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Brief report

Comparison of the Aptima MG and Cobas TV/MG tests for the detection of *Mycoplasma genitalium* in urogenital and extragenital samples

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ABSTRACT

Background: *Mycoplasma genitalium* (*M. genitalium*) is an emerging sexually transmitted pathogen of increasing importance. The objective of this study was to compare two tests for the detection of *M. genitalium*; the Aptima® MG test (Hologic® Inc., San Diego, CA) and the Cobas® TV/MG test (Roche® Diagnostics, Mannheim, Germany).
Methods: This is a prospective descriptive study where a total of 489 genital and extragenital samples were analyzed in parallel and in random order by both systems. The samples were collected from patients attending the Sexually Transmitted Infections Center in Seville and the Infectious Diseases consultation of the Virgen de Valme Hospital.
Results: The overall agreement between both trials was very good ($k > 0.91$). The sensitivity and specificity of the Aptima® MG test were 100% and 98.7% respectively for the Cobas® TV/MG test.
Conclusion: Both systems showed excellent performance for the detection of *M. genitalium*.

Comparación de los test Aptima MG y Cobas TV/MG para la detección de *Mycoplasma genitalium* en muestras urogenitales y extragenitales

Palabras clave:
Mycoplasma genitalium
Aptima® MG
Cobas® TV/MG
Muestras urogenitales y extragenitales

RESUMEN

Introducción: *Mycoplasma genitalium* (*M. genitalium*) es un patógeno de transmisión sexual emergente de importancia creciente. El objetivo de este estudio fue comparar dos *test* para la detección de *M. genitalium;* el *test* de Aptima® MG (Hologic® Inc., San Diego,CA) y el test Cobas® TV/MG (Roche® Diagnostics, Mannheim, Alemania).
Métodos: Se trata de un estudio descriptivo prospectivo donde se analizaron en paralelo y en orden aleatorio por ambos sistemas un total de 489 muestras genitales y extragenitales de pacientes procedentes del Centro de Infecciones de Transmisión Sexual en Sevilla y de las Consultas de Enfermedades Infecciosas del Hospital Virgen de Valme.
Resultados: La concordancia global entre ambos ensayos fue muy buena ($k > 0.91$). La sensibilidad y la especificidad del test Aptima® MG fue del 100% y 98,7% respectivamente y del 100% y del 99,8% respectivamente, para el test Cobas® TV/MG.
Conclusión: Ambos sistemas mostraron un rendimiento excelente para la detección de *M. genitalium*.

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Introduction

Mycoplasma genitalium (*M. genitalium*) is an emerging sexually transmitted pathogen of increasing significance involved primarily in nongonococcal urethritis in men and inflammatory diseases of the female reproductive tract[1]. In most cases, they course as asymptomatic infections and any symptoms are indistinguishable from other sexually transmitted infections (STI)[2,3], rendering a microbiological diagnosis essential.

Currently, nucleic acid amplification techniques (NAAT) are the only diagnostic healthcare method available for the detection of *M. genitalium*[4,5]. In recent years, numerous NAATs based mainly on real-time polymerase chain reaction (qPCR) or transcription-mediated amplification (TMA) have been marketed[6–9]. However, the evaluation and comparison of the different marketed tests is difficult because the theoretical *gold standard,* which would be culture, is not possible. Therefore, in these cases, the so-called *patient infection status* (PIS) is accepted, which consists of conducting a third test in discrepant cases so that it is accepted as a valid result when at least two of the tests conducted yield the same result[10].

The objective of this study was to compare tests for the detection *of M. genitalium*; the Aptima® MG (AMG) test (Hologic® Inc., San Diego, CA, USA), based on TMA, and the Cobas®TV/MG test (C6800) (Roche® Diagnostics, Mannheim, Germany), based on qPCR. Both tests have been approved by the FDA for genital samples. Our study also included extragenital samples.

Materials and methods

This is a prospective descriptive study carried out at the Microbiology Laboratory of the Hospital Virgen de Valme (Seville, Spain). Over a period of one month, all the samples in which the molecular detection of *M. genitalium* was requested were processed in parallel with both tests. The samples came from patients treated at the Centro de Infecciones de Transmisión Sexual de Sevilla (CITSS) and at the hospital's STI Infectious Diseases Consultations.

The type of sample taken from each patient was left up to the physician's discretion according to the patient's sex, sexual practices and clinical manifestations. The samples were sent in specific collection tubes for each test.

Laboratory methods

All the samples were processed on the same day that they were received at the laboratory by means of both systems following the manufacturer's instructions. The main distinguishing characteristics of both systems are shown in Table 1.

