

## Detection of Urogenital Mycoplasmas Using Culture and PCR: A Descriptive Pilot Study

E. Aghabalaee and M.H. Hedaiati  
Department of Microbiology, Faculty of Basic Sciences,  
Islamic Azad University, Zanzan Branch, I.R. Iran

**Abstract:** Mycoplasmas as human urogenital tract pathogens are associated with infections, reproductive failures and adverse pregnancy outcomes and thus very important to diagnose. Several methods have been used to detect genitourinary Mycoplasmas, each having their own limitations, advantages and disadvantages. In the present study, researchers used microbial culture and PCR to detect Mycoplasmas in urogenital specimens with the aim of comparing detection rate, sensitivity and specificity of the two methods. It was a descriptive cross sectional pilot study. About 30 urogenital samples including 17 vaginal swabs, 7 male urine and 6 female urine samples were collected from patients referring to hospitals regardless of their disease and studied for Mycoplasmas by culture (using two media: PPLO agar and PPLO broth) and PCR. Of total 30 specimens, Mycoplasmas were detected in 14, 11 and 11 of them using PPLO broth culture medium, PPLO agar culture medium and PCR, respectively. Accordingly, the sensitivity and specificity of the PCR Method was determined 100 and 95%, respectively while culture was found to have a sensitivity of 77% and specificity of 66%. Researchers found PCR based on 16S rRNA sequences to be highly sensitive and specific offering a rapid, easy and cost benefit method for detection of Mycoplasmas in comparison to Microbial Culture Method.

**Key words:** Urogenital, mycoplasmas, culture, PCR, urine, Iran

---

### INTRODUCTION

Mycoplasmas as the smallest free-living organisms are widely spread in the nature. Human Mycoplasma species are mainly colonized in genitourinary tract and although, some of them are normal commensals, others are proven pathogens of urogenital tracts (Taylor-Robinson, 2002). According to many studies they are known to have a role in infections of genitourinary tract (Keane *et al.*, 2000; Nassar *et al.*, 2008; Patel and Nyirjesy, 2010; Moi *et al.*, 2009), cervicitis (Manhart *et al.*, 2003; McIver *et al.*, 2009; Schlicht *et al.*, 2004) endometritis (Cohen *et al.*, 2002), tubal infertility (Clausen *et al.*, 2001) and Pelvic Inflammatory Disease (PID) (Taylor-Robinson, 2002). They can also cause adverse pregnancy outcomes such as abortion, preterm delivery and preterm birth and may lead to neonatal mortality and morbidity (Taylor-Robinson, 2007; Larsen and Hwang, 2010; Cassell *et al.*, 1991; Govender *et al.*, 2009). In addition, it is well established that they are associated with non-gonococcal non-chlamydial urethritis in men (Hooton *et al.*, 1988; Horner *et al.*, 1993; Taylor-Robinson *et al.*, 1993; Takahashi *et al.*, 2006). Accumulating evidence exists considering genitourinary

Mycoplasmas as sexually transmitted infections (Ross and Jensen, 2002; Uuskula and Kohl, 2002; Manhart *et al.*, 2007; Manhart and Kay, 2010).

Several methods have been used to detect genitourinary Mycoplasmas, each having its own limitations, advantages and disadvantages. The common method of microbial culture is not routine now since it requires special media that are complex and expensive and also the process is very time consuming as it takes at least 2-5 days to culture *U. urealyticum* and almost 8 weeks to culture *M. genitalium* (Razin *et al.*, 1998; Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009). Serological methods are not also widely used due to cross-reactivity with other species (Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009; Agbakoba *et al.*, 2006). With the revolutionary development of PCR Methods in the early 1990s, detection of such organisms that are difficult to cultivate became more feasible. These methods have been widely used to diagnose Mycoplasmas in the studies (Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009; Agbakoba *et al.*, 2006; Jensen *et al.*, 1991; Timenetsky *et al.*, 2006; Svenstrup *et al.*, 2005; Dhawan *et al.*, 2006; Stirling, 2003). In the present study, researchers examined 30 urogenital samples (from both

male and female patients) for *Mycoplasmas* using culture (with two media: PPLO broth and PPLO agar) and PCR. The main purpose of the study was to determine and compare the sensitivity and specificity of these two methods for detection of urogenital *Mycoplasmas*.

