Automated purification of DNA from urine for sensitive PCR of *Chlamydia trachomatis* amplicons

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DNA was purified from 130 urine research samples using a fully automated protocol on the BioRobot® M48 workstation together with the MagAttract® DNA Mini M48 Kit. Purified DNA performed well in a real-time PCR assay being developed for the amplification of *Chlamydia trachomatis* DNA. Comparison of this easy-to-use procedure with a commercial *C. trachomatis* assay and manual DNA purification showed identical sensitivity.

Chlamydia trachomatis is a major cause of sexually transmitted disease in humans (1). Infection in women is frequently asymptomatic and untreated infections may progress to infertility and pelvic inflammatory disease (PID) (2). Development of sensitive and reproducible assays for clinical and life science research of *C. trachomatis* is essential to monitor and help prevent further spread of this pathogen.

False-negative results are a risk due to both the low pathogen titers obtained from asymptomatic individuals, and the PCR inhibitors found in urine. Reliable and efficient DNA purification and PCR systems limit the possibility for false-negative results, and an internal control DNA (ICD) for co-amplification makes it possible to distinguish between PCR failure and true negative results (3).

Material and methods

Aliquots of urine (1 ml) were centrifuged for 5 minutes at 3000 rpm to pellet cells and debris. Pellets were resuspended in 200 µl Buffer G2 and purification of DNA was automated using the BioRobot M48 workstation in combination with the MagAttract DNA Mini M48 Kit using App. Package, M48, Infectious Disease and the Bact_200ul protocol.

C. trachomatis DNA was amplified in 25 µl real-time PCR using 5 µl purified DNA as template. A *C. trachomatis*-specific probe (FAM™) and a probe specific for the internal amplification control (Yakima Yellow™) were used to detect and identify amplified DNA. Real-time PCR of DNA purified using the BioRobot M48 workstation was performed in duplicate to demonstrate reproducibility.

Sensitive and Reproducible Assay and Control PCR Analysis

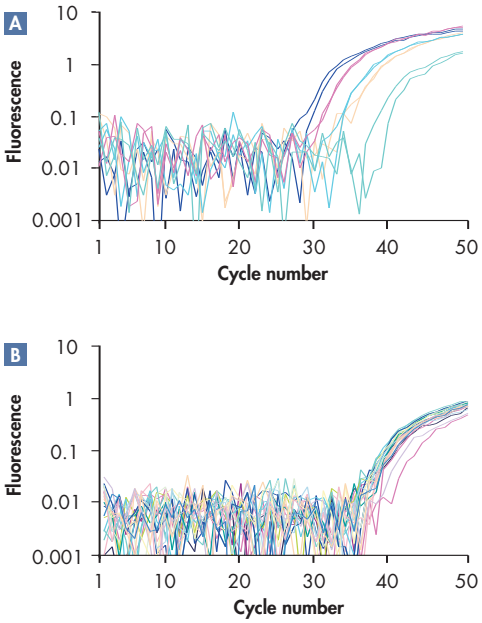

Figure 1 Amplification plots of DNA purified using the BioRobot M48 workstation. Data shown was generated from samples 1–16 (Table 1). A 5 of 16 samples testing positive for *C. trachomatis* genomic DNA B 11 of 16 samples testing negative for *C. trachomatis* genomic DNA, but showing positive amplification of control DNA.

Automated DNA purification using the BioRobot M48 workstation and real-time PCR of *C. trachomatis* DNA was compared against manual DNA purification and an established, commercial assay (Supplier R) for the amplification and detection of *C. trachomatis* DNA.

Results

Of the 130 samples tested and compared to the established *C. trachomatis* assay system, only a representative selection of data from 16 samples is shown (Table 1). The 2 systems gave identical results for all samples, as did duplicate amplifications.

Internal control DNA is only amplified when template DNA is not, as the amplicon is larger and control DNA is present at a very low concentration. A true negative test result is confirmed by a positive control-amplification. Negative results in both test and control amplifications indicate the presence of PCR inhibitors. Both test and control amplicons are amplified using the same primers (3).

DNA purified using the BioRobot M48 workstation performed well in real-time PCR and gave results identical to those obtained using the established assay system (Figure 1). Positive results were observed from samples with both high and low counts of *C. trachomatis*, and all negative test results were accompanied with positive amplification of control DNA, indicating efficient purification and a lack of PCR inhibitors.

Table 1. High PCR Sensitivity Equivalent to a Commercial Assay with DNA Purified Using the BioRobot M48 Workstation

	Real-time PCR analysis*		
Urine sample	Manual DNA purification and commercial *C. trachomatis* assay†	BioRobot M48 DNA purification method and real-time PCR of *C. trachomatis* DNA	BioRobot M48 DNA purification method and real-time PCR of control DNA
1	–	–	+
2	–	–	+
3	–	–	+
4	+	+	–
5	–	–	+
6	–	–	+
7	–	–	+
8	+	+	–
9	+	+	–
10	–	–	+
11	–	–	+
12	+	+	–
13	+	+	–
14	–	–	+
15	–	–	+
NTC	–	–	+

* + = positive (amplicon detected); – = negative (amplicon not detected); NTC = control amplification.

† Supplier R.

Conclusions

This study demonstrates an efficient automated system for the isolation of *C. trachomatis* DNA from urine samples. The BioRobot M48 workstation and the MagAttract DNA Mini M48 Kit offer:

- **High-performance DNA** — suited to downstream applications such as real-time PCR
- **Reproducible real-time PCR** — using inhibitor-free template DNA
- **Equivalent sensitivity** — compared to manual purification and an established commercial assay
- **Reduced workload** — with less hands-on time and a more efficient workflow compared to manual methods

References

1. Centers for Disease Control (1985) *Chlamydia trachomatis* infection. Policy guidelines for prevention and control. Morb. Mortal. Wkly. Rep. **35**, 535.
2. Brunham, R.C., Maclean, I.W., Binns, B., and Peeling, R.W. (1985) *Chlamydia trachomatis*: its role in tubal infertility. J. Infect. Dis. **152**, 1275.
3. Betsou, F., Beaumont, K., Sueur, J.M., and Orfila, J. (2003) Construction and evaluation of internal control DNA for PCR amplification of *Chlamydia trachomatis* DNA from urine samples. J. Clin. Microbiol. **41**, 1274.

Ordering Information

Product	Contents	Cat. no.
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps: MagAttract Suspension B, Buffers, Proteinase K	953336
App. Package, M48, Infectious Disease	Software protocol package for infectious disease applications, v. 2.0, on the BioRobot M48 workstation	9016145
BioRobot M48	Robotic workstation for automated purification of nucleic acids using MagAttract M48 Kits; installation, 1-year warranty on parts and labor	9000708

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