Short Communication

Assessment of *Chlamydia trachomatis* infection of semen specimens by ligase chain reaction

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Diagnostic potential of the *Chlamydia trachomatis* ligase chain reaction system (LCx) to assess the presence of *C. trachomatis* in urine and semen specimens was evaluated. Paired urine and semen specimens from 153 asymptomatic male partners of subfertile couples attending our Center for Reproductive Medicine were examined by LCx. As controls, 19 semen samples from four donors who were participating in the programme for artificial insemination were used. Of these, 12 samples had previously been shown to be *C. trachomatis*-positive by an in-house PCR. *C. trachomatis* was detected by LCx in seven of 153 (5 %) urine samples. None of the 153 semen samples tested positive by LCx. Also, none of the 12 *C. trachomatis*-containing control semen samples were positive by LCx. By in-house PCR, seven urine specimens and two of 153 (1 %) semen samples tested positive. The corresponding urine samples of these male partners were also *C. trachomatis*-positive, as well as the 12 *C. trachomatis*-containing samples from donors. In conclusion, LCx is not sensitive enough to assess the presence of *C. trachomatis* in semen specimens; therefore, this method is not recommended to routinely screen semen specimens from donors who participate in programmes for artificial insemination or male partners of subfertile couples for *C. trachomatis*.

Received 22 January 2003

Accepted 27 May 2003

Introduction

Chlamydia trachomatis is the cause of the most prevalent sexually transmitted bacterial disease worldwide and is responsible for an estimated 90 million infections (Gerbase *et al.*, 1998). If not treated at an early stage, infection can lead from initial urethritis or cervicitis to severe sequelae, such as prostatitis or epididymitis among men or pelvic inflammatory disease, ectopic pregnancy or tubal infertility in women (Marrazzo & Stamm, 1998). As infection runs an asymptomatic course in 50–80 % of cases, many infected individuals are not treated and continue to spread bacteria among the population, usually via sexual contacts (Fish *et al.*, 1989; Zimmerman *et al.*, 1990; Marrazzo & Stamm, 1998). Considering the high worldwide prevalence of *C. trachomatis* infection, artificial insemination by donor (AID) is a potential route for the spread of *C. trachomatis*. Indeed, transmission of *C. trachomatis* through AID has been reported (Nagel *et al.*, 1986; van den Brule *et al.*, 1993). By using an in-house PCR, we detected *C. trachomatis* DNA in 12 cryopreserved ejaculates from four of 97 donors who had been accepted for the AID program, indicating 4 % pre-

valence of *C. trachomatis* infection (Pannekoek *et al.*, 2000). Evidently, screening of donors for *C. trachomatis* infection is of paramount importance to guarantee the microbiological quality of semen. The in-house PCR, which detects elementary bodies (EBs) at a lower limit of 2.5–5.0 EBs (μl semen)⁻¹, was shown to be a highly sensitive and specific assay for the detection of *C. trachomatis* DNA in semen. A disadvantage of this test is that it requires DNA isolation from duplicate samples (one of which is spiked with EBs) and Southern hybridization; these are time-consuming procedures that also require a lot of hands-on time. We therefore questioned whether the commercially available and widely used LCx assay (Abbott Diagnostics) would be appropriate for routine screening of semen specimens. The LCx assay has been developed to assess *C. trachomatis* infection in urine, urethral or cervical specimens and has been shown to be highly sensitive and specific (Chernesky *et al.*, 1994; Rumpianesi *et al.*, 1996; Watson *et al.*, 2002).

Methods

Source of specimens. One hundred and fifty-three male partners of subfertile couples were enrolled in this study. Their urine and semen specimens were collected, with approval from our institutional review board, after informed consent was given. In addition, four semen

Abbreviations: AID, artificial insemination by donor; EB, elementary body; LCx, ligase chain reaction system.

donors for AID were included in the study. All male partners and semen donors were asymptomatic of infection.

