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A novel gel-based method for self-collection and ambient temperature postal transport of urine for PCR detection of *Chlamydia trachomatis*

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ABSTRACT

Objectives: The aim of this study was to develop a novel urine transport method to be used in self-collection-based screening for *Chlamydia trachomatis*. The method needed to be suitable for *C trachomatis* PCR detection, be economical and suitable for transport by standard envelope mailing.

Methods: An anhydrous gel composed of super-absorbent polymer and buffering agent was used to desiccate urine into a dry granular state, which could subsequently be reconstituted upon arrival at a laboratory. DNA was then extracted from the reconstituted solution using the Roche MagNA Pure protocol for the detection of *C trachomatis* by PCR. Collections of urine specimens from three populations with widely differing chlamydia prevalence (100%, n = 56; 47%, n = 70; 3%, n = 97) were used. We determined the gel method's impact on *C trachomatis* PCR sensitivity and specificity using neat and gel-processed urine specimens. An equine herpes virus PCR was used to test for assay inhibition.

Results: Overall, the sensitivity of the gel-based method ranged from 94.6–100% compared with neat urine, with a specificity of 100%. No PCR inhibition or decrease in analytical sensitivity was observed using the gel-processed extracts.

Conclusions: The gel-based method was found to be suitable for the detection of *C trachomatis* by PCR. In addition, its ease of use, effectiveness at ambient temperature and low cost makes it well-suited for self-collection kits used in population-based *C trachomatis* screening, particularly for geographically and socially isolated individuals.

Despite advances in diagnostic technology, *Chlamydia trachomatis* screening and disease control is still limited in some populations due to a variety of factors, including geographic isolation or an inability or unwillingness to access healthcare centres for diagnostic testing.^{1–4} Unfortunately, this has maximum impact upon the groups most at risk of chlamydial infections, including young people under the age of 25 years, indigenous populations and men who have sex with men.^{1–4} Over the past few years, an annual 20% increase in *C trachomatis* cases has been reported in Australia.¹ In response, the Australian National Sexually Transmitted Infections Strategy 2005–2008 report identified as a priority the need for increased testing of these at-risk populations, prompting the need to investigate alternative collection and testing methodologies.^{1–5}

The use of self-collected specimens, such as urine, vaginal swabs or tampons, which can be collected by individuals in their own homes can help increase the numbers of people using sexually transmitted infections (STIs) testing.⁶ Several studies have successfully evaluated the use of home-collected and mailed urines for *C trachomatis* screening.^{7–9} Such non-traditional methods of collection and transport would be ideal for facilitating the extension of existing *C trachomatis* testing programmes.^{1–5} However, there may be unforeseen obstacles that impair programme implementation and, consequently, need to be overcome, such as a need to refrigerate the sample, complexity of the collection method or additional costs associated with transport and mailing of liquids.

We describe the development and evaluation of a novel super-absorbent polymer-based method for the self-collection and ambient temperature transport of urine, which is economical, easy to use and retains the high sensitivity of *C trachomatis* real-time polymerase chain reaction (rtPCR) detection.

METHODS

Gel matrix

The gel matrix consisted of two reagents in equal parts: 0.5 g of the super-absorbent polymer Poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide) (C₅H₇NaO₃) (Sigma-Aldrich, New South Wales, Australia) and 0.5 g of the buffering agent Tris base (NH₂C(CH₂OH)₃) (Sigma-Aldrich) in equal parts, the whole of which is further referred to as the "gel". In this evaluation, 3 ml of each urine specimen was added to a 10 ml tube containing 1 g of dry gel.

In its anhydrous form, the polymer exists as complex folded chains of molecules. When urine is introduced, the water is adsorbed to the polymer, expanding and opening up the chains' structures, which results in the gel swelling into a dry granular form while desiccating the urine. Consequently, the cells, proteins and DNA previously suspended within the urine are sequestered within the swollen gel matrix; thus, the transformation of the urine into a dry granular state allows urine samples to be packaged for standard envelope mailing without fear of leakage.

