CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

ORIGINAL ARTICLE

Diagnosis of *Chlamydia trachomatis* infections in a sexually transmitted disease clinic: evaluation of a urine sample tested by enzyme immunoassay and polymerase chain reaction in comparison with a cervical and/or a urethral swab tested by culture and polymerase chain reaction

I. P. Jensen[1], *H. Fogh*[2] *and J. Prag*[3]

[1]Department of Virology, Statens Serum Institut, [2]Department of Dermato-Venerology, Rigshospitalet and [3]Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

Objective To evaluate the value of a urine sample for diagnosing *Chlamydia trachomatis* infection in an STD clinic in a prospective study of samples collected from 410 consecutive STD patients (167 female and 243 male).

Methods Urine samples were tested by enzyme immunoassay (EIA) and polymerase chain reaction (PCR) in comparison with cervical and/or urethral swabs tested by PCR and cell culture. A questionnaire was completed for a total of 320 patients concerning symptoms, and determining whether they were controls, contacts or were being tested subsequent to legal abortion.

Results The overall prevalence of *C. trachomatis* infection was 11.5%. At least 40% of patients were asymptomatic. Of the *C. trachomatis*-positive patients, 85% were diagnosed by testing urine, compared to 91% by testing swabs. For urine tests, the sensitivities of PCR were 66.7% and 71.9% for female and male patients, respectively, and the sensitivities of EIA were 40.0% and 62.5%, or 46.7% and 71.9%, for female and male patients, respectively, by including a 30% gray zone below the cut-off value. For swabs, the sensitivities of PCR were 93.3% and 87.5% for female and male patients, respectively, and equal to the sensitivities of culture. In total, 3.3% of controls and 35% of contacts were found to be *C. trachomatis* positive.

Conclusion The use of urine samples for the diagnosis of *C. trachomatis* infections was effective, but urine samples should be additional to conventional swab(s) instead of replacing. Partner notification and a confirmation of cure is recommended.

Keywords Urine, diagnosis, *Chlamydia trachomatis*, STD

Accepted 21 February 2002

Clin Microbiol Infect 2003; 9: 194–201

INTRODUCTION

Chlamydia trachomatis is today the most frequent sexually transmitted microorganism in the Western World, causing serious complications and sequelae. In Denmark, almost 13 000 *C. trachomatis* infections were diagnosed in 1998 (25% from male patients and 75% from female patients), corresponding to a prevalence of 4.7%, based on approximately 270 000 analyses. A decrease in the prevalence of at least 3.5% was observed from 1989 to 1998, but the number of analyses in the same period of time was 2.7 times higher, which could explain some of the observed decrease in prevalence [1–3]. Because many infections are asymptomatic [4–7], the only way to reduce the

Corresponding author and reprint requests: I. P. Jensen, Department of Virology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark
Tel: +45 32683341
Fax: +45 32683148
E-mail: ipj@ssi.dk

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases

number of cases is by screening and treating selective groups [8–12]. A non-invasive method using a urine sample seems suitable for this. Besides being non-invasive, a urine sample needs no physician for sampling, and is suitable for 'home collection' [9].

In this prospective study of patients attending a sexually transmitted disease (STD) clinic in Copenhagen, Denmark, we compared a first-catch urine sample tested by two commercially available tests—an enzyme immunoassay (EIA), Syva Microtrak II EIA (Syva Company, San Jose, CA, USA), and a polymerase chain reaction (PCR), Amplicor (Roche Diagnostic Systems, F. Hoffman-La-Roche Ltd, Basel, Switzerland)—with a cervical and/or urethral swab tested by culture and PCR. The aim of the study was to clarify whether a urine sample could replace the conventional swabbing technique in this population.

MATERIALS AND METHODS

Study population

In total, 410 consecutive patients (243 males, median age 28 years (range 17–60 years) and 167 females, median age 25 years (range 16–69 years)) visiting a dermatovenerologic 'walk-in' clinic at University Hospital, Rigshospitalet in Copenhagen from January 1995 to February 1996 were enrolled in the study after written informed consent according to the Helsinki Declaration II. Approval of the study was obtained from the regional scientific ethical committee.

