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Improved PCR Detection of *Chlamydia trachomatis* by Using an Altered Method of Specimen Transport and High-Quality Endocervical Specimens

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Duplicate endocervical swabs were collected for detection of *Chlamydia trachomatis* by PCR (Roche Diagnostics). One swab was swirled in Specimen Transport Medium (Roche) for PCR testing and discarded. A saline aliquot from the other specimen, sent as a dry swab to the laboratory, was Papanicolaou stained to determine specimen adequacy, and the remainder was PCR tested. Significantly more (24%) PCR-positive results (118 versus 95; $P < 0.001$) were obtained with the dry specimens than with the swirled specimens when first tested. In addition, PCR-positive results were obtained with 107 (10.6%) of 1,007 microscopically adequate specimens but with only 3 (0.9%) of 341 inadequate specimens ($P < 0.001$).

One of the most sensitive and specific assays for detection of *Chlamydia trachomatis* in endocervical or urethral specimens is the Amplicor *C. trachomatis* Test (Roche Diagnostic Systems, Branchburg, N.J.) (2–4, 6, 9, 10, 15) employing PCR technology. Both the sensitivity (7, 8, 12, 13) and the specificity (7, 8, 14) of other, less-sensitive chlamydia detection tests have been shown to be directly related to the relative adequacy of endocervical specimens. Such a specimen is one that contains metaplastic cells and/or columnar or cuboidal endocervical cells (the cells infected by *C. trachomatis*) or large numbers of erythrocytes (1, 7, 8, 13). This laboratory has previously determined that an aliquot of an endocervical specimen can be stained by the Papanicolaou (Pap) method and microscopically analyzed to determine specimen adequacy without reducing enzyme-linked immunosorbent assay detection of *C. trachomatis* antigen from the remainder of the specimen (7, 8). The present study had two main objectives. The first was to determine whether endocervical specimens could be collected and processed (without the initial use of Specimen Transport Medium [STM; Roche]) for Pap stain determination of specimen adequacy without jeopardizing PCR detection of *C. trachomatis* DNA from those specimens. Detergents in STM will lyse the cells that must be microscopically detected in order to indicate an adequate specimen (unpublished findings). The second objective was to establish the relative importance of endocervical specimen quality for the reliable detection by PCR of *C. trachomatis* from the specimens.

Specimen collection and testing. From 11 July 1994 until 31 December 1994, duplicate endocervical specimens for PCR testing were obtained from each patient by using Dacron swabs supplied with the STD Swab Specimen Collection and Transport Kit (Roche). Specimens were collected from asymptomatic females attending a prenatal clinic and from females with symptoms suggestive of sexually transmitted diseases. All patients in the study were 13 years of age or older. The exocervical mucus was removed and specimens were collected as previously described (7).

From 11 July 1994 until 30 September 1994, the first of two swab specimens from each patient was swirled for 15 s in 1 ml of STM and then discarded. STM thus inoculated was stored at room temperature and sent within 2 to 24 h to the laboratory, where it was stored at 4 to 8°C for up to 96 h. PCR testing was usually performed daily, Monday through Friday. Swirled STM samples were tested for the presence of *C. trachomatis* DNA by PCR according to the manufacturer's instructions (10). The swirled specimen was not Pap stained, because of the cell-lysing action of detergents in the STM. The second swab specimen from each patient was placed into an empty polypropylene tube (12 by 5 mm), which was sealed with a polyethylene cap and sent to the laboratory. Both the tubes and the caps were purchased from Baxter Diagnostics, Inc., McGaw Park, Ill. This swab, designated the dry specimen, was stored alongside the swirled specimen. Prior to testing, 300 μl of 0.9% saline was added to each dry specimen. After the specimens were vortexed for 10 s, the swabs were discarded, 30 μl of sample was smeared on slides, and the slides were Pap stained by an automated procedure in the hospital's cytology laboratory as previously described (8). One milliliter of STM was then added to the remaining specimen. The dry specimen from each patient was PCR tested at the same time and in the same manner as the swirled specimen. All Pap stains were read by one of us (J.A.K., J.W.S., or J.L.K.) without knowledge of the patient population or PCR results. From 1 September 1994 until 31 December 1994, the order of collection of swirled and dry specimens was reversed. Whenever possible, the centrifuged sediments from those swirled and dry specimens which had tested positive by PCR were also tested as previously described with a direct fluorescent antibody (DFA) reagent (MicroTrak; Syva Co., Palo Alto, Calif.) (7) to determine the practicality of the DFA assay as a rapid confirmatory assay for PCR-positive results. The presence of three or more fluorescent elementary bodies, confirmed by a second experienced microscopist, was considered a positive result.

