Dry swabs or collected in UTM and semen can be used for the detection of *Chlamydia trachomatis* by the cobas® 4800

**Authors:**
Chloé Le Roy¹ ², Aline Papaxanthos³, Oliver Liesenfeld⁴, Virginie Mehats¹ ², Maïthé Clerc¹ ², Cécile Bébéar¹ ² ⁵ and Bertille de Barbeyrac¹ ² ⁵

¹Univ. de Bordeaux, USC Mycoplasmal and Chlamydial Infections in Humans, French National Reference Centre for Chlamydial Infections, 33076 Bordeaux, France.
²INRA, USC Mycoplasmal and Chlamydial Infections in Humans, French National Reference Centre for Chlamydial Infections, 33076 Bordeaux, France.
³CHU de Bordeaux, Biologie de la Reproduction, Bordeaux, France.
⁴Medical and Scientific Affairs, Roche Molecular Systems, 4300 Hacienda Drive, Pleasanton, CA 94588, USA.
⁵CHU de Bordeaux, Laboratoire de Bactériologie, Bordeaux, France.

**Corresponding Author:**
Bertille de BARBEYRAC

✉️ : Université Bordeaux Segalen, USC Infections humaines à mycoplasmes et à chlamydiae, Centre National de Référence des Infections à chlamydiae, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France
✉️ : bertille.de.barbeyrac@u-bordeaux2.fr
☎️ : +33 5 57 57 16 33
☎️ : +33 5 56 93 29 40

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cobas® 4800 test for *Chlamydia trachomatis* detection.
ABSTRACT

In this prospective study we compared the fully automated cobas® 4800 CT/NG and the cobas® TaqMan CT tests for Chlamydia trachomatis detection in urines and in genital specimens collected with Copan flocked swabs in culture media. Moreover, we established a protocol for the highly sensitive detection of C. trachomatis in semen specimens using the cobas® 4800 CT/NG test. A total of 708 consecutive urogenital (293 male urines, 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 analyzed. Positive and negative percent agreements between the cobas® 4800 CT/NG and cobas® TaqMan CT tests were 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 of the cobas® 4800 CT/NG assay for C. trachomatis ranged from 90.9 to 100% according to the specimen type, with an overall prevalence of 7.2% (51/708). The clinical specificity ranged from 99.1 to 100% according to the specimen type. Dilution of 25 µl semen sample in cobas® PCR media 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 UTM culture media.

Key words: C. trachomatis, urogenital, semen, real-time PCR, Roche cobas 4800
INTRODUCTION

Sexually transmitted infections (STIs) are a major public health concern. *Chlamydia trachomatis* infections are the most prevalent bacterial STIs 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 (PID), 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 because these pathogens can cause serious reproductive complications in the recipients of semen donations and infection in their offspring (Peeling, 2005; Eley & Pacey, 2011).

A variety of nucleic acid amplification tests (NAAT) methods to detect *C. trachomatis* in urethral, urine and cervical specimens are currently available with far higher sensitivity than cell culture or antigen detection (Semeniuk *et al.*, 2002; Bébéar & de Barbeyrac, 2009). Recently, an increasing number of laboratories are 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 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 urines are cleared by the Federal Drug Administration (FDA) for use with commercial NAATs. According to the manufacturer’s instructions for the cobas®
4800 CT/NG test (Roche Diagnostics, France) dry swabs should be discharged into cobas® PCR media containing guanidine hydrochloride. This step makes it impossible to perform bacterial culture. In our routine practice, we used swab material discharged in bacterial transport media to be tested by cell and standard culture and 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 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 (Pannekoek *et al.*, 2000; Gdoura *et al.*, 2008; Eley & Pacey, 2011). In addition to the issue of NAAT inhibitors, sensitivity of the tests may also be affected since a significant proportion of men with infection of the upper genital tract will be asymptomatic and harbor fewer bacteria (Witkin, 2002).

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

**MATERIALS AND METHODS**

**Clinical specimens**

Consecutive urogenital specimens from the Bacteriology Department of the Bordeaux University Hospital were included from July to September 2010. The Copan flocked swabs used for cervical and urethral sample collection were discharged in the Universal Transport
Medium (UTM, COPAN Diagnostics) while Copan flocked 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 catch urine was self-collected in a sterile plastic urine bottle. All specimens were transported to the Bacteriology Department of the Bordeaux University Hospital within 24 hours for testing.