Materials

From all patients, a 50-mL first-void urine sample was collected, at least 30 min after last urination from which two aliquots of 10 mL were taken, one for EIA and one for PCR. A urethral swab from male patients, and a cervical swab together with a urethral swab from female patients, were collected for culture and PCR. Swabs (for PCR and culture) were kept in culture transport media, and the same swabs were used for PCR and culture. In order to avoid bias for one specimen type, half of the patients collected a urine sample before swabbing, and the other half were swabbed before the urine sample was collected.

In total, 320 questionnaires were completed, concerning symptoms of urethritis (dysuria, urethral discharge) and cervicitis (vaginal discharge, bleeding), and determined whether a patient was a control, was being tested 4 weeks after a legal abortion, or had been in contact with a *C. trachomatis*-positive patient or had came for follow-up examination 4 weeks after treatment for *C. trachomatis* infection with a single oral 1.0-g dose of azithromycin.

METHODS

EIA and direct fluorescent assay on urine samples

Urine samples were kept at 4 °C for a maximum of 3 days before reaching the laboratory. When the urine samples had reached room temperature, they were mixed and an aliquot of 4 mL was taken for Syva Microtrak II Chlamydia EIA (Syva Company). The rest of the urine sample (6 mL) was kept for direct fluorescent assay (DFA) by the Syva Microtrak *Chlamydia trachomatis* Direct Specimen Test (Syva Company). The urine samples for EIA as well as for DFA were centrifuged (3000 *g* for 30 min), and for the EIA the pellet was processed according to the manufacturer's instructions.

For DFA, the pellet was washed by another centrifugation with phosphate-buffered saline (PBS) (pH 7.4), and the pellet was then resuspended in 100 μL of PBS, from which 10–25 μL was used for the slide preparation. Fixation and staining of the slide was performed according to the manufacturer's instructions.

All positive EIA results (OD value $\geq$ cut-off) and EIA gray-zone positive results (a gray zone was defined as a 30% zone below the cut-off value) were tested by DFA on the EIA specimen remnants or on the separate urine samples for DFA, as described above.

Observation of one or more elementary bodies (EBs) was considered as confirmation of a positive or a gray-zone-positive EIA result. All slides were read by the same person throughout the whole study.

PCR

The Amplicor *Chlamydia trachomatis* Test (Roche Diagnostic Systems) was used for the PCR testing of urine samples, urethral swabs and cervical swabs. The urine samples for PCR were kept at 4 °C, and swabs for PCR were kept at −80 °C, before further processing twice a week.

The PCR detects plasmid DNA. The automatic COBAS System was used for the samples collected

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

from the last 61 patients. An internal control to detect inhibitors in the sample causing a false-negative result was incorporated in the COBAS system, but not in the Amplicor *Chlamydia trachomatis* Test.

A gray-zone result, according to the manufacturer's instructions, was an OD value between 0.2 and 0.5. All gray-zone-positive results were later retested, and a PCR detecting major outer membrane protein (MOMP PCR) was performed blind by F. Hoffmann-La Roche, Ltd, Diagnostic Division, Basel, Switzetland. The samples were stored at $-80\,^{\circ}C$ until testing.

Urine samples

Urine samples were tested according to the instructions of the manufacturer. Prior to PCR testing, the urine sample was centrifuged at 1500 *g* for 10 min, and the pellet was used for further testing.

Swabs

Swabs were tested according to the instructions of the manufacturer.

Culture

The swabs were cultured in cycloheximide-treated McCoy cells [13] without blind passage, and inclusion-forming units (IFUs) were detected by iodine staining. The culture was read after 48 h and 72 h.

To achieve optimal culture result, the swabs were transported on ice to the laboratory. Samples were inoculated twice weekly. The samples were kept at $-80\,^{\circ}C$ prior to inoculation.

If one or more IFUs were observed, the sample was considered positive.

Criteria for 'true' positive results

A culture-positive result was considered as 'true', and needed no further confirmation. A positive EIA and PCR result or a gray-zone-positive EIA or PCR result was considered a 'true' positive if it could be confirmed by a urine DFA or by a MOMP PCR or if a parallel test result was a 'true'positive.

Evaluation of a negative result

For every 15 sets of negative sample results (negative urine EIA result, negative urine PCR result, negative swab PCR result, and negative swab culture result), a separate urine sample was tested by DFA. In total, 23 urine samples (representing 23 sets of negative samples) were tested by DFA. This confirmation was not performed blind.

Testing in case of discordant results

According to the criteria for a 'true' positive result, only a positive MOMP PCR, a positive culture or a positive DFA could confirm another positive result from the same site of sampling.

