Comparison of urine, first and second endourethral swabs for PCR based detection of genital *Chlamydia trachomatis* infection in male patients

H Sugunendran, H D L Birley, H Mallinson, M Abbott, C Y W Tong

**Objectives:** To compare endourethral swabs and urine as diagnostic specimens for the detection of genital *Chlamydia trachomatis* infection using the polymerase chain reaction (PCR), in male patients attending a genitourinary clinic and to assess whether the first endourethral swab used solely for diagnosing gonococcal infection could be used for *C trachomatis* detection as well.

**Methods:** Two endourethral swabs were taken from 80 male patients, in whom the likelihood of genital *C trachomatis* infection was high. The first swab was used for microscopy and culture for *Neisseria gonorrhoeae*, before being used for *C trachomatis* detection. First voided urine specimens were collected from 61 of these patients. All three specimens were processed for *C trachomatis* DNA detection using the Roche Cobas Amplicor PCR. A diagnosis of genital *C trachomatis* infection was made if any one of the specimens tested reproducibly positive. Samples from 13 patients showing discrepant PCR results between swabs and/or urine were retested by ligase chain reaction (LCR).

**Results:** *Chlamydia trachomatis* DNA was detected in 35 (43.8%) of the 80 patients. In 17 of the 35 patients (48.6%), all the genital specimens were positive. However, in 18 (51.4%) patients, one or more of the genital specimens had negative PCR results. Among the 18 patients with discrepant results, urine was found to be a more sensitive diagnostic specimen than the second urethral swab picking up 13 out of 16 positives (81.3%) as opposed to five out of 18 (27.8%). There was no significant difference between the two swabs. Retesting by LCR, of the samples from 13 of the 18 patients with discrepant PCR results confirmed them all as true positives, although as with PCR, not all specimens in the set were concordantly positive. LCR detected all the 13 positives in urine, while there was no difference in the detection rate between the first and the second urethral swabs.

**Conclusions:** Urine appeared to be a better diagnostic specimen than the conventional second endourethral swab for *C trachomatis* detection by PCR in this cohort of male patients. There was no difference between the first swab, intended primarily for *N gonorrhoeae* testing and the second swab intended for *C trachomatis* detection. This raises questions over the need for the conventional second swab for detecting *C trachomatis*.

**Keywords:** endourethral swabs; urine; polymerase chain reaction; *Chlamydia trachomatis*

**Introduction**

Genital *Chlamydia trachomatis* infection is the most prevalent bacterial sexually transmitted infection in the United Kingdom. It is estimated that 5–12% of sexually active adults in the age group of 16–34 in the United Kingdom may be infected with this organism. In Merseyside, UK, rates of 5–10% have been reported among asymptomatic young women attending clinics for contraception. The sequelae of chlamydial infection—pelvic inflammatory disease, ectopic pregnancy, and tubal infertility are well known and the effectiveness of community based screening in reducing these is well described. Recently, DNA amplification tests such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) have become available and are currently being used for diagnosis of genital chlamydial infection. Their superiority over the conventional techniques such as culture or ELISA has been shown. One advantage of DNA amplification tests may be that chlamydia can be detected from urine avoiding the need for invasive samples. With the initiation of community based chlamydia screening in women in the United Kingdom, there is likely to be a need to evaluate the best method for screening male partners of women in whom chlamydia is detected.

As in most GUM clinics in the United Kingdom, in the Royal Liverpool University Hospital, male patients are screened by using two endourethral swabs in order to diagnose urethritis. The first swab, after being used for microscopy and culture for *Neisseria gonorrhoeae*, is discarded. The second swab is used solely for the detection of *Chlamydia trachomatis*. Endourethral swabs are intensely disliked by patients and may be a factor in the non-attendance at GUM clinics. Thirty five out of 39 consecutive patients attending our clinic complained of mild to moderate discomfort while having the swabs. After the endourethral swabs, a first voided urine sample is examined macroscopically for threads which, if present, are Gram stained and examined...
microscopically for polymorphonuclear leucocytes. The presence of inflammatory cells is taken as evidence of urethritis. This practice presented an opportunity to audit the relative sensitivity of the two urethral swabs, as compared with urine samples for chlamydia detection.

