

Swabs (dry or collected in universal transport medium) and semen can be used for the detection of *Chlamydia trachomatis* using the cobas 4800 system

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In this prospective study, the fully automated cobas 4800 CT/NG and the cobas TaqMan CT tests were compared for *Chlamydia trachomatis* detection in urine and in genital specimens collected with Copan flocced swabs in culture media. A protocol was also established for the highly sensitive detection of *C. trachomatis* in semen specimens using the cobas 4800 CT/NG test. A total of 708 consecutive urogenital samples (293 male urine samples and 356 vaginal, 45 cervical and 14 urethral swabs) obtained from the Bacteriology Department, as well as 100 consecutive semen samples collected from patients attending the Reproduction Biology Department, Bordeaux University Hospital, France, from July to September 2010, were analysed. Positive and negative agreements between the cobas 4800 CT/NG and cobas TaqMan CT tests were 92.7% [95% confidence interval (CI), 82.7–97.1%] and 99.2% (95% CI, 98.2–99.7%), respectively, with an overall agreement of 98.7% (699/708). The clinical sensitivity of the cobas 4800 CT/NG assay for *C. trachomatis* ranged from 90.9 to 100% depending on specimen type, with an overall prevalence of 7.2% (51/708). The clinical specificity ranged from 99.1 to 100% depending on specimen type. Dilution of 25 µl semen samples in cobas PCR medium proved to be the most sensitive protocol with the lowest inhibition rate. In conclusion, the cobas 4800 CT/NG test was found to be an effective method for detection of *C. trachomatis* in semen, male urine and genital swab samples collected dry or in universal transport medium.

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INTRODUCTION

Sexually transmitted infections (STIs) are a major public health concern. *Chlamydia trachomatis* infections are the most prevalent bacterial STI diagnosed worldwide (Peipert, 2003; Manavi, 2006; Bébéar & de Barbeyrac, 2009). In men, *C. trachomatis* has been linked to genitourinary tract inflammation, urethritis and epididymitis (Bhalla *et al.*, 2007). In women, this bacterial infection can lead to pelvic

inflammatory disease, subsequent ectopic pregnancy and tubal factor infertility (Manavi, 2006; Bhalla *et al.*, 2007). In addition, most women and men with chlamydial infection are often asymptomatic (Peipert, 2003). The role of *C. trachomatis* in male infertility is still controversial (Ochsendorf, 2008). Current screening for this bacterial STI pathogen among infertile men and semen donors is strongly recommended, as it can cause serious reproductive complications in the recipients of semen donations and infection in their offspring (Peeling & Embree, 2005; Eley & Pacey, 2011).

A variety of nucleic acid amplification test (NAAT) methods to detect *C. trachomatis* in urethral, urine and

Abbreviations: CI, confidence interval; C_t, cycle threshold; IC, internal control; NAAT, nucleic acid amplification test; STI, sexually transmitted infection; UTM, universal transport medium.