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

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

The vaginal Copan flocked swab was re-suspended in 1 mL of a home-prepared 2SP medium (Sucrose 0.2 M, K₂HPO₄ 15 mM, KH₂PO₄ 6 mM and water pH=7) prior to DNA extraction. Urine samples were frozen at -20°C overnight to destroy NAAT inhibitors effects and 500 µL of thawed urine was centrifuged; 200 µL of lysis buffer from the MagNaPure LC DNA isolation kit I (Roche Diagnostics, France) was then added to the pellet. Nucleic acid extraction was fully automated using the MagNaPure LC DNA isolation kit I on the extractor MagNaPure LC (Roche Diagnostics, France) according to the manufacturer’s instructions. The detection of *C. trachomatis* was performed using the cobas® TaqMan CT test v2.0 on the cobas® TaqMan 48 (Roche Diagnostics, France) 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 of each urine sample were added to 4.5 mL of cobas® PCR media. The Copan flocked swabs initially discharged in 2SP or UTM were resuspended in 1 mL of cobas® PCR media. Samples were then tested on the cobas® 4800 system. Briefly, 22 samples, 3 controls and reagents were loaded per run onto the cobas® x480 analyser which performed the DNA extraction using magnet bead technology similar to
that of the Magna Pure LC. Internal controls were added to each sample to check 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 specimen using the cobas® 4800 CT test

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

In order to determine the LOD, C. trachomatis-negative semen samples were pooled; 450 µL of pooled semen or 2SP medium solution was spiked with 50 µL of a 10^5 inclusion forming unit/mL (IFU/ml) C. trachomatis-serovar D cell-culture and then each was ten-fold serial diluted to the dilution of 10^{-6}. For each dilution, a volume of 50, 40 or 25 µL was added to 4.5 mL of cobas® PCR media and tested in duplicate on the cobas® 4800 system.

Data analysis

Specimens that were 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. Clinical sensitivity and clinical specificity of C. trachomatis were calculated on the basis of the reference test and by specimen type (Table 1). Positive and negative-percent agreements, along with their 95% confidence intervals, and overall-percent agreement were calculated based on the initial
results (Simel et al., 1991). To compare ct values obtained in different specimen types using the cobas® 4800 CT/NG and TaqMan CT Test, and to compare C. trachomatis or IC ct values between 2SP-spiked and semen-spiked samples in the cobas 4800 CT/NG test two way analysis of variance (ANOVA) 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 (3 male urine and 6 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 percent 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 clinical 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 3 cervical swabs) were flagged by the cobas® 4800 as “failed”, indicating a failure of DNA extraction from the specimen; one urine specimen was flagged as “invalid”, indicating that the internal control 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 (6 vaginal and 1 cervical swabs and 17 urine samples) were flagged as “invalid”.

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

**Chlamydia trachomatis detection in semen samples**

All 100 semen samples provided negative results for *C. trachomatis*. Means of IC ct values obtained with 1 to 40 µL of semen samples did not significantly differ from ct values obtained with urogenital swab and urine samples. The mean IC ct obtained with 50 µL of semen sample showed a strong increase compared to ct values of lower sample volumes indicating the presence of NAAT inhibitors (Figure 2). Based on this result, we tested 25, 40, and 50 µL of spiked semen sample to determine the limit of detection for *C. trachomatis*. The semen samples processed with *C. trachomatis*-infected cells yielded positive results, indicating successful DNA extraction and amplification. At a semen volume of 25 µL, no significant difference in *C. trachomatis* or IC ct values was observed between 2SP-spiked and semen-spiked samples (Table 2). Nevertheless, with a volume of 40 and 50 µL of semen-spiked samples, ct values were higher or even negative (Table 2).