Patients and methods

Local ethics committee approval was obtained. The district audit monies supported this study. Eighty male patients presenting to the GUM clinic, either with symptoms suggestive of urethritis (n = 48) or whose sexual partners had been diagnosed as having genital chlamydial infection (n = 32) or both (n = 16), were entered into the study. Two separate endourethral swabs were taken, by gently passing each cotton tipped swab 1–4 cm inside the urethral meatus and rotating it by 360°. The first swab, after being smeared on a glass slide for microscopy and plated for N. gonorrhoeae culture, was not discarded, but was placed in 2SP (sucrose phosphate) transport medium. The second urethral swab was placed directly in another bottle of 2SP. A 10 ml sample of first voided urine was collected after the swabs. The Roche Cobas Amplicor PCR (Roche diagnostics, Basle, Switzerland) was used to detect C. trachomatis DNA in the three samples according to protocols recommended by the manufacturer.

A diagnosis of genital chlamydial infection was made if any one of the specimens tested positive by PCR. The results were considered concordant if both the swabs and urine gave the same results by chlamydia PCR. When one or more of the diagnostic specimens were negative and other(s) positive, results were considered discrepant. Samples from 13 of the 18 patients with discrepant PCR results were further tested by ligase chain reaction (LCR, Abbott Laboratories Ltd, LCx) to provide an independent check of chlamydia status. The McNemar test and the Fisher’s exact test were used for statistical analysis.

A diagnosis of urethritis was made by microscopic examination of a Gram stained urethral smear showing the presence of ≥5 polymorphonuclear leucocytes (PMNL) per high power (×1000) microscopic field (averaged over five fields). The presence of threads in urine was also taken as evidence of urethritis, if ≥10 PMNL per high power field (averaged over five fields) were seen in a Gram stained preparation of a thread from a first void urine specimen.

All patients with microscopically proved urethritis and all patients whose partners were diagnosed as having chlamydia infection were given doxycycline 100 mg twice daily for 7 days. The patients who turned out to be positive for chlamydia in any one of the genital specimens by PCR were given the same treatment subsequently. All patients were reviewed after 2–4 weeks and given a test of cure by PCR, if appropriate.

### Results

Eighty patients were enrolled in the study. The mean age of the patients was 26 years (range 16–53 years). All of them had two urethral swabs taken but the urine samples of only 61 patients were available for PCR.

Out of the 80 patients, 62 (77.5%) had concordant results—that is, all the genital specimens were either positive or negative. C. trachomatis DNA was detected in 35 (43.8%) out of the 80 patients. Seventeen (48.6%) of these had concordant positive results; however the results were discrepant in 18 patients (51.4%). The pattern of results obtained from all the 80 patients is shown in Table 1.

If the 35 chlamydia positive patients’ results are scrutinised, 27/35 (77.1%) had a positive result from the first swab, 22/35 (62.8%) had a positive result from the second swab, and 25/28 (89.3%) had a positive result from the first swab, 22/35 (62.8%) had a positive result from the second swab, and 25/28 (89.3%) had a positive result from urine as show in Table 2. Among these patients, urine PCR seems to have a significantly better detection rate for C. trachomatis than the second swab (p = 0.02). There was no significant difference in the detection rates between the first and second swab (p = 0.26) or between the first swab and urine (p = 0.22).

Among the patients with discrepant samples, 10/18 (55.6%) had a positive result from the first swab, 5/18 (27.8%) had a positive result from the second swab and 13/16 (81.3%) had a positive result from urine. If the overall positive rates of the three genital specimens are analysed, there was no statistically significant difference between the detection rate of the three sample types.

### Table 1 — Detection rate of C. trachomatis in the three genital specimens in 35 of the 80 patients who tested positive

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Positive Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab 1</td>
<td>77.1%</td>
</tr>
<tr>
<td>Swab 2</td>
<td>62.8%</td>
</tr>
<tr>
<td>Urine</td>
<td>89.3%</td>
</tr>
</tbody>
</table>

CI = confidence interval.
Comparison of urine, first and second endourethral swabs for PCR based detection of genital C trachomatis infection in male patients

There was no difference in the sensitivities between the two swabs.

Out of the 80 patients enrolled in the study, 32 were partners (contacts) of chlamydia positive patients. C trachomatis could be detected only in 19 of these patients (59.4%) and 11 of these 19 patients (57.9%) had discrepant results. Of the 35 chlamydia positive patients, 13 (37.1%) had no microscopic evidence of urethritis.

A diagnosis of gonococcal urethritis was made by microscopy in 11 patients tested in this sample and N gonorrhoeae was cultured from all these patients. All 11 presented with a urethral discharge. Two were partners of women who had been diagnosed as having chlamydia. Five out of these 11 patients (45.4%) were found to be co-infected with chlamydia. Three (60%) of them had discrepant results, one with positive urine and negative swabs, one with positive swabs and negative urine, and one in whom no urine was analysed but who had a positive first swab but a negative second swab.

