

An Automated Sample Preparation System for *Chlamydia trachomatis* in Urine Samples

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Genital infection of *Chlamydia trachomatis* is the most common bacterial sexually transmitted disease in the western world.¹ *C. trachomatis* is an obligate intracellular bacteria of which certain serotypes (D–K) may cause genital tract infections in both men and women. This highly contagious disease has its highest prevalence among teenagers and young adults under the age of 25. In the United States, as many as 3 million new cases are reported every year.² However, the number infected may actually be much higher, since approx. 50% of infected men and 85% of infected women do not show any obvious symptoms.³ The largely asymptomatic nature of many *C. trachomatis* infections is likely the main factor contributing to the wide distribution of this bacterium.⁴ When diagnosed, *C. trachomatis* can be easily treated and cured with antibiotics. Untreated, genital chlamydia may have serious consequences for women, causing chronic pelvic pain, pelvic inflammatory disease (PID), ectopic pregnancy, and infertility regardless if any symptoms are experienced.⁵ Statistics show that the chance of infertility increases with the number of infections, and in addition, transmission from mother to child during labor can cause pneumonia or trachoma in the infant.⁶ Clearly, screening for *C. trachomatis* is important to reduce the number of infections. Thus, in many countries, various screening programs, focusing on young adolescents in particular, are now being initiated in an attempt to control and reduce the number of cases.

Diagnostic system for *C. trachomatis*

Although *C. trachomatis* elementary bodies are found in urine, the use of cervical or urethral swabs is still the preferred type of sampling method for chlamydia diagnosis.⁷ However, collecting cervical and urethral swab samples requires the assistance of a physician and involves a considerable amount of discomfort for the patient. For women, the use of vaginal swabs represents a potential solution to this problem. However, to reduce the occurrence of *C. trachomatis* infections, a surveillance program also needs to include men. Consequently, using a less invasive sampling method such as urine samples instead of swabs for diagnosis is an obvious advantage or even a requirement to obtain control over this disease. In recent years, several nucleic acid

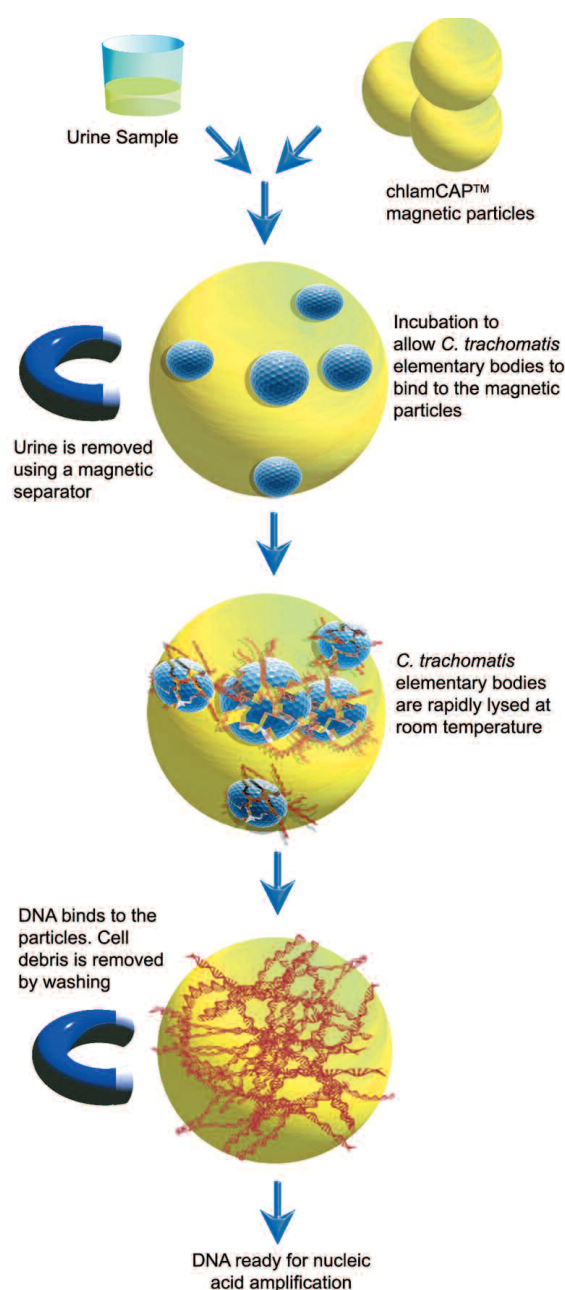


Figure 1 Principle of the chlamCAP sample preparation system for *C. trachomatis* elementary bodies in urine samples.

