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article

Combined cervical swab and urine specimens for PCR diagnosis of genital *Chlamydia trachomatis* infection

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Objectives: Sampling of both the cervix and urine increases the chance of detection of *Chlamydia trachomatis* compared with sampling either site alone. We determined the effect of combining urine and cervical swab specimens in the clinic setting on the sensitivity of *C trachomatis* polymerase chain reaction (PCR) testing.

Methods: For each of 100 women attending a genitourinary medicine clinic with high likelihood of genital *C trachomatis* infection, one endocervical swab was placed in transport medium and another in one of two aliquots of first void urine. Four PCR assays per patient (urine + swab, swab alone, and urine alone both pre- and post-freeze-thawing) were processed by automated *C trachomatis* PCR (Cobas, Amplicor). An inhibition control was included with each assay to identify specimens containing PCR inhibitors.

Results: 71% of women were Amplicor *C trachomatis* PCR positive (according to the results of at least one specimen). PCR test results were concordant for 95/100 patients, and of the five discordant result sets there was only one major discrepancy. Inhibitors of PCR were present in 22/400 specimens from 20 patients, and 16/22 were cervical swabs ($p < 0.001$).

Conclusions: Combining a cervical swab with a urine specimen is acceptable for PCR testing for genital *C trachomatis* infection, and has the potential to increase further the cost effectiveness of DNA based screening for *C trachomatis* genital infection.

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Keywords: polymerase chain reaction; *Chlamydia trachomatis*

Introduction

Nucleic acid amplification methods, such as the polymerase chain reaction (PCR), are significantly more sensitive, and should therefore be used in preference to other tests for the detection of genital *Chlamydia trachomatis* infection.¹⁻³ In women, the sensitivity of *C trachomatis* PCR testing is increased by approximately 12% if both cervical swab and urine specimens are examined as opposed to urine alone.¹ However, this approach is expensive, particularly considering the relatively high cost of PCR tests versus enzyme immunoassays. We investigated the utility of PCR testing of combined urine and cervical swab specimens, for female genitourinary medicine clinic attendees, in order to maximise the sensitivity of *C trachomatis* PCR testing while minimising specimen processing costs. However, we were concerned to determine whether combining specimens reduced test sensitivity because of naturally occurring inhibitors of the enzyme used to amplify DNA, which may be more prevalent in cervical specimens than in urine.⁴

Patients and methods

Samples were obtained from 100 women attending the genitourinary medicine (GUM) clinic at Leeds General Infirmary who had a high likelihood of genital *C trachomatis* infection. These comprised women who had tested positive elsewhere for genital *C trachomatis* infection and who had been referred for treatment and partner notification; women who had been recalled to clinic for follow up with unex-

pected, untreated genital *C trachomatis* infection; and female contacts of men with *C trachomatis* infection. A routine vaginal speculum examination was performed and two endocervical swabs (Medical Wire, UK) were taken simultaneously before other cervical sampling. One swab was placed in transport medium (IDEIA, Dako Diagnostics, UK). The other was placed in one of two aliquots of first void urine (patients voided urine immediately after examination). Specimens were transported to the laboratory within 4 hours of collection.

Specimens (urine + swab, urine alone, and swab alone) were processed by automated *C trachomatis* PCR (Cobas Amplicor using standard urine or cervical swab protocols (Roche, version 3). Aliquots of 500 μ l of urine were used for both the urine and combined urine + swab specimens. The combined urine + swab specimen was tested both before and after freezing at -20°C and then thawing at room temperature. Hence, four PCR assays per patient were performed. An inhibition control supplied by the manufacturer was included with each assay in order to identify specimens containing PCR inhibitors.

