Diagnosis of urogenital *Chlamydia trachomatis* infection in women based on mailed samples obtained at home: multipractice comparative study

Lars Østergaard, Jens K Møller, Berit Andersen, Frede Olesen

Abstract

Objective—To compare urine and vaginal flush samples collected by women at home with endocervical and urethral swabs obtained by general practitioners for their efficacy in the diagnosis of urogenital *Chlamydia trachomatis* infection.

Design—Multipractice comparative study.

Setting—33 general practices and a central department of clinical microbiology in Aarhus County, Denmark.

Subjects—222 women aged 18–25 years who for any reason had a gynaecological examination.

Interventions—Endocervical and urethral swabs were obtained by the women's general practitioners. The same women when at home then collected a first void urine sample, a midstream urine sample, and a vaginal flush sample (using a vaginal pipette) and mailed them to the laboratory.

Main outcome measures—*C trachomatis* detected by the polymerase chain reaction and the ligase chain reaction. Eight tests for *C trachomatis* were performed for every woman. When two of the eight yielded positive results the patient was considered infected.

Results—The overall prevalence of *C trachomatis* infection was 11.2% (23/205 women). Test sensitivities in samples obtained by general practitioners, samples obtained at home subjected to polymerase chain reaction, and samples obtained at home subjected to ligase chain reaction were 91%, 96%, and 100% respectively. The corresponding specificities were 100%, 92.9%, and 99.5%.

Conclusions—The diagnostic efficacy of samples obtained by women at home and mailed to the laboratory was as good as for samples obtained by a general practitioner when using the ligase chain reaction. This may have important implications for the practicability of screening for this common, often asymptomatic, and treatable infection.

Introduction

Chlamydia trachomatis is the leading cause of bacterial sexually transmitted diseases, occurring in 3–14% of women attending family planning clinics.^{1,2} Women are affected most by the consequences of infection, with pelvic inflammatory disease, ectopic pregnancy, and tubal infertility.^{3,4} As about half of infected women are asymptomatic^{3,5} screening of clinically healthy women is also necessary in order to reduce the prevalence of the infection and subsequent complications.^{6,7} Screening procedures formerly required gynaecological examinations during which both endocervical and urethral swabs were taken in order to achieve high diagnostic

sensitivity.⁸ This need for gynaecological examinations in clinically healthy women may limit screening for *C trachomatis* because of cost and feasibility.

The introduction of DNA amplification methods—namely, the polymerase chain reaction assay⁹ and the ligase chain reaction assay^{10,11}—has increased sensitivity for the detection of *C trachomatis*. This increased sensitivity allows male urine to be used as sample material,^{12,13} and recently the ligase chain reaction assay has successfully been applied to urine samples from women.^{1,10,14,15} However, sensitivity in detecting urogenital *C trachomatis* infections in single urine samples from women is not optimal,^{1,10,14,15} and also the evaluation of diagnostic specificity might be influenced by the lack of optimal comparators.

We assessed whether urogenital samples could be obtained at home by the women themselves and subsequently mailed to the laboratory for *C trachomatis* DNA analysis. The diagnostic efficacy of these samples was compared with that of urethral and endocervical swabs obtained from the same patients by their general practitioners.

Patients and methods

Women consulting one of 33 general practitioners in Aarhus County from 15 January 1995 to 15 February 1996 were considered for the study by the general practitioner. For inclusion the women had to be white Danish citizens aged 18–25 years in whom a gynaecological examination was performed for any reason—for example, for prescription of oral contraceptives or screening for cervical cancer. Patients who had received antibacterials in the four weeks before examination and pregnant women were excluded. Written informed consent was obtained from all women and the study approved by the local ethics committee. A standard questionnaire concerning current partner, previous *C trachomatis* infection, symptoms and signs, and reason for the examination was completed by the general practitioner. The examination was performed without lubricants. Cervical and urethral swabs were placed in Amplicor transport medium (Roche Molecular Systems) and mailed with the questionnaire to the Department of Clinical Microbiology, Aarhus, for detection of *C trachomatis*.

