Optimal Method of Collection of First-Void Urine for Diagnosis of Chlamydia trachomatis Infection in Men

Craig A. Wisniewski, John A. White, Claude-Edouard C. Michel, Lourdes Mahilum-Tapay, Jose Paolo V. Magbanua, Elpidio Cesar B. Nadala, Jr., Penelope J. Barber, Beng T. Goh, and Helen H. Lee

Department of Haematology, University of Cambridge, Cambridge CB2 2PT, Department of Genitourinary Medicine, St. Thomas’ Hospital, Lambeth Palace Road, London SE1 7EH, Brook Advisory Centre, Birmingham B1 1BL, and Barts and the Royal London NHS Trust, Whitechapel, London E1 1BB, United Kingdom

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First-void urine (FVU) is the preferred specimen for the diagnosis of urogenital Chlamydia trachomatis infection in men. We have developed FirstBurst, a urine collection device that collects the first 4 to 5 ml of FVU and yields a specimen with a sixfold higher C. trachomatis organism load than the regular urine cup by quantitative PCR (32,533 versus 5,271 plasmids/ml; P < 0.0001). Consequently, the use of FirstBurst to collect a urine sample improved the sensitivity of a rapid test for Chlamydia over testing of samples collected with a urine cup (82 versus 47% sensitivity using PCR as a reference; P < 0.0015).

Urogenital infection with Chlamydia trachomatis is the most commonly reported bacterial sexually transmitted infection and continues to be a major public health problem worldwide (5, 17). Untreated C. trachomatis infections can lead to serious sequelae such as pelvic inflammatory disease in women and epididymitis in men (5). A large proportion of infected individuals either have mild symptoms or are asymptomatic (2). Thus, screening of high-risk populations has been recommended (4).

The use of the invasive and painful urethral swab is a major barrier to screening of asymptomatic men and is a key factor in male nonattendance at genitourinary medicine clinics (7, 16). However, the development of highly sensitive nucleic acid amplification tests (NAATs) has allowed the use of first-void urine (FVU) for C. trachomatis diagnosis. FVU has become the specimen of choice for the diagnosis of urethral C. trachomatis infection in men by NAATs, since it is noninvasive and yet allows the detection of infected epithelial cells and associated C. trachomatis particles (3, 9). The volume of FVU has been defined arbitrarily as the first 20 to 30 ml of the initial flush of urine, which is thought to contain the highest concentration of diagnostically relevant components, such as C. trachomatis elementary bodies or antigens or inflammation-related enzymes (6). However, the optimal volume for an FVU specimen is unknown, and early attempts to study the organism load in FVU fractions have encountered difficulties due to the lack of a suitable sample collection device. At present, collection volumes for FVU ranging from 5 to 40 ml for men (8, 15) and from 10 to 50 ml for women (13) are deemed to be acceptable samples.

We have developed a novel FVU collection device called FirstBurst (10), which allows the convenient collection of the first 4 to 5 ml of FVU without dilution by the subsequent urine stream (Fig. 1A). We compared the C. trachomatis organism loads of FirstBurst- and urine cup-collected specimens and evaluated the sensitivity of a novel rapid test for Chlamydia using these two urine specimen types. In addition, we quantified the organism loads in four fractions of FVU from C. trachomatis-infected men by using a tandem of FirstBurst devices to further define the C. trachomatis load of the FVU specimen.

Participants for the study comparing C. trachomatis loads between the FirstBurst device and the urine cup and for the study comparing the performances of FirstBurst- and cup-collected specimens on the Chlamydia Rapid Test (CRT) were recruited between September 2005 and December 2006 from the Ambrose King Centre at the Royal London Hospital and from the Brook Advisory Centre, a sexual health service for young people in Birmingham, United Kingdom. Male patients at least 16 years old, who had not been on antibiotics for the previous month and who were able to understand the instructions, were recruited to the study. Participants from both sites were surveyed about the two urine collection methods by use of a written questionnaire administered after the provision of both urine specimens.

Participants for the FVU fractionation study were recruited in London between November 2005 and December 2006 from the Lydia Clinic at St. Thomas’ Hospital, the Lloyd Clinic at Guy’s Hospital, and the Ambrose King Centre. The participants were asymptomatic male patients at least 16 years old whose FVU specimens had tested positive for C. trachomatis by the ProbeTec ET assay (Becton Dickinson Diagnostic Systems, Sparks, MD) within the previous 6 weeks but who had not yet been treated for their infections and were not taking antibiotics.
Ethical approval for the three studies was granted by the Guy’s and St. Thomas’ Hospital Research Ethics Committee, the Moorfields and Whittington Research Ethics Committee, and the Brook in Birmingham Research Ethics Committee. Written informed consent was obtained from all participants, and the clinical research guidelines for the relevant institutions were followed in the conduct of this research. The number of patients who were ineligible or declined participation in each study was not recorded.