Results

Of 100 patients tested, 71 were Amplicor *C trachomatis* PCR positive (according to the results of at least one specimen). The Amplicor *C trachomatis* PCR test results were concordant for 95/100 patients. The five discordant result sets (table 1) included only one major discrepancy (1/100, 1%, 95% CI $-0.01-3\%$). In this

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Table 1 Specimen findings for five patients with discordant results

	PCR result on			
	urine	cervical swab	urine + cervical swab (pre-freeze-thaw)	urine + cervical swab (post-freeze-thaw)
Patient 1	negative	positive	negative	negative
Patient 2	negative	positive	positive	positive
Patient 3	negative	positive	positive	positive
Patient 4	positive	negative	positive	positive
Patient 5	positive	positive	negative	positive

case (patient 1, table 1) the cervical swab was Amplicor *C trachomatis* PCR positive, whereas the other three PCR assays were PCR negative. In each of these three, inhibitors of PCR were not detected, as internal control amplification occurred. Hence, the pre- and post-freeze-thaw combined samples yielded false negative results. On further investigation, this patient was found to have received 5 days of treatment with doxycycline immediately before testing, but on examination a purulent cervicitis was still present. In the remaining discordant result sets, one specimen yielded a negative result while the other three were *C trachomatis* PCR positive.

Inhibitors of PCR were present in 22/400 specimens from 20/100 patients; 20/22 of these specimens were from *C trachomatis* infected patients ($p < 0.1$). Of these 22 specimens, 16 were cervical swabs ($p < 0.001$). One pre-freeze-thaw combined sample was *C trachomatis* PCR negative, presumably due to PCR inhibitors as the specimen was positive after freeze-thawing.

Discussion

Although nucleic acid amplification methods permit non-invasive sampling for *C trachomatis*, additional sampling of the cervix can increase diagnostic sensitivity.¹ Cervical specimens are easily obtained in GUM clinics where female patients are routinely offered a vaginal speculum examination. We have demonstrated that acceptable sensitivity is retained by performing a *C trachomatis* PCR test on a combined urine and cervical swab specimen (sensitivity 98.6%, specificity 100%), as opposed to testing separate samples. We examined specimens from women with a high probability of *C trachomatis* infection as these may be more likely to contain PCR inhibitors secondary to tissue inflammation.⁴ We did not aim to prove that testing both cervical and urine samples for *C trachomatis* would increase the chance of a positive result as this has been shown previously,¹ and requires a considerably larger patient cohort, preferably including individuals with a low *C trachomatis* burden.

We found no evidence of PCR inhibition as a result of combining cervical and urine specimens. By contrast, in *C trachomatis* positive women PCR inhibitors were significantly more common in cervical swabs than in other specimens (11/72 versus 4/216, $\chi^2 p < 0.001$). Inhibition was detected almost three times more commonly in specimens from *C trachomatis*

positive women than from uninfected females ($p < 0.1$). This is probably due to the higher concentration of serum/tissue products in cervical swab specimens, particularly those taken from women with cervicitis. PCR inhibitors present on cervical swabs ($n=16$) appeared to be diluted to non-detectable levels when swabs were placed in urine, as inhibitors were still evident in only 2/16 cases when combined specimens were tested. In these two cases inhibitors could not be detected post-freeze-thawing. Freeze-thawing of combined swab and urine specimens is advantageous in terms of laboratory processing, allowing batching and aiding specimen transport, as specimens can be frozen if delay is expected, and also reduces the prevalence of PCR inhibitors.^{4,5} Freeze-thawing also reduces the prevalence of PCR inhibitors. For example, *C trachomatis* PCR inhibitors can be found in 20% of urine specimens from pregnant women, but approximately half of these are rendered non-inhibitory following freeze-thawing.⁵ Alternative methods of removing *C trachomatis* PCR inhibitors include specimen dilution,^{4,6,7} but this is relatively cumbersome, particularly when large numbers of specimens are processed, and may cause DNA contamination of samples and/or false negative results.

Combining a cervical swab with a urine specimen is acceptable for PCR testing for genital *C trachomatis* infection. Such an approach maximises test sensitivity while minimising costs, and indeed has the potential to increase further the cost effectiveness of DNA based screening for *C trachomatis* genital infection.²

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Contributors: All authors actively participated in the study, and no one else fulfils the criteria for authorship; MW and MR devised the study and wrote the manuscript; MR organised patient recruitment; and MW analysed the data; CH contributed to study design and JB carried out laboratory tests.

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