A kit comprising two 10 ml sterile sample tubes, a vaginal pipette containing 5 ml sterile isotonic sodium chloride, written instructions, and a stamped addressed envelope was given to every patient by the general practitioner. Patients were instructed not to wash the urogenital region before collecting the samples. A first void urine sample was obtained by collecting the first 10 ml, then a 10 ml midstream urine specimen was obtained. The pipette was then introduced about 5 cm into the vagina and the vagina flushed with the contents of the pipette. The fluid was sucked back into the

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BMJ 1996;313:1186–9

Table 1—Diagnostic efficacy of samples obtained by general practitioners and women at home

Sample and test procedure (205 patients examined)	No of positive results	% Sensitivity (95% confidence interval)	% Specificity (95% confidence interval)	% Positive predictive value (95% confidence interval)	% Negative predictive value (95% confidence interval)
General practitioner combined*	21	91 (72 to 99)	100.0 (98.0 to 100.0)	100 (84 to 100)	98.9 (96.0 to 99.9)
Polymerase chain reaction home obtained combined†	35	96 (78 to 100)	92.9 (88.1 to 96.1)	63 (45 to 79)	99.4 (96.8 to 100.0)
Ligase chain reaction home obtained combined†	24	100 (85 to 100)	99.5 (97.0 to 100.0)	96 (79 to 100)	100.0 (98.0 to 100.0)
Endocervical swab	18	78 (56 to 93)	100.0 (98.0 to 100.0)	100 (82 to 100)	97.3 (93.9 to 99.1)
Urethral swab	13	57 (35 to 77)	100.0 (98.0 to 100.0)	100 (75 to 100)	94.8 (90.6 to 97.5)
First void urine (polymerase chain reaction)	19	74 (52 to 90)	98.9 (96.1 to 99.9)	89 (67 to 99)	96.8 (93.1 to 98.8)
Midstream urine (polymerase chain reaction)	19	83 (61 to 95)	100.0 (98.0 to 100.0)	100 (82 to 100)	97.9 (94.6 to 99.4)
Vaginal pipette (polymerase chain reaction)	30	83 (61 to 95)	94.0 (89.4 to 96.9)	63 (44 to 80)	97.7 (94.3 to 99.4)
First void urine (ligase chain reaction)	18	78 (56 to 93)	100.0 (98.0 to 100.0)	100 (82 to 100)	97.3 (93.9 to 99.1)
Midstream urine (ligase chain reaction)	19	83 (61 to 95)	100.0 (98.0 to 100.0)	100 (82 to 100)	97.4 (94.6 to 99.4)
Vaginal pipette (ligase chain reaction)	22	91 (72 to 99)	99.5 (97.0 to 100.0)	96 (77 to 100)	98.9 (96.1 to 99.9)

*Combination of endocervical and urethral swabs.

†Combination of first void urine, midstream urine, and vaginal pipette samples.

pipette and the pipette sealed. The patient then mailed the pipette and the two urine samples to the department of clinical microbiology.

PROCESSING SAMPLES

Endocervical and urethral swabs were assayed by the Amplicor polymerase chain reaction for the presence of *C trachomatis* DNA. A specimen was considered positive for infection only if positivity was confirmed on retesting. The pipette sample, the first void urine sample, and the midstream urine sample were divided by means of disposable pipettes into aliquots for the Amplicor *C trachomatis* urine polymerase chain reaction assay (Roche Diagnostics) and ligase chain reaction *C trachomatis* assay for urine (Abbott Laboratories). Urine was tested according to the manufacturer's instructions. The procedure for examining the vaginal pipette fluid (approximately 1.0–1.5 ml) was as follows. For both chain reactions equal amounts of the sample were added to 1.5 ml Eppendorf tubes and centrifuged at 10 000 *g* for 30 minutes, after which the supernatant was removed. For the polymerase chain reaction assay the pellet was resuspended in 500 µl urine resuspension buffer (Amplicor). After 60 minutes at room temperature 500 µl urine diluent was added to the sample, which was stored at room temperature for a minimum of 10 minutes. The polymerase chain reaction assay was then carried out according to the manufacturer's instructions. For the ligase chain reaction assay 1 ml ligase chain urine specimen resuspension buffer was added and the assay performed according to the manufacturer's instructions.