All these results indicate that a volume of 25 µL of semen sample reveals 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 in culture media and extracted using the MagNaPure LC (De Martino *et al.*, 2006). The interest to discharge swabs in culture media is to allow testing 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 if we could use the cobas® 4800 platform using our usual sampling with swabs collected dried or in culture media. The preparation of specimens was not done according to the manufacturer’s instructions provided with the cobas® 4800 CT/NG test. Swab material was discharged in 2SP medium or UTM instead of being discharged directly in the cobas® PCR media. Our results are in agreement with those reported by Rockett et al. (Rockett et al., 2010). In their study, swab specimens were initially resuspended in 1.0 ml of phosphate-buffered saline (PBS), 500 µl of which was then added to 500 µl of cobas® PCR media. The cobas® 4800 CT/NG test showed a simple and short workflow sequence, allowing prompt and specific 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 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 to that of the MagNaPure LC with which 24 specimens were flagged “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 infertility setting is semen. Moreover, semen analysis may provide additional information on C. trachomatis infection, but 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 ones to detect *C. trachomatis* in semen. Protocols need to be developed for optimal detection of *C. trachomatis* in this specimen. The optimal amount of semen that should be used for such testing is not known. Surveys of the literature showed that 10 to 100 µL have been used with PCR tests (Wolff *et al.*, 1994; Pannekoek *et al.*, 2000; Peeling, 2005). In our study, we show that a volume of 25 µL of 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 that may interfere with the ability of the primers to access the target DNA in order to initiate the annealing step in the NAAT amplification cycle (Peeling, 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 which uses an internal control 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 some *C. trachomatis* infections may be missed if urine is the only test specimen and semen is not tested as well. Those studies evaluating the *C. trachomatis* prevalence in male infertility populations have had relatively small sample sizes and short study duration (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 program, further evaluations are needed to assess the clinical sensitivity to detect *C. trachomatis* in semen samples.

In conclusion, our results show 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.

TRANSPARENCY DECLARATION

This study was supported by a grant received from Roche Molecular Diagnostics (USA).

REFERENCES


test for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male urine.


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

<table>
<thead>
<tr>
<th>cobas® 4800 results</th>
<th>Sens. (%)</th>
<th>Spec. (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
<th>Failed, n (%)</th>
<th>Invalid, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas samples</td>
<td>[95% CI]</td>
<td>[95% CI]</td>
<td></td>
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<tr>
<td>Male urines (n=293)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pos Pos (n)</td>
<td>94.1 [73.0-98.9]</td>
<td>88.8 [95% CI]</td>
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<td>1 (0.34)</td>
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</tr>
<tr>
<td>Neg Neg (n)</td>
<td>99.2 [97.4-99.8]</td>
<td>99.6 [95% CI]</td>
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<tr>
<td>Urethral swabs (n=14)</td>
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<tr>
<td>Pos Pos (n)</td>
<td>100 [20.6-100]</td>
<td>100 [95% CI]</td>
<td></td>
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<td>0 (0.0)</td>
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<td>Male urines (n=293)</td>
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<tr>
<td>Neg Neg (n)</td>
<td>100 [77.1-100]</td>
<td>100 [95% CI]</td>
<td></td>
<td></td>
<td>3 (6.7)</td>
<td>0 (0.0)</td>
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<td>Cervical swabs (n=45)</td>
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<tr>
<td>Pos Pos (n)</td>
<td>100 [51.0-100]</td>
<td>100 [95% CI]</td>
<td></td>
<td></td>
<td>15 (4.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Neg Neg (n)</td>
<td>100 [91.4-100]</td>
<td>100 [95% CI]</td>
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<tr>
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<td>90.9 [76.4-96.9]</td>
<td>90.9 [95% CI]</td>
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<td>15 (4.2)</td>
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<tr>
<td>Neg Neg (n)</td>
<td>99.1 [97.3-99.7]</td>
<td>99.1 [95% CI]</td>
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</tbody>
</table>

aReference results were obtained with the MagNaPure LC and the cobas® TaqMan 48 CT Test.

bPos, positive; Neg, negative; CI, confidence interval; Sens., sensitivity; Spec., specificity; NPV, negative predictive value; PPV, positive predictive value.
Figure 1: Comparison of the cycle threshold values of the *C. trachomatis* detection obtained with the cobas® 4800 CT/NG (C4800) and cobas® TaqMan CT (CTM) tests on 708 urogenital samples.

- Cycle threshold value means; ○ Cycle threshold value obtained for each specimens.
Figure 2: Comparison of mean cycle threshold values of the internal control for semen and urogenital specimens.

- Cycle threshold value means; error bars represent standard deviations.
Table 2: Comparison of cycle threshold values of *C. trachomatis* detection in semen or 2SP spiked with *C. trachomatis*-infected cells.

<table>
<thead>
<tr>
<th>C. trachomatis - infected cells dilution</th>
<th>Cycle threshold values of <em>C. trachomatis</em></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>50 µL 2SP-spiked samples</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>29.8</td>
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<tr>
<td>10⁻²</td>
<td>32.5</td>
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<td>10⁻³</td>
<td>35.6</td>
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<tr>
<td>10⁻⁴</td>
<td>37.3</td>
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<tr>
<td>10⁻⁵</td>
<td>40.2</td>
</tr>
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</table>

Neg: negative.