Reconstitution of urine from the gel was achieved by adding 1.0 ml of 100% isopropanol followed by several vigorous inversions of the tube, which acted to liberate the bound water and re-suspend the cells and DNA. After 5 minutes

resting, 200 µl of supernatant was drawn off and the DNA extracted by the magnetic-bead-based MagNA Pure automated extraction machine using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, New South Wales, Australia).

Study design and patient specimens

C trachomatis PCR results were compared using de-identified paired samples comprising neat urine and urine processed through the gel. Except where indicated, DNA was extracted from 200 µl of neat urine or 200 µl of urine reconstituted from gel, and detection of *C trachomatis* was performed at the Molecular Diagnostic Unit, Pathology, Queensland on a Roche COBAS TaqMan 48 rtPCR analyser using the COBAS TaqMan *C trachomatis* Test (Roche Diagnostics).

Following the recommendation by Zhou *et al.*,¹⁰ the evaluation of the new method was performed in three stages: exploratory, challenge testing and clinical evaluation.

Exploratory stage

An initial laboratory-based retrospective evaluation designed to evaluate the principal usefulness of the gel was performed using specimens from Pathology Queensland Central Laboratory (PQCL). Altogether, 56 de-identified urine specimens (32 male, 24 female) previously *C trachomatis* positive by the TaqMan 48 *C trachomatis* assay during routine STI diagnostic testing were assessed using the gel-based method. The original urine samples were stored at 4°C for 2 weeks following collection and then at -20°C prior to being divided into aliquots. The fractions were added to gel and incubated at ambient room temperature in the laboratory for approximately 7 days prior to processing and testing in order to simulate the estimated time a sample may take to be delivered by mail to a diagnostic laboratory.

Challenge stage

Paired neat and gel-absorbed urine fractions were collected prospectively from patients attending the Townsville Sexual Health Clinic (TWNSHC). Specimens (36 male, 34 female) were selected on the basis of clinical symptoms and history to ensure a high proportion positive for *C trachomatis*. The paired samples were blinded prior to being transported to Brisbane by road and air courier where they were processed on arrival.

Clinical evaluation stage

A prospective evaluation was conducted to determine the gel's clinical sensitivity and specificity compared with neat urine as well as with results from the Roche Amplicor *C trachomatis* diagnostic assay (Roche Diagnostics), which is widely used for routine *C trachomatis* PCR testing in many pathology laboratories. Aliquots from urine samples of all patients (66 male, 31 female) presenting to the Gold Coast Sexual Health Clinic (GCSHC) for routine *C trachomatis* testing were collected over a 2-week period. Two de-identified paired aliquots from each patient sample were drawn off prior to the urine sample's transport to the Gold Coast Hospital for routine *C trachomatis* testing using the Amplicor *C trachomatis* assay. One aliquot was applied to the anhydrous gel and the second was left as a neat urine fraction. Both fractions were incubated at ambient room temperatures for approximately 7 days before processing and were blinded prior to *C trachomatis* testing.

Impact of variable temperature

To investigate the effect of increased storage temperatures we incubated five *C trachomatis* positive PQCL neat and gel urine

fractions at 37°C over a period of 8 days prior to processing and testing. To address the impact of freezing temperatures, 10 *C trachomatis* positive urines from the main evaluation stage were stored overnight, each in three fractions: a neat urine at room temperature, a neat urine at -20°C and a gel urine at -20°C, prior to processing and testing.

Limit of detection

To assess the limit of detection, *C trachomatis* positive urine was serially diluted 10-fold in *C trachomatis* negative urine. Each dilution was tested in triplicate using neat and gel-based methods in the TaqMan 48 rtPCR *C trachomatis* assay.