Thus for every patient the following eight tests for *C trachomatis* DNA were performed: endocervical swab polymerase chain reaction assay, urethral swab polymerase chain reaction assay, first void urine polymerase chain reaction and ligase chain reaction assays, midstream urine polymerase and ligase chain reaction assays, and vaginal pipette polymerase and ligase chain reaction assays.

DEFINITION OF INFECTION

A patient was considered infected with *C trachomatis* if at least two of the eight assays gave positive results; a positive result in one of the eight assays had to be confirmed by a positive result in one of the remaining seven.

STATISTICAL ANALYSIS

Sensitivity, specificity, and positive and negative predictive values were calculated according to Griner *et al.*¹⁶ and 95% confidence intervals for these values calculated based on the binomial distribution. Fisher's exact test was used for categorical variables and the Mann-Whitney test for continuous variables.

Results

Of 222 patients who were examined, 205 (92.3%; 95% confidence interval 88.0% to 95.5%) mailed all three samples obtained at home. The other 17 patients did not differ from the 205 with respect to age, having a steady partner, and the presence of symptoms or signs.

Twenty three patients were considered to have *C trachomatis* infection according to the criteria for positivity, giving a prevalence of 11.2% (7.2% to 16.4%). Table 1 shows the diagnostic efficacy with respect to samples obtained by the general practitioners and by the women at home. The low specificity of the polymerase chain reaction assay of home obtained combined samples was due to a low specificity of the reaction in vaginal pipette samples; results in 11 samples from the 182 patients who were test negative (6.0%) could not be confirmed. Testing the content of 114 unused vaginal pipettes by polymerase chain reaction yielded three (2.6%) positive results. None of the 114 pipette samples yielded a positive result when assayed by the ligase chain reaction. False positive results in vaginal pipette samples but not in urine samples therefore occurred with the polymerase chain reaction assay but apparently not with the ligase chain reaction assay.

When only two samples collected by the women at home were considered (that is, the same number obtained by the general practitioners) a sensitivity of 96% was achieved by applying the ligase chain reaction to the vaginal pipette sample and either the first void or midstream urine specimen.

Sensitivities for endocervical swab, first void urine polymerase chain reaction assay, and first void urine ligase chain reaction assay were 78%, 74%, and 78% respectively (table 1). Thus regardless of material sampled or system of testing, obtaining only one sample from women for the detection of *C trachomatis* was not enough to achieve optimal sensitivity.

The relation between anatomical sampling site—that is, endocervix and urethra—and the corresponding results for urine and vaginal pipette samples is shown in table 2 for patients with a test positive urethral or endocervical swab. Urine samples were test positive even in women who had positive findings only in the endocervix, and vaginal pipette samples were test positive even in women who had positive findings only in the urethra. Thus *C trachomatis* was detectable in urine and vaginal pipette samples despite apparently restricted anatomical sites of infection.

Only two (8.7%) of the 23 *C trachomatis* positive patients reported previous urogenital *C trachomatis* infection compared with 34 (18.7%) of the 182 women who were test negative. This difference was not explained by a difference in mean age (data not shown). A regular sexual partner was reported by 17 (73.9%) *C trachomatis* positive women and 144 (79.1%)

Table 2—Correlation between positive sampling site and results of testing urine and vaginal pipette samples

Anatomical sampling site	Positive only in vaginal pipette sample	Positive only in urine sample(s)	Positive in both vaginal pipette and urine sample(s)
Positive only from endocervix	0	0	8
Positive only from urethra	0	1*	2
Positive from both endocervix and urethra	1†	0	9

*Patient with urethral syndrome. Midstream urine sample was positive in both ligase chain reaction and polymerase chain reaction assays, but first void urine sample tested negative in both reactions.