Specimens. First-void urine from the 153 male partners was stored at -20°C . After first-void urine had been collected, semen specimens were obtained and also stored at -20°C . All male partners had at least 5 days sexual abstinence before donation of semen. Nineteen semen specimens from four donors were used, of which 12 were previously shown to be *C. trachomatis*-positive by in-house PCR (Pannekoek *et al.*, 2000), as positive and negative controls in the detection assays and were blindly re-examined.

Assessment of *C. trachomatis* DNA in urine and corresponding semen specimens by LCx. First-void urine was processed and tested by LCx (Abbott Diagnostics) according to the manufacturer's urine protocol. Assessment of *C. trachomatis* in semen specimens from the subjects and controls was done as follows. An aliquot (100 μl) of each semen specimen was vortexed and centrifuged at room temperature at 12 000 g for 10 min. The pellet was resuspended in 100 μl urine resuspension buffer (Abbott Diagnostics) and incubated at 95°C for 15 min. After cooling, the suspension was mixed with 100 μl LCx reaction mixture (Abbott Diagnostics) and processed further according to the manufacturer's protocol.

DNA extracted from the equivalent of 10 μl specimen (see below) was precipitated, resuspended in 100 μl urine resuspension buffer and incubated at 95°C for 15 min. After cooling, the suspension was mixed with 100 μl LCx reaction mixture and processed further according to the manufacturer's protocol.

Assessment of *C. trachomatis* DNA in urine and semen by in-house PCR. Urine and corresponding semen specimens from the male partners and semen specimens from the four controls were assessed for *C. trachomatis* infection by an in-house PCR as described previously (Pannekoek *et al.*, 2000). In brief, duplicate 50 μl samples of each specimen were processed and, prior to DNA extraction, 250 EBs [quantified by using direct immunofluorescence (Microtrak; Syva)] were added to one of the duplicate samples, which was used to monitor the efficiency of DNA extraction and PCR. Both samples were then subjected to DNA extraction by using the silica-guanidinium thiocyanate procedure in combination with buffer L6 and size-fractionated silica particles, as described previously (Boom *et al.*, 1990; Pannekoek *et al.*, 2000). Primers that targeted the cryptic chlamydial plasmid were used for amplification and the equivalent of 10 μl specimen was used as template in a final PCR mixture of 50 μl . The equivalent of 4 μl specimen was analysed for the presence of *C. trachomatis* amplified DNA by Southern hybridization with a specific internal end-labelled probe (Pannekoek *et al.*, 2000).

Results

Assessment of *C. trachomatis* DNA in urine and corresponding semen specimens by LCx

In total, 153 urine and corresponding semen specimens from the male partners were assessed for the presence of *C. trachomatis* DNA by LCx. *C. trachomatis* was detected in 7 (5%) urine samples. None of the 153 semen samples tested positive for *C. trachomatis*.

Assessment of *C. trachomatis* DNA in urine and corresponding semen specimens by in-house PCR

To exclude a false-positive outcome, the urine samples of the seven LCx *C. trachomatis*-positive male partners were re-tested for *C. trachomatis* infection by in-house PCR. In addition, the 153 semen samples were also assessed for *C. trachomatis* infection by using the in-house PCR (Pannekoek

et al., 2000). The urine samples of the seven male partners who were *C. trachomatis*-positive by LCx were also positive by in-house PCR. Of the 153 semen samples, two (1%) were *C. trachomatis*-positive by in-house PCR. The corresponding urine samples of these two male partners were also *C. trachomatis*-positive by in-house PCR and LCx. All spiked samples (urine and semen) were positive by in-house PCR (not shown). These results indicate a low sensitivity of LCx for the assessment of *C. trachomatis* infection of semen specimens.