Statistical methods

Fisher's exact test (two-tailed) was used for the statistical calculations. $P < 0.05$ was considered statistically significant.

RESULTS

In total, 47 patients (32 males and 15 females) were found to be *C. trachomatis* positive, corresponding to a prevalence in this population of 11.5% (47/410). The prevalence in male patients was 13.2% (32/243), and that in female patients 9% (15/167). Forty of 47 patients (85%) were diagnosed by testing a urine sample, compared to 43 of 47 (91%) by testing swabs (cervical and/or urethral swab). The difference in sensitivity between testing urine samples and testing swabs did not reach statistical significance (Fisher's exact test (two-tailed), $P = 0.5$).

In four *C. trachomatis*-positive cases, the diagnosis was based on urine testing alone; two were asymptomatic male patients tested as contacts, one a male patient with dysuria and urethral discharge, and one a female with dysuria who was tested as a contact of a *C. trachomatis*-positive partner. Therefore, the detection rate was increased by 9% by testing a urine sample in conjunction with testing swabs. In total, 14% (2/14) of 15 *C. trachomatis*-positive female patients had an isolated urethral infection, as two patients were positive only by sampling from the urethra (one patient was positive by urine PCR, and one by urine PCR as well as urethral swab PCR and urethral swab culture). One patient did not have a cervical swab taken at all.

The results of comparing the two different specimen types are presented in Table 1. The results are presented in relation to the 'true' positive patient,

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

Table 1 Sensitivity, specificity, positive predictive value and negative predictive value of EIA, PCR and culture for each specimen type calculated in relation to the 'true' *C. trachomatis*-positive patient among the female/male patients ($n = 167/243$) and after resolving discordant results

	Sensitivity Female/male (%/%)	Specificity Female/male (%/%)	PPV Female/male (%/%)	NPV Female/male (%/%)
EIA (urine)	40/62.5 (46.7/71.9)	94.1/95.3 (94.1/95.3)	40/66.7 (46.7/69.7)	94.1/94.4 (94.7/95.7)
PCR (urine)	66.7[a]/71.9	100/100	100/100	96.8/95.9
PCR (swab)	93.3/87.5	100/100	100/100	99.3/98.1
Culture (swab)	93.3/87.5	100/100	100/100	99.3/98.1

[a]Included two PCR-positive urine results, negative by retesting and negative by MOMP PCR.
The figures in parentheses are the values calculated by including gray-zone-confirmed EIA results (one female urine and three male urine were gray-zone EIA positive, confirmed by DFA or MOMP PCR).

with calculation of sensitivity, specificity and predictive values, after resolution of discordant results. The difference between urine EIA and urine PCR did not reach statistical significance ($P = 0.4$ and $P = 0.7$ ($P = 0.5$ and $P = 1.0$), respectively, Fisher's exact test (two-tailed)).

Upon testing urine samples by EIA, only 26 of 45 (58%) of 45 EIA-reactive urines (OD value $\geq$ cut-off) could be confirmed by DFA.

Of 24 urine samples that were EIA gray-zone positive, three could be confirmed by DFA and one by MOMP PCR, and these four were considered 'true' positive results.

Of the tests on urine with PCR, 14 of 47 (30%) were false negative according to the criteria for a 'true' positive result (nine samples were from male and five from female patients). Eight were later found to be MOMP PCR positive. Six were EIA as well as DFA positive (five were MOMP PCR positive), and one urine was EIA gray-zone positive, DFA negative, and MOMP PCR positive.

On discrepancy testing, four of 17 PCR results (24%) could not be reproduced by retesting (three urine PCR results (two positive results and one negative result) and one positive urethral swab PCR).

Of two female patients with an initial positive urine PCR, negative by retesting, negative by MOMP PCR, and negative by urine EIA and DFA, one had a positive urethral swab PCR, and a positive cervical and urethral swab culture, and the other had a positive urethral swab PCR and culture. Both were considered urine PCR 'true' positive according to the criteria for a 'true' positive result. Apart from these two cases, there were no false-positive PCR results.

Three gray-zone PCR-positive samples (two urethral swabs and one urine sample) were negative by a repeated PCR and by a MOMP PCR, and were considered true negative according to the criteria for results.