†Vaginal pipette sample was positive in both ligase chain reaction and polymerase chain reaction assays.

C trachomatis negative women. Twelve (52.2%) *C trachomatis* positive women and 99 (54.4%) *C trachomatis* negative women were asymptomatic. The prevalence of *C trachomatis* infection among asymptomatic women was therefore 10.8% (12/111).

Discussion

Our study did not show better efficacy for doctor obtained samples compared with samples obtained by women at home for the diagnosis of urogenital *C trachomatis* infection. A non-significant trend for better diagnostic efficacy of samples obtained at home could not be confirmed because of the small number of women with infection.

Home obtained samples may have important implications for large scale screening of young women. This is required because the infection is treatable, common,^{2,4} and often asymptomatic^{3,5} and has no useful predictors.^{2,17,18} Recent studies successfully subjected first void urine samples from women to ligase chain reaction assay for the detection of *C trachomatis*.^{1,10,14,15} Lee *et al*¹⁴ examined 1937 women and Chernesky *et al*¹⁰ 420 and found sensitivities of 94% and 96% with the ligase chain reaction assay of first void urine samples. These values were higher than the 78% in our study.

The main problem in comparative studies is that no reference standard exists. In the studies by Lee *et al*¹⁴ and Chernesky *et al*¹⁰ a culture technique estimated to have sensitivities of only 65% and 56%, respectively, was used as comparator for calculating sensitivities of the ligase chain reaction assay. We previously applied the polymerase chain reaction assay used in this study to swabs and found a sensitivity of about 95% when compared with four unrelated tests.¹⁹ Furthermore, only endocervical swabs were included for comparison in the studies by Lee *et al* and Chernesky *et al*, and as shown in this and other studies a urethral swab is also needed in order to achieve high diagnostic sensitivity for samples obtained by doctors.^{5,8,20} When culture results of urethral and endocervical swabs were compared with the results of applying the ligase chain reaction assay to first void urine the assay had a sensitivity of 86%.²¹ Another explanation for the lower sensitivity of the urine ligase chain reaction assay in this study could be that samples were not refrigerated in the mail.

A possibility of false positive results with the polymerase chain reaction assay of vaginal pipette samples could have affected the number of patients diagnosed as infected. This did not occur, however, because the polymerase chain reaction assay of the vaginal sample was not decisive for positivity in any patient.

The 100% specificity obtained by general practitioners was almost equalled by the 99.5% specificity of the ligase chain reaction assay of home obtained samples. The high specificity of the ligase chain reaction assay of first void urine samples has also been found in other studies,^{1,10,14,15} but the algorithm used for analysis of discrepant results has been questioned.²²

Key messages

- Samples obtained by women at home and mailed to the laboratory can replace samples obtained by general practitioners for the diagnosis of urogenital *Chlamydia trachomatis* infection
- A combination of mailed samples obtained at home is necessary in order to obtain optimal diagnostic sensitivity; a single urine sample is not enough
- Mailed samples obtained at home may also be appropriate for diagnosis in young asymptomatic women with regular partners; these women have a high prevalence of urogenital *C trachomatis* infection
- The use of mailed self obtained samples in women might limit costs and increase the practicability of screening programmes for urogenital *C trachomatis* infection

HAVING SEVERAL SAMPLES IS IMPORTANT

The rationale for combining urine and vaginal pipette samples in our study was that an infection restricted to the endocervix was probably more likely to be detected in a vaginal pipette sample and infection restricted to the urethra was probably more likely to be detected in a urine sample. However, this held for only one patient, who had the urethral syndrome. This patient had positive findings only in the urethral swab and with ligase and polymerase chain reaction assays of midstream urine samples (table 2). A 10.8% prevalence of *C trachomatis* infection in clinically healthy young women and the fact that 74% of infected women had a regular sexual partner strongly suggest that home obtained samples have a place in screening young asymptomatic women with a regular sexual relationship.