For the FirstBurst-versus-cup studies, participants were randomized to provide the first FVU specimen with either FirstBurst or the routine clinic 50-ml urine cup (Fisher Scientific, Loughborough, United Kingdom) after not having urinated for at least 2 h. After another 2-h interval, the same participants provided a second urine sample with the alternative collector. Specimens were capped, sealed in separate bags, and then stored at 4°C prior to transport to the laboratory within 3 days. At the laboratory, the specimens were stored at 4°C before being tested for *C. trachomatis* both by the Amplicor CT/NG PCR assay (Roche Molecular Systems, Branchburg, NJ) and by the CRT within 2 days of collection. Portions (0.5 ml) of the PCR-positive samples were stored at −20°C prior to quantitative PCR (qPCR) analysis.

For the FVU fractionation study, the collection system (Fig. 1B) comprised three FirstBurst devices that were aligned in tandem vertically and that drained into a 500-ml urine container (Sarstedt, Leicester, United Kingdom). After not having urinated for at least 1 h, each participant was verbally instructed to urinate directly into the funnel of the first (top) collector and to continue urinating without interruption until he had voided his bladder. The individual specimen containers were capped and sealed in separate bags and were then stored at 4°C until their transport to the laboratory within 6 days of collection. When they arrived at the laboratory, the volume of urine in each fraction was measured, divided into 0.5-ml portions in microcentrifuge tubes, and stored at −80°C. Each of the three FVU fractions as well as the residual urine was tested initially for the presence of *C. trachomatis* DNA by the Amplicor CT/NG PCR assay prior to qPCR analysis.

The stored urine samples, which had been frozen only once, were thawed to room temperature prior to qPCR analysis. The FVU specimens for qPCR analysis were extracted as described by Magbanua et al. (11). qPCR was performed using the method of Pickett et al. (14) using amplification conditions described previously (12). All qPCR analysis was performed within 30 days of specimen collection.

The *Chlamydia* Rapid Test is a dipstick immunoassay developed at the University of Cambridge that targets *Chlamydia* lipopolysaccharide. Three milliliters of FVU were assayed with the CRT as described previously (11), except that the FVU sample was diluted with 3 ml instead of 6 ml of water (Sigma, St. Louis, MO).

Statistical analysis was performed with SAS (version 9.1) software. Confidence intervals (CI) were calculated as exact binomials. The geometric mean of the *C. trachomatis* load and its respective standard deviation and 95% CI for the various specimen types were calculated from the natural log transformation of the organism load obtained for each corresponding sample. Group comparisons were performed by analysis of variance or the chi-square test. A *P* value of <0.05 was considered statistically significant.

We analyzed the *C. trachomatis* load by qPCR for 85 infected men selected by screening 1,002 individuals who provided both FirstBurst- and cup-collected urine. The distribution of organism loads according to the method and order of FVU collection is shown in Fig. 2. The mean organism loads were 32,533 (95% CI, 19,536 to 54,176) and 5,271 (95% CI, 3,103 to 8,955) plasmids/ml for the FirstBurst device and the urine cup, respectively. FirstBurst-collected specimens had an organism load 6.3 (95% CI, 5.0 to 7.9) times that of cup-collected specimens (*P* < 0.0001). Whether the FirstBurst sample was collected first (*n* = 38) or second (*n* = 47) did not significantly affect the geometric mean of the organism load (*P* = 0.5337). A correlation in the organism load was observed between samples collected from the same individual, in that those with high organism loads in the first specimen also tended to have high loads in the second specimen, regardless of the collection method.

Amplicor CT/NG PCR data were also available for both FirstBurst and urine cup samples in 62 of the 85 *C. trachomatis*-positive specimen pairs. The mean optical densities obtained by Amplicor CT/NG PCR with these 62 samples were 3.07 (95% CI, 2.79 to 3.36) and 2.50 (95% CI, 2.17 to 2.82), respectively (*P* = 0.0005).

In a further study to assess the performance of the CRT relative to the Amplicor CT/NG PCR assay, we performed *C. trachomatis* testing on an additional 534 randomly collected pairs of FirstBurst and cup specimens from male attendees at the Brook Advisory Centre. *C. trachomatis* was detected by the PCR assay in specimens from 34 individuals (positivity rate, 6.4%; 95% CI, 4.3 to 8.4%); for 33 of these 34 individuals, both the FirstBurst- and the cup-collected urine were PCR positive, whereas for the remaining individual, the FirstBurst specimen was PCR positive but the cup specimen was PCR negative. The
CRT yielded positive results for 16 of the 34 urine cup samples from PCR-positive participants, giving a sensitivity of 47% (95% CI, 30 to 64%) for cup-collected urine. In contrast, it yielded positive results for 28 of the 34 FirstBurst-collected samples from the PCR-positive subjects, giving a sensitivity of 82% (95% CI, 70 to 95%). The difference in CRT sensitivity between cup-collected and FirstBurst-collected specimens was statistically significant (P = 0.0015). The overall specificity of the CRT was 98.8% (95% CI, 97.9 to 99.8%) with reference to the PCR positivity of either type of specimen (494/500).