In one case (a male patient), only urine EIA and DFA were positive. The parallel samples were negative. The patient was tested as a contact of a *C. trachomatis*-positive partner without urethritis symptoms according to the questionnaire.

For the confirmation of a negative result, DFA was performed on 23 urine samples representing every 15th set of negative sample results, and found to be negative for all 23 urine samples.

Data from the analysis of 320 completed questionnaires are presented in Table 2.

Information was available from 36 *C. trachomatis* patients (information was not available for six positive female patients and five *C. trachomatis*-positive male patients). The median ages of *C. trachomatis*-positive patients were 24.5 years (range 18–43 years) and 21 years (range 10–41 years) for male and female patients, respectively.

In total, 23 of 36 patients (64%) complained of symptoms compatible with a *C. trachomatis* infection. Eighteen of 27 (67%) male patients and five of nine (56%) female patients were symptomatic, corresponding to an asymptomatic carriage rate of *C. trachomatis* of approximately 40%. Among symptomatic *C. trachomatis*-positive patients, 80% (4/5) of female patients and 88% (16/18) of male patients were diagnosed by urine testing. In total, of 117 patients (48 female and 69 male) complaining of symptoms compatible with *C. trachomatis* infection, 20% (23/117) were actually diagnosed as having a *C. trachomatis* infection.

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

Table 2 Medical and socio-demographic data correlated with *C. trachomatis* status and gender

	Female patients			Male patients		
	Total number	***C. trachomatis* positive $n=15$**	***C. trachomatis* negative $n=152$**	**Total number**	***C. trachomatis* positive $n=32$**	***C. trachomatis* negative $n=211$**
Dysuria[a]	18	2	16	60	16	44
Urethral discharge[a]	11	0	11	37	13	24
Vaginal discharge[a]	34	4	30	–	–	–
Bleeding[a]	4	1	3	–	–	–
Abortion	3	0	3	–	–	–
Contact	14	4[b]	10	17	7[b]	10
Control	35	0	35	25	2[c]	23

Information was not available for six *C. trachomatis*-positive female patients and for five *C. trachomatis*-positive male patients.
In total, information was not available for 90 of 410 patients (22%) (36 female patients and 54 male patients).
In total, 69 male patients and 48 female patients had one or more symptoms compatible with a *C. trachomatis* infection.
[a]Of *C. trachomatis*-positive contacts, two female contacts and four male contacts had symptoms compatible with a *C. trachomatis* infection.
[b]Two *C. trachomatis*-positive male controls were asymptomatic.

Thirty-one contacts with a *C. trachomatis*-positive individual were tested, and 11 (four female and seven male) were *C. trachomatis* positive (35%), six of whom were symptomatic (two female and four male).

Sixty patients (35 female and 25 male) were tested as controls 4 weeks after treatment. In total, 3.3% (2/60) asymptomatic male patients were positive. During the study period, only six of 36 *C. trachomatis*-positive patients had a later follow-up test, of whom five were negative and one was positive. One of these patients, a female, was again positive 5 months later, after a negative follow-up test 6 weeks after a positive one and treatment.

DISCUSSION

We demonstrated a prevalence of *C. trachomatis* infection of 11.5% among dermatovenerologic patients, corresponding to the prevalence shown by other studies from a similar study group [6,14–18]. At least 40% of the patients or more had an asymptomatic *C. trachomatis* infection.

By testing urine samples, we could diagnose 85% of the infections, compared to 91% diagnosed by testing swabs.

Four more *C. trachomatis*-positive patients (three male and one female, or 9%) were diagnosed by testing urine in conjunction with swab(s). This may indicate insufficiently rigorous urethral swabbing, especially for male patients.

Up to 24% of female patients have isolated urethral infection [19], and we demonstrated an isolated urethra *C. trachomatis* infection in 14% of the female population, indicating the importance of supplying a cervical swab in addition to a urethral swab or a urine sample.

In our study, culture and PCR performed equally and very well on swabs, probably due to a well-established culture laboratory and rapid, cooled transport to the laboratory. The use of indirect immunofluorescence staining of inclusions in cell culture instead of the iodine staining we used in our study might have shown the number of cases to be even higher [20].

Although culture is highly specific and, until recently, was considered to be the 'reference standard' test, it is costly and laborious, and, for the evaluation of the diagnostic efficacy of the very sensitive nucleic acid amplification assays, an expanded standard has been introduced [21].