Women at home collected three different samples whereas general practitioners obtained only two. Using a strategy of home obtained samples therefore increases the cost of diagnostic testing per patient but saves the cost of a visit to the doctor; in addition, the woman loses no earnings. Cost-benefit analyses are needed to assess the most beneficial strategy. We emphasise, however, that using home obtained samples alone limits the possibility of examining for other pathogens or disease and rules out opportunistically educating patients about sexually transmitted diseases.

In our study 92% of women mailed all three samples collected at home. This, however, does not mean that women in the general population will comply. Further studies are needed to evaluate the efficacy of screening programmes using the various home obtained samples and to assess women's attitudes to these sampling methods.

We conclude that the diagnostic sensitivity and specificity of urine and vaginal flush samples collected by women at home and mailed to the laboratory for the detection of *C trachomatis* infection are as good as for samples taken by a general practitioner during a gynaecological examination. This may have important implications for the feasibility and costs of screening programmes for young asymptomatic women.

We acknowledge the technical work of Gitte Høj, Mette Jensen, and Jonna Guldberg and the secretarial help of Anne Marie Andersen. We also acknowledge all the general practitioners who participated.

Funding: The Scandinavian Society for Antimicrobial Chemotherapy and Lægekredsforeningen in the County of Aarhus.

Conflict of interest: None.

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(Accepted 26 September 1996)

Catchment areas in general practice and their relation to size and quality of practice and deprivation: a descriptive study in one London borough

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Abstract

Objective—To relate the sizes of general practice catchment areas in one London borough to list size, deprivation payments, medical staffing, and locally and nationally recognised measures of quality.

Design—Study of general practice catchment area maps.

Setting—London borough of Lambeth.

Subjects—60 out of the 71 general practices in Lambeth.

Main outcome measures—Practice catchment area size with corrections for numbers of doctors and patients.

Results—Catchment area size varied greatly between practices, showing an almost 150-fold difference between the largest and smallest practices. This size differential was even more marked when the size of the catchment area was corrected for the number of general practitioners in the practice, where a 300-fold difference was found. Substantial differences existed between practices in each of the four locally assigned quality bands. The weakest practices had catchment areas three times as large as those of the strongest practices. When corrected for medical staffing, the difference was eight times as great. A calculated measure of patient dispersion showed that the practice population of the strongest practices was four times as densely clustered as that of the weakest practices, whose patients were more widely geographically dispersed.

Conclusions—Large variations exist in the size of catchment areas of inner city practices even when corrected for numbers of doctors and

patients. These differences are associated with variations in quality of care.

Introduction

All NHS general practitioners are required to provide a map of the geographical boundaries of their practices,¹ which should be reproduced in the practice leaflet. While the location of a practice is controlled to some extent by the Medical Practices Committee,² there is no such control over the practice's boundaries. Research interest has tended to focus on administrative characteristics such as list size, partnership size, length of consultation, and staffing and activities such as prescribing and referrals, while the issue of catchment areas has received little attention. General practitioners may set their boundaries wherever they choose, with no legislative constraints and a dearth of published research findings to guide their choice. We performed a study of general practices in one London borough to describe the size of practices' catchment areas and examine the relation between their size and other practice characteristics.

Method

All 71 practices in the London borough of Lambeth were invited to contribute to the study by submitting an up to date map of their catchment areas. Non-responders were followed up by letter, telephone, and visit if necessary. The catchment area boundaries were digitised and catchment area size calculated using the MAPINFO mapping package. Practice location within the borough was classified into three groups—northern, middle, and southern—on the basis of postcode. Further information was obtained from Lambeth,

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BMJ 1996;313:1189-92