Comparison of the performances of the LCx assay and in-house PCR in the detection of *C. trachomatis* in semen specimens from donors for AID

The apparently low sensitivity of LCx when testing semen specimens was further investigated by using 19 control semen specimens that originated from four donors for AID. The infection status of these semen specimens was determined previously by in-house PCR (Pannekoek *et al.*, 2000). These specimens served as positive and negative controls to monitor the performance of LCx and were blindly assessed for *C. trachomatis* infection by LCx and in-house PCR. None of the 19 samples, whether spiked with EBs or not, tested positive for *C. trachomatis* by LCx. In contrast, 12 of the 19 samples, as well as all spiked samples, tested positive by in-house PCR. Decoding of the specimens revealed that these 12 samples were also previously shown to be *C. trachomatis*-positive by in-house PCR (Pannekoek *et al.*, 2000). To further investigate the performance of LCx with semen, the same amount of DNA as that used as template for in-house PCR (the equivalent of 10 μl specimen) was extracted from the 19 semen specimens and tested by LCx. Four of 19 samples (non-spiked) tested positive. These four samples were also positive by in-house PCR. Of the spiked samples, only eight of 19 tested positive by LCx.

Discussion

We evaluated the diagnostic potential of LCx to detect *C. trachomatis* in semen specimens. From 153 asymptomatic male partners of subfertile couples, paired urine and semen samples were tested by LCx. *C. trachomatis* was detected in 7 (5%) urine samples, whereas none of the corresponding semen specimens tested positive by LCx. In addition, none of 12 *C. trachomatis*-infected semen specimens from four donors for AID that served as controls tested positive by LCx. The presence of *C. trachomatis* in these controls was assessed by in-house PCR (Pannekoek *et al.*, 2000); *C. trachomatis* was detected in two semen specimens from male partners, which corresponded to their respective LCx-positive urine specimens.

Detection of *C. trachomatis* in semen specimens collected for AID is of importance as detection of *C. trachomatis* in semen specimens, but not in corresponding urine samples, has been reported (Krieger *et al.*, 1996; Fujisawa *et al.*, 1999). Although a variety of commercial DNA amplification methods is currently available to detect *C. trachomatis* in urethral, urine and cervical specimens, none of these tests provides a

protocol that is validated for screening of semen specimens for *C. trachomatis* infection. In the present study, we evaluated the performance of LCx with semen specimens in comparison to an in-house PCR that is appropriate for semen specimens (Pannekoek *et al.*, 2000). Our results clearly demonstrate that the LCx assay is not a sensitive enough method to detect *C. trachomatis* in semen, although detection of *C. trachomatis* in semen specimens by LCx has been reported: Bollmann *et al.* (2001) processed 10 µl or a 1:5 dilution of 10 µl semen for LCx and used spiked samples as inhibitor controls. *C. trachomatis* was detected in three of 105 (3 %) specimens, but no data were reported about inhibition. Other studies either lack technical data (Eggert-Kruse *et al.*, 2002a, b) or the number of positive samples is very low and the results are not confirmed by a second test (Fujisawa *et al.*, 1999).

The low sensitivity of LCx to detect *C. trachomatis* in semen specimens compared to urine specimens may be due to the fact that semen might contain inhibitory components that interfere negatively with the ligase chain reaction. This is strongly supported by the observation that none of the 19 *C. trachomatis*-spiked samples from donors tested positive by LCx. In contrast, by using the equivalent of only 10 µl specimen, an in-house PCR detected *C. trachomatis* in 12 naturally infected specimens. In addition, all spiked samples gave a positive signal by in-house PCR. DNA extraction from semen samples may remove LCx-inhibitory factors; indeed, some improvement in LCx performance was observed after DNA isolation. By using DNA extracted from 10 µl specimen as input in the LCx assay, *C. trachomatis* infection was detected in four of 12 samples and 11 of 19 spiked samples.

Results were not confirmed with a second test in any of the studies in which *C. trachomatis* was detected in semen specimens, but not in corresponding urine samples, by LCx (Krieger *et al.*, 1996; Fujisawa *et al.*, 1999). In our study population, we did not find *C. trachomatis* in semen only. Thus, it is obvious that more systematic studies are needed to investigate the diagnostic value of first-void urine screening to assess *C. trachomatis* infection among donors for AID. In summary, our results strongly indicate that LCx should not be used to detect *C. trachomatis* in semen specimens from donors for AID. To ensure the microbiological quality and safety of AID, assessment of *C. trachomatis* infection among donors might require both urine and semen specimens at each time of donation. Therefore, an automated amplification test that enables sensitive detection of *C. trachomatis* in semen specimens is urgently needed.