## MATERIALS AND METHODS

This descriptive cross sectional pilot study was performed during 2011-2012. Urogenital specimens were collected from patients referring to four major hospitals in Karaj with various chief complaints who needed urinary or vaginal tests according to their physicians' diagnosis. As a result, a total of 30 specimens including 13 urine samples (7 from male and 6 from female patients) and 17 vaginal swabs were gathered and analyzed. All patients gave informed consent agreements.

**Specimens:** Clean catch urine samples were collected in sterile plastic containers. Vaginal swabs were placed in test tubes containing Stuart Transport Medium (Merck, Germany) consisting of Pleuropneumoniae-Like Organism (PPLO) broth, yeast extraction, bovine serum, penicillin or streptomycin. Both swabs and urine samples were stored at 4°C and immediately transported to the lab. Vaginal swabs for PCR were frozen at -70°C until PCR.

**Culture for urogenital mycoplasmas:** All specimens were cultured within 12 h of collection. Filtration Method was used to separate *Mycoplasmas* from the specimens. Specimens were cultured in two culture media: PPLO broth (liquid culture medium) and PPLO agar (semi-solid culture medium) (DIFCO, USA). Specimens were filtered by syringe filter 0.45 µm in order to reduce bacterial and fungal contaminations. Then, 100 µL of each sample was inoculated into PPLO agar plates and 300 µL of each specimen was poured into tubes containing 2.7 µL of PPLO broth. All cultured tubes and plates were then incubated at 35°C with 80% humidity and 5% CO<sub>2</sub> for 14 days and examined daily for color change and fried-egg colonies characteristic of *Mycoplasmas*.

At days 10 and 14, 200 µL of the PPLO broth in the cultured tubes was inoculated into separate plates containing PPLO agar medium and incubated at 35°C, 80% humidity and 5% CO<sub>2</sub>. All plates were examined by microscope ×10 at days of 3, 7, 10 and 14.

**PCR for urogenital Mycoplasmas:** First, sample vials containing urine and vaginal specimens were filtered by 0.45 µm syringe filters. After vortexing for 5-10 sec, 100 µL of each sample vial was transferred to microtubes and centrifuged at 12000 rpm for 5 min at room temperature.

After centrifugation, the supernatant was discarded and the pellet was suspended in 1 mL of DBPS or PBS buffer. All microtubes were centrifuged twice more at 12000 rpm for 2 min with the pellet washed using 100 µL of the buffers. Then, they were heated for 10 min at 95-100°C bain-marie and again centrifuged at 12000 rpm for 5 min. The supernatants were transferred to additional sterile 0.5 mL microtubes and kept at 4°C until PCR.

PCR was performed using two specific primers (270 bp) including GPO3 (5' GGGAGCAAACACGAT AGATACCCT 3') and MGSO (5' TGCACCATCTGT CACTCTGTAAACCTC 3') in order to detect *Mycoplasma* genus in the specimens (Sinagene, Iran). Final PCR buffer included: 10 µL of the extracted DNA, 0.2 µM of each primer, 1.5 µM MgCl<sub>2</sub>, 0.2 mM dNTP5, 5000 units of Taq polymerase and 5 µL 10×Amplitaq buffer. PCR reaction included an initial 5 min denaturation at 94°C followed by 35 cycles of 30-sec denaturation at 95°C, a 45 sec annealing at 56°C and a 60 sec extension at 72°C. A final extension was performed for 10 min at 72°C. Electrophoresis was performed on 15 µL of the reaction in a 3% agarose gel stained with Ethidium Bromide Solution (10 mg mL<sup>-1</sup>). DNA bands were detected at 245-312 nm using UV transilluminator (Uvitec, UK).

**Statistical analysis:** Data was analyzed using SPSS Ver. 17. Statistical significance was determined using Chi-square and Exact Fisher tests. The p<0.05 was set.