Inhibition control

An equine herpes virus (EHV) rtPCR method (kindly provided by Dr Gerry Harnett, PathCentre, Western Australia) was used to test for PCR inhibitors. Briefly, DNA extracts from gel fractions were tested in EHV rtPCR reaction mixes spiked with EHV DNA. Using this system, the presence of DNA inhibition is indicated by a significant delay in cycle threshold value. The EHV rtPCR consisted of 10 pmol each primer (EQHSV-330F 5'-GATGACACTAGCGACTTCGA-3', EQHSV-410R 5'-CAGGGCAGAAACCATAGACA-3'), 4 pmol probe (EquHSV-360 FAM-5'-TTTCGCGTGCCTCCTCCAG-3'-BHQ-1), 12.5 µl of Quantitect Probe master mix (Qiagen, Victoria, Australia), 1 µl of cultured EHV DNA in a 25 µl reaction, with 2 µl of gel fraction extract or water acting as the input template. Ten replicates of water and DNA extract from ten gel-processed urines were tested in the EHV rtPCR on a Corbett RotorGene 3000 (Corbett Robotics, Sydney, Australia) under the following conditions: 15 min incubation at 95°C, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. The cycle threshold values from the water and gel extracts were then compared.

Data analysis

Intercooled Stata (v9.2) software (Stata Corp, Texas, USA) was used to compare groups and calculate CIs.

RESULTS

Main evaluation

Exploratory stage

Of 56 *C trachomatis* positive urine specimens received from PQCL, 53 were positive results using the gel method (table 1). The three discrepant specimens (2 male, 1 female) were further investigated by retesting fresh DNA extractions of the original neat urine specimens, with two specimens returning positive results.

Challenge stage

Of the TWNSHC paired specimens, 69 of 70 were in agreement (table 1) with one urine sample from a male patient testing *C trachomatis* positive in the neat urine fraction but negative in the gel fraction.

Clinical evaluation stage

There were no discrepant results in the paired specimens from GCSHC and these matched the routine diagnostic result using the Cobas Amplicor *C trachomatis* assay (table 1).

Impact of variable temperature

The cycle threshold values from the neat and gel fractions incubated at 37°C were found to be similar, with mean cycle threshold values of 35.9 and 34.9, respectively. Similarly, the

Table 1 Comparison of *Chlamydia trachomatis* detections in three sample populations of neat urine and the corresponding gel-processed urine

| Evaluation stage/location | NU-/GU- | NU+/GU+ | NU+/GU- | NU-/GU+ | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
|----------------------------|---------|---------|---------|---------|-------------------------|-------------------------|
| Exploratory, PQCL (n = 56) | N/A | 53 | 3 | 0 | 94.6 (85.1 to 98.9) | N/A |
| Female (n = 24) | N/A | 23 | 1 | 0 | 95.8 (78.9 to 99.9) | N/A |
| Male (n = 32) | N/A | 30 | 2 | 0 | 93.8 (79.0 to 99.2) | N/A |
| Challenge, TWNSHC (n = 70) | 37 | 32 | 1 | 0 | 96.9 (84.2 to 99.9) | 100.0 (90.5 to 100) |
| Female (n = 34) | 15 | 19 | 0 | 0 | 100.0 (82.4 to 100) | 100.0 (78.2 to 100) |
| Male (n = 36) | 23 | 13 | 1 | 0 | 92.9 (66.1 to 99.8) | 100.0 (85.2 to 100) |
| Clinical, GCSHC (n = 97) | 94 | 3 | 0 | 0 | 100.0 (29.3 to 100) | 100.0 (96.2 to 100) |
| Female (n = 31) | 29 | 2 | 0 | 0 | 100.0 (15.8 to 100) | 100.0 (88.1 to 100) |
| Male (n = 66) | 65 | 1 | 0 | 0 | 100.0 (2.5 to 100) | 100.0 (94.5 to 100) |

GSHC, Gold Coast Sexual Health Clinic; GU+/GU-, gel-processed urine positive/gel-processed urine negative; NA, not applicable; NU+/NU-, neat urine positive/neat urine negative; PQCL, Pathology Queensland Central Laboratory; TWNSHC, Townsville Sexual Health Clinic.

frozen gel samples produced comparable results with both the neat frozen and room temperature urines with mean cycle threshold value differences 0.7 and 0.1, respectively.