The samples with discrepant results were stored at −80 °C and were later analysed with the Anyplex™ II STI-7 (ASTI-7) test (Seegene® Inc, Seoul, South Korea). This is a qPCR-based test that detects up to seven main pathogens that cause STIs (*Chlamydia trachomatis* [*C. trachomatis*], *Neisseria gonorrhoeae* [*N. gonorrhoeae*], *Trichomonas vaginalis* [*T. vaginalis*], *Mycoplasma hominis*, *M. genitalium*, *Ureaplasma urealyticum* and *Ureaplasma parvum)* in a single reaction. For this purpose, the nucleic acids were extracted first using the MagCore® HF16 plus system (RBC Bioscience, USA). The nucleic acids were eluted to a final volume of 60 μl. Subsequently, qPCR was performed on the CFX96 equipment.

Statistical analysis

The clinical performance of the equipment for the detection of *M. genitalium* was evaluated by comparing the results of the test using the PIS, which consists of conducting a third test in discrepant cases so that it is accepted as a valid result when at least 2 of the tests yield the same result. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated overall and according to the type of sample. 95% confidence intervals were provided for the estimates of sensitivity, specificity, PPV and NPV. Statistical significance was calculated using the p value, with $p < 0.05$ being statistically significant.

The concordance between the two tests was analysed using Cohen's kappa coefficient.

Ethical considerations

The study was approved by the Ethics Committee of Hospital de Valme, Seville.

Results

A total of 489 consecutive samples obtained from 384 patients were processed in parallel, of which 255 (66%) were from men and 129 (33%) from women, with a median age of 33.4 years (range 18 to 71 years). The samples processed were from: first morning urine 248 (50.7%), cervical swabs 122 (25%), rectal swabs 96 (19.6%) and pharyngeal swabs 23 (4.7%). In total, *M. genitalium* was detected in 34 patients in the following samples: 17 urine, 9 cervical swabs and 8 rectal swabs, with a prevalence of 6.9%, 7.4% and 8.3%, respectively. *M. genitalium* was not detected in any pharyngeal swabs. The rest of the processed samples, 455, were negative. The overall prevalence of *M. genitalium* in the patients studied was 8.9%, and was higher in men compared to women, 9.8% vs. 7%, respectively.

The result of both tests matched in 482 of the 489 processed samples (98.6%). Overall agreement was very good ($K > 0.91$). The difference between the two tests was not statistically significant ($p < 0.001$). Table 2 shows the results of sensitivity, specificity, PPV and NPV overall and by type of sample.

A total of 7 samples (1.4%) yielded discrepant results; 6 (1.2%) were positive for the AMG test and negative for the C6800 test, and 1 (0.20%) was positive for the C6800 test and negative for the AMG *test*. These samples were analysed using the Anyplex™ II STI-7 test and *M. genitalium* was not detected in any of them (Table 3).

Table 1
Characteristics of the two marketed systems compared.

Characteristics	Panther System (Hologic Inc., San Diego, CA, USA)	Cobas® 6800/8800 system (C6800; Roche Diagnostics, Mannheim, Germany)
Amplification	Transcription-mediated amplification	Real-time PCR
Detection of *M. genitalium*	16 S rRNA	*mgpB* DNA
RNA/DNA load	Relative Light Units (results ≥50,000 were considered positive)	Cycle threshold (results between 10 and 36 were considered positive)
Sample capacity	Up to 100 sample tubes	Up to 96 sample tubes
Time to first result	3.5 h	3.5 h
Automated	Yes	Yes
Allows primary tubes	Yes	Yes
Individual doses of lyophilised reagents	Yes	No

Table 2
Clinical performance of the two systems compared according to sample type.

Samples	Test	TP	TN	FP	FN	S	Sp	PPV	NPV	Prevalence	Kappa
Urine (n = 248)	AMG	17	229	2	0	100% (77.8%–99.5%)	99.1% (96.6%–99.9%)	89.47% (65.5%–98.2%)	100% (97.9%–99.9%)	6.9%	0.97
	C6800	17	231	0	0	100% (77.1%–99.5%)	100% (97.9%–99.9%)	100% (77.1%–99.5%)	100% (97.9%–99.9%)		
Cervical swab (n = 122)	AMG	9	110	3	0	100% (62.9%–98.9%)	97.4% (91.9%–99.3%)	75% (42.8%–93.3%)	100% (95.8%–99.9%)	7.4%	0.80
	C6800	9	112	1	0	100% (62.9%–98.9%)	99.1% (94.5%–99.9%)	90% (54.1%–99.5%)	100% (95.9%–99.9%)		
Rectal swab (n = 96)	AMG	8	87	1	0	100% (59.8%–98.5%)	98.9% (93%–99.9%)	88.9% (50.7%–99.4%)	100% (94.7%–99.9%)	8.3%	0.94
	C6800	8	88	0	0	100% (59.8%–98.5%)	100% (94.8%–99.9%)	100% (59.8%–98.5%)	100% (94.8%–99.9%)		
Pharyngeal swab (n = 23)	AMG	0	23	0	0	–	100% (82.2%–99.6%)	–	100% (82.2%–99.6%)	–	–
	C6800	0	23	0	0	–	100% (82.2%–99.6%)	–	100% (82.2%–99.6%)		
Total (n = 489)	AMG	34	449	6	0	100% (87.4%–99.7%)	98.7% (97%–99.5%)	85% (68.8%–93.8%)	100% (98.9%–99.9%)	8.9%	0.91
	C6800	34	454	1	0	100% (87.4%–99.7%)	99.8% (98.54%–99.9%)	97% (82.9%–99.8%)	100% (98.9%–99.9%)		

FN: false negative; FP: false positive; NPV: negative predictive value; PPV: positive predictive value; S: sensitivity; Sp: specificity; TN: true negative; TP: true positive.

