Volume Effect on Sensitivity of Nucleic Acid Amplification Tests for Detection of *Chlamydia trachomatis* in Urine Specimens from Females

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Nucleic acid amplification tests (NAATs) for the detection of *Chlamydia trachomatis* are routinely used on first-catch urine (FCU) specimens. We analyzed data from a head-to-head comparison of NAATs on female FCU specimens and found that the volume of urine collected could affect test performance.

*Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen worldwide. Approximately 90 million new cases of infection occur each year, with more than 3 million of them in the United States (3). Asymptomatic infections are common in both men and women. Currently, the Centers for Disease Control and Prevention recommend that sexually active teens and adults of ≥24 years of age be routinely screened for chlamydia. Thus, accurate laboratory tests that use noninvasive procedures for the diagnosis of chlamydia are needed.

Following the introduction of nucleic acid amplification tests (NAATs), the use of male and female first-catch urine (FCU) to detect *C. trachomatis* has become routine. Obtaining specimens of FCU for the detection of *Chlamydia* is both convenient and noninvasive. Ligase chain reaction (LCR; Abbott Laboratories, Abbott Park, Ill.), PCR (Roche Molecular Systems, Branchburg, N.J.), transcription-mediated amplification (TMA; Gen-Probe Inc., San Diego, Calif.), and strand displacement amplification (Becton Dickinson Inc., Sparks, Md.) are NAATs for the detection of chlamydia (2, 4, 5, 6). These NAATs have high sensitivity and specificity with urogenital specimens. However, the amount of FCU obtained from a patient can be a limitation. The package insert for each test indicates the manufacturer’s requirement for a specific FCU volume. These amounts are 15 to 20 ml for LCR, 10 to 50 ml for PCR, and 30 to 50 ml for TMA. Because of these specimen criteria, laboratories have either rejected FCU based on the amount of urine collected or performed the assay with a disclaimer about the reliability of results due to the submission to the laboratory of an incorrect FCU volume. We analyzed data from a head-to-head comparison of three NAATs to examine the effect of FCU volume on assay performance (1).

A total of 655 females were seen at a San Francisco sexually transmitted disease clinic. Patients were either symptomatic or asymptomatic and had not urinated within the previous 2 h. This study was conducted in 1997 as part of the Centers for Disease Control and Prevention 455 multicenter NAAT evaluation. Females were given sterile collection cups that were marked at 30 ml with a black line. Each patient was instructed not to clean her urethral-vulval area and to collect approximately 30 ml or less of FCU (i.e., to fill the collection cup to a level at or below the indicated line). All samples were held at 4°C until they were transported to the laboratory. LCR and PCR were done within 4 and 7 days of collection, respectively. FCU specimens for TMA were frozen at −70°C and tested 6 months later. Specimens were not tested for NAAT inhibitors. We followed the manufacturer’s instructions for each of the NAATs (LCR, PCR, and TMA). All tests were performed under blind conditions.

Test results that were positive with at least two of three NAATs were defined as true positives. We examined the sensitivities of the tests and prevalence of *C. trachomatis* as stratified by FCU volume ranges of ≤20, 21 to 30, 31 to 50, and 51 to 90 ml. Cochran-Armitage one-sided tests for trend were used to examine NAAT sensitivity and prevalence stratified by urine volume categories (SAS version 8.2e; SAS Institute, Inc., Cary, N.C.). *C. trachomatis* prevalence by NAAT was 6.3% (41 of 655 specimens). Mean FCU volume was 44.4 ml (±18.1 ml), with a range of 10 to 90 ml and a median of 40 ml for 655 specimens. Only 31% (201 of 655) of patients provided <30 ml of FCU. The sensitivity of the NAATs was 92.7, 100, and 87.8% for LCR, PCR, and TMA, respectively. Table 1 shows NAAT sensitivities stratified by FCU volume. PCR performed equally well at all volumes. In contrast, low LCR performance was observed at ≤20 ml (60% sensitivity). The opposite trend was seen with TMA; sensitivity decreased as FCU volume increased. At high FCU volumes (≥50 ml), TMA sensitivity was 57%. For all NAATs, the highest number of *C. trachomatis* infections were found in the 21- to 30-ml FCU volume range. With TMA, *C. trachomatis* prevalence decreased significantly as urine volume increased.

We found that only 31% of females were able to give <30 ml of FCU despite our request for that volume. Although the number of positives analyzed in this study was small, our data show some interesting trends. LCR had lower sensitivity with samples with ≤20 ml of FCU collected and improved sensitivity as the FCU volumes increased. This conflicts with the LCR product insert requirement of 15 to 20 ml of FCU for testing.
This FCU volume criterion might not be optimal for the LCR test. With TMA, higher FCU volumes (>50 ml) yielded lower sensitivity. Consequently, for TMA the rejection of specimens on the basis of excess FCU volume may be prudent. While a requirement for 30 to 50 ml of FCU is stated in the package insert, we obtained a sensitivity of 89% with this amount and saw better performance at lower FCU volumes (<30 ml) for TMA. Whether false-negative results are due to dilution of target or inhibition remains to be answered. But it is tempting to speculate that when an assay’s sensitivity decreases as the FCU volume increases, it is due to target dilution. If the reverse result is observed, it may be that the assay is more sensitive to inhibitors that are diluted out as volume increases. Internal controls were not used in these assays; therefore, we could not assess inhibition. This evaluation is a post hoc analysis and the original Centers for Disease Control and Prevention 455 study was not designed to assess the effect of urine volume on test sensitivity. Further studies are needed to validate our results and also to determine whether FCU volume variation has similar effects on NAAT performance for males. Because FCU is a standard specimen for screening, it will be important to know whether the volume of this specimen affects assay sensitivity. This concept holds true for all NAATs and is still relevant in the face of the recent withdrawal of LCR.

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REFERENCES


