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Diff-Quik Stain as a Simplified Alternative to Papanicolaou Stain for Determination of Quality of Endocervical Specimens Submitted for PCR Detection of *Chlamydia trachomatis*

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The simple, rapid, two-step Diff-Quik stain procedure (Baxter Diagnostics) was compared with the Papanicolaou stain for microscopic determination of endocervical specimen quality. Results from 230 (98.7%) of 233 specimens stained by both methods indicated agreement between the two staining methods for detection of the endocervical cells or erythrocytes indicating specimen adequacy. By using the Amplicor *Chlamydia trachomatis* Test (Roche Diagnostic Systems) to detect *C. trachomatis* and the Diff-Quik stain to assess specimen adequacy, PCR-positive results were obtained from 147 (9.1%) of 1,615 microscopically adequate specimens but from only 13 (2.2%) of the 583 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 *Chlamydia trachomatis* Test (Roche Diagnostic Systems, Branchburg, N.J.). Although this PCR test can amplify target DNA fragments by more than 1,000,000-fold (2), the collection and testing of poorly obtained endocervical specimens, which were evaluated for specimen adequacy by the Papanicolaou (Pap) stain, have been associated with a significant reduction in PCR detection of the chlamydial pathogen (5). An adequate endocervical specimen is one which contains metaplastic cells, columnar or cuboidal endocervical cells, and/or large numbers of erythrocytes (1, 4, 6, 8).

Because of the impact of specimen quality on detection of *C. trachomatis* by a variety of different tests, the Centers for Disease Control and Prevention has recommended periodic cytologic evaluation of specimen quality when non-direct fluorescent-antibody assays are used to help improve specimen collection techniques (3). The Pap stain has been reported to be a reliable means of differentiating and identifying cells in endocervical specimens submitted for detection of *C. trachomatis* (4, 6). However, the procedure for Pap staining smears is time-consuming, involving multiple steps. When the slides are stained in a cytology laboratory, as they have been at this hospital, stain interpretations may not be available for 1 to 2 days after the smears are made and after the PCR tests have been completed. The overall cost associated with the preparation and reading of each Pap stain is approximately $2.07 (5).

The purpose of the present study was to evaluate the accuracy, speed, and relative costs of using the Diff-Quik procedure (Baxter Diagnostics, Inc., McGaw Park, Ill.) for determining endocervical specimen quality. A further objective of the study was to determine whether high-quality endocervical specimens, as determined by microscopic examination of a large number of Diff-Quik-stained slides, were associated with the significantly increased detection of *C. trachomatis* DNA that was previously reported for Pap-stained, high-quality specimens, compared with specimens lacking appropriate cells (5).

Collection and testing of specimens. Following removal of exocervical mucus, duplicate endocervical 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. Specimens were collected with Dacron swabs supplied with the STD Swab Specimen Collection and Transport kit (Roche). The two swabs were combined and sealed within a dry polypropylene tube for transport to the laboratory (5). Specimens were stored in the laboratory at 4 to 8°C for as long as 96 h prior to testing.

To establish the comparability of the Diff-Quik and Pap stains, 0.5 ml of 0.9% saline was added to each tube containing a pair of dry endocervical swabs. After being vortexed for 10 s, the swabs were discarded, 25 μl of each sample was smeared onto one well of two four-well glass slides (4), and the duplicate smears were air dried. Dried smears were fixed with the Pro-Fixx cytology fixative (Baxter). One of the duplicate smears from each specimen was Pap stained in the cytology division of the laboratory as previously described (6). The other smear was stained in the immunology division of the laboratory by the Diff-Quik method. For this procedure, each slide was dipped first into the Diff-Quik stain solution I (xanthene dye) five times for 1 s each and then into Diff-Quik stain solution II (thiazine dye) twice for 1 s each time. The slides were rinsed by being dipped five times in each of two jars of deionized water. The slides were air dried and coverslipped after application of mounting medium. All stains were microscopically analyzed at a magnification of 200× for the presence of endocervical cells or large numbers of erythrocytes that indicate specimen adequacy, as previously described (4, 6). One of the present authors read the Pap stains, while another read the Diff-Quik stains. When the stains were first read, neither microscopist was aware of the results of the microscopic analysis of the duplicate slides or the results from the PCR assays. When one of the duplicate stains from a specimen was interpreted as being microscopically adequate and the other was interpreted as being inadequate, a third microscopist evaluated both slides without knowing the initial findings. To determine whether endocervical specimen quality, as indicated from analyses of Diff-Quik-stained slides, had a significant impact on PCR detection of *C. trachomatis* DNA, the Diff-Quik stains were prepared as described above, except that 50 μl of each sample was smeared onto a slide.

