Feasibility of patient-collected vulval swabs for the diagnosis of *Chlamydia trachomatis* in a family planning clinic: A pilot study

Susan Macmillan, MRCOG, DFFP, *Clinical Research Fellow, Department of Obstetrics and Gynaecology*; **Hamish McKenzie**, BSc, MB ChB, PhD, FRCPath, *Senior Lecturer and Honorary Consultant, Department of Medical Microbiology, University of Aberdeen, Aberdeen, Scotland*

Gillian Flett, FRCOG, MFFP, *Consultant in Family Planning and Reproductive Health Family Planning Services, Grampian Healthcare NHS Trust, Aberdeen, Scotland*

Allan Templeton, MD, FRCOG, *Professor, Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen, Scotland*

Correspondence: Dr Susan Macmillan, Clinical Research Fellow, Department of Obstetrics and Gynaecology, Aberdeen Maternity Hospital, Foresterhill, Aberdeen AB25 2ZD, Scotland

(Accepted June 12th, 2000)

Summary

This pilot study set out to determine the feasibility of using patient-collected vulval swabs, instead of urine, for the diagnosis of female *Chlamydia trachomatis* infection. Main outcome measures included prevalence of infection and sensitivity, specificity, and acceptability of both test methods. An assessment was also made of those who declined to be tested. Consecutive women under 25 years of age attending a single urban family planning clinic were invited to participate. Sixty-eight percent (103/152) agreed to undergo testing. Overall prevalence was 11.7%. The sensitivity/specificity for the ligase chain reaction (LCR) assayed patient-collected vulval swabs and urine was 100%/100% and 92%/100%, respectively. The acceptability of self-collection was high with 93% characterising the test as 'not bad', 79% recommending it to a friend, and 79% choosing the test next time. Significantly more women, however, would choose urine for testing on a subsequent occasion ($p < 0.001$). Less than 1/5 of the patients who declined did not take part because of concerns regarding the vulval swab. Patient-collected vulval swabs assayed by LCR represent a non-invasive, sensitive, and acceptable way to detect genital *C. trachomatis* infection in women attending a family planning clinic. Compared with urine testing, benefits in terms of transport and processing should encourage more widespread use of this approach.

Key words

acceptability, *Chlamydia trachomatis*, ligase chain reaction, patient-collection, vulval swabs

Introduction

Chlamydia trachomatis is the most common sexually transmitted infection in the United Kingdom (UK). Most female infections are asymptomatic and consequences include pelvic inflammatory disease,^{1,2} tubal infertility,^{3,4} and ectopic pregnancy.⁵ Infection risks are highest in women under 25 years of age^{6,7} and complications may be more severe.⁸⁻⁹ In response, the CMO's Expert Advisory Group on *Chlamydia*⁶ has recommended opportunistic screening, targeting asymptomatic, sexually active women under 25 years of age, especially teenagers. There is a need now to increase the uptake and acceptability of testing.

Despite a clear case made for screening, the optimum diagnostic test to satisfy both patients and health care professionals is still not known. Until recently, accurate

Key message points

- *C. trachomatis* is the most common sexually transmitted bacterial infection in the UK. While most female infections are asymptomatic, the risk of acquiring the infection is related to young age and complications may be more severe in younger women.
- The CMO's Expert Advisory Group on *Chlamydia* has recommended opportunistic screening, targeting asymptomatic, sexually active women under 25 years of age, especially teenagers.
- Nucleic acid amplification tests are highly sensitive and specific and have the ability to test non-invasively using either urine or vulval swabs.
- Most recent studies have focused largely on urine testing, despite concerns regarding provision of samples, inhibitors, and transport issues. Vulval swabs, particularly when patient-collected, may be a better alternative.
- This study found that patient-collected vulval swabs assayed by LCR represent a non-invasive, sensitive, and acceptable way to detect genital chlamydial infection and more widespread use should be encouraged.

