

Brief Original Article

Comparison of Mycoplasma IES, Mycofast Revolution and Mycoplasma IST2 to detect genital mycoplasmas in clinical samples

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Abstract

Introduction: Culture is regarded as the gold standard for the detection of genital mycoplasma in clinical samples. Commercially available diagnostic kits, based on liquid broth cultures, provide interesting alternatives to conventional culture. We assessed the laboratory performances of Mycoplasma IES (IES), the Mycofast Revolution (REV) and Mycoplasma IST 2 (IST2) compared to A7 agar plates for the detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in clinical samples.

Methodology: From April to July 2013, endocervical or vaginal samples were collected from sexually active women with abnormal vaginal discharge. Each specimen was tested in parallel using the three commercial kits and the A7 agar plates.

Results: A total of 303 samples were included in this study, 35.6% (108/303) of which were positive on A7 plates. Sensitivities for the detection of *U. urealyticum* of IES, REV and IST2 were 100%, 96.2% and 95.3%, respectively while those for *M. hominis* were of 92.8%, 92.8% and 85.7%, respectively. Specificity was 100% for the 3 methods. Concerning antimicrobial susceptibility testing, full agreement between IES and REV was documented.

Conclusions: The Mycoplasma IES kit was found to be equivalent or superior compared to other commercial culture-based assays for a rapid and accurate identification of *U. urealyticum* and *M. hominis* and detection of resistance. It might be considered a cost-effective tool for detection of these organisms, particularly attractive in developing countries.

Key words: mycoplasma; culture; diagnostic; rapid assay.

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Introduction

Ureaplasma species (*Ureaplasma parvum* and *Ureaplasma urealyticum*) and *Mycoplasma* species (*Mycoplasma hominis* and *Mycoplasma genitalium*), generally referred to as “genital mycoplasmas”, have been associated to a large variety of infections in adults and infants, in particular may be responsible of non-gonococcal urethritis, cervicitis, pelvic inflammatory disease and adverse pregnancy outcome [1-6]. However, since they are found in the vagina of up to 80% of pregnant and non-pregnant women [7], their pathogenic role is difficult to prove.

Tetracyclines, macrolides, and quinolones can be used for treatment of infections caused by genital mycoplasmas [6]. While ureaplasmas are generally susceptible to macrolides, they are resistant to lincosamides except in high concentrations. *M. hominis*, in contrast, is naturally resistant to C14 and C15 macrolides (*e.g.* clarithromycin, erythromycin, azithromycin and roxithromycin), but susceptible to 16-membered macrolides (josamycin and miocamycin)

and lincomycin [6]. Increasing resistance to fluoroquinolones have been documented in several reports [8,9]. Therefore, rapid detection and susceptibility determination are important to start adequate treatment and to reduce the risk of complications.

Genital mycoplasmas can be detected by several methods. Culture on semisolid media is still regarded as the “gold standard” for the detection of *M. hominis* and *U. urealyticum* from clinical samples. Especially in low to moderate test volume laboratories it is still considered the most cost effective strategy. Nucleic acid amplification-based tests have higher specificity and sensitivity than culture and are the only means for detection of *Mycoplasma genitalium* and for discriminating between *U. urealyticum* and *U. parvum* [10-12]. They can be also completed in few hours, despite cost of equipment and reagents is significant and molecular diagnosis-trained personnel is required. Nonetheless, culture is still performed to monitor the antimicrobial susceptibility profiles of these pathogens

[8,13]. The development of commercially available diagnostic assays, which are based on liquid broth cultures, offer a simpler alternative to conventional culture [11,14]. Mycofast Revolution (EliTech Diagnostic, Puteaux, France) and MycoplasmaIST2 (BioMérieux, Marcy l'Etoile, France) are two widely used assays for the detection of *M. hominis* and *U. urealyticum*, similar with regards to identification, antimicrobial susceptibility testing and turnaround time.

The Mycoplasma IES (Autobio, Zhengzhou, China) is a new commercially available diagnostic assay for the detection of *M. hominis* and *U. urealyticum* from genital samples and determination of their antimicrobial susceptibility profiles within 24 hours. The kit is based on the culture method, biochemistry identification and antibiotic susceptibility. Urea can be cleaved by urease for *U. urealyticum* and release NH_3 and arginine can be decomposed by arginase for *M. hominis* and release NH_3 . Then NH_3 causes an increased pH of the liquid medium. The corresponding color change of the indicator is used to read the result.