based tests (NAT) have been successfully applied for the diagnosis of *C. trachomatis*. The most commonly used systems are the BDProbeTec™ ET (Becton Dickinson, Sparks, MD), which uses strand displacement amplification (SDA); the Cobas Amplicor (Roche Diagnostic Systems, Basel, Switzerland), which uses polymerase chain reaction (PCR); and the APTIMA Combo 2 (Gen-Probe Inc., San Diego, CA), which uses transcription-mediated amplification (TMA). All

of these systems have a sensitivity between 87 and 99% and a specificity of over 99%.⁸ Protocols for isolating *C. trachomatis* nucleic acids from both swab and urine samples exist for all of these systems. However, in general, the processing of urine samples requires more hands-on time and is characterized by multistep procedures to isolate bacterial nucleic acid prior to amplification, as compared to swab samples. More importantly, a major obstacle to using urine samples for diagnostic purposes is the presence of NAT inhibitors in urine, which may produce false-negative results for the detection of *C. trachomatis* nucleic acids.⁸ For instance, for the BDProbeTec ET system, an inhibition rate from 1.9 to 8% using urine samples has been reported. Thus, an efficient method for sample preparation of urine samples that is less labor intensive and simultaneously avoids problems with inhibition of the amplification process has long been needed within chlamydia diagnostics.

Automated sample preparation of *C. trachomatis* from urine

Genpoint AS (Oslo, Norway) has developed a sample preparation system for the isolation of bacteria and their DNA from complex samples, with subsequent analysis using various nucleic acid amplification techniques.^{9,10} The system, in which cell and DNA isolation are integrated into one process using uniquely coated magnetic particles, has been automated and further developed to efficiently capture *C. trachomatis* elementary bodies (the chlamCAP™ system). Using the specially developed particles, it is possible to capture elementary bodies directly from urine. Initially, *C. trachomatis* elementary bodies are adsorbed to the magnetic particles and magnetically separated from the sample matrix, removing inhibiting substances. Following rapid lysis at room temperature, released DNA is adsorbed onto the same particles. After washing, the purified DNA is transferred to new tubes for NAT analysis. Figure 1 illustrates the principle of the system.

Since cell isolation and DNA purification are integrated in a single process, and isolation is based on paramagnetic particles, the procedure is well suited for automation. This increases reproducibility and throughput of the diagnostic

