

Evaluation of Two Commercial Real-Time PCR Assays for Detection of *Mycoplasma genitalium* in Urogenital Specimens

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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. Concordances of 96% and 93% were 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 found primarily in the human urogenital tract. Originally isolated from men with urethritis, *M. genitalium* has been convincingly linked to nongonococcal 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, PathoFinder, 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 Communauté Européenne (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 μ l of clinical specimens containing the extraction and inhibition real-time PCR internal control (Dia-EIC;

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

Extract no.	Result ^a			
	In-house PCR vs:		TIB MOLBIOL vs cobas z 480	Diagenode vs cobas z 480
	LC480	cobas z 480		
1	Pos (38.6)	Neg	Pos (40.7)	Pos (39.3)
2	Pos (38)	Pos (38.1)	Neg	Neg
3	Pos (39)	Pos (39)	Neg	Neg
4	Pos (38.1)	Pos (38.2)	Neg	Neg
5	Pos (40)	Pos (40)	Neg	Pos (39.2)
6	Pos (38.5)	Pos (38.5)	Pos (38.2)	Neg
7	Pos (37.3)	Pos (37.5)	Pos (43.9)	Neg
8	Pos (38.3)	Pos (38.6)	Pos (38.1)	Neg
9	Pos (39.3)	Pos (37.3)	Pos (38.9)	Neg

^a Pos, positive; Neg, negative. Cycle threshold values are indicated in parentheses.

Diagenode, Belgium) using the MagNa Pure liquid chromatography (LC) DNA isolation kit I on the MagNa Pure LC extraction system (Roche Diagnostics, France) according to the manufacturer's instructions. The elution volume was 100 μ l. 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 50 *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 reevaluated with the in-house real-time PCR assay using two real-time PCR instruments: the LC480 and the cobas z 480 analyzer. The 20- μ l PCR mixture consisted of 0.3 μM each

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TABLE 2 Clinical performance characteristics of the two commercial PCR assays performed on the cobas z 480 analyzer for the detection of *M. genitalium*

Assay	Result	No. of samples by in-house PCR result		% sensitivity ^a (95% CI)	% specificity ^a (95% CI)	NPV ^b (%)	PPV ^b (%)
		Positive	Negative				
TIB MOLBIOL	Positive	50	0	92.6 (82.4–97.1)	NA	92.6	100
	Negative	4	50	NA	100 (92.9–100)		
Diagenode	Positive	47	0	87 (75.6–93.4)	NA	87.7	100
	Negative	7	50	NA	100 (92.9–100)		

^a The results of the commercial assays were compared to the in-house results using McNemar's test. *P* values were >0.05. CI, confidence interval; NA, not applicable.

^b NPV, negative predictive value; PPV, positive predictive value.

MgPa-355F and MgPa-432R primers (8), 0.2 μM MgPa-380 Taq-Man 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 with an excitation wavelength range of 483 to 533 nm for the LightCycler 480 software or with the filter combination with a range of 465 to 510 nm 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 *Mycoplasma genitalium* 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 that with a range of 498 to 645 nm for the LightMix kit and that with a range of 465 to 510 nm for the Diagenode kit. The filter combinations were that with a range of 498 to 700 nm for the internal control LightMix kit and that with a range of 498 to 580 nm 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 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 of 98.1%, 95% confidence interval [CI] of 90.2 to 99.7; specificity of 100%, 95% CI of 92.9 to 100) compared to those of the LC480. This discrepant result could be considered 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 (C_T) 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 internal control 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. C_T 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% ($\kappa = 0.92$; 95% CI, 0.905 to 0.985) for the LightMix kit and of 93% ($\kappa = 0.86$; 95% CI, 0.867 to 0.967) for the Diagenode kit, indicating a very good agreement between these kits and the in-house PCR assay. The mean $C_T \pm$ 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, LightMix, and Diagenode kits on cobas z 480, were not significantly different ($P > 0.05$).

In conclusion, the LC480 instrument and the cobas z 480 analyzer used with the UDF software presented identical performances 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 z 480 instrument, validated by the use of an internal amplification control.

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