Comparison of three real-time PCR assays for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in young pregnant women

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**Abstract**

We compared 3 commercial real-time PCR assays, the Abbott RealTime CT/NG, the cobas® 4800 CT/NG, and the Cepheid Xpert® CT/NG, for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in vaginal swabs collected prospectively from pregnant women aged <25 years. The overall agreement among 2 assays ranged from 98.9% to 99.5% with a kappa score between 0.94 and 0.97 for *C. trachomatis*. For *N. gonorrhoeae*, the overall agreement was 100%. All kits allowed prompt and specific results for *C. trachomatis* and *N. gonorrhoeae* in young pregnant women.

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**1. Introduction**

*Chlamydia trachomatis* is the most common pathogen causing sexually transmitted infections especially in young people. *Neisseria gonorrhoeae* is much less prevalent, but coinfections with *C. trachomatis* are common. The majority of these infections are asymptomatic in women. Today, nucleic acid amplification tests (NAATs) are widely used for high-throughput screening of *C. trachomatis* and *N. gonorrhoeae*. However, the detection of *N. gonorrhoeae* using NAATs has been compromised historically by unacceptably high rates of nonspecific reactivity with non-gonococcal *Neisseria* species, and international guidelines recommended that *N. gonorrhoeae*-positive NAAT results should be confirmed by using supplementary assays with different target if the positive predictive value is <90% (Bignell et al., 2012). At the Bordeaux University Hospital, all pregnant women requesting abortion and aged <25 years are screened for *C. trachomatis* and *N. gonorrhoeae* infections in vaginal swabs, using the Abbott RealTime CT/NG test (Abbott, Rungis, France). Indeed, the prevalence of *C. trachomatis* and *N. gonorrhoeae* was evaluated at 12.5% and 2.4%, respectively, in this population in 2012 (de Barbeyrac, personal data). In a previous study realized among pregnant women attending for routine antenatal care and aged <25 years, we reported a prevalence of 7.9% for *C. trachomatis*, whereas no gonorrhoea was detected (Peuchant et al., 2015). These results were obtained using vaginal swabs and the cobas® 4800 CT/NG test (Roche diagnostics, Meylan, France). The gap in *N. gonorrhoeae* prevalence in these 2 populations was surprising and could be due to differences in the performances of tests used or differences link to the studied population.

The aim of this study was to compare the performances of the Abbott RealTime CT/NG, the cobas® 4800 CT/NG, and the Cepheid Xpert® CT/NG (Cepheid, Maurens-Scopont, France) assays, tested simultaneously, for *C. trachomatis* and *N. gonorrhoeae* detection in vaginal swabs collected from pregnant women aged <25 years.

**2. Materials and methods**

**2.1. Samples analyzed**

This study was conducted prospectively from September 2012 to February 2013 on pregnant women aged <25 years and presenting for the first visit prior to abortion or attending for routine antenatal care at the Bordeaux University Hospital, France. Vaginal swabs were collected before abortion or during pregnancy follow-up.

**2.2. Sample process**

All dry vaginal swabs were first discharged in 2 mL of 2SP (saccharose-phosphate) media. According to the manufacturer’s package insert instructions, the volume of specimens tested was 400 μL for the Abbott RealTime CT/NG and the cobas® 4800 CT/NG tests and 1 mL for the Cepheid Xpert® CT/NG assay. Each NAAT contains an internal control, as well as positive and negative controls. Results were interpreted...
according to the manufacturer’s instructions, including recommended procedures for resolution of indeterminate results. For each vaginal swab, the 3 NAATs were performed at the same time.

2.3. Data analysis

Concordant results for 1 specimen were defined as positive if all three NAATs gave positive results and negative if all 3 NAATs gave negative results. For discordant results, a true-positive result was assigned if at least 2 out of the 3 NAATs gave a positive result and a true-negative was assigned if at least 2 out of the 3 NAATs gave a negative result.

Patient infected status was defined as being infected with *C. trachomatis* or *N. gonorrhoeae* if all 3 NAATs or 2 out of 3 NAATs gave positive results and as being not infected if all 3 NAATs or 2 out of 3 NAATs gave negative results for the collected vaginal swab, as previously described (Martin et al., 2004).

Overall percent agreement was calculated based on initial results using head-to-head comparison of the 3 assays (Feinstein and Cicchetti, 1990; Simel et al., 1991). The unweighted kappa statistic was calculated and interpreted as follows: ≤0.20, poor agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, good agreement; ≥0.80, excellent agreement.

