

## **Comparison of Two Laboratory Techniques for Detecting Mycoplasmas in Genital Specimens**

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**Objective:** To compare Mycoplasma 1<sup>st</sup> kit and a conventional culture system in the detection of genital mycoplasmas.

**Design:** Comparative Prospective study.

**Setting:** Department of Microbiology and Genito-Urinary-Medicine Clinic.

**Method:** Mycoplasma 1<sup>st</sup> kit and a conventional culture system were compared; One hundred specimens from males and females were allocated to each method. Swabs were inoculated in the broth medium for the conventional culture system and in the Mycoplasma 1<sup>st</sup> kit cupules. All inoculated broths were incubated aerobically at 37°C and cultures showing pH shift were sub cultured in agar plates and incubated anaerobically in a candle jar at 37°C up to 7 days. The agar plates were examined for the characteristic *ureaplasma* and *mycoplasma hominis* colonies.

**Result:** Detection of the genital mycoplasmas obtained from each method was very similar with high sensitivities. Seventeen out of 100 specimens screened for *mycoplasma hominis*, yielded positive results by the conventional culture method and were detected in 26 specimens by Mycoplasma 1<sup>st</sup> kit. Fifty-eight out of 100 patients' specimens, *ureaplasma urealyticum* were isolated by the culture method and detected in 55 specimens by Mycoplasma 1<sup>st</sup> kit. The commercial kit provides additional information on antimicrobial susceptibilities.

**Conclusion:** Both systems were easy to perform. While broth-agar culture could be less expensive per test for routine screening of specimens than the kit, the commercial kit provides additional information on antimicrobial susceptibilities.

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Commercial kits designed for isolation and identification of *mycoplasma hominis* and *ureaplasma urealyticum* have become available for routine diagnostic use<sup>1,2</sup>.

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The unavailability of commercially prepared media has prevented many laboratories from offering mycoplasma culture on site. Recently, a variety of products and kits designated for detection of mycoplasmas in clinical laboratories has become available<sup>3</sup>. The kits for the quantitative detection and identification of *ureaplasma urealyticum* and *mycoplasma hominis* from urogenital specimens are commercially available in France (Sanofi Diagnostic Pasteur, bioMérieux).

They consist of strips with wells containing specific dried or lyophilized substrates and inhibitors. Specimens are placed in a suspension medium that is used to inoculate the wells. The presence and identification of mycoplasma are based on color change of specific wells<sup>4</sup>.

High rates of genital mycoplasma infections have been determined both by culture and by a commercially available kit. The Mycoplasma 1<sup>st</sup> kit (bioMérieux) is available for the detection of *mycoplasma hominis* and *ureaplasma urealyticum*<sup>5</sup>.

The aim of this study was to compare The Mycoplasma 1<sup>st</sup> kit and conventional culture system in the detection of genital mycoplasmas.

## **METHOD**

Two hundred random genital specimens from male and female patients were included in the study; one hundred specimens were allocated for each method. The Mycoplasma 1<sup>st</sup> kit and conventional culture system were used for the analysis. The swabs were placed into transport media (10B broth) and (R1, medium), for culture and the Mycoplasma 1<sup>st</sup> kit, respectively.

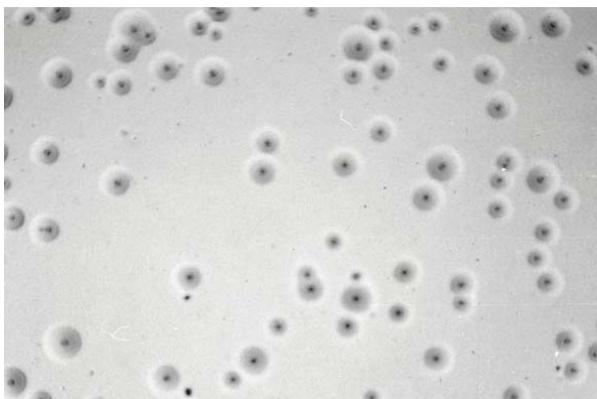
For conventional culture, Shepard's growth medium was used for recovery of *mycoplasma hominis* and *ureaplasma urealyticum*<sup>6</sup>. For commercial detecting kit system, the swab in Ri (transport liquid medium) was processed according to the manufacturer's instructions. The kit, antimicrobial susceptibility was tested against 6 major antibiotics including Doxycycline, Erythromycin and Tetracycline.

## **RESULT**

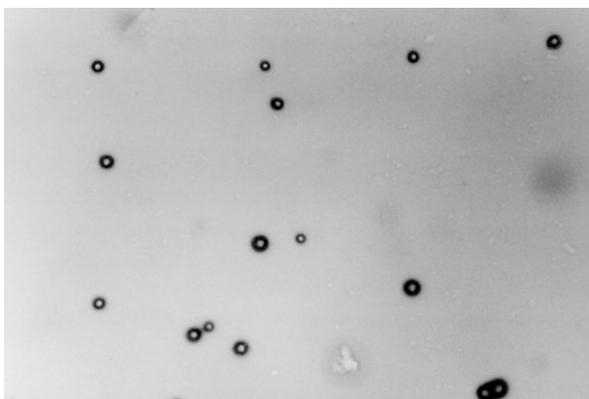
The results of the commercial kit compared to the conventional culture system for the detection of *mycoplasma hominis* and *ureaplasma urealyticum* are shown in Table 1. Seventeen specimens yielded positive result for *mycoplasma hominis* by conventional culture method and was detected in 26 specimens by Mycoplasma 1<sup>st</sup> kit. *Ureaplasma urealyticum* was isolated on A8 agar plates from 58 by culture method and detected in 55 specimens by Mycoplasma 1<sup>st</sup> kit, see figure 1 and 2.