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

The much higher sensitivity of PCR and ligase chain reaction (LCR) on urine samples compared to culture on swabs reported in other studies could be caused by a suboptimal culture technique with low sensitivity or different culture techniques [14–16,22,23].

PCR on urine performed better than EIA on urine in relation to the 'true' positive patient for females, and equally well for males, by including a 30% negative gray zone for EIA (Table 1). However, there was a need for confirmation of a positive EIA result by DFA, as only 58% of EIA-positive results could be confirmed by DFA. Our study is similar to a study in which only 49% of EIA-positive results (Syva Microtrak) could be confirmed by DFA [17].

The sensitivity of EIA was increased by 10% by including a negative gray zone of 30%, but to achieve this increase 24 gray-zone-positive urines had to be tested by a DFA, which was quite laborious. The advantages of EIA are ease of performance, the possibility of running a large number of samples, and the fact that special laboratory equipment is not necessary.

The only male patient diagnosed as 'true' *C. trachomatis* positive by EIA confirmed by DFA might be a collection failure (see Results).The large number of false-negative urine PCR results (30%) could possibly be a result of inhibitors in the urine samples, as repeated testing after freezing and thawing turned one PCR-negative urine to PCR positive, and five PCR-negative urines to MOMP PCR positive.

Stary et al. found 33% false-negative PCR (Amplicor) results when testing fresh urine samples [17].

Labile inhibitors in cervical mucus cannot be removed by freezing and thawing, but can be inactivated by a brief warming to 95 °C, or by leaving at room temperature for 2 days [24–26]. A dilution of the sample, together with a brief warming, was recommended in order to eliminate inhibitors not removed by freezing and thawing [26], or simply by diluting the sample prior to testing [27–29].

A low number of *C. trachomatis* particles in the sample may also give rise to a false-negative PCR result [30], but only two of 14 patients with a PCR false-negative urine result had less than 10 IFUs in their corresponding urethral swabs (data not shown). A false-positive PCR result could very likely be true positive, as the confirmatory MOMP PCR is much less sensitive than plasmid PCR [31].

The PCR results did not reproduce very well corresponding with results from other studies [24,32].

The fact that 35% of contacts tested positive emphasizes the importance of contact tracing.

Two asymptomatic male controls (3.3%) tested *C. trachomatis* positive, 4 weeks after a single 1.0-g dose of azithromycin. Only six of the *C. trachomatis*-positive patients diagnosed during the study returned for a later follow-up test after treatment.

A single 1.0-g dose of azithromycin is as effective as a standard 7-day cure of doxycycline, and gives a higher compliance rate [33–35]. Our cases could be treatment failures or reinfections. We must therefore recommend a confirmation of cure 4 weeks after treatment in a high-risk population in order to minimize the spread of *C. trachomatis*. A home-collected, mailed urine sample tested by PCR/LCR could be suggested for this purpose [9]. A fear of false-positive results after treatment, especially by antigen tests and amplification tests, has been expressed, but positive results are turning negative at least 2 weeks after a treatment was initiated [36,37]. A confirmation of cure might not be cost-effective, and instead a follow-up visit was recommended about 2 weeks after treatment was initiated, with a new test only in cases of possible reinfection, lack of compliance or treatment failure [38–40].

In conclusion, we found that a urine sample worked well in this population of symptomatic and asymptomatic STD patients for the diagnosis of urogenital *C. trachomatis* infection. However, testing a urine sample is not straightforward. Urine EIA gave a large number of false-positive results, whereas urine PCR was characterized by too many false-negative results, most likely caused by inhibitors. The optimal way to diagnose a *C. trachomatis* infection is to add a urine sample to conventional swab(s) for assessment, instead of replacing a swab by a urine sample. When a urine sample was tested in addition to a swab, the detection rate was increased by 9%. As two (3.3%) asymptomatic male follow-up patients were found to be *C. trachomatis* positive 4 weeks after therapy was initiated, we think that a confirmation of cure is advisable to minimize the spread of *C. trachomatis* infections, together with partner notification, as 35% of contacts tested positive.

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

ACKNOWLEDGMENTS

Our special thanks go to laboratory technician Bente Andersen, Department of Virology, Statens Serun Institut, Copenhagen, and laboratory technician Hanne Kubert, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, for their excellent work on this study.

