Evaluation of two commercial real-time PCR assays for the detection of

*Mycoplasma genitalium* in urogenital specimens

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Real-time PCR detection of *Mycoplasma genitalium*

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ABSTRACT

The performance of two commercial real-time PCR kits for the detection of *Mycoplasma genitalium* was evaluated in comparison to an in-house real-time PCR assay. A concordance of 96% and 93% was found for the TIB MOLBIOL and the Diagenode assays, respectively, compared to the results of the in-house assay.
Mycoplasma genitalium is a sexually transmitted organism that is primarily found in the human urogenital tract. Originally isolated from men with urethritis, M. genitalium has been convincingly linked to non-gonococcal urethritis in men (1, 2). In women, it has been shown that the M. genitalium infection spectrum is similar to that caused by Chlamydia trachomatis and Neisseria gonorrhoeae: cervicitis, pelvic inflammatory disease and tubal factor infertility (1-3).

M. genitalium is very fastidious, and although culture techniques have improved in the recent years, its isolation and cultivation remain extremely difficult and time consuming (4). Consequently, the routine detection of M. genitalium is entirely dependent on nucleic acid amplification tests (NAATs). Several real-time PCR assays have been described for the molecular detection of M. genitalium (2, 3), yet no FDA-approved commercial assay has been made available. Recently, several companies (Bio-Rad, Patho Finder and Seegene) have commercialized multiplex PCR for the detection of sexually transmitted pathogens, including M. genitalium (5, 6). TIB MOLBIOL distributed by Roche Diagnostics and Diagenode have recently developed monoplex real-time PCR kits for the detection of M. genitalium. These kits are commercially available in various countries, mainly in Europe, but not in the United States. In addition, Roche Diagnostics has commercialized the cobas® 4800 System (7), including the cobas® z 480 analyzer developed for in vitro diagnostic (IVD) applications, and has recently developed a User-Defined-Workflow (UDF) software that allows the use of the cobas® z 480 analyzer as a real-time PCR instrument.

The aim of this study was (i) to compare the performance of the LC480 and the cobas® z 480 analyzer, as applied to our M. genitalium in-house real-time PCR, and (ii) to assess the performance of the research-use-only TIB MOLBIOL/Roche and the CE-marked Diagenode...
assays for the detection of *M. genitalium* in urogenital samples in comparison to our in-house real-time PCR assay. A total of 104 DNA samples extracted from urogenital specimens collected between January 2010 and May 2011 in the Department of Bacteriology, University Hospital of Bordeaux (France) were retrospectively selected according to the results of an in-house TaqMan assay targeting the MgPa adhesin gene (8) performed on the LC480 analyzer. This selected sample panel included 54 consecutive *M. genitalium*-positive and 50 consecutive *M. genitalium*-negative samples. The nucleic acid extraction had been performed on 200 µl of clinical specimens containing the extraction & inhibition real-time PCR internal control (Dia-EIC, Diagenode, Belgium) using the MagNaPure LC DNA isolation kit I on the MagNaPure LC extraction system (Roche Diagnostics, France) according to the manufacturer’s instructions. The elution volume was 100 µl. The limit of the in-house assay detection was unchanged in the presence of the Dia-EIC (data not shown).

The 54 *M. genitalium*-positive DNA extracts were obtained from 31 women and 23 men and included 31 vaginal and 5 urethral samples and 18 male urine samples. The fifty *M. genitalium*-negative DNA extracts were collected from 34 women and 16 men and included 34 vaginal and 2 urethral samples and 14 male urine samples. All of the tests were performed on the same DNA extracts, which had been stored at -80°C and thawed. The 104 DNA extracts were re-evaluated with the in-house real-time PCR assay using two real-time PCR instruments: the LC480 and the cobas® z 480 analyzer. The 20-µl PCR mixture consisted of 0.3 µM of each MgPa-355F and MgPa-432R primers (8), 0.2 µM of MgPa-380 TaqMan probe (8), 1.5 µl of Universal Internal Control primers/probe (Diagenode, Belgium), 12.5 µl of LightCycler® 480 Probe Master Mix (Roche diagnostics, France) and 5 µl of template DNA. The same amplification conditions were
performed using the LC480 and the cobas® z 480 analyzer in accordance with Jensen et al. (8), and the data analysis was performed using the “absolute quantification-fit point method” with the filter combination 483-533 for the LightCycler® 480 software or with the filter combination 465-510 for the UDF software. The experimenter was blinded to the results of the different assays.

Two commercial *M. genitalium* detection kits, the TIB MOLBIOL LightMix® kit targeting a 224-bp gap gene fragment, and the Diagenode *Mycoplasma genitalium* real-time PCR kit (DIA-MG-050 vs2) targeting a 251-bp *mg219* gene fragment, were evaluated in comparison to the in-house real-time PCR using the cobas® z 480 analyzer. PCR mixtures of 25 µl were prepared using the LightCycler® 480 Probe Master Mix and 5 µl of template DNA according to each manufacturer’s instructions. The data were analyzed with the UDF software using the “absolute quantification-fit point method” and required the use of a color compensation file generated by the TIB MOLBIOL LightMix-universal color compensation 530/640/690 or the Diagenode Color Compensation Set (DIA-DAF(Lc480)-005). The filter combinations used for *M. genitalium* detection were 498-645 for the LightMix® kit and 465-510 for the Diagenode kit. The filter combinations were 498-700 for the internal control LightMix® kit and 498-580 for Universal Internal Control Diagenode (DIA-UIC-050).