Discussion

A number of different tests and genital specimens are currently being used for the diagnosis of genital chlamydial infection and there is some uncertainty as to the cross reliability of these tests. Ampicillic PCR has been relatively well evaluated for both urogenital and urine specimens, with an overall sensitivity and specificity of 90% and 99–100% respectively. LCR (Abbott Laboratories) has shown to have an overall sensitivity of 94% and a specificity of 99–100%.

The results from our study suggest that when using PCR, neither swab nor urine alone can find all positives. Patients whose samples yield discrepant results, some negative and some positive for chlamydia, may have a lower level of infection, which is near to the limit of detection by the test. Among the discrepant samples, urine and the first swab appear to be good diagnostic specimens for detecting chlamydia. The second urethral swab, which is the one conventionally used for the diagnosis of genital chlamydial infection, had the lowest detection rate, failing to pick up chlamydia in 13/18 (72.2%) of the discrepant specimens.

Studies have shown the variation in sensitivities in different genital specimens. Some studies have suggested that urine is a better specimen than urethral swabs for chlamydial detection by PCR. Young and colleagues studied 244 men who attended a GUM clinic and found that urine PCR (96%) was more sensitive than urethral swab PCR (89%). Toye et al showed that in men, urine PCR had a sensitivity of 90.9% compared with a sensitivity of 72.3% for PCR on urethral swabs. The higher sensitivity of urine PCR may reflect the difficulty of obtaining sufficient C trachomatis DNA from urethral swabs, which are inserted only 1–4 cm proximal to the meatus. Other studies are at odds with these findings. Crotchett et al showed that when using PCR, the sensitivity of urine was 91.1% compared with 99.3% for a urethral swab.

The study done by Wiesenfeld and colleagues gave a sensitivity of 98.4% for urethral swab and 87.1% for first void urine. None of these studies compared urine with two endourethral swabs.

Our data suggest that urine had a higher detection rate than the second swab, which is routinely used for chlamydial detection. There was no statistically significant difference between the first swab, which is routinely not used for chlamydial detection, and the second swab, which is the conventional swab used for this purpose. Thus, if endourethral swabs are to be used for chlamydial diagnosis, it seems that the use of a second swab may not be necessary.

In current clinical practice we rely on the endourethral swabs for the diagnosis of gonorrhoea and chlamydia. Urine can be used for the detection of both these infections by the use of DNA amplification methods. However, for a rapid diagnosis of urethritis, urine alone may not be sufficient. Even though a leucocyte esterase test could be done on first passed urine specimens for diagnosing urethritis, it has a low sensitivity. In the absence of visible threads in the urine, endourethral swabs for a Gram stained smear may be necessary for a rapid diagnosis of urethritis. Moreover, endourethral swabs are needed for culture and antimicrobial sensitivity testing of N gonorrhoeae.

There is a wealth of research data demonstrating the superiority of DNA amplification techniques for the diagnosis of genital chlamydial infection. This study shows a single endourethral swab can be used for microscopy and culture of N gonorrhoeae, as well as C trachomatis detection by PCR. This causes us to question the value of the second urethral swab in the diagnosis of chlamydial infection in men.

Conflict of interest: None.

The study was supported by the district audit monies. We are indebted to our nursing staff who assisted with sample collection. We are also indebted to Steve Taylor of Liverpool University for statistical support.

Contributors: HS was involved in patient recruitment, specimen collection, data analysis and wrote the paper; HDLB was responsible for the study design and was involved in patient recruitment, specimen collection, data interpretation and was the co-author; HM supervised the ligase chain reaction, helped in data analysis, and commented on the manuscript; MA participated in study design and commented on the manuscript; CYWT supervised the polymerase chain reaction and was involved in data analysis, data interpretation, and helped in preparing the final manuscript.

Comparison of urine, first and second endourethral swabs for PCR based detection of genital *Chlamydia trachomatis* infection in male patients

H Sugunendran, H D L Birley, H Mallinson, M Abbott and C Y W Tong

*Sex Transm Infect* 2001 77: 423-426
doi: 10.1136/sti.77.6.423

Updated information and services can be found at:
http://sti.bmj.com/content/77/6/423

These include:

**References**

This article cites 12 articles, 3 of which you can access for free at:
http://sti.bmj.com/content/77/6/423#BIBL

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Chlamydia (841)
- Ophthalmology (680)
- Gonorrhoea (806)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/