REFERENCES

1. Epi-News. Danish National Surveillance of Communicable Diseases. 1999; 24.
2. Epi-News. Danish National Surveillance of Communicable Diseases. 1996; 38.
3. World Health Organization. *Guidelines for the prevention of genital Chlamydia infections.* Uppsala: WHO Collaborating Center for Sexually Transmitted Diseases and their Complications, University of Uppsala, 1990.
4. Stary A, Steyrer K, Heller-Vitouch C, Muller I, Mårdh PA. Screening for *Chlamydia trachomatis* in military personnel by urine testing. *Infection* 1991; 19: 9–11.
5. Jensen IP. A comparison of urine sample to urethral swab for detection of *Chlamydia trachomatis* in asymptomatic young men using two enzyme immunoassays. *Sex Transm Dis* 1992; 19: 165–9.
6. Chernesky MA, Lee H, Schachter J *et al.* Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine by a ligase chain reaction assay. *J Infect Dis* 1994; 170: 1308–11.
7. Søgaard P, Møller BR, Thorsen P *et al.* Prevalence of *Chlamydia trachomatis* among young men and women in military service. Comparison of urethral swab with a urine sample. *Ugeskr Læger* 1996; 158: 759–63.
8. Rahm VA, Gnarpe H, Odlind V. *Chlamydia trachomatis* among sexually active teenage girls. Lack of correlation between chlamydial infection, history of the patient and clinical signs of infection. *Br J Obstet Gynecol* 1988; 95: 916–19.
9. Østergaard L, Møller JK, Andersen B, Olesen F. Diagnosis of urogenital *Chlamydia trachomatis* infection in women based on mailed samples obtained at home: multipractice comparative study. *BMJ* 1996; 313: 1186–9.
10. Moi H, Danielsson D. Diagnosis of genital *Chlamydia trachomatis* infection in males by cell culture and antigen detection test. *Eur J Clin Microbiol* 1986; 5: 563–8.
11. Brady M, Baker C, Neinstein LS. Asymptomatic *Chlamydia trachomatis* infections in teenage males. *J Adolesc Health Care* 1988; 9: 72–5.
12. Nagy B, Corradi G, Vajda Z, Gimes R, Csomok S. The occurrence of *Chlamydia trachomatis* in semen of men participating in an IVF programme. *Human Reprod* 1989; 4: 54–6.
13. Ripa KT, Mårdh PA. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cell. *J Clin Microbiol* 1977; 6: 328–31.
14. Chernesky MA, Chong S, Jang D, Luinstra K, Sellors J, Mahony JB. Ability of commercial ligase chain reaction and PCR assays to diagnose *Chlamydia trachomatis* infections in men testing first-void urine. *J Clin Microbiol* 1997; 35: 982–4.
15. Barbeyrac B, Rodriguez P, Dutilh B, Le Roux P, Bebear C. Detection of *Chlamydia trachomatis* by ligase chain reaction compared with polymerase chain reaction and cell culture in urogenital specimens. *Genitourin Med* 1995; 71: 382–6.
16. Doorum GJJ, Buimer M, Prins M *et al.* Detection of *Chlamydia trachomatis* infection in urine samples from men and women by ligase chain reaction. *J Clin Microbiol* 1995; 33: 2042–7.
17. Stary A, Choueiri B, Hörting-Müller I, Halisch P, Teodorowicz L. Detection of *Chlamydia trachomatis* in urethral and urine samples from symptomatic and asymptomatic male patients by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1996; 15: 465–71.
18. Buimer M, van Doorum GJJ, Ching S *et al.* Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction based assays with clinical specimens from various sites: implications for diagnostic testing and screening. *J Clin Microbiol* 1996; 34: 2395–400.
19. Morris RE, Legault J, Baker C. Prevalence of isolated urethral asymptomatic *Chlamydia trachomatis* infection in the absence of cervical infection in incarcerated adolescent girls. *Sex Transm Dis* 1993; 20: 198–200.
20. Zapata M, Chernesky M, Mahony J. Indirect immunofluorescence staining of *Chlamydia trachomatis* inclusions in microculture plates with monoclonal antibodies. *J Clin Microbiol* 1984; 19: 937–9.
21. Thejls H, Gnarpe J, Gnarpe H *et al.* Expanded gold standard in the diagnosis of *Chlamydia trachomatis* in a low prevalence population: diagnostic efficacy of tissue culture, direct immunofluorescence, enzyme immunoassay, PCR and serology. *Genitourin Med* 1994; 70: 300–3.
22. Pate MS, Hook EW III. Laboratory to laboratory variation in *Chlamydia trachomatis* culture practice. *Sex Transm Dis* 1995; 22: 322–6.
23. Schepetiuk S, Tuckweng K, Martin L, Waddell R, Higgins R. Detection of *Chlamydia trachomatis* in urine samples by nucleic acid tests: comparison with culture and enzyme immunoassay of genital swab specimens. *J Clin Microbiol* 1997; 35: 3355–7.