diagnosis of genital *C. trachomatis* infection could be achieved only by invasive collection, and in the UK the majority of women still undergo endocervical testing using enzyme immunoassay (EIA). With advances in DNA technology, however, nucleic acid amplification assays have been introduced. Advantages include high sensitivity and specificity, and the ability to test non-invasively using either urine¹⁰ or vulval swabs.¹¹

Most recent studies have focused largely on urine testing. Despite advantages in terms of familiarity and non-invasiveness, disadvantages include the inability to produce a sample on demand. One study¹² found that 25% of the participants were unable to produce a urine specimen. There also exist concerns regarding inhibitors,¹³ transport issues relating to bulkiness and refrigeration,¹⁴⁻¹⁶ and the need for additional laboratory processing steps. In contrast, vulval swabs can be performed on demand, are compact, do not appear to be as susceptible to temperature influences, and require fewer processing steps. Furthermore, patient-collected swabs have the potential to decrease both patient embarrassment and clinic costs.

The aims of this study were to determine the feasibility, in terms of sensitivity and acceptability, of using LCR assayed patient-collected vulval swabs, instead of urine, for the diagnosis of female genital *C. trachomatis* infection in a family planning population. Assessment of those who declined to undergo screening was also undertaken.

Method

All screening was undertaken at Square 13, a single urban family planning clinic. Consecutive women under 25 years of age, were invited to participate in the study by means of an information sheet. Antibiotic use in the previous month and symptoms of pelvic infection were the exclusion criteria. Approval for the study was received from the local Ethical Committee.

Study population

One hundred and three apparently healthy women were recruited during January to May 1998.

Specimen collection

Instructed not to clean their vulval region prior to sampling, the women collected the samples in one of the clinic's toilets. Advice regarding vulval swabbing was given both verbally and pictorially (Figure 1). The vulval swab was collected by rotating the swab 0.5 cm within the vaginal introitus, against the posterior fourchette. Approximately 20 ml of first void urine was then collected in a universal container. Specimens were either transported directly to the microbiology lab, or were refrigerated overnight for collection the following day. As the clinic is off-site from the main laboratories, specimens were transported for approximately 2 hours un-refrigerated.

Specimen testing

The LCR assays were performed by a dedicated technician with the LCx probe system (Abbott Diagnostics, Maidenhead, UK) in accordance with manufacturer's instructions.

Resolution of LCR discrepancies

Any set of patient specimens was considered discrepant if the two test results were not unanimous. All initially positive specimens were re-tested by LCR for both samples. In those women whose paired results remained discrepant, confirmation testing of the positive LCR sample was performed by sending the specimen to Abbott Laboratories, Abbott Park, Illinois, USA for assay by LCR of a target sequence in the gene coding for the major outer membrane protein (MOMP). This was done in a blind fashion with

coded samples. Known negative samples were also included.

Analysis

For calculation of test performance, a woman was considered to be truly positive if both specimens were positive, or if the positive specimen in a discrepant pair was confirmed by LCR MOMP. Uninfected patients were defined as those with negative LCR tests for both the vulval and urine specimens. False positive results were defined as positive results that were not confirmed on re-testing, and discrepant positive LCR results that could not be confirmed by LCR MOMP. Following resolution of discrepant results, the sensitivity, specificity, and predictive values (positive and negative) for each specimen was calculated, as described by Griner et al.¹⁷

Acceptability

Following testing, participants were asked, by semi-structured interview, questions relating to acceptability of both test methods. Answers were recorded on a data sheet along with basic demographics.

Decliners

By semi-structured interview, basic demographics and the reason for declining to participate was recorded.

Statistics

Data were stored in a personal computer and the results were analysed using the Statistical Package for Social Services (SPSS). Characteristics of the participants and decliners were compared using Chi-square statistic with Yates' continuity correction or Fisher's exact test where greater than 25% of the expected cells were less than five. For calculation purposes, appropriate groups with small or no representation were combined or the group was omitted. Chi-square statistic with Yates' continuity correction was used to compare vulval and urine testing in terms of acceptability. $P < 0.05$ was considered statistically significant.