cervical specimens are currently available with far higher sensitivity than cell culture or antigen detection (Semeniuk *et al.*, 2002; Bébéar & de Barbeyrac, 2009). An increasing number of laboratories are now offering combinatorial NAATs for the diagnosis of both *C. trachomatis* and *Neisseria gonorrhoeae* infections (Gaydos *et al.*, 2010; Hopkins *et al.*, 2010; Rockett *et al.*, 2010; Cheng *et al.*, 2011; Kerndt *et al.*, 2011). The Roche Diagnostics cobas CT/NG test, performed on the cobas 4800 system, is a new commercialized diagnostic assay using an automated workstation to isolate nucleic acids from clinical specimens and a real-time instrument for the detection of *C. trachomatis* and *N. gonorrhoeae* (Rockett *et al.*, 2010; Taylor *et al.*, 2012; Van Der Pol *et al.*, 2012). Self-collected vaginal swabs in women and male urine samples have been cleared by the US Food and Drug Administration for use with commercial NAATs. According to the manufacturer's instructions for the cobas 4800 CT/NG test, dry swabs should be discharged into cobas PCR medium containing guanidine hydrochloride. This step makes it impossible to perform bacterial culture. In our routine practice, we use swab material discharged in bacterial transport medium to be tested by cell and standard culture and by PCR. In men, first-void urine is currently the specimen of choice for the routine detection of *C. trachomatis* and *N. gonorrhoeae*, with little or no emphasis placed on testing of semen because the urine sample is easy to collect, is a non-invasive sample and contains a high organism load (Eley, 2011). In France, however, all semen specimens for *in vitro* fertilization are screened for *C. trachomatis*. It is noteworthy that semen specimens contain more NAAT inhibitors than other urogenital samples and specific protocols have to be developed for the optimal detection of *C. trachomatis* in this specimen type (Pannekoek *et al.*, 2000; Gdoura *et al.*, 2008; Eley & Pacey, 2011). In addition to the problem of NAAT inhibitors, the sensitivity of the tests may also be affected, as a significant proportion of men with infection of the upper genital tract will be asymptomatic and harbour fewer bacteria (Witkin, 2002).

Therefore, the objectives of our study were to: (i) compare the fully automated cobas 4800 CT/NG and cobas TaqMan CT tests for detection of *C. trachomatis* in urine samples and genital specimens collected with Copan flocced swabs discharged in culture medium, and (ii) develop a protocol for the highly sensitive detection of *C. trachomatis* in semen specimens using the cobas 4800 CT/NG test.

METHODS

Clinical specimens. Consecutive urogenital specimens from the Bacteriology Department of Bordeaux University Hospital, France, were included from July to September 2010. The Copan flocced swabs used for cervical and urethral sample collection were discharged in universal transport medium (UTM; Copan Diagnostics), whilst Copan flocced vaginal swabs were self-collected and mailed dry to the laboratory. Dry-shipped urogenital swab samples were recently validated for use with NAATs (Gaydos *et al.*, 2012). First-void urine was self-collected in a sterile plastic urine bottle. All specimens were

transported to the Bacteriology Department of Bordeaux University Hospital within 24 h for testing.

Semen specimens were obtained from 100 consecutive patients attending the Reproduction Biology Department of Bordeaux University Hospital from July to September 2010.

Detection of *C. trachomatis* using the cobas TaqMan CT test.

Each vaginal Copan flocced swab was resuspended in 1 ml home-prepared 2SP medium (0.2 M sucrose, 15 mM K_2HPO_4 , 6 mM KH_2PO_4 and water, pH 7) prior to DNA extraction. Urine samples were frozen at $-20^\circ C$ overnight to destroy NAAT inhibitors and 500 μ l thawed urine was centrifuged. Lysis buffer (200 μ l) from a MagNaPure LC DNA Isolation kit I (Roche Diagnostics) was then added to the pellet. Nucleic acid extraction was fully automated using a MagNaPure LC DNA Isolation kit I on an extractor MagNaPure LC system (Roche Diagnostics) according to the manufacturer's instructions. The detection of *C. trachomatis* was performed using a cobas TaqMan CT test v2.0 on a cobas TaqMan 48 (Roche Diagnostics) instrument according to the manufacturer's instructions.

Detection of *C. trachomatis* using the cobas 4800 CT/NG test.

Urine specimens were processed using the cobas 4800 CT/NG test following the manufacturer's instructions. In brief, 4.5 ml urine sample was added to 4.5 ml cobas PCR medium. The Copan flocced swabs initially discharged in 2SP or UTM were resuspended in 1 ml cobas PCR medium. Samples were then tested on the cobas 4800 system. Briefly, 22 samples, three controls and reagents were loaded in each run onto the cobas x480 analyser, which performed the DNA extraction using magnetic bead technology similar to that of the MagNA Pure LC. Internal controls were added to each sample to check the extraction and amplification steps. This instrument then loaded extracted DNAs, controls and amplification reagents into a 96-well PCR microplate. Following this process, a technician sealed the microwell plate and placed it into the z480 real-time PCR instrument. Results were described as positive, negative, invalid or failed by the cobas 4800 software.

