There were a total of 34 (10.7%) positive specimens by the DFA and/or the PCR assay (Table 1). Twenty-nine (85.3%) of the positive assay results were from specimens graded as adequate, while only 5 (14.7%) were from specimens graded as inadequate by examination of the cellular composition on the DFA slide ($P < 0.001$). The prevalence of chlamydia in the adequate specimens was 14.2% (29/204), compared to 4.3% (5/115) for the inadequate specimens (Table 1).

Of the 204 adequate specimens, 15 (4.7%) were positive by both PCR and DFA, 10 were PCR positive and DFA negative, and 4 were PCR negative and DFA positive. Of the 115 inadequate specimens, 4 were PCR positive and DFA negative and 1 was PCR negative and DFA positive (Table 1).

Of the 204 specimens graded as adequate, 56.4% (115 of 204) contained columnar epithelial cells only, 13.2% (27 of 204) contained columnar epithelial cells and erythrocytes, and 30.4% (62 of 204) contained more than 100 erythrocytes per high-power field (Table 2). The positivity rates for these three categories of adequacy were 13.9, 22.2, and 11.3%, respectively. If only columnar cells (with or without erythrocytes) were used as a measure of adequacy, the chlamydia prevalence was 15.5% (22 of 142), while a prevalence of 14.6% (13 of 89) was obtained with specimens containing erythrocytes with or without columnar cells. The prevalence for those specimens containing erythrocytes present at more than 100 cells per high-power field was 11.3% (7 of 62).

When clinic type was considered, the clinicians in the STD and family planning clinics obtained adequate specimens 72.9 and 72.6% of the time, respectively; the high school clinicians had an adequacy rate of 39.3% (Table 3). The prevalence of chlamydia in the adequate specimens obtained from the STD clinics was 17.0%, compared to 14.7% for all specimens (adequate and inadequate). In the family planning clinics, the prevalence for those specimens containing cells indicative of adequacy was 14.3%, in contrast to 12.3% for all specimens. Similarly, the prevalence in the adequate specimens from the school clinics was higher (6%) than the prevalence (2.4%) for the group containing both adequate and inadequate specimens (Table 3).

TABLE 2. Cellular components versus chlamydial positivity for 204 adequate endocervical specimens[a]

Cell type category	No. (%) of specimens in category	No. (%) of chlamydia-positive specimens for:	
		Cell type category	Entire population
Columnar only	115 (56.4)	16/115 (13.9)	16/204 (7.8)
Columnar plus erythrocytes	27 (13.2)	6/27 (22.2)	6/204 (2.9)
>100 erythrocytes	62 (30.4)	7/62 (11.3)	7/204 (3.4)

DISCUSSION

This study indicated that the cellular quality of the cervical specimen significantly affects the prevalence of chlamydia identified by both the DFA and PCR assays ($P < 0.0001$). The prevalence of chlamydia in adequate specimens was 14.2%, while the prevalence in inadequate specimens was 4.3%. The DFA, which has a reported sensitivity of 70 to 85% (1), was the assay most affected by specimen quality, while the relatively more sensitive PCR was affected to a lesser degree. By DFA, the prevalence of chlamydia in the adequate specimens was 9.3% versus only 0.9% for the inadequate specimens. Considering PCR alone, the prevalence of chlamydia in adequate specimens was 12.3% (25 of 204) versus 3.5% (4 of 115) for inadequate specimens. The more sensitive PCR test was less significantly affected by specimen quality but still performed in a vastly superior manner when the specimen was adequate. These findings are in accordance with those of the study by Phillips et al., who reported that the sensitivity of the DFA was 40% when smears contained less than five columnar cells and 92% when the slides contained five or more such cells (24). Our study also confirmed the findings of Kellogg et al., who found that the prevalence of chlamydia among 1,007 adequate specimens was 10.6% by PCR, compared to 0.9% for 341 inadequate specimens (20). In an earlier study by this same group, the prevalence of confirmed chlamydial antigen, as determined by Chlamydiazyme (Abbott), in 706 adequate specimens was 13.2% versus 1.7% for 918 inadequate specimens (18).

TABLE 3. Impact of specimen adequacy and clinic type on chlamydia prevalence

Clinic type	% Adequate specimens	% Chlamydia prevalence for:		
		All specimens	Adequate specimens	Inadequate specimens
STD	72.9 (94/129)	14.7 (19/129)	17.0 (16/94)	8.6 (3/35)
Family planning	72.6 (77/106)	12.3 (13/106)	14.3 (11/77)	6.9 (2/29)
High school	39.3 (33/84)	2.4 (2/84)	6 (2/33)	0 (0/51)
Total	63.9 (204/319)	10.7 (34/319)	14.2 (29/204)[a]	4.3 (5/115)[a]

[a] $P > 0.0001$.

Our finding that 11.3% of specimens which contained more than 100 erythrocytes per high-power field were positive for chlamydial DNA or antigen is similar to those of the study done by Kellogg et al., which reported that the prevalence among 245 specimens containing "too many red blood cells to analyze microscopically" was 13.1%, compared to a prevalence of 13.3% for 661 specimens containing endocervical cells (18). The presence of such a high number of erythrocytes may be indicative of friability of the chlamydia-infected cervix, which is induced to bleed when swabbed for a clinical specimen.

The use of the same swab for the adequacy test and the PCR test did not appear to adversely affect the positivity rate for the PCR test, as there were 25 (12.3%) PCR-positive swab specimens after rolling the swab 180° or more on the DFA slide. This prevalence was similar to that reported for the PCR test in our population at this time. However, four specimens deemed adequate and one classified as inadequate were PCR negative and DFA positive. These specimens may have contained PCR inhibitors, which have been reported to be present in some cervical specimens (2, 5, 23). Additionally, these positives may have been missed by PCR because all of the specimen may have been deposited on the DFA slide.

The fact that the clinicians involved in this study obtained adequate specimens only 63.9% of the time indicated that training and periodic retraining of clinicians who obtain cervical specimens for chlamydial testing are necessary. Another study found adequacy rates of 49.7%, which also indicated the necessity of training (18). Such training could help ensure that cervical specimens submitted for diagnostic assays contain the proper cellular components necessary for the detection of organisms. While more-sensitive tests can help detect more infections, the quality of the specimen is probably the most important facet of the diagnostic test. To monitor the performance of clinicians in obtaining adequate specimens, clinical laboratories should periodically ascertain the cellular quality of cervical specimens as a routine quality assurance procedure. This study appeared to indicate, but did not definitively prove, that the same swab could be used to ascertain the adequacy of the cervical specimen before it is placed into the PCR transport medium for swirling. Other methods besides the DFA test have been used to assess the cellular components of the specimen; these include the Papanicolaou stain and Diff-Quick (Baxter Diagnostics, Inc., McGaw, Ill.) (18–21). These methods are less costly and easier to use in a routine clinical laboratory than the DFA test, which requires an experienced microscopist. One advantage of the DFA test is its ability to confirm positives obtained in the PCR test or positives or gray zone values obtained by an enzyme immunoassay test and to monitor for the presence of inhibitors in the PCR assay.

Monitoring the adequacy rates of clinicians over time can achieve positive results, as indicated by the studies of Kellogg et al., who have improved their clinician specimen adequacy rate from 49.7 to 74.7% (18, 20). Targeted training for those clinicians who continually collect inadequate specimens can provide the progress necessary to improve the diagnostic yield of chlamydial screening and testing programs in the future.

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