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TABLE 1. PCR detection of *C. trachomatis* from microscopically adequate and inadequate specimens as determined by the Diff-Quik procedure

Population (*n*)	No. (%) of patients with the following stain interpretations		No. (%) of patients with PCR-positive results	
	Inadequate specimen	Adequate specimen	Inadequate specimen	Adequate specimen
Asymptomatic females from prenatal clinic (622)	139 (22.3)	483 (77.7)	4 (2.9)	36 (7.5)
Symptomatic females				
Outpatients (non-obstetrics and gynecology) (342)	132 (38.6)	210 (61.4)	1 (0.8)	21 (10.0)
Hospitalized (83)	28 (33.7)	55 (66.3)	1 (3.6)	2 (3.6)
Emergency department (603)	196 (32.5)	407 (67.5)	5 (2.6)	55 (13.5)
Family practice (258)	49 (19.0)	209 (81.0)	1 (2.0)	19 (9.1)
Private obstetrics and gynecology patients (290)	39 (13.4)	251 (86.6)	1 (2.6)	14 (5.6)
Total (2,198)	583 (26.5)	1,615 (73.5)	13 (2.2)	147 (9.1)

For PCR detection of *C. trachomatis*, 1 ml of Specimen Transport Medium (Roche) was added to each specimen tube after the 50-μl aliquot had been removed for staining (5). After incubation at 25°C for at least 10 m, 1 ml of specimen diluent (Roche) was added to each specimen tube and the specimens were PCR tested according to the manufacturer's instructions (7). The PCR test, including amplification, was repeated on all specimens with PCR-positive or borderline results. All PCR tests were carried out in the immunology division of the laboratory.

Statistical analysis of the results was performed by the Z test for differences in proportions for independent samples (9). A P value of <0.05 was selected as the minimum level of significance.

Comparability of the Diff-Quik and Pap stains. Of 260 endocervical specimens which were stained by both the Pap and the Diff-Quik methods, smears from 27 (10.4%) could not be compared because one or both smears were too thick or did not adhere to the slides. Of the 233 specimens which could be microscopically analyzed in duplicate, results from 230 (98.7%) indicated agreement between the Diff-Quik and Pap stain analyses as to specimen adequacy. Two specimens were interpreted as being microscopically adequate by the Pap stain but inadequate by the Diff-Quik procedure, while one specimen was found to be adequate by Diff-Quik analysis but inadequate by the Pap stain. In all, 195 (83.7%) and 196 (84.1%) of the 233 analyzed specimens were microscopically adequate by the Diff-Quik and Pap stain interpretations, respectively.

Specimen adequacy as determined from Diff-Quik stains versus PCR detection of *C. trachomatis*. Duplicate endocervical specimens were Diff-Quik stained and PCR tested from an additional 2,380 patients. Smears from 182 (7.6%) of the patients could not be microscopically analyzed because they did not adhere to the slides or were too thick. Of the 2,198 specimens which could be microscopically analyzed, 1,615 (73.5%) were microscopically adequate (Table 1). However, the adequacy of collected endocervical specimens ranged from only 61.4% for outpatients of non-obstetrics and gynecology physicians to 86.6% for the patients of obstetrics and gynecology physicians. Significantly more PCR-positive results were detected from the Diff-Quik-stained, microscopically adequate specimens than from specimens which were determined to be inadequate ($P < 0.001$ [Table 1]). If the rate of chlamydia detection from inadequate specimens had been the same as that (9.1%) from adequate specimens, then approximately 53 rather than 13 PCR-positive results should have been detected from the microscopically inadequate specimens. This indicates that the PCR sensitivity for *C. trachomatis* from inadequate specimens was only approximately 25% (or 13 of 53), compared with PCR detection of the pathogen from adequate specimens. Because of the low sensitivity of PCR for detection of *C. trachomatis* from microscopically inadequate specimens when the Pap stain was used to assess specimen adequacy (5), negative PCR results from inadequate specimens during the present study were not reported. Instead, the report indicated that the specimens were microscopically inadequate and that recollection was recommended.

The Diff-Quik staining procedure on batches of from 4 to 10 four-well slides was usually completed within 3 min. Because of the procedure's speed and simplicity, stains were made by the same group of immunology division technologists who performed the PCR assays, obviating the need to send slides to the cytology laboratory for Pap staining. Microscopic analysis of each Diff-Quik-stained smear usually required no more than 30 s, in part because erythrocytes were better preserved by this method than they were by the Pap stain procedure. The cost of material and labor for preparing and reading Diff-Quik stains was $1.44 per smear, or approximately 30% lower than the costs associated with the Pap stains.

The Diff-Quik stain proved to be a simple, rapid, cost-effective alternative to the Pap stain which was previously routinely used by this laboratory for determination of the quality of endocervical specimens submitted for detection of *C. trachomatis*. Diff-Quik staining allowed the laboratory to report the specimen quality, along with the PCR results, 1 to 2 days earlier than when slides were Pap stained in our cytology laboratory. The speed of reporting is particularly important when the PCR result is negative and the specimen quality is inadequate. The results of the current study also reemphasize that high-quality endocervical specimens are associated with significantly greater PCR detection of *C. trachomatis* than specimens lacking appropriate cells. It is not surprising that the skills of physicians and nurses who collect the specimens vary considerably, as documented from the current results, on the basis of the collectors' training and experience. For reasons of quality improvement, it is helpful to identify groups of specimen collectors who routinely obtain high-quality specimens and those who do not. Groups which are doing poorly can be targeted for additional training, which can be supplied, in our experience, by some of the individuals from groups which have consistently collected adequate specimens. Considering the costs of material and labor for PCR technology ($10.78 per test), the insensitivity of PCR when used on microscopically inadequate endocervical specimens, and the costs to society for undiagnosed, untreated *C. trachomatis* infections, routine or periodic microscopic analysis of endocervical specimens submitted for detection of the pathogen is an appropriate investment.

Statistical analysis of the results was done by Sally H. Cavanaugh.

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