Our study aimed to evaluate this assay's performances for the detection of *M. hominis* and *U. urealyticum* in a hospital cohort. The results were compared to those of the Mycofast Revolution and Mycoplasma IST 2.

Methodology

The study was conducted prospectively from April to July 2013 in the clinical microbiology laboratory of a 1200-bed university hospital in Rome, the Catholic University of the Sacred Heart Medical Center. Duplicate endocervical or vaginal swabs (Eswab, Copan Diagnostics, Inc, Brescia, Italy) had been collected from sexually active women with abnormal vaginal discharge attending to the outpatient clinic of the Laboratory of Microbiology. Patients that received antibiotics or used vaginal medications for at least one week prior to enrolment were excluded. All specimens were cultured within 24 hours of collection. Swabs were suspended into 1.2 ml of saline solution, and vortexed rapidly. Prior to inoculation, all media were allowed to come to room temperature for 30 min.

Mycoplasma IES testing was performed by putting 300 μl of the seeded suspension into the reconstitute medium of manufacturer's collection kits. Following, 100 μl of the suspension were inoculated into the wells of the gallery. One drop of mineral oil was added to each well. Strips were incubated at 37°C for 24 hours.

For Mycoplasma IST 2 testing, 200 μl of seeded saline suspension were put into R1 broth (BioMérieux,

Marcy l'Etoile, France), which was combined with a vial of R2 (BioMérieux, Marcy l'Etoile, France) and vortexed. Fifty-five μl of the solution was used to inoculate each of the 22 test wells on the strip. Two drops of mineral oil were added to each well. The strips were incubated at 37°C for 24-48 hours.

For Mycofast Revolution testing, 300 μl of the seeded saline suspension was dispensed on UMMt (Ureaplasma Mycoplasma medium transport) medium (EliTech Diagnostic, Puteaux, France); 100 μl were dispensed into wells 1 to 20 of the tray, as previously described [11]. Fifty μl of Mueller Hinton supplement (S. Mh) (EliTech Diagnostic, Puteaux, France) were dispensed into wells 6 and 7. The wells were covered with two drops of sterile mineral oil and the tray was incubated at 37°C for 24-48 hours.

The A7 agar plates (Remel, Lenexa, USA) were used as comparison method in order to assess the sensitivities and the specificities of each commercial assay. Samples inoculated onto A7 plates by streaking the surfaces of the plates in two directions, perpendicular to each other. Plates were incubated in a moist chamber to avoid drying of the agar and examined after 48 h of incubation as previously described [15]. Growth was quantitated as follows: 1+, 1 to 10 colonies; 2+, 11 to 100 colonies; and 3+, >100 colonies.

Three ATCC strains (ATCC27813 UU, ATCC27618™ UU, ATCC15488 MH) (BioLife Solutions, Bothell, USA), 4 clinical confirmed positive strains from Autobio (2 *Ureaplasma* spp., 2 *M. hominis*) (Autobio, Zhengzhou, China) and 2 ELITECH strains (UU7101 PSF, MH7102 PSF) (EliTech Diagnostic, Puteaux, France) were included in each assay as positive controls [16-17]. Sensitivity, specificity, positive predictive value and negative predictive value of the Mycofast Revolution, Mycoplasma IES, and Mycoplasma IST 2 assays were determined by comparing identification results of each system to those produced by the A7 agar. Breakpoints (in $\mu\text{g}/\text{mL}$) used for defining susceptibility or resistance to antimicrobial agents of Mycoplasma IES and Mycofast Revolution assays were those provided by the Clinical and Laboratory Standards Institute (CLSI, document M43-A) [16]. Strains were regarded as resistant when growth was inhibited by the higher critical concentration of the antimicrobial agent, but not the lower critical concentration or when growth was not inhibited by either the higher or lower critical concentrations of the antimicrobial agents. Result of the antibiotic susceptibility test (AST) was evaluated only when the inoculum was 10^4 - 10^5 colony forming units/ml and for isolates grew in pure culture. It was not

possible to evaluate the result of antimicrobial susceptibility testing purchased by Mycoplasma IST 2 as the concentrations of antimicrobial used in the assay are different from those indicated by CLSI.