Interpretation of PCR results. A clinical specimen with an optical density (A_{450}) reading of less than 0.100 was considered negative, while one with an A_{450} of greater than 0.500 was considered positive. In an attempt to detect low levels of chlamydial DNA and to improve PCR sensitivity, an A_{450} range of

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TABLE 1. Effects of method of endocervical specimen processing on PCR detection of *C. trachomatis*

Endocervical specimen processing method	PCR test	No. (%) of 129 chlamydia-positive patients with the following PCR result:		
		Positive	Borderline	Negative
Swirled swab processed according to manufacturer's PCR instructions	Initial screen	95 (73.6)[a]	9 (7.0)[b]	25 (19.4)
	Repeat test	121 (93.8)	0	8 (6.2)
Dry swab used first for Pap stain and then for PCR	Initial screen	118 (91.5)	6 (4.6)	5 (3.9)
	Repeat test	122 (94.6)	0	7 (5.4)

[a] None of the positive results for swirled specimens but one of the positive results for dry specimens reverted to negative when the initial PCR test was repeated.
[b] All nine borderline results for swirled specimens and five of six borderline results for dry specimens during the initial PCR screen became PCR positive on retesting.

0.100 to 0.500 was considered borderline, in contrast to the range of 0.200 to 0.500 that was called equivocal by the manufacturer. If a PCR-positive result was obtained with only one of the duplicate specimens from any patient, both specimens were reamplified and retested singly. Whenever a borderline result was obtained, the specimen was reamplified and retested in duplicate. PCR retesting was undertaken within 48 to 72 h of the original assays. When specimens were retested, an A_{450} of $\geq$0.250 was considered a positive result, as indicated by the manufacturer. The Z test for differences in proportions for independent samples was used for statistical analysis of results (16). A P value of $<$0.05 was selected as the minimum level of significance.

Duplicate endocervical specimens were collected during the study from 1,420 patients. PCR-positive results were obtained with one or both specimens from 129 (9.1%) of the patients. Patients with positive results ranged in age from 14 to 35 years (mean, 20.7 years; median, 20 years).

Effects of specimen collection and processing methods on detection of *C. trachomatis*. When the specimens were first tested by PCR, the dry specimens used for Pap staining were unexpectedly associated with significantly more (24%) PCR-positive results (118 versus 95) than the swirled specimens which had been processed according to the manufacturer's instructions ($P < 0.001$; Table 1). This finding was true whether the dry specimens were the first (52 dry specimens versus only 40 swirled specimens were positive [$P < 0.05$]) or the second (66 dry specimens versus 55 swirled specimens were positive [$P < 0.05$]) to be collected. When the specimens were first tested, false-negative results occurred with 17 (14.0%) of the 121 swirled specimens but with none of the 122 dry specimens which were ultimately PCR positive when the specimens were retested ($P < 0.001$). Of 17 swirled specimens whose PCR results changed from negative to positive when retested, 11 (65%), 3 (18%), 2 (12%), and 1 (6%) were first tested by PCR after 1, 2, 3, and 4 days of storage, respectively. On PCR retesting, both the swirled and dry specimens yielded similar detection of *C. trachomatis* (Table 1). One dry specimen, which was PCR positive (optical density = 3.405) when first tested, became negative on retesting. The swirled specimen from the same patient was repeatedly PCR positive.

In all, only 19 (1.3%) of the initial PCR results for the 1,420 swirled specimens were within the expanded borderline range, and 9 (47.4%) of these specimens became positive on retesting. Similarly, only 18 (1.3%) of the initial PCR results for dry specimens were within the borderline range and five (27.8%) of these specimens reverted to positive when retested. However, five (55.6%) of the nine swirled specimens with initial borderline results that became positive after retesting had low A_{450} readings of only 0.100 to 0.200 (below the manufacturer's equivocal range) when first tested. In contrast, none of the five dry specimens whose borderline results ultimately reverted to positive were in the low borderline range when initially tested. For the five chlamydia-positive patients whose swirled specimens initially gave low borderline results, four of the accompanying dry specimens had repeatedly PCR-positive results and fluorescent elementary bodies were detected in one or both specimens from two individuals.