Results

A total of 103 women under 25 years of age were tested for *C. trachomatis* infection by LCR assay of vulval swabs and first void urine. Mean age was 19.9 (SD 2.6) years with a range from 14 to 25 years. Sixty-eight percent (103/152) of those approached agreed to participate in this study. Those declining were no different from those accepting in relation to age, marital status, parity, occupation, reason for visit, and contraceptive use (Table 1). Table 2 states the main reasons given by the decliners for not participating. More than one reason was given in some cases.

Specimens from 91 participants tested negative by both tests, and these women were considered uninfected. Eleven participants tested positive by both tests and were considered infected. One woman tested positive by vulval swab but negative by urine. This was confirmed on re-testing of both samples by LCR. The vulval swab was subsequently confirmed positive by LCR MOMP assay. On the basis of our definition of an infected woman, 12 of the 103 women were infected with *C. trachomatis*, resulting in a prevalence of 11.7%. Of the 12 infected women, 11 (91.7%) were positive by LCR of FVU and all (100%) by vulval LCR. The sensitivity, specificity, and predictive values (positive and negative) of the vulval swabs were 100%, 100%, 100%, and 100%, respectively. The sensitivity, specificity, and predictive values (positive and negative) of urine were 91.7%, 100%, 100%, and 98.9%, respectively.

Figure 1 How to do a vulval swab

Table 1 Patient characteristics of the study participants (n = 103) and decliners (n = 49)

Characteristic	Participants n (%)	Decliners n (%)	P value (95% confidence interval)
Mean age (SD)	19.88 (2.6)	19.8 (2.82)	0.863 ¹ (-0.997 to 0.836)
Marital status:			
Single	81 (78.6)	38 (77.6)	0.879 ²
Married or cohabiting	22 (21.4)	11 (22.4)	
Parity:			
Nulliparous	100 (97.1)	47 (95.9)	0.658 ³
Parous	3 (2.9)	2 (4.1)	
Occupation:*			
Professional / Mgmt ⁴	8 (7.8)	2 (4.1)	0.186 ²
Skilled	13 (12.6)	4 (8.2)	
Semi-skilled	22 (21.4)	8 (16.3)	
Unskilled	5 (4.9)	2 (4.1)	
Student	50 (48.5)	32 (65.3)	
Housewife	2 (1.9)	1 (2.0)	
Unemployed	3 (2.9)	0	
Reason for visit:**			
First visit	4 (3.9)	0	0.372 ²
Repeat prescription	53 (51.5)	33 (67.3)	
Emer contraception ⁵	30 (29.1)	12 (24.5)	
Advice	8 (7.8)	3 (6.1)	
Smear/IUCD ⁶	2 (1.9)	0	
Pregnancy test	6 (5.8)	0	
Contraception:***			
None	3 (2.9)	1 (2.0)	0.161 ²
COCP	45 (43.7)	30 (61.2)	
Condom	37 (35.9)	11 (22.4)	
Double Dutch	5 (4.9)	2 (4.1)	
POP	1 (1.0)	0	
Depo	11 (10.7)	4 (8.2)	
Cap	1 (1.0)	1 (2.0)	

* For calculation purposes, groups 1 and 2, and 3 and 4 were combined. Groups 6 and 7 were omitted.
** For calculation purposes, only groups 2 and 3 were compared.
*** For calculation purposes, groups 2 and 4, 3 and 7, and 5 and 6 were combined. Group 1 was omitted.
¹ Student t-test
² Chi-square statistic with Yates' continuity correction
³ Fisher's exact test
⁴ Professional or management
⁵ Emergency contraception
⁶ Attending for smear or pre-insertion of intrauterine contraception device

With regard to acceptability, the women were asked how they found the tests- 'not too bad' or 'didn't like it'. Both tests were highly acceptable with only 6.8% (7/103) and 1.9% (2/103) stating they didn't like the vulval swabs or urine tests, respectively. This was not significantly different (p = 0.17). The women were then asked whether, if a female relative or friend was being offered the tests, which, if any, would they recommend. The vulval swab was recommended by 10.7% (11/103), urine by 21.4% (22/103), and both by 68% (70/103). Combining these found the vulval swab recommended by 78.6% (81/103) and urine by 89.3% (92/103). These were not significantly different (p = 0.058) Finally, the participants were asked 'if testing for chlamydial infection was offered, would you choose a urine test' and '... would you choose a vulval swab?' The women in this study were significantly more likely to choose testing by urine - 95.1% (98/103), than by vulval swab - 78.6% (81/103) (p = 0.001).