3. Results

A total of 377 pregnant women were included in the study, 265 from women requesting abortion (median age = 21 years [15–24]) and 112 from routine antenatal care (median age = 21 years [14–24]). Overall, the prevalence of *C. trachomatis* infection was 10.1% (38/377), ranging from 7.1% (8/112) for women with routine antenatal care to 11.3% (30/265) for those requesting abortion. For *N. gonorrhoeae*, the prevalence was similar among both population studied (1.8% (2/112) and 1.9% (5/265), respectively).

3.1. *C. trachomatis* results

The first analysis on *C. trachomatis* results obtained with the 3 NAATs showed concordant positive results for 36 vaginal swabs and concordant negative results for 336 specimens (Table 1). Only 5 vaginal swabs provided discordant results between the 3 assays. Three discrepant specimens were Cepheid Xpert® CT/NG and cobas® 4800 CT/NG negative and assigned as true-negative. Two specimens were Abbott RealTime CT/NG and Cepheid Xpert® CT/NG positive and Abbott RealTime CT/NG and cobas® 4800 CT/NG positive, respectively, and were assigned as true-positive. Using head-to-head comparison, the overall agreement between the Abbott RealTime CT/NG and the cobas® 4800 CT/NG test was designed to target a conserved chromosomal sequence of *C. trachomatis* and 2 highly conserved, noncontiguous chromosomal sequences of *N. gonorrhoeae* (Gaydos et al., 2013). Targeting at least 2 different genetic sequences reduce the potential for false-positive results and enhance specificity.

Among discrepant results for *C. trachomatis*, there were 3 Abbott RealTime CT/NG positive/cobas® 4800 CT/NG and Cepheid Xpert® CT/NG negative samples. With the Abbott RealTime CT/NG assay, these specimens were initially tested equivocal because of a cycle number beyond the cutoff but were scored as *C. trachomatis* positive based on a positive repeat testing using the same specimen as recommended by the manufacturer. According to the specimen status, these vaginal swabs were considered as Abbott false-positive result. However, for discordant results, one limitation of our study is that we could not sequence amplicons obtained as sequence of primers used in each assay was not available. These data may challenge the proposed algorithm for the Abbott RealTime CT/NG assay and raises the question of interpretation of equivocal result.

We reported a prevalence of 7.1% for *C. trachomatis* infections among pregnant women aged ≥25 years and attending for routine antenatal care. These results confirmed those obtained in the same population in a previous study performed in 2011 in the same hospital, using the cobas® 4800 CT/NG test (Peuchant et al., 2015), and in agreement with those reported in the literature (Borges-Costa et al., 2011). Among women requesting abortion, the prevalence of *C. trachomatis* was 10.4% in our study. Similar findings were reported in 2 French

<table>
<thead>
<tr>
<th>Test result</th>
<th>Number of specimens</th>
<th>Specimen status</th>
<th>Patient infected status</th>
</tr>
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<tr>
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<td>cobas® 4800 CT/NG</td>
<td>Cepheid Xpert® CT/NG</td>
<td>Infected status</td>
</tr>
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<td>+</td>
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TP = true-positive; TN = true-negative.
recent studies (Lavoué et al., 2012; Toyer et al., 2012) and in other worldwide works (Baczynska et al., 2008; Garland et al., 2000; Patel et al., 2008), the highest C. trachomatis infection rates being described in women <25 years.

For N. gonorrhoeae, we found a prevalence of 1.8% among pregnant women aged <25 years and attending for routine antenatal care, while no gonorrhea infection was found in our previous study in the same population (Peuchant et al., 2015). This difference, not statistically significant (P = 0.16, Fischer’s exact test), could be likely due to the low prevalence of this infection in pregnant women. Among subjects consulting for induced abortion, our prevalence of N. gonorrhoeae infection was 1.9%, slightly higher than that reported by Toyer et al. (2012) using the Abbott Real-Time CT/NG test. A weakness of our study is the limited number of patients infected with N. gonorrhoeae.

In conclusion, this study demonstrated that the cobas® 4800 CT/NG, the Abbott RealTime CT/NG, and the Cepheid Xpert® CT/NG assays had excellent performances for C. trachomatis and N. gonorrhoeae detection in vaginal samples, without need of confirmatory testing for N. gonorrhoeae. In view of the high prevalence of C. trachomatis infection and the current PCR techniques which simultaneously detect C. trachomatis and N. gonorrhoeae, systematic screening for pregnant women aged <25 years could be beneficial.

Competing interest

The authors declare that they have competing interests with Roche Diagnostics and Cepheid, which provided the cobas® 4800 CT/NG and the Cepheid Xpert® CT/NG kits for this study, respectively.

Contributors

BB contributed to the design of the study, SD and CLR collected samples and data and performed the real-time PCRs. OP, SD, CLR, CB, and BB performed the data analysis. OP, SFB, CH, CB, and BB wrote the paper and contributed to the interpretation of the results.

References