## RESULTS

A total of 30 urogenital specimens including 13 urinary samples and 17 vaginal swabs were gathered from patients (7 male, 23 female; mean age: 31.7±7.2) and tested for *Mycoplasmas* using culture (PPLO broth and agar) and PCR. Most patients were married (83.3%) and referred to the hospitals with chief complaints suggestive of urogenital infection (63.3%). Studying PPLO broth cultures showed that 14 cases (46%) were positive for *Mycoplasmas* including 6 vaginal samples and 8 urine samples (color change was considered positive). PPLO agar culture medium found 11 (30%) positive samples including 5 vaginal swabs and 6 urine samples (colony morphology was considered sufficient to identify *Mycoplasmas*). Using PCR Detection Method, *Mycoplasmas* DNA was found in 11 samples (30%) including 5 vaginal swabs and 6 urine samples. Table 1 shows the number of positive samples using different detection methods.

PPLO broth medium culture showed highest detection rate (46%) with a high false positive rate. Using

Table 1: Positive samples for Mycoplasmas using different detection methods

Specimen type	No. of specimens			
	Tested	PPLO broth positive (%)	PPLO agar positive (%)	PCR positive (%)
Vaginal swabs	17	6 (35)	5 (29)	5 (29.0)
Urine (female)	6	3 (50)	2 (33)	3 (50.0)
Urine (male)	7	5 (71)	4 (57)	3 (42.0)
Total	30	14 (46)	11 (30)	11 (33.3)

PPLO agar medium culture, Mycoplasmas were found in 33% of the samples, giving the method a sensitivity of 77% and specificity of 66%. In comparison to culture, PCR Detection Method with 33.3% detection rate the sensitivity and specificity were determined to be 100 and 95%, respectively improving test sensitivity and specificity by 23 and 29%, respectively in comparison to PPLO agar culture.

**DISCUSSION**

In this study, researchers used two culture media (PPLO broth and PPLO agar) and PCR to detect Mycoplasmas in 30 urogenital specimens and compared the detection rates and sensitivity and specificity of the tests. In general, the results finding Mycoplasmas in 11 of 30 urogenital specimens by PCR indicates a relatively higher infection rate compared with reports from other cities of Iran. However, the infection rate with Mycoplasmas in Iran is generally lower than other countries probably due to social and cultural factors.

Several other methods have been suggested to detect Mycoplasmas; however, using PCR along with microbial culture seems to be the most acceptable method in order to reduce false results (Amirmozafari *et al.*, 2009). With considering the limitations of a pilot study, the results were generally in consistence with previous studies comparing PCR and culture for detection of Mycoplasmas (Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009). In the study, researchers found 30% Mycoplasma positive specimens using culture (PPLO agar and PPLO broth) while PCR detected Mycoplasmas in 3.3% of the samples. Although, it has been reported that using both PPLO broth and agar media together might enhance the ability to isolate Mycoplasmas (Stellrecht *et al.*, 2004), adding broth medium in the study didn't increase the ability to isolate Mycoplasmas and mostly increased the false positive results.

A previous study in Iran by Amirmozafari *et al.* (2009) compared PCR and culture for detection of Mycoplasmas in cervical specimens of 312 women and found detection rates of 16 and 2.8% for PCR and culture, respectively. Another study by Kathleen found detection rates of 25 and 33% for culture and PCR for diagnosing genital

Mycoplasmas (Stellrecht *et al.*, 2004). In several studies it has been shown that culture is not only time consuming and costly but also it has high false negative rates in comparison to PCR Methods and also there are some strains of Mycoplasmas which can't be detected by culture and besides PCR has the advantage of detecting nonviable organisms (Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009; Stirling, 2003; Young *et al.*, 2010). Of course PCR Methods might have some limitations and despite all the surveillance and precise environment settings, there is still a chance of DNA contamination in molecular detection systems leading to false positive results. This chance of contamination can be reduced by assigning appropriate environment settings and facilities as well as using internal controls in different phases of PCR (Potts, 2008). Previous studies have shown that diagnosing the strains of Mycoplasmas by PCR is superior to classic methods, proving it to be of higher sensitivity, specificity and reliability (Timenetsky *et al.*, 2006; Stirling, 2003; Young *et al.*, 2010).