Table 3
Results of the alternative test for the detection of *M. genitalium* in cases with discrepancy between the AMG test and the C6800 test.

No. of patients	Sex	Age (years)	Signs and symptoms	Sample type	AMG (RLU)	C6800 (CT)	Seegene (CT)
1	Male	27	Yes	Urine	P (1800618)	N	N
2	Female	36	Yes	Cervical	N	P (19.68)	N
3	Female	20	No	Cervical	P (1354899)	N	N
4	Male	28	No	Urine	P (1604014)	N	N
5	Male	29	No	Rectal	P (1781613)	N	N
6	Female	22	No	Cervical	P (1639282)	N	N
7	Female	27	No	Cervical	P (1880001)	N	N

N: negative; P: positive.

Discussion

The current European guidelines already recommend the detection of *M. genitalium* in the case of persistent or recurrent urethritis[2], but not in the screening of asymptomatic patients to avoid the development of resistances and because its usefulness is not well defined. In the near future, requests for its diagnosis in microbiology laboratories will probably increase, meaning that highly-automated platforms that can process a large number of samples may be necessary.

Recently, both tests analysed were validated for the detection of *M. genitalium* in urogenital samples[8,9], with excellent sensitivity and specificity results. The results derived from our study indicate that the clinical performance of both tests was excellent in terms of sensitivity, specificity, PPV and NPV. In our study, the sensitivity of the AMG test was higher than that described by Gaydos et al.[9] both in urine samples (100% vs. 90.9%) and in endocervical samples (100% vs. 81.5%). In the case of the C6800 test, we found the same sensitivity as in the study of van der Pol et al.[8] for urine samples (100%), while sensitivity in endocervical samples was higher in our study (100% vs. 83.3%).

In recent years, several studies have been published comparing TMA *with* non-automated PCR for the diagnosis of infections by *M. genitalium*[11]. However, to our knowledge, no study has directly compared two automated platforms using the primary tube. The discordant results occurred mainly with the AMG test, which more frequently presented positive results compared to the C6800. The 7 discrepant samples were all negative for the detection of *M. genitalium* when they were analysed with the Anyplex™ II test. Not having a suitable *gold standard*, such as culture, prevents us from knowing whether these results correspond to false positives from the AMG test or to greater analytical sensitivity, since it is based on a TMA that detects *M. genitalium* from ribosomal RNA (rRNA), unlike the other two systems, based on qPCR, that detect DNA.

One noteworthy aspect of these commercial tests is that they only analyse *M. genitalium*, which means that the samples would require processing with new determinations to detect other STI aetiological agents (*N. gonorrhoeae, C. trachomatis, T. vaginalis*). However, there are other commercial tests that can detect several targets in the same reaction, although these kits' performance is usually lower in terms of the number of samples that can be processed per day.

Our study included extragenital samples (rectal and pharyngeal swabs), which were not considered in the validation studies. The prevalence of *M. genitalium* was greater in the rectal samples, and all of these patients were asymptomatic. The usefulness of *M. genitalium* screening in rectal samples from asymptomatic patients is not entirely clear, although in at-risk patients, treatment to halt the chain of transmission would appear to be justified[11–13]. On the other hand, *M. genitalium* was not detected in any pharyngeal swabs. This result is similar to what is described in the literature[14] and therefore does not seem to constitute a reservoir, and we think that this sample delivers low performance.

Finally, and although it was not the subject of this article, it should be noted that in recent years the studies carried out on resistance have observed that *M. genitalium* is evolving towards becoming a so-called superbug and may become resistant to all the antimicrobials available for its eradication. Thus, *M. genitalium*'s resistance to azithromycin, the first-line treatment[15], has been increasing rapidly, as has the number of reported cases[16]. The same is happening with the alternative treatment, moxifloxacin, with an increase in resistance worldwide[3]. This may all be expected to continue in the coming years, whereby we believe that detection and a sensitivity study are necessary at the same time.

Our study provides data on the usefulness of both tests for the detection of *M. genitalium* in rectal samples. The main limitation is the small sample size. The prevalence in rectal samples may be overestimated since the population studied is mainly MSM. Therefore, further studies are needed to confirm the results obtained.

The results indicate that both systems demonstrated excellent performance for the detection of *M. genitalium*, both in genital and extragenital samples, whereby the choice of one system or another will depend on the laboratory's preferences.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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