Detection of *C. trachomatis* in semen specimens using the cobas 4800 CT test.

Initially, we determined inhibition rates and limits of detection for different semen sample volumes by a cycle threshold (C_t) value analysis using a cobas 4800 CT test. In this study, semen specimens were obtained from 100 consecutive patients. Volumes of 50 μ l ($n=67$), 40 μ l ($n=27$), 30 μ l ($n=36$), 25 μ l ($n=36$), 10 μ l ($n=7$), 5 μ l ($n=12$), 2.5 μ l ($n=5$) or 1 μ l ($n=5$) were each added to 4.5 ml cobas PCR medium. Each sample was homogenized by vortexing and then tested on the cobas 4800 system. The inhibition rate was determined by comparing the C_t value of the internal control (IC) with that obtained in 708 urogenital samples.

To determine the limit of detection, *C. trachomatis*-negative semen samples were pooled, and 450 μ l pooled semen or 2SP medium was spiked with 50 μ l of a *C. trachomatis*-serovar D cell culture (10^5 inclusion-forming units ml^{-1}) and each was then serially diluted tenfold to a dilution of 10^{-6} . For each dilution, a volume of 50, 40 or 25 μ l was added to 4.5 ml cobas PCR medium and tested in duplicate on the cobas 4800 system.

Data analysis. Specimens that tested positive by both the cobas 4800 CT/NG and the cobas TaqMan CT tests were considered consensus positive for *C. trachomatis*. Similarly, specimens that were tested negative by both assays were considered consensus negative. The clinical sensitivity and specificity of *C. trachomatis* were calculated on the basis of the reference test and by specimen type (Table 1). Positive and negative percentage agreements, along with their 95% confidence intervals (CI), and overall percentage agreement were calculated based on the initial results (Simel *et al.*, 1991). To compare C_t values obtained from different specimen types using the cobas 4800 CT/NG

Table 1. Performance characteristics of the cobas 4800 CT/NG test for detection of *C. trachomatis* in urine and swab specimens

| Sample | cobas 4800 <i>C. trachomatis</i> results | | | | | | | | | |
|-----------------------|--|----------|--------------------------|--------------------------|-------------------------|---------|----------|----------------|-------------------------------|--|
| | Reference results* | | | | cobas 4800 performance† | | | | Additional cobas 4800 results | |
| | Pos. (n) | Neg. (n) | Sensitivity [% (95% CI)] | Specificity [% (95% CI)] | NPV (%) | PPV (%) | PPV (%) | Failed [n (%)] | Invalid [n (%)] | |
| Male urines (n=293) | 16 | 2 | 94.1 (73.0–98.9) | | 88.8 | | | 0 (0.0) | 1 (0.34) | |
| Urethral swabs (n=14) | 1 | 274 | 100 (20.6–100) | 99.2 (97.4–99.8) | 100 | 99.6 | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Cervical swabs (n=45) | 0 | 13 | 100 (51.0–100) | 100 (77.1–100) | 100 | 100 | 3 (6.7) | 0 (0.0) | 0 (0.0) | |
| Vaginal swabs (n=356) | 0 | 41 | 90.9 (76.4–96.9) | 100 (91.4–100) | 90.9 | 100 | 15 (4.2) | 0 (0.0) | 0 (0.0) | |
| | 30 | 3 | | 99.1 (97.3–99.7) | | 99.1 | | | | |
| | 3 | 320 | | | | | | | | |

*Reference results were obtained with the MagNaPure LC system and the cobas TaqMan 48 CT test.