**CONCLUSION**

The comparison between PCR and microbial culture showed a sensitivity and specificity of 100 and 95% for PCR and 77 and 66% for culture, respectively. This improvement of sensitivity and specificity by PCR methods for detection of urogenital Mycoplasmas is consistence with the literature (Stellrecht *et al.*, 2004; Amirmozafari *et al.*, 2009; Dhawan *et al.*, 2006). In this study, researchers found PCR based on 16S rRNA sequences to be highly sensitive and specific offering a rapid, easy and cost benefit method for detection of Mycoplasmas in comparison to microbial culture method. Further studies with larger sample sizes are needed to confirm the results and they might focus on more issues such as using PCR for distinction of Mycoplasma strains in larger scales and comparing it with more detection methods other than culture.

**REFERENCES**

Agbakoba, N.R., A.I. Adetosoye and I.F. Adewole, 2006. Biochemical and serological characterization of mycoplasma strains isolated from the genital tracts of humans in Nigeria. *Afr. J. Med. Med. Sci.*, 35: 125-129.

Amirmozafari, N., R. Mirnejad, B. Kazemi, E. Sariri, M.R. Bojari and F.D. Darkahi, 2009. Comparison of polymerase chain reaction and culture for detection of genital *Mycoplasma* in clinical samples from patients with genital infections. *Saudi. Med. J.*, 30: 1401-1405.

- Cassell, G.H., K.B. Waites and D.T. Crouse, 1991. Perinatal *Mycoplasma* infections. Clin. Perinatol., 18: 241-262.
- Clausen, H.F., J. Fedder, M. Drasbek, P.K. Nielsen and B. Toft *et al.*, 2001. Serological investigation of *Mycoplasma genitalium* in infertile women. Hum. Reprod., 16: 1866-1874.
- Cohen, C.R., L.E. Manhart, E.A. Bukusi, S. Astete and R.C. Brunham *et al.*, 2002. Association between *Mycoplasma genitalium* and acute endometritis. Lancet, 359: 765-766.
- Dhawan, B., V. Gupta, N. Khanna, M. Singh and R. Chaudhry, 2006. Evaluation of the diagnostic efficacy of PCR for *Ureaplasma urealyticum* infection in Indian adults with symptoms of genital discharge. Jpn. J. Infect Dis., 59: 57-58.
- Govender, S., G.B. Theron, H.J. Odendaal and L.J. Chalkley, 2009. Prevalence of genital *Mycoplasmas*, *Ureaplasmas* and *Chlamydia* in pregnancy. J. Obstet. Gynaecol., 29: 698-701.
- Hooton, T.M., P.L. Roberts, W.E. Stamm, M.C. Roberts, K.K. Holmes and G.E. Kenny, 1988. Prevalence of *Mycoplasma genitalium* determined by DNA probe in men with urethritis. Lancet, 331: 266-268.
- Horner, P.J., C.B. Gilroy, B.J. Thomas, R.O. Naidoo and D. Taylor-Robinson, 1993. Association of *Mycoplasma genitalium* with acute non-gonococcal urethritis. Lancet, 342: 582-585.
- Jensen, J.S., S.A. Uldum, J. Sonderfard-Anderson, J. Vuust and L. Klaus, 1991. Polymerase chain reaction for the detection of *Mycoplasma genitalium* in clinical samples. J. Clin. Microbiol., 29: 46-50.
- Keane, F.E., B.J. Thomas, C.B. Gilroy, A. Renton and D. Taylor-Robinson, 2000. The association of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium* with bacterial vaginosis: Observations on heterosexual women and their male partners. Int. J. STD AIDS, 11: 356-360.
- Larsen, B. and J. Hwang, 2010. *Mycoplasma*, *Ureaplasma* and adverse pregnancy outcomes: A fresh look. Infect Dis. Obstet. Gynecol., 2010: 521921-521921.
- Manhart, L.E. and N. Kay, 2010. *Mycoplasma genitalium*: Is it a sexually transmitted pathogen?. Curr. Infect. Dis. Rep., 12: 306-313.