Table 3 shows the reasons why participants didn't like a test. Comments from the participants formed three themes. The first related to the non-invasive nature of both tests: 'A

Table 2 Reasons given by decliners (n = 49)

Reason	Decliners n (%)
I don't have the time	17 (34.7)
I have my period	14 (28.6)
Unnecessary as I'm not at risk	10 (20.4)
Didn't want to do a vulval swab	8 (16.3)
I would worry about a positive result	5 (10.2)
Not important to have done	2 (4.1)
Recently tested for <i>Chlamydia</i>	1 (2.0)
Don't like to take part in research	1 (2.0)
Too many tests	1 (2.0)
Didn't want to produce a sample of urine	0

lot more pleasant than previous methods' and 'Excellent, not embarrassing.' The second related to negative aspects of the vulval swab: 'Not sure if it (swab) was done properly', 'The swab was: difficult to break ...a bit awkward ...easy to drop.' The third related to positive aspects of vulval swabbing: 'Vulval swab was very easy and straightforward, less messy than a urine test', 'I thought it was better than having someone else to do it ', and 'The vulval swab was much easier to do'.

Discussion

This study found that opportunistic testing of young women for genito-urinary *C. trachomatis* infection using self-collected vulval swabs assayed by LCR compared favourably with urine testing in terms of sensitivity and acceptability.

The study population was chosen to reflect those women who will be targeted if the CMO's Expert Advisory Group's recommendations are accepted. The refusal rate of almost one in three reflects the difficulties in encouraging asymptomatic young Scottish women to be screened. In contrast, an American study comparing self-sampled vulval swabs to clinician-sampled vulval swabs and endocervical samples assayed by polymerase chain reaction reported a decline rate of only 2%.¹⁸ Their population were likely to accept testing, however, as they were selected by having risk factors for sexually transmitted infection (STI) or had recently been diagnosed with *C. trachomatis* infection. Furthermore, American women have annual smears.

The importance of assessing why women withhold consent to participate in clinical trials has recently been highlighted in the pregnant population, highlighting issues of 'It could never happen to me', poor communication, and method of recruitment.¹⁹ These are all issues relevant to STIs.

It is encouraging that no demographic differences were found between those who participated and those who declined, and that two thirds of those who refused to participate stated a time factor or menstruation as the reason. One would hope that they would agree to screening at a subsequent visit. The aforementioned American study found that 43% and 36% of their women never used tampons or never looked at their own genitals, respectively.¹⁸ This

Table 3 Reasons for not liking vulval and urine tests

Reason	Vulval swab n (%)	Urine n (%)
Embarrassing	1 (1.0)	0
Uncomfortable	5 (4.9)	0
Difficult to do	4 (3.9)	2 (1.9)
Time consuming	0	1 (1.0)

information was not collected from our women, so we are unable to confirm whether the 16% of those who declined to participate because of the vulval swab aspect represented those who are more introspective regarding their genitalia.

Of concern, however, were the 35% who felt that they did not need to be tested for *C. trachomatis* infection. Overall, knowledge of the infection and its sequelae in this group of women is known to be poor²⁰ and targeted health education is urgently required so that individual risk is recognised.

Our definition of an infected patient has been used by other researchers.^{18,21-22} We defend our definition by acknowledging that there is now abundant evidence supporting superior sensitivity and specificity of LCR in comparison to older diagnostic tests.^{10,11,22} Specificity, however, is a concern with DNA amplification methods because even slight contamination can be potentiated by the amplification reaction. To increase specificity, retesting of the initially positive specimens was done. This left one woman with discrepant results. The MOMP LCR was chosen as the confirmatory test as it has been shown to have greater sensitivity compared to direct immunofluorescence,²³ particularly with female urine.²⁴ Furthermore, the MOMP assays were blinded, with four negative controls accurately confirmed. The results confirmed the validity of the positive vulval specimen in the patient with a negative urine.