†NPV, Negative predictive value; PPV, positive predictive value.

and TaqMan CT tests, and to compare *C. trachomatis* or IC C_t values between 2SP-spiked and semen-spiked samples in the cobas 4800 CT/NG test, two-way analysis of variance models were used. *P* values of <0.05 were considered significant.

RESULTS

Detection of *C. trachomatis* in urogenital specimens

A total of 708 urogenital samples were collected: 293 male urine, 14 urethral swab, 356 vaginal swab and 45 cervical swab samples. Nine specimens (three male urine and six vaginal swab samples; Table 1) provided discrepant results for *C. trachomatis* between the cobas 4800 and the cobas TaqMan CT tests, resulting in positive and negative agreements of 92.7% (95% CI, 82.7–97.1%) and 99.2% (95% CI, 98.2–99.7%), respectively, with an overall agreement of 98.7% (699/708). The clinical sensitivity and specificity of the cobas 4800 CT/NG test for *C. trachomatis* ranged from 90.9 to 100% and from 99.1 to 100%, respectively, depending on the specimen type (Table 1). Overall, the *C. trachomatis* prevalence was 7.2% (51/708).

Eighteen of the 708 specimens (15 vaginal and three cervical swabs) were flagged by the cobas 4800 as 'failed', indicating a failure of DNA extraction from the specimen, and one urine specimen was flagged as 'invalid', indicating that the IC results were negative (Table 1). This provided an overall extraction failure rate of 2.54% and an inhibition rate of 0.14% for the 708 urogenital specimens tested. With the reference test, 24 specimens (six vaginal swabs, one cervical swab and 17 urine samples) were flagged as 'invalid'.

The mean C_t values were not statistically different between the two methods, regardless of the origin of the specimen (Fig. 1). This indicated that the bacterial load in swab specimens resuspended in cobas PCR medium was not affected by the previous resuspension of the same swab in 2SP medium or UTM. The C_t difference was in favour of the cobas TaqMan 48 for female swabs (C_t mean of 29.1 vs 30.8) and in favour of the cobas 4800 for urine (C_t mean 30.14 vs 32.8).

C. trachomatis detection in semen samples

All 100 semen samples provided negative results for *C. trachomatis*. Means of IC C_t values obtained with 1–40 μ l of semen sample did not significantly differ from C_t values obtained with urogenital swab and urine samples. The mean IC C_t obtained with 50 μ l semen sample showed a strong increase compared with C_t values of lower sample volumes, indicating the presence of NAAT inhibitors (Fig. 2). Based on this result, we tested 25, 40 and 50 μ l spiked semen samples to determine the limit of detection for *C. trachomatis*. The semen samples processed with *C. trachomatis*-infected cells yielded positive results, indicating

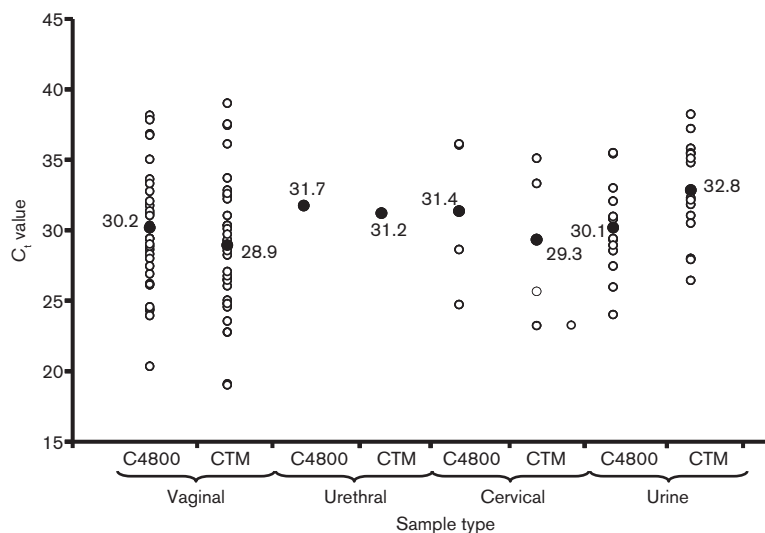


Fig. 1. Comparison of the C_t values of *C. trachomatis* detection obtained with the cobas 4800 CT/NG (C4800) and cobas TaqMan CT (CTM) tests on 708 urogenital samples. ○, C_t value obtained for each specimen; ●, mean C_t value.