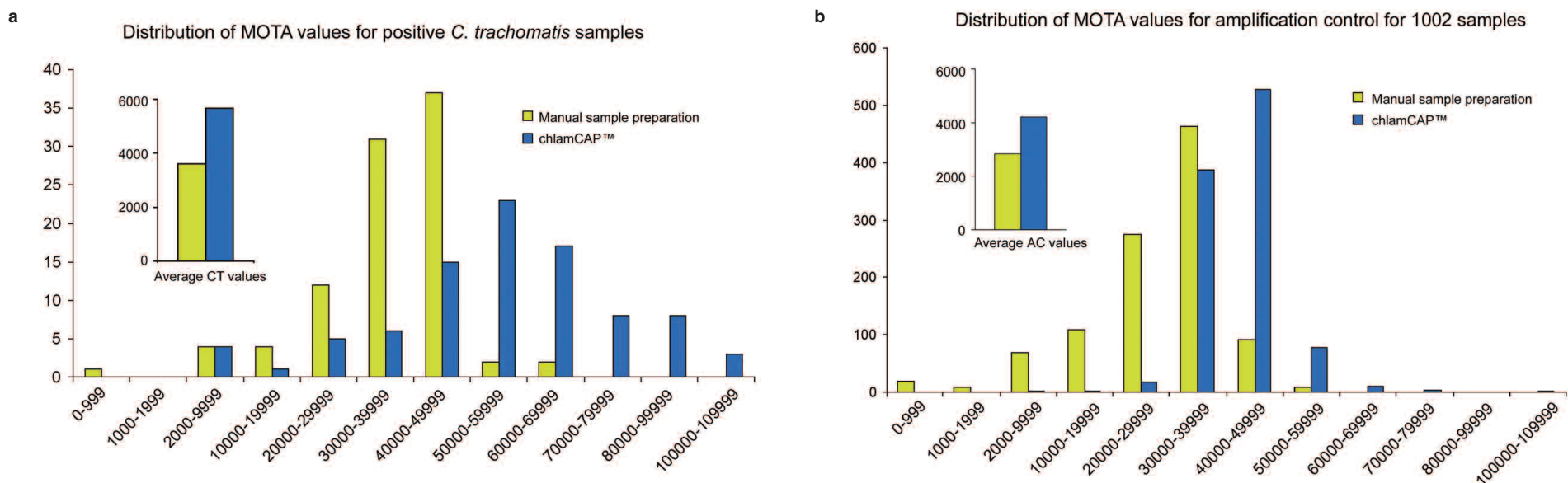


Figure 2 Histograms showing the distribution of MOTA values obtained using the chlamCAP automated system compared to manual sample preparation combined with detection by strand displacement using the BD ProbeTec ET system. a) Distribution of the MOTA values obtained for samples positive for *C. trachomatis*. b) Distribution of MOTA values obtained for the AC for all urine samples analyzed for *C. trachomatis*.

procedure and at the same time reduces hands-on time for laboratory personnel considerably. In collaboration with Tecan Systems (San José, CA), Genpoint has adapted a pipetting robot (the Miniprep 75) with the capacity of processing 48 samples in approx. 2 hr. The robot is equipped with a magnetic separator that allows the instrument to perform the entire chlamCAP isolation procedure. The flexibility of the robotic platform allows for adaptation to virtually any downstream system for NAT detection. Currently, the robot is set up to deliver isolated DNA directly into reaction wells for strand displacement amplification (BDProbeTec ET) or PCR.

Experimental

In an extensive study, 1002 urine samples were analyzed for the presence of *C. trachomatis* using the automated chlamCAP isolation system combined with the BDProbeTec ET final detection system. All samples were also analyzed using full BDProbeTec ET analysis with manual sample preparation. In the BDProbeTec ET, instrument values are given as MOTA (Method Other Than Acceleration) values. Thus, samples with MOTA values $\geq 10,000$ are considered positive, samples with MOTA values < 2000 are considered negative, and samples with values between 2000 and 9,999 are in the gray zone and were rerun for verification. For urine samples, it is common practice to also run an external amplification control (AC) for each sample to detect any potential inhibition of the SDA reaction. Figure 2a presents a histogram showing the MOTA values for *C. trachomatis*-positive urine samples obtained by SDA in the BDProbeTec ET instrument following the automated chlamCAP sample preparation compared to the manual procedure. The number of positive samples obtained with chlamCAP was in accordance with the results using the manual sample preparation. However, average MOTA values obtained using chlamCAP were on average 57% higher than with manual sample preparation. Moreover, no inhibition of the amplification reaction (MOTA value for amplification control below 1000) was observed using the chlamCAP system (Figure 2b), whereas an inhibition rate of

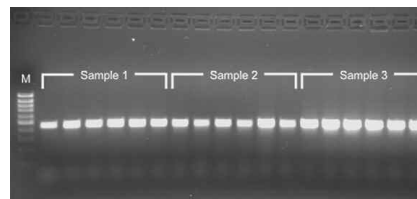


Figure 3 Agarose gel electrophoresis of PCR products generated by amplification using the automated chlamCAP system for isolation of *C. trachomatis* DNA from urine samples.

1.9% was observed for the manual sample preparation. Average MOTA values obtained for the amplification control were 42.030 and 28.351 for chlamCAP and manual sample preparation, respectively. These results strongly suggest that an amplification control for each sample may be omitted when combining the BDProbeTec ET detection system with this magnetic particle based system for sample preparation.

The chlamCAP system for sample preparation may also be readily combined with other downstream NAT-based detection systems such as PCR. Figure 3 shows an example of PCR products generated from positive *C. trachomatis* samples prepared using the automated system. The figure shows three different urines samples isolated in six parallels using the Miniprep 75, demonstrating the high level of reproducibility provided by the system.

Conclusion

The automated chlamCAP sample preparation system provides diagnostic laboratories with a robust method for testing *C. trachomatis* using urine as sample material. Automation of DNA preparation prior to NAT analysis considerably reduces hands-on time, eliminates the need for centrifugation and the hazards associated with certain manual sample preparation methods, and minimizes the possibilities for variability in test results due to errors in manual handling. These clear improvements will help promote the choice of urine as sample material for *C. trachomatis* testing, alleviating the need for a physician performing sample collection, therefore improving the feasibility of population screening. Thus, the automated chlamCAP system can contribute to reducing the extent of the *C. trachomatis* epidemic.

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