Of the 129 patients whose specimens ultimately had PCR-positive results, both the swirled and dry specimens were positive for 114 (88.4%), while repeatedly positive results were obtained for 8 (6.2%) more only with the dry specimens and for 7 (5.4%) only with the swirled specimens. DFA confirmation was performed on 113 and 112 of the dry and swirled specimens, respectively, that had PCR-positive results, including both specimens from 14 of the 15 patients for whom only one specimen was repeatedly PCR positive. Positive DFA results were obtained with 89 (78.8%) of 113 dry, PCR-positive specimens and with 80 (71.4%) of 112 swirled, PCR-positive specimens which were also DFA tested. Of the 14 patients who had repeatedly PCR-positive results for only one specimen and whose specimens could also be DFA tested, 6 (43%) had DFA evidence of chlamydial elementary bodies in one or both specimens.

Impact of dry specimen quality on detection of *C. trachomatis*. Of the dry specimens from 1,420 patients, 72 (5.1%) could not be microscopically analyzed to determine specimen quality because of the thickness of smears or loss of cellular material. Of the remaining 1,348 specimens, 1,007 (74.7%) contained endocervical-metaplastic cells and/or large numbers of erythrocytes and were therefore determined to be microscopically adequate. Of 122 PCR-positive results which were obtained with dry endocervical specimens, 110 (90.2%) came from those specimens which could be microscopically analyzed to determine the specimen quality. *C. trachomatis* DNA was detected by PCR for 107 (10.6%) of the 1,007 microscopically adequate specimens but for only 3 (0.9%) of the 341 inadequate specimens ($P < 0.001$). For the seven dry specimens with repeatedly false-negative PCR results, Pap stains of four showed microscopic evidence of adequacy and stains of the remaining three could not be analyzed. The cost associated with the preparation and reading of each Pap stain was about $2.07. Approximately 30 to 60 s was required to read each smear.

The PCR testing of endocervical specimens submitted on dry swabs to the laboratory had two major advantages for the diagnosis of *C. trachomatis* infections compared with the testing of swabs which were swirled in STM, as recommended by the manufacturer. First, significantly more PCR-positive results were obtained with dry specimens than with swirled specimens when the specimens were first tested by PCR. The failure of 4 to 25% of the positive specimens to give positive PCR results when first tested has been reported for numerous previous studies (2, 3, 6, 10, 11, 15). In the present study, false-negative specimens which reverted to PCR positive when re-

tested were more likely to occur when the specimen had been stored for only 1 or 2 days prior to testing. Further studies of the role that endocervical specimen components, the Roche specimen transport tube, or STM may play in inhibition of PCR-positive results should be undertaken.

The second advantage of the PCR testing of endocervical specimens submitted on dry swabs was that these specimens, unlike those submitted in STM, could be easily, quickly, and reliably stained to microscopically determine specimen adequacy without jeopardizing PCR test sensitivity. Endocervical specimen adequacy is often substantially lower than may be suspected, and it depends heavily on the training, motivation, and skills of the collectors (7, 8). Despite our use of the very sensitive PCR assay, evidence of infection due to *C. trachomatis* was detected in significantly more patients during the present study with microscopically adequate endocervical specimens than with specimens lacking appropriate cells. Improved collection of high-quality specimens and periodic cytologic evaluation of specimen adequacy, as currently recommended by the Centers for Disease Control and Prevention (5), should help to increase the cost-effectiveness of the PCR test. An evaluation of a simpler, less expensive alternative to the Pap stain for determining specimen adequacy is currently in progress.

The results of the present study also indicate that if a swirled endocervical specimen is submitted to the laboratory in STM, a low borderline A_{450} range of 0.100 to 0.500 (compared with the manufacturer's recommended range of 0.200 to 0.500) may help to improve PCR sensitivity. Such an expansion of the borderline range for use with specimen swabs submitted dry for testing appears to be unnecessary. In addition, DFA testing of centrifuged specimen sediments can provide a rapid, relatively simple means of confirmation of PCR-positive results in many (but not all) cases, with patients or populations for whom confirmation is desired. The Centers for Disease Control and Prevention has recommended confirmation of positive *Chlamydia* results from other nonculture tests in specific clinical situations, but it has not yet addressed the need to confirm positive PCR results (5).

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