**Table 1: *Mycoplasma Hominis* and *Ureaplasma Urealyticum* Detection Using the Commercial Kit and Culture System Methods**

	<i>Mycoplasma hominis</i> (n=100)		<i>Ureaplasma urealyticum</i> (n=100)	
	Positive	Negative	Positive	Negative
Mycoplasma 1 <sup>st</sup> kit	26	74	55	45
Culture System Method	17	83	58	42



**Figure 1: Colonial Morphology of *Mycoplasma hominis* on A8 Agar Medium**



**Figure 2: Colonial Morphology of *Ureaplasma urealyticum* on A8 Agar Medium**

Isolates were identified as *mycoplasma hominis* or *ureaplasma urealyticum* by growth inhibition test (disc method), as described by Clyde<sup>7</sup>. Using the antisera, zones of inhibition less than 1.5 mm in diameter were considered negative. The sensitivity was similar between the culture method and the Mycoplasma 1<sup>st</sup> kit for the detection of *ureaplasma urealyticum*.

The two methods did not compare favorably for the detection of *mycoplasma hominis*; the sensitivity of culture method was 17% compared to 26% of Mycoplasma 1<sup>st</sup> kit.

Antimicrobial susceptibilities as determined by Mycoplasma 1<sup>st</sup> kit are shown in Table 2. All genital mycoplasma isolates showed resistance to Erythromycin. All genital mycoplasma isolates were susceptible to Doxycycline, Tetracycline and Pristinamycin, three exceptions (2 strains resistances to Doxycycline and one strain resistance each to the Tetracycline and Pristinamycin, respectively).

**Table 2: Antibiotic Susceptibilities of the Genital Mycoplasma Isolates as Determined by Mycoplasma 1<sup>st</sup> Kit**

Percentage of Isolates, n (%) with Antibiotic Susceptibilities				
Antibiotic	Susceptible	Intermediate	Resistant	
Doxycycline	96.5		00.0	3.4
Josamycin	60.3		37.9	1.7
Ofloxacin	24.1		72.4	3.4
Erythromycin	00.0		00.0	100.0
Tetracycline	98.2		00.0	1.7
Pristinamycin	98.2		00.0	1.7

Thirty-five strains of the genital mycoplasma isolates as detected by Mycoplasma kit were susceptible to Josamycin (60.3%) and 14 strains to Ofloxacin (24.1%), two exceptions (22 strains intermediately resistant, one strain resistant to the first antibiotic and 42 strains intermediately resistant and two strains resistant to the second antibiotic respectively).

## DISCUSSION

Detection of mycoplasmas in the genital tract usually depends on culturing the specimen on appropriate media and identifying the isolates.

This study showed that the number of genital mycoplasmas detected by commercial kit and culture system was approximately similar. Both methods were suitable for clinical laboratory use and they were easy to perform.

However, Mycoplasma 1<sup>st</sup> kit could be used for identification as well as providing antimicrobial susceptibilities<sup>3,5,8,9</sup>. The test is expensive for routine screening of specimens, but did not require prior media preparation and the reagents; the test kit has a shelf-life of up to 12 months. The result of the Mycoplasma kit could read within 24-48 hours.

The conventional broth-agar culture method requires time for media preparation, storage of media, supplements and antibiotics. Broth medium (10B) and Agar medium (A8- in sealed plastic bags) could be stored for up to 3 months at 4°C. Although reading the results in this method require an additional incubation time due to inoculation of serially diluted specimens followed by subculturing of broth-to-agar. We have found that the conventional is the most sensitive method for the isolation of both *mycoplasma hominis* and *ureaplasma urealyticum*. The finding of this study is similar to other studies where it was found that the conventional method is the most sensitive for isolation of many members of class Mollicutes including genital mycoplasmas<sup>1,2</sup>.

In this study, *mycoplasma hominis* was resistant to Erythromycin compared to *ureaplasma urealyticum*, which was sensitive to that antibiotic<sup>3,8,10</sup>.

The Erythromycin-resistant strains of *ureaplasma urealyticum* could be a problem in neonatal infection since Erythromycin is considered the first choice for treatment in neonatal infection<sup>11</sup>. However, it is suggested that the resistance to Erythromycin is due to reduced activity because of the acidity of the growth medium used for genital mycoplasmas<sup>5</sup>. Other factors have been considered in vaginal secretion<sup>3,12</sup>.

Tetracycline and/or Doxycycline should always be tested for susceptibility against genital mycoplasmas because resistance is known to occur in both *mycoplasma hominis* and *ureaplasma urealyticum*<sup>4</sup>.

## CONCLUSION

**The detection of the genital mycoplasmas by culture system and a commercially available kit were similar with high sensitivities. The conventional culture requires preparation of the media while the Mycoplasma kit does not. Mycoplasma 1<sup>st</sup> kit could be used for antimicrobial susceptibilities of the isolates.**

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