Comparisons were made using McNemar’s and one-way analysis of variance (ANOVA) tests for qualitative and quantitative variables, respectively. The kappa statistic (κ) was used to evaluate the agreement between the commercial kits and the in-house PCR results. The clinical sensitivity and specificity of the commercial tests were calculated on the basis of the results obtained from the in-house PCR assay that we used as a proxy for a gold standard for these patients. Statistical analysis used the biostaTGV website (marne.u707.jussieu.fr/biostatgv/). A *P* value of <0.05 was considered as statistically significant.
Among the 54 *M. genitalium*-positive and 50 *M. genitalium*-negative DNA extracts, one extract (no. 1, Table 1) provided a discrepant result, which was positive with the LC480 analyzer and negative with the cobas® z 480 analyzer. However, there was no significant difference in the sensitivity and the specificity of the cobas® z 480 (sensitivity 98.1%, 95% confidence interval [CI] 90.2-99.7; specificity 100%, 95% CI 92.9-100) compared to those of the LC480. This discrepant result could be considered as a false-negative result on cobas® z 480 because extract no. 1 was *M. genitalium*-positive 3 times out of 4. Furthermore, the cycle threshold (CT) values indicated a sample with a low *M. genitalium* DNA load and the z480 false-negative result could likely be due to a sampling error (Table 1).

Both commercial kits accurately detected the 50 negative specimens and 46 of the 54 positive specimens (Table 2). For each kit, the IC was properly amplified in all extracts. Eight extracts provided discrepant results: three were negative according to both commercial tests, one was negative with the LightMix® kit only, and four were negative with the Diagenode kit only. CT values for each of the assays in these 8 discrepant samples (no. 2 to 9) are listed in Table 1.

The clinical sensitivity was 92.6% for the LightMix® kit and 87% for the Diagenode kit. The clinical specificity was 100% for both commercial kits (Table 2). There was no statistically significant difference between the clinical sensitivity and specificity of each of the commercial assays compared to those of the in-house method (*P* > 0.05). We found a concordance of 96% (κ = 0.92, 95% CI, 0.905-0.985) for the LightMix® kit and of 93% (κ = 0.86, 95% CI 0.867-0.967) for the Diagenode kit indicating a very good agreement between these kits and the in-house PCR assay. The mean CT ± standard deviation (SD) values of 33.8 ± 3.9, 34.5 ± 4.0 and 33.8 ± 3.5, respectively obtained from the in-house PCR, the LightMix® and the Diagenode kits on cobas® z 480, were not significantly different (*P* > 0.05).
In conclusion, the LC480 instrument and the cobas® 480 analyzer used with the UDF software presented identical performance when applied to the *M. genitalium* in-house real-time PCR assay. Both of the commercial kits allowed similar sensitive and specific results when used with the LightCycler® 480 Probe Master Mix and the cobas 480 instrument, validated by the use of an internal amplification control.

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TRANSPARENCY DECLARATION

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REFERENCES


TABLE 1. *Mycoplasma genitalium* real-time PCR results for the discrepant extracts.

<table>
<thead>
<tr>
<th>Extract number</th>
<th>In-house PCR</th>
<th>TIB MOLBIOL</th>
<th>Diagenode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC480</td>
<td>cobas® z 480</td>
<td>cobas® z 480</td>
</tr>
<tr>
<td>1</td>
<td>Pos (38.6)</td>
<td>Neg</td>
<td>Pos (40.7)</td>
</tr>
<tr>
<td>2</td>
<td>Pos (38)</td>
<td>Pos (38.1)</td>
<td>Neg</td>
</tr>
<tr>
<td>3</td>
<td>Pos (39)</td>
<td>Pos (39)</td>
<td>Neg</td>
</tr>
<tr>
<td>4</td>
<td>Pos (38.1)</td>
<td>Pos (38.2)</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>Pos (40)</td>
<td>Pos (40)</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>Pos (38.5)</td>
<td>Pos (38.5)</td>
<td>Pos (38.2)</td>
</tr>
<tr>
<td>7</td>
<td>Pos (37.3)</td>
<td>Pos (37.5)</td>
<td>Pos (38.1)</td>
</tr>
<tr>
<td>8</td>
<td>Pos (38.3)</td>
<td>Pos (38.6)</td>
<td>Pos (38.9)</td>
</tr>
<tr>
<td>9</td>
<td>Pos (39.3)</td>
<td>Pos (37.3)</td>
<td>Pos (38.9)</td>
</tr>
</tbody>
</table>

*a* Pos, positive; Neg, negative.

*b* Cycle threshold values are indicated in brackets.
TABLE 2. Clinical performance characteristics of the two commercial PCR assays performed on the cobas® z 480 analyser for the detection of *M. genitalium*.

<table>
<thead>
<tr>
<th>In-house PCR results</th>
<th>Sensibility (%)a [95% CI]c</th>
<th>Specificity (%)a [95% CI]c</th>
<th>NPVb (%)</th>
<th>PPVb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<td></td>
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<tr>
<td>TIB</td>
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<td>0</td>
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<td>[82.4-97.1]</td>
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<tr>
<td>MOLBIOL</td>
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<td>NA</td>
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<td></td>
<td>[75.6-93.4]</td>
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<td></td>
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<tr>
<td>Diagenode</td>
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<td>0</td>
<td>87.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>[75.6-93.4]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>50</td>
<td>100</td>
<td>[92.9-100]</td>
</tr>
</tbody>
</table>

aThe results of the commercial assays were compared to the in-house results using the Mc Nemar’s test. *P* were >0.05.

bNPV, negative predictive value; PPV, positive predictive value.

cCI, confidence interval.