successful DNA extraction and amplification. At a semen volume of 25 μ l, no significant difference in *C. trachomatis* or IC C_t values was observed between 2SP-spiked and semen-spiked samples (Table 2). However, with a volume of 40 and 50 μ l semen-spiked samples, C_t values were higher or even negative (Table 2).

All these results indicated that a volume of 25 μ l semen sample revealed the lowest detection limit (10^{-5}) and a low inhibition rate for *C. trachomatis*.

DISCUSSION

The cobas 4800 CT/NG test proved to be highly suitable for high-throughput identification of *C. trachomatis* nucleic acids and gave good agreement with an established method like the cobas TaqMan 48 CT test using swabs initially discharged into culture medium and extracted using a MagNaPure LC system (De Martino *et al.*, 2006). The

reason for discharging swabs into culture medium is to allow testing of specimens by culture techniques for *C. trachomatis* or *N. gonorrhoeae*. For *N. gonorrhoeae*, the advantage is to confirm its presence and to determine antimicrobial susceptibility.

The first objective of our study was to determine whether we could use the cobas 4800 platform using our usual sampling with swabs collected dry or in culture medium. Preparation of specimens was not carried out according to the manufacturer's instructions provided with the cobas 4800 CT/NG test. Swab material was discharged into 2SP medium or UTM instead of being discharged directly into the cobas PCR medium. Our results were in agreement with those reported by Rockett *et al.* (2010). In their study, swab specimens were initially resuspended in 1 ml PBS, 500 μ l of which was then added to 500 μ l cobas PCR medium. The cobas 4800 CT/NG test showed a simple and short workflow sequence, allowing prompt and specific

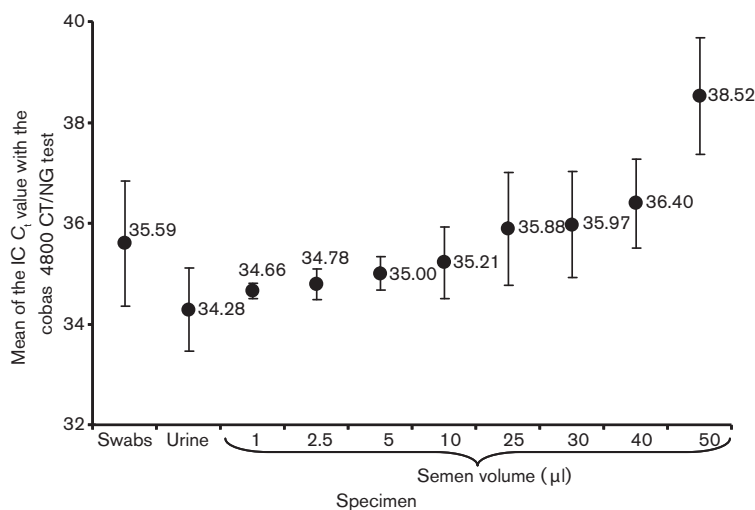


Fig. 2. Comparison of mean C_t values of the IC for semen and urogenital specimens. Error bars represent SD.