To further understand the distribution of organism loads within the FVU fraction, we analyzed the C. trachomatis loads of the four specimen fractions from 31 male participants. The mean volumes collected for fractions FVU1, FVU2, and FVU3 were 4.6 ml (range, 3.9 to 5.6 ml), 4.6 ml (range, 3.8 to 5.4 ml), and 4.6 ml (range, 3.7 to 5.4 ml), respectively. The volume of residual voided urine (RVU) ranged from 20 to 405 ml (geometric mean, 105 ml). The mean total C. trachomatis load of the entire voided specimen was 32,533 (95% CI, 19,536 to 54,176) and 5,271 (95% CI, 3,103 to 8,955) plasmids/ml for the FirstBurst device and the urine cup, respectively.

A user survey (n = 249) conducted after the completion of specimen collection showed that 90% of the participants preferred FirstBurst to a urine cup for collection of their FVU samples, primarily because of the ease of use of the device.

FirstBurst is a patented disposable urine collection device that was developed to facilitate the consistent and convenient collection of the first 4 to 5 ml of FVU into a separate specimen tube without subsequent mixing or dilution by the rest of the urine stream. The excess urine is diverted through an overflow system that allows the patient to finish urination without moving the device out of the way. The device was developed to optimize the performance of the CRT and to make the sampling process as simple as possible. The present study showed that the device allowed easy and accurate collection of the first 4 to 5 ml of the urine stream, which contained the highest load of C. trachomatis. Collection of this small volume of FVU for testing substantially improved the sensitivity of the immunoassay-based CRT from 47% to 82%; the performance of this test is directly related to the concentration of the chlamydial organisms in the urine specimen. This finding shows that, for C. trachomatis, the initial flush of the urethra with the first few milliliters of urine provides a diagnostic sample with a target concentration significantly higher than that obtained after dilution with the subsequent 5 to 45 ml of voided urine. The fractionation data demonstrate that the C. trachomatis load decreases substantially beyond the first 4 to 5 ml of FVU. Furthermore, this initial fraction contained ~60% of the total chlamydial plasmid load in the entire voided specimen. The qualitative results of highly sensitive NAATs are less affected by organism load; nevertheless, the higher C. trachomatis load seen in FirstBurst device-collected FVU might provide a diagnostic advantage for specimens with organism loads near the cutoff detection limits of individual NAATs. Indeed, in the present CRT performance study, 1 of 34 specimens collected with the FirstBurst device tested positive for C. trachomatis by the Amplicor CT/NG PCR assay, whereas the matched urine cup specimen from the same infected individual tested negative by the PCR assay. The significantly higher signals we obtained by Roche Amplicor PCR using FirstBurst-collected urine compared to those for cup-collected urine further demonstrate this diagnostic advantage. A more efficient urine collection method such as the FirstBurst device could potentially improve the sensitivity of certain C. trachomatis NAATs, particularly for samples with low organism loads that are at the detection limit.

The C. trachomatis positivity rates of 8.5 and 6.4% for the participants in the two FirstBurst-versus-cup studies are consistent with values reported previously for similar populations in the United Kingdom (1). Participants in the FVU fractionation study were recruited on the basis of their untreated C. trachomatis infections. All had been asymptomatic at the time of initial testing, and ~40% of these individuals had urethral smears performed that showed no urethritis. Given that the load of C. trachomatis in FVU from men is positively associated with symptoms, clinical signs, and positive urethral smears (12), it might not be possible to extrapolate the FVU fractionation data directly to all men with symptomatic infections.
However, given that most men with urethral *C. trachomatis* infections are asymptomatic, our findings are likely applicable to screening of the general population. In addition, the mean *C. trachomatis* load in the FVU1 fraction from the fractionation study did not differ significantly from that in the FVU sample collected with the same device from infected men in the FirstBurst-versus-cup quantitation study (38,561 versus 32,533 plasmids/ml, respectively; \( P = 0.7334 \)), suggesting that the two study populations had similar overall organism loads.

The FVU specimens were collected in the present study with the requirement that participants hold their urine for \( \geq 1 \) or \( \geq 2 \) h since the last voiding. Further study is needed to ascertain whether or not this wait is necessary with FirstBurst-collected specimens in order to maintain optimal sensitivity for diagnosis of urogenital *C. trachomatis* infections in men, either with rapid tests or with NAATs. Finally, the organism loads of other urethral pathogens, such as *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Mycoplasma genitalium*, may also be maximal in the first few milliliters of urine. Further studies are thus warranted to determine whether collection of FVU specimens with devices such as FirstBurst improves the performance of diagnostic tests for these infections.

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REFERENCES