Effect of Endocervical-Specimen Adequacy on Detection of *Chlamydia trachomatis* by the APTIMA COMBO 2 Assay

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Six hundred one endocervical specimens were analyzed for *Chlamydia trachomatis* by the APTIMA Combo 2 assay and evaluated for columnar epithelial cell adequacy by direct fluorescent-antibody staining. With 5.5% positive adequate and 7.8% positive inadequate specimens (*P* = 0.27), the study suggested no difference in positivity rates due to specimen adequacy when this amplified technology was used.

*Chlamydia trachomatis* infection is the most common bacterial sexually transmitted disease in the United States (7). In women, symptoms of *C. trachomatis* infection are usually mild or absent (15). The Centers for Disease Control and Prevention (CDC) has recommended widespread screening of women in an effort to control this epidemic (3). Many improvements in test technologies have evolved, including nucleic acid amplification tests (NAATs) (2, 5, 6, 8, 14, 16). However, since chlamydial intracellular organisms which infect columnar epithelial cells, inadequate specimens with few or no columnar cells have substantially impacted the sensitivity of testing for *C. trachomatis* antigens (9, 11). The value of increased sensitivity when using amplified testing to overcome inadequate specimens has not been confirmed in other studies with older NAATs (1, 9–13, 18). As a result, CDC has recommended monitoring of columnar cell content in endocervical specimens to assess specimen quality (4).

This study assessed whether specimen adequacy, based on the traditional presence or absence of columnar epithelial cells, significantly affected the detection of *C. trachomatis* by a more recent NAAT, the APTIMA Combo 2 assay (Gen-Probe, Incorporated, San Diego, CA). We believed the increased sensitivity of this second-generation amplification test, APTIMA Combo 2, could potentially overcome the necessity to measure cellular adequacy.

Six hundred one specimens were collected from women at two family planning clinics, two sexually transmitted disease clinics, and a community health center (sites A, B, and C). Asymptomatic and symptomatic females who presented at the centers were eligible based on an age criterion (age of 14 to 25 years) (17).

Following removal of exocervical mucus, two endocervical swabs were rotated simultaneously during collection. One swab was placed in an APTIMA swab transport tube (Gen-Probe, Incorporated, San Diego, CA). The second swab was used to prepare a Microtrak slide for a determination of cellular adequacy (Trinity Biotech, Jamestown, NY). Endocervical swab specimens were tested at the Wyoming Public Health Laboratory (WPHL) by APTIMA Combo 2 assay, the NAAT currently used by WPHL. Slides were identity unlinked and forwarded to Johns Hopkins University, Baltimore, MD, for evaluation by direct fluorescent-antibody (DFA) staining.

The Combo 2 assay was performed on 601 endocervical swab specimens according to the assay instructions. Specimens testing positive initially were retested a second time. Additionally, since the WPHL was participating in a separate but concurrent regional *C. trachomatis* study, aliquots of positive specimens were sent for testing with the stand-alone APTIMA CT assay (Gen-Probe, Incorporated, San Diego, CA), which uses an alternative gene target.

Slides were stained with a fluorescein-conjugated monoclonal antibody (Kallestad, Chaska, MN) and read by epifluorescence microscopy by medical technologists proficient in DFA microscopy. All 601 slides were assessed for the presence of chlamydial elementary bodies, columnar 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 cells, with or without other cells (9–11).

Based on slide evaluation, 422 (70%) were graded as adequate and 179 (30%) as inadequate. A total of 37 (6%) specimens were positive by Combo 2. Of 422 adequate specimens, 23 (5.5%) were *C. trachomatis* positive by Combo 2. Of 179 inadequate specimens, 14 (7.8%) were *C. trachomatis* positive by Combo 2 (Table 1). No significant difference in the positivity rates for adequate and inadequate specimens was found (*P* = 0.27). Only 7 of 601 (1.2%) were DFA slide positive, demonstrating typical fluorescent elementary bodies. These 7 were included in the 37 Combo 2-positive results. There were no discordant specimens in which Combo 2 was negative and DFA was positive. All 37 (100%) Combo 2 positives repeated positive. Thirty-four aliquots of the Combo 2-positive specimens were available and sent for testing with the stand-alone APTIMA CT assay. All 34 aliquots confirmed as positive for *C. trachomatis* by the APTIMA CT assay. A remnant sample was unavailable for 3 of the 37 Combo 2-positive specimens.

Specimen adequacy by clinic site averaged 70%. When examining the positivity rates of adequate versus inadequate groups within individual sites, numbers in each category were...
too small for statistical analyses. Site C routinely uses a large proctoscopic-type swab for a more thorough removal of exocervical mucus, exposing more of the cellular components. There was no increase in specimen adequacy resulting from this practice.

The accepted definition of adequacy has previously been based upon columnar epithelial cell presence (1, 9, 10, 18). However, the presence of erythrocytes (RBCs) may indicate cervical friability, a common finding for C. trachomatis infection (9, 18). Given this scenario, the study looked at the additional presence of RBCs in adequate and inadequate groups. There was no significant change in positivity for groups containing RBCs from that for groups without RBCs ($P = 0.28$).

The use of two swabs rotated simultaneously during collection eliminated the discrepancy which can occur when a single swab is used to collect a specimen for two testing platforms. Additionally, this collection method resulted in a total sample positivity of 6% (37/601), which is similar to the general 6% prevalence rate in the current population.

CDC has suggested supplemental testing after an initial positive NAAT screening in certain cases and in populations with a low prevalence of infection (4). One such approach suggested by CDC involves repeating the original test on the original specimen. In this study, all 37 Combo 2-positive specimens were repeat positive by the original test and the original specimen, thus confirming positive results by repeat testing. Another CDC option suggested additional testing of the original specimen with a different NAAT or one that uses an alternative target or format. Of the 34 Combo 2 samples with sufficient quantity for allocation and testing with the stand-alone APTIMA CT assay, all 34 (100%) verified as positive with this alternative-target method. Verification of adequate and inadequate positive specimens using both approaches further supported the finding of this study and suggested that specimen adequacy confirmation may not be necessary when the APTIMA Combo 2 assay is used, even in this low-prevalence population.

Our results differed from earlier studies with amplification tests, PCR (10, 18), and ligase chain reaction (12), which concluded that cellular adequacy was a determining factor when testing for the presence of C. trachomatis. We were able to detect C. trachomatis even when the specimen was graded as inadequate, based on the same measurement of cellular adequacy.

A potential limitation of the finding of no statistical difference in positivity rates between specimens graded adequate and those graded inadequate could be due to the lack of enough statistical power due to the small sample size. However, we feel our results point to the general conclusion that measurement of cellular adequacy may not be required when detecting C. trachomatis with this NAAT.

In conclusion, this study suggests that the cellular adequacy of endocervical specimens, when tested for the presence of Chlamydia trachomatis by using the APTIMA Combo 2 assay, did not appear to influence the positivity of the results. Regardless, submission of the best possible cervical specimen should always be a priority for clinicians who obtain endocervical samples for the diagnosis of C. trachomatis infection.

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