Limit of detection

The TaqMan 48 *C trachomatis* assay detected *C trachomatis* target DNA to the same 10-fold dilution in both the neat and gel fractions with little cycle threshold value variation (table 2).

Inhibition control

The mean cycle threshold values of the gel-fraction extracts and water templates in the EHV rtPCR were similar: 24.1 and 24.4, respectively.

DISCUSSION

With this study, we demonstrate a simple and sensitive gel-transport method for the PCR detection of *C trachomatis*, which can be used for self-collected and mailed urine specimens. The gel material is non-hazardous and is widely available and inexpensive, costing approximately AUD\$0.13 per specimen. Furthermore, by converting the urine into the solid phase, the method eliminates the possibility of accidental leakage and, thus, preventing both contamination of the sample and limiting the risk of exposure to postal workers. These features make this method ideally suited to self-collected population-based screening using the postal system and should be particularly useful for difficult to reach populations or for follow-up and recall programmes of people previously tested for *C trachomatis*.

The initial exploratory stage with known positive specimens showed that use of the gel method has limited impact on the sensitivity of *C trachomatis* PCR. This was supported by the similar high sensitivity and specificity rates obtained in the subsequent challenge and clinical evaluation stages, although due to the small number of positives in the GCSHC population the sensitivity value had a lower confidence limit of 29.3%. The gel method's sensitivity and specificity in the female portion of the study populations was found to be equal or greater than that of the male portion (table 1) suggesting that the method is suitable for use by both genders. Testing 10-fold dilutions of

C trachomatis DNA showed that the use of the gel did not appear to impact the analytical sensitivity of the TaqMan 48 CT assay when compared with neat urine. Further, the gel did not introduce PCR inhibitors into the nucleic acid extracts.

A total of four specimens from the exploratory and challenge stages were negative in the gel fraction but positive in the neat urine fraction suggesting that use of the gel may slightly impact the clinical sensitivity of *C trachomatis* PCR. However, at least one of these false-negative results may have been due to deterioration of the sample prior to use in this study given the previously positive neat urine in question was negative when re-extracted and retested. Nevertheless, we believe any minor decrease in the sensitivity of the gel method would be outweighed by the increased access to *C trachomatis* testing provided by this method, particularly to geographically and socially isolated populations. Due to its simplicity, the gel method could also be potentially applied synergistically to other programmes or protocols. For example, Wisniewski *et al*¹¹ recently reported development of the FirstBurst urine collection device to collect the first 4–5 ml of first void urine from men, and showed that this approach provided higher organism yields compared with regular urine cup collection and, hence, improved the performance of both point of care and PCR tests for *C trachomatis*. The volumes used by FirstBurst are compatible with our gel method and so the two approaches could potentially be used in combination to improve clinical sensitivity.

The gel method was shown to provide suitable *C trachomatis* detection within a 7 day window between collection and testing, which was considered to be a likely maximum time frame for mail handling and processing. In a further limited experiment, we were able to successfully detect *C trachomatis* in gel fractions that had been left at room temperature for 6 weeks (data not shown). The bulk of the evaluation was performed under ambient room temperatures of approximately 25°C. However, the challenge stage (TWNSHC) neat and gel fractions, coming from a warmer, tropical climate, would have been subjected to a wider variation of temperatures during their road and air transport, with little impact seen on sensitivity. Furthermore, there was no observable impact to the gel's overall