With regard to comparative studies, specimens described as vaginal swabs relate to sampling from the same area. Hook et al²⁵ compared patient-obtained vaginal swabs assayed by LCR to culture in a genito-urinary medicine population. A similar prevalence (12.9%) and sensitivity (91.8%) was found. Gray et al²⁶ screened 373 rural Africans using paired self-collected vaginal swabs and urine. Prevalence was lower (3.5%), but the two tests performed equally, identifying 15/17 positive women. They also found a discordant rate of 1% but these samples were not re-tested.

Why would vulval swabs have an apparently higher sensitivity in this study? Female chlamydial infection may involve the cervix, urethra or both and one theory is that exfoliated cellular debris and organisms from both the infected cervix and urethra are present at the vaginal introitus.²⁷ As most women are infected at the cervix, an area remote from the urethra, urine testing may miss some lower genital tract infections.

No studies, to our knowledge, have assessed patient acceptability of non-invasive testing for *C. trachomatis* infection. There was no significant difference found regarding preference for a particular test or specific recommendation to a female friend or relative. More women found the vulval swab 'embarrassing', 'uncomfortable' and 'difficult to do' than with urine testing, but the numbers were small. The finding that urine was significantly more likely to be chosen over vulval swabs was not surprising owing to its familiarity. However, vulval testing compared well, with almost 80% stating they would chose this method again. If not compared directly to urine, patient-collected swabs may have a comparable acceptability, but this is beyond the conclusions of this study.

Patient counselling, highlighting swabbing method, possibly using a model, could potentially eliminate the 9% who found the test uncomfortable or difficult to do. However, we acknowledge that those who are more introspective of their genital area may never feel comfortable with this method of screening.

The ideal diagnostic test for *C. trachomatis* infection would combine patient acceptability, ease of sampling and

testing, high sensitivity and specificity, and competitive costs. The main limitations of endocervical specimens are that the speculum examination is uncomfortable, the procedure limits where the test can be performed, and the need for trained healthcare personnel increases costs. In comparison, by avoiding a speculum examination, non-invasive testing may increase a patient's willingness to undergo a screening test and provide the opportunity to expand screening to individuals who might not otherwise be offered or choose to undergo testing. Furthermore, it limits the need for skilled personnel, thereby decreasing costs, and may permit screening at locations where there would be difficulties in conducting pelvic examinations i.e. school, home, and mobile units. The only criticism of this type of approach has been the loss of the vaginal examination. We argue that those who are asymptomatic of pelvic complaints, those who do not require or have undergone cervical cytology, and those who screen negative for *C. trachomatis* infection, do not need a vaginal examination. In contrast, those with symptoms will by and large accept examination; those requiring a smear should undergo endocervical testing, and those positive for *C. trachomatis* infection should proceed to examination and a complete STI screen.

Conclusion

This study found that opportunistic testing for genito-urinary *C. trachomatis* infection on women using LCR assay of patient-collected vulval swabs was as sensitive as LCR of urine, with high acceptability. Compared to urine testing, advantages in terms of specimen provision, transport, and processing should encourage more widespread use of this approach. Vulval swabbing must not be over-shadowed by the familiarity of urine as, ultimately, it may be the most acceptable screening method to both patients and health professionals. Further research is required to assess the acceptability of this method alone, the effects of pregnancy and menstrual blood on non-invasive testing, and why women decline screening.

Acknowledgements

We wish to thank all the women who volunteered to be tested. We thank Mrs J Skinner and Mrs L Ford for excellent technical assistance and Abbott Diagnostics, UK for performing the LCR-MOMP assays. We are grateful for the statistical assistance provided by Ann Fitzmaurice.

Statements on funding and competing interests

Funding. None

Competing interests. None

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