- Manhart, L.E., C.W. Critchlow, K.K. Holmes, S.M. Duro, D.A. Eschenbach, C.E. Stevens and P.A. Totten, 2003. *Mucopurulent cervicitis* and *Mycoplasma genitalium*. J. Infect. Dis., 187: 650-657.
- Manhart, L.E., K.K. Holmes, J.P. Hughes, L.S. Houston and P.A. Totten, 2007. *Mycoplasma genitalium* among young adults in the United States: An emerging sexually transmitted infection. Am. J. Public Health, 97: 1118-1125.
- McIver, C.J., N. Rismanto, C. Smith, Z.W. Naing and B. Rayner *et al.*, 2009. Multiplex PCR testing detection of Higher-than-expected rates of cervical *Mycoplasma*, *Ureaplasma* and *Trichomonas* and viral agent infections in sexually active Australian women. J. Clin. Microbiol., 47: 1358-1363.
- Moi, H., N. Reinton and A. Moghaddam, 2009. *Mycoplasma genitalium* in women with lower genital tract inflammation. Sex Transm Infect., 85: 10-14.
- Nassar, F.A., F.H. Abu-Elamreen, M.E. Shubair and F.A. Sharif, 2008. Detection of *Chlamydia trachomatis* and *Mycoplasma hominis*, *Genitalium* and *Ureaplasma urealyticum* by polymerase chain reaction in patients with sterile pyuria. Adv. Med. Sci., 53: 80-86.
- Patel, M.A. and P. Nyirjesy, 2010. Role of *Mycoplasma* and *Ureaplasma* species in female lower genital tract infections. Curr. Infect. Dis. Rep., 12: 417-422.
- Potts, B., 2008. *Mycoplasma* PCR Assay: Pre Validation studies and regulatory approval. J. Applied Microbiol., 111: 417-425.
- Razin, S., D. Yogev and Y. Naot, 1998. Molecular biology and pathogenicity of *Mycoplasmas*. Microbiol. Mol. Biol. Rev., 62: 1094-1156.
- Ross, J.D.C. and J.S. Jensen, 2002. *Mycoplasma genitalium* as a sexually transmitted infection: Implications for screening, testing and treatment. Sex Transm. Infect., 82: 269-271.
- Schlicht, M.J., S.D. Lovrich, J.S. Sartin, P. Karpinsky, S.M. Callister and W.A. Agger, 2004. High prevalence of genital *Mycoplasmas* among sexually active young adults with urethritis or *Cervicitis symptoms* in la crosse, wisconsin. J. Clin. Microbiol., 42: 4636-4640.
- Stellrecht, K.A., A.M. Woron, N.G. Mishrik and R.A. Venezia, 2004. Comparison of multiplex PCR assay with culture for detection of genital *Mycoplasmas*. J. Clin. Microbiol., 42: 1528-1533.
- Stirling, D., 2003. A short history of the polymerase chain reaction. J. Methods Mol. Biol., 226: 3-6.
- Svenstrup, H.F., J.S. Jensen, E. Bjornelius, P. Lidbrink, S. Birkelund and G. Christiansen, 2005. Development of a quantitative Real-Time PCR assay for detection of *Mycoplasma genitalium*. J. Clin. Microbiol., 43: 3121-3128.
- Takahashi, S., K. Takeyama, S. Miyamoto, K. Ichihara and T. Maeda *et al.*, 2006. Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. J. Infect. Chemother, 12: 269-271.

- Taylor-Robinson, D., 2002. *Mycoplasma genitalium*-an up-date. *Int. J. STD AIDS* 13: 145-151.
- Taylor-Robinson, D., 2007. The role of *Mycoplasmas* in pregnancy outcome. *Best Practice Res. Clin. Obstet. Gynaecol.*, 21: 425-438.
- Taylor-Robinson, D., C.B. Gilroy and P.E. Hay, 1993. Occurrence of *Mycoplasma genitalium* in different populations and its clinical significance. *Clin. Infect. Dis.*, 17: S66-S68.
- Timenetsky, J., L.M. Santos, M. Buzinhan and E. Mettifofo, 2006. Detection of multiple *Mycoplasma* infection in cell cultures by PCR. *Braz. J. Med. Biol. Res.*, 39: 907-914.
- Uuskula, A. and P.K. Kohl, 2002. Genital *Mycoplasmas*, including *Mycoplasma genitalium*, as sexually transmitted agents. *Int. J. STD AIDS*, 13: 79-85.
- Young, L., J. Sung, G. Stacey and J. Masters, 2010. Detection of *Mycoplasma* in cell cultures. *J. Nature Protocols*, 5: 929-934.