Table 2. Comparison of C_t values of *C. trachomatis* detection in semen or 2SP medium spiked with *C. trachomatis*-infected cells

–, Negative.

| Dilution of <i>C. trachomatis</i> -infected cells | C_t value of <i>C. trachomatis</i> detection | | | | | |
|---|--|--------------|-------------------|--------------|-------------------|--------------|
| | 50 μ l sample | | 40 μ l sample | | 25 μ l sample | |
| | 2SP spiked | Semen spiked | 2SP spiked | Semen spiked | 2SP spiked | Semen spiked |
| 10^{-1} | 29.8 | 32.6 | 30.3 | 30.6 | 31.0 | 30.2 |
| 10^{-2} | 32.5 | 35.8 | 33.1 | 32.8 | 33.2 | 33.3 |
| 10^{-3} | 35.6 | 37.5 | 34.9 | 35.3 | 35.7 | 36.8 |
| 10^{-4} | 37.3 | 40.5 | 37.3 | 38.0 | 37.9 | 37.1 |
| 10^{-5} | 40.2 | – | 38.7 | – | 40.0 | 40.0 |

results to be validated through the use of an internal extraction and amplification control. In total, 18 female swabs were flagged by the cobas 4800 software as ‘failed’, indicating a failure to extract the specimen. These specimens were homogenized by vortexing and retested. All retested specimens were negative. No extraction problem was observed with urethral swabs and urine samples. This problem was probably due to clots of cells in the vaginal or cervical samples and/or the low sample volumes used in this study (1 ml instead of 4.5 ml). Just one urine specimen was flagged as ‘invalid’ by the cobas 4800, indicating a superior extraction step compared with that of the MagNaPure LC system for which 24 specimens were flagged as ‘invalid’ by the cobas TaqMan 48.

For the routine detection of *C. trachomatis* in males, first-void urine is currently the specimen of choice (Bébéar & de Barbeyrac, 2009; Taylor *et al.*, 2012). However, an obvious alternative test specimen to consider in an infertility setting is semen. Moreover, semen analysis may provide additional information on *C. trachomatis* infection. However, there are still no approved or recommended methods for detecting *C. trachomatis* in semen. From a practical standpoint, commercial NAAT methods are the most appropriate to detect *C. trachomatis* in semen. Protocols need to be developed for optimal detection of *C. trachomatis* in this specimen type. The optimal amount of semen that should be used for such testing is not known. Surveys of the literature show that 10–100 μ l has been used with PCR tests (Wolff *et al.*, 1994; Pannekoek *et al.*, 2000; Peeling & Embree, 2005). In our study, we showed that a volume of 25 μ l semen sample was optimal for *C. trachomatis* detection with the cobas 4800 CT/NG test. A fundamental difficulty with the testing of semen samples for *C. trachomatis* is the viscosity and the abundance of human DNA, which may interfere with the ability of the primers to access the target DNA to initiate the annealing step in the NAAT amplification cycle (Peeling & Embree, 2005). It is essential that, in every NAAT for semen, there is an in-built inhibitor control (Skidmore *et al.*, 2006). Consequently, we established a validated protocol with the cobas 4800 CT/NG test that used an IC for inhibitors to

assess *C. trachomatis* infection in semen specimens. Another difficulty is the possibility that, during ejaculation, semen may become contaminated with elementary bodies in the urethra (Ochsendorf *et al.*, 1999). This further complicates any comparison between testing of semen and urine. Furthermore, it is clear that some *C. trachomatis* infections may be missed if urine is the only test specimen and semen is not tested as well. These studies evaluating the prevalence of *C. trachomatis* in male infertility populations have had relatively small sample sizes and short study durations (Eley & Pacey, 2011). In our study, the new semen protocol performed for *C. trachomatis* detection using the cobas 4800 CT/NG test had a high analytical sensitivity and showed low inhibition rates. Before establishing a successful screening programme, further evaluations are needed to assess the clinical sensitivity to detect *C. trachomatis* in semen samples.

In conclusion, our results showed that the fully automated cobas 4800 CT/NG test is sensitive and specific for the detection of *C. trachomatis* in urogenital specimens. In addition, using the cobas 4800 CT/NG test and our semen sample protocol, *C. trachomatis* could be detected routinely in semen specimens.

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