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201

24. Bauwens JE, Clark AM, Stamm WE. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. *J Clin Microbiol* 1993; 31: 3023–7.
25. Clad A, Naudasher I, Flecken U, Freidank HM, Petersen EE. Evidence of labile inhibitors in the detection of *Chlamydia trachomatis* in cervical specimens by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1996; 15: 744–7.
26. Verkooyen RP, Luijendijk A, Huisman WM *et al*. Detection of PCR inhibitors in cervical specimens by using the Amplicor *Chlamydia trachomatis* assay. *J Clin Microbiol* 1996; 34: 3072–4.
27. Chernesky MA, Jang D, Sellors J *et al*. Urinary inhibitors of polymerase chain reaction and ligase chain reaction and testing of multiple specimens may contribute to lower assay sensitivities for diagnosing *Chlamydia trachomatis* infected women. *Mol Cell Probes* 1997; 11: 243–9.
28. Chernesky MA, Chong S, Jang D, Luinstra K, Faught M, Mahony J. Inhibition of amplification of *Chlamydia trachomatis* plasmid DNA by ligase chain reaction associated with female urines. *Clin Microbiol Infect* 1998; 4: 397–400.
29. Mahony J, Chong S, Luinstra K *et al*. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. *J Clin Microbiol* 1998; 36: 3122–6.
30. Thomas BJ, Macload EJ, Taylor-Robinson D. Evaluation of a commercial polymerase chain reaction for *Chlamydia trachomatis*: suggestions for improving sensitivity. *Eur J Clin Microbiol Infect Dis* 1995; 14: 719–23.
31. Mahony JB, Luinstra KE, Sellors JW, Chernesky MA. Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J Clin Microbiol* 1993; 31: 1753–8.
32. Peterson EM, Darrow V, Blanding J, Aarnaes S, De La Maza LM. Reproducibility problems with the Amplicor PCR *Chlamydia trachomatis* Test. *J Clin Microbiol* 1997; 35: 957–9.
33. Martin DH, Mroczkowski TF, Dalu ZA *et al*. A controlled trial of a single dose of azithromycin for the treatment of chlamydial urethritis and cervicitis. *N Engl J Med* 1992; 327: 921–5.
34. Worm AM, Østerlind A. Azithromycin levels in cervical mucus and plasma after a single 1.0 g oral dose for chlamydial cervicitis. *Genitourin Med* 1995; 71: 244–6.
35. Hillis SD, Coles FB, Litchfield B *et al*. Doxycycline and azithromycin for prevention of chlamydial persistence or recurrence one month after treatment in women. *Sex Transm Dis* 1998; 25: 5–11.
36. Claas HC, Wagenvoort JH, Niesters HG, Tio TT, Van Rijsoort-Vos JH, Quint WG. Diagnostic value of the polymerase chain reaction for *Chlamydia* detection as determined in a follow-up study. *J Clin Microbiol* 1991; 29: 42–5.
37. Workowski KA, Lampe MF, Wong KG, Watts MB, Stamm WE. Long-term eradication of *Chlamydia trachomatis* genital infection after antimicrobial therapy. Evidence against persistent infection. *JAMA* 1993; 270: 2071–5.
38. Radcliffe KW, Rowen D, Mercey DE, Ridgway GL, Robinson AJ, Bingham JS. Is a test of cure necessary following treatment for cervical infection with *Chlamydia trachomatis*? *Genitourin Med* 1990; 66: 444–6.
39. Ali SM, Booth G, Monteiro E. Test of cure following treatment of genital *Chlamydia trachomatis* infection in male and female patients. *Genitourin Med* 1997; 73: 223.
40. White DJ, Mann CH, Matthews RS, Leeming JG, Clay JC. The value of tests of cure following cervical chlamydial infection. *Int J STD AIDS* 1993; 4: 5–7.

© 2003 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **9**, 194–201