Table 2 Mean Taqman 48 *Chlamydia trachomatis* assay cycle threshold values of 10-fold *C trachomatis* positive urine dilutions processed neat or through gel in triplicate

| Urine fraction | <i>C trachomatis</i> template dilution | | | | |
|----------------|----------------------------------------|----------------------|----------------------|--------------------|--------------------|
| | 1.0E ⁻¹ | 1.0E ⁻² | 1.0E ⁻³ | 1.0E ⁻⁴ | 1.0E ⁻⁵ |
| Neat | 34.60 (34.3 to 34.8) | 37.77 (37.3 to 38.3) | 40.37 (40.2 to 40.6) | ND | ND |
| Gel | 34.97 (34.8 to 35.1) | 37.90 (37.7 to 38.2) | 41.80 (41.0 to 42.5) | ND | ND |

Replicate threshold ranges are shown in parentheses. ND, not detected.

Key messages

- ▶ A novel method was developed that turns self-collected urine into a solid, dry gel, which can then be used to detect chlamydia by PCR.
- ▶ The method is cheap, safe, easy to use and does not impact the sensitivity or specificity of the Roche Taqman *Chlamydia trachomatis* diagnostic assay.
- ▶ This method prevents urine leakage during transport, minimising sample contamination and risk to people handling the package.
- ▶ The method is ideally suited for population screening, especially for hard to reach populations since urine samples could be easily sent through the mail.

sensitivity when subject to warmer or below freezing incubation temperatures. This suggests the gel's performance is independent of temperature variations potentially encountered during different modes of transport. Notwithstanding the results of this study, we recommend that the gel method be evaluated in any prospective patient population before routine use to investigate whether differences in local conditions impact upon its performance.

During the course of the evaluation, one sample was identified by routine diagnostic testing as being dually infected with *C trachomatis* and *Neisseria gonorrhoeae*, affording an opportunity to test the gel's performance with other target organisms. The original extracts of the sample's neat and gel urine fractions were screened with a previously published *N gonorrhoeae* rtPCR assay¹² and similar cycle threshold values were obtained (data not shown). The results suggest that the gel may be suited for the PCR detection of *N gonorrhoeae* as well; however, a comprehensive evaluation would need to be performed to establish the suitability of the gel for use in *N gonorrhoeae* screening.

We have optimised the gel method, including volumes used, for use with the Roche MagNA Pure extraction and COBAS TaqMan 48 *C trachomatis* protocols, and so re-evaluation and modification of the gel method may be necessary if alternate nucleic acid extraction and detection protocols are used. The data from this evaluation support the use of the gel for diagnostic purposes; however, a study with larger sample numbers is needed to fully validate diagnostic applicability and performance. Currently, the gel is being evaluated as part of a self-collected and mailed specimen kit used in an Australian government-funded Chlamydia Screening Pilot Trial of at-risk and regional populations.

We have developed a novel method for use in mailing urine that is inexpensive, easy to collect and process, and has been demonstrated to be suitable for *C trachomatis* detection by PCR. The gel has been created as a supplementary sampling method for situations in which traditional collection and *C trachomatis* screening cannot be achieved, and is not intended to replace

conventional collection and testing protocols. It is our hope that this gel and other novel approaches will facilitate an increase in accessible and widespread *C trachomatis* screening, with the ultimate aim of reducing *C trachomatis* prevalence and disease burden within high-risk, isolated or disadvantaged populations.

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Competing interests: None.

Ethics approval: Approved by the Human Research Ethics Committee for the Townsville Health Service District and James Cook University.

Contributors: SB: conceptualisation, study design, sample processing, data collection and analyses, and drafting of manuscript; DMW: conceptualisation, study design and drafting of manuscript; MBS: study design, sample collection, data analyses and drafting of manuscript; CB: sample processing and testing and drafting of manuscript; KB: sample collection and drafting of manuscript; SA: sample collection and drafting of manuscript; RG: study design, sample collection and data analyses; RM: data analyses and drafting of manuscript; SBL: study design, data analyses and drafting of manuscript; JD: study design and drafting of manuscript; MDN: study design and drafting of manuscript; TPS: conceptualisation, study design and drafting of manuscript.

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