References

- Bollmann, R., Engel, S., Petzoldt, R. & Gobel, U. B. (2001). *Chlamydia trachomatis* in andrologic patients – direct and indirect detection. *Infection* 29, 113–118.

Boom, R., Sol, C. J., Salimans, M. M., Jansen, C. L., Wertheim-van Dillen, P. M. & van der Noordaa, J. (1990). Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 28, 495–503.

Chernesky, M. A., Jang, D., Lee, H., Burczak, J. D., Hu, H., Sellors, J., Tomazic-Allen, S. J. & Mahony, J. B. (1994). Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. *J Clin Microbiol* 32, 2682–2685.

Eggert-Kruse, W., Neuer, A., Clussmann, C., Boit, R., Geissler, W., Rohr, G. & Strowitzki, T. (2002a). Seminal antibodies to human 60kd heat shock protein (HSP 60) in male partners of subfertile couples. *Hum Reprod* 17, 726–735.

Eggert-Kruse, W., Zwick, E. M., Batschulat, K., Rohr, G., Armbruster, F. P., Petzoldt, D. & Strowitzki, T. (2002b). Are zinc levels in seminal plasma associated with seminal leukocytes and other determinants of semen quality? *Fertil Steril* 77, 260–269.

Fish, A. N., Fairweather, D. V., Oriel, J. D. & Ridgway, G. L. (1989). *Chlamydia trachomatis* infection in a gynaecology clinic population: identification of high-risk groups and the value of contact tracing. *Eur J Obstet Gynecol Reprod Biol* 31, 67–74.

Fujisawa, M., Nakano, Y., Matsui, T., Okada, H., Arakawa, S. & Kamidono, S. (1999). *Chlamydia trachomatis* detected by ligase chain reaction in the semen of asymptomatic patients without pyospermia or pyuria. *Arch Androl* 42, 41–44.

Gerbase, A. C., Rowley, J. T., Heymann, D. H., Berkley, S. F. & Piot, P. (1998). Global prevalence and incidence estimates of selected curable STDs. *Sex Transm Infect* 74 (Suppl. 1), S12–S16.

Krieger, J. N., Riley, D. E., Roberts, M. C. & Berger, R. E. (1996). Prokaryotic DNA sequences in patients with chronic idiopathic prostatitis. *J Clin Microbiol* 34, 3120–3128.

Marrazzo, J. M. & Stamm, W. E. (1998). New approaches to the diagnosis, treatment, and prevention of chlamydial infection. *Curr Clin Top Infect Dis* 18, 37–59.

Nagel, T. C., Tagatz, G. E. & Campbell, B. F. (1986). Transmission of *Chlamydia trachomatis* by artificial insemination. *Fertil Steril* 46, 959–960.

Pannekoek, Y., Westenberg, S. M., de Vries, J., Repping, S., Spanjaard, L., Eijk, P. P., van der Ende, A. & Dankert, J. (2000). PCR assessment of *Chlamydia trachomatis* infection of semen specimens processed for artificial insemination. *J Clin Microbiol* 38, 3763–3767.

Rumpianesi, F., Donati, M., Negosanti, M., D'Antuono, A., La Placa, M. & Cevenini, R. (1996). Detection of *Chlamydia trachomatis* by a ligase chain reaction amplification method. *Sex Transm Dis* 23, 177–180.

van den Brule, A. J., Hemrika, D. J., Walboomers, J. M., Raaphorst, P., van Amstel, N., Bleker, O. P. & Meijer, C. J. (1993). Detection of *Chlamydia trachomatis* in semen of artificial insemination donors by the polymerase chain reaction. *Fertil Steril* 59, 1098–1104.

Watson, E. J., Templeton, A., Russell, I., Paavonen, J., Mardh, P.-A., Stary, A. & Pederson, B. S. (2002). The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol* 51, 1021–1031.

Zimmerman, H. L., Potterat, J. J., Dukes, R. L., Muth, J. B., Zimmerman, H. P., Fogle, J. S. & Pratts, C. I. (1990). Epidemiologic differences between chlamydia and gonorrhea. *Am J Public Health* 80, 1338–1342.