Results

A total of 303 samples were included in this study. Genital mycoplasmas were detected in 35.6% (108/303) of samples with A7 plates. *U. urealyticum* was detected in 106 (35%) cultures, and *M. hominis* in 14 (4.6%). Twelve (4%) cultures were positive for both.

The Mycoplasma IES assay detected *U. urealyticum* in all 106 culture positive and *M. hominis* in all but one positive cultures while the Mycofast Revolution detected *U. urealyticum* in 102 samples and *M. hominis* in 13 samples. Finally, Mycoplasma IST 2 assay detected 101 *U. urealyticum* and 12 *M. hominis*. As shown in Table 1 sensitivities for the detection of *U. urealyticum* of Mycoplasma IES, Mycofast and Mycoplasma IST 2 were 100%, 96.2% and 95.3%, respectively while those for *M. hominis* were of 92.8%, 92.8% and 85.7%, respectively.

Regarding antimicrobial susceptibility testing, since only two pure cultures of *M. hominis* were obtained, only results for *U. urealyticum* grown as single species were taken into account. The following rates of resistance were determined using the Mycoplasma IES assay: levofloxacin, 6.4% (6/94), erythromycin, 3.2% (3/94), and tetracycline 1.1% (1/94). The overall agreement between Mycoplasma IES and Mycofast Revolution assay was 100%, being the same strains categorized as resistant by the last assay. Similarly, to the other Italian data, fluoroquinolones showed the lowest efficacy while resistance to tetracyclines was very low also in our study [18].

Discussion

National and international studies have documented a prevalence of genital mycoplasma among symptomatic patients frequently over 30-40% [2,4]. In our study 108 women (35.6%) in the patient group were positive for *U. urealyticum*, *M. hominis* or both mycoplasmas. Our findings are different to those of two Italian studies. In particular, according to a recent Italian survey performed on a population of 331 women, the prevalence of positive samples for genital mycoplasmas was 43.5% [18]. Another national study including 9956 patients in a 8-year period showed a 18.6% prevalence [19].

Because of the pathogenic role of *M. hominis* and *U. urealyticum* is increasingly accepted, fast and efficient diagnostic methods are imperative to treat infections and minimize complications. This study is the first to compare the Mycoplasma IES assay with other commercially available kit assays for mycoplasma detection in genital clinical specimens. The test showed to be highly sensitive and specific. In particular, sensitivity of Mycoplasma IES was higher than sensitivities of Mycoplasma IST 2 and Mycofast (100% versus 95.3% and 96.2%, respectively). Concerning antibiotic susceptibility, the agreement rate between Mycoplasma IES and Mycofast Revolution was 100%.

Although this study is limited by being performed in a single clinic as well as conducted over a relatively short period of time, our results suggest that the Mycoplasma IES assay allows for a rapid and accurate identification of *M. hominis* and *U. urealyticum* and detection of resistance.

The method has two additional advantages. In terms of the simplicity of specimen processing and inoculation, the kit was very easy to perform. At an estimated cost of EUR 2.50 for the Mycoplasma IES per

Table 1. Comparison of Mycoplasma IES, Mycofast Revolution and Mycoplasma IST 2 tests for the detection of genital mycoplasmas.

Microorganism (n)	Comparison with reference results			
	Sensitivity (%) (TP/TP+ FN)	Specificity (%) (TN/TN+FP)	PPV (%) (TP/TP+FP)	NPV (%) (TN/TN+FN)
<i>Ureaplasma urealyticum</i> (106)				
Mycoplasma IES	100	100	100	100
Mycofast Revolution	96.2	100	100	98
Mycoplasma IST 2	95.3	100	100	97.5
<i>Mycoplasma hominis</i> (14)				
Mycoplasma IES	92.8	100	100	99.6
Mycofast Revolution	92.8	100	100	99.6
Mycoplasma IST 2	85.7	100	100	99.3

Abbreviations. PPV, positive predictive value; NPV, negative predictive value; TP, true positive; FP, false positive; TN, true negative; FN, false negative.

patient recruited in this study, the assay could be a cost-effective diagnostic tool in developing countries with budget limitations.

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