Diagnostic parameters of the AF Genital System® for detection of *Mycoplasma hominis* and *Ureaplasma urealyticum*

Enty Tjoa1,*  Shikha Joon2,3,* and Lucky H. Moehario1

Abstract

**Objective:** The prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* (genital mycoplasma) amongst Indonesian women is poorly understood because of limited availability of diagnostic techniques. We sought to compare the diagnostic parameters of the AF Genital System® with those of culture methods and PCR as the gold standard for identification of *M. hominis* and *U. urealyticum* in vaginal swab specimens.

**Methods:** This was an observational diagnostic study. Eighty-eight specimens were collected from patients with abnormal vaginal discharge. Detection of *M. hominis* and *U. urealyticum* was performed using the AF Genital System®, culture methods, and PCR.

**Results:** Compared with PCR and culture methods, respectively, the AF Genital System® had sensitivities of 66.6% and 57% (*M. hominis*) and 55.5% and 77.8% (*U. urealyticum*). Compared with PCR and culture methods, respectively, the AF Genital System® had specificities of 82.9% and 86.5% (*M. hominis*) and 82.3% and 84.8% (*U. urealyticum*).

**Conclusion:** The sensitivity of the AF Genital System® for detection of *M. hominis* and *U. urealyticum* from vaginal swab samples was lower than that of PCR, but specificity was reasonably good (82% to 83%).

1Department of Microbiology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia
2Institute for Systems Genetics, Frontiers Science Center for Disease-Related Molecular Network, West China Hospital, Sichuan University, Chengdu, China
3iGlobal Research and Publishing Foundation, New Delhi, India
*These authors contributed equally to this work.

**Corresponding author:** Enty Tjoa, Department of Microbiology, School of Medicine and Health Sciences, Atma Jaya Catholic, University of Indonesia, Jalan Pluit Raya No. 2, Jakarta 14440, Indonesia.

Emails: enty@atmajaya.ac.id; entytjoa@gmail.com
Introduction

Genital mycoplasmas (*Mycoplasma hominis* and *Ureaplasma urealyticum*) are commonly found in the urogenital tracts of healthy, asymptomatic, and symptomatic individuals (both men and women). Symptomatic patients may present with severe urogenital complications such as pelvic inflammatory disease, prostatitis, bacterial vaginosis, amnionitis, postpartum infections, non-gonorrheal urethritis, and even infertility. However, the presence of any other sexually transmitted infection-causing pathogens must be excluded before proceeding to genital mycoplasma-specific diagnosis and treatment in asymptomatic or symptomatic individuals. Data regarding the prevalence of *M. hominis* and *U. urealyticum* among Indonesian women with abnormal vaginal discharge are lacking, potentially because of limited availability of diagnostics for these infections. Routine diagnosis largely relies on culture methods, polymerase chain reaction (PCR), and rapid tests. Conventional culture methods for microbiological detection are time-consuming, laborious, cumbersome, and less sensitive while PCR-based methods are costly and require considerable expertise. Here, we compared the diagnostic parameters of the AF Genital System®, a biochemical test-based diagnostic tool, with those of culture methods and PCR as the gold standard for identification of *M. hominis* and *U. urealyticum* in vaginal swab specimens.

Materials and methods

Study protocol and specimens

This observational and diagnostic study was performed to compare the diagnostic parameters of the AF Genital System® (https://www.liofilchem.com; LIOFILCHEM® s.r.l., Roseto, Italy) with those of culture methods and PCR as the gold standard for identification of *M. hominis* and *U. urealyticum* in vaginal swab specimens. Clinical specimens (vaginal and urine samples) were procured from patients who complained of abnormal vaginal discharge and presented at the obstetrics and gynecology and/or genital clinics at The Atma Jaya Hospital and The Royal Taruma Hospital, Jakarta. The inclusion criteria were age 21 to 38 years, no children after 2 years of marriage or longer, and suspected pelvic inflammatory disease. Patients who fulfilled the inclusion criteria but were unwilling to participate or did not sign informed consent forms were excluded from the study. The reporting of this study conformed to STROBE guidelines.

Ethics and informed consent

Clinical specimens were obtained with prior written informed consent of the patient. The study was performed according to the standards and guidelines outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the School of Medicine and Health Sciences, Atma Jaya Catholic University.
of Indonesia (approval number: 11/05/KEP-FKUAJ/2017).

**Microbiology work-up and identification**

The collected samples were subjected to Gram staining\(^3\)–\(^5\) and analysis using the AF Genital System\(^R\), culture methods,\(^3\)–\(^5\) and PCR\(^6\) at the microbiology laboratory of The Atma Jaya Hospital. In this study, PCR was used as the gold standard as it is a highly sensitive molecular biology technique that aids in the rapid diagnosis of genital mycoplasmas (e.g., *M. hominis* and *U. urealyticum*) using organism-specific primers.

**Detection of *M. hominis* and *U. urealyticum using the AF Genital System\(^R\)**

The AF Genital System\(^R\) comprises a panel of 24 wells, each containing a biochemical substrate (growth indicators) and antibiotics (selection markers). The purpose of the system is to detect, presumptively identify, and assess antimicrobial susceptibility in microorganisms from urogenital samples (urethral and vaginal swabs, urine, and seminal fluid).

*M. hominis* and *U. urealyticum* were detected using the AF Genital System\(^R\) according to the manufacturer’s instructions. Accordingly, swabs (containing vaginal and urine samples) were immersed in physiological saline (provided with the kit) for 5 minutes. Next, 0.2 mL of clinical specimen suspensions were transferred into each well of the AF Genital System\(^R\). The inoculated wells were then overlaid with a drop of Vaseline oil and incubated at 36 ± 1°C for 18 to 24 hours. The test results were monitored via the change in color of the inoculated wells.

**Detection of *M. hominis* and *U. urealyticum by culture methods.** Urogenital swabs were transported in Amies transport medium containing phosphate-buffered saline (Thermo Fisher Scientific, Waltham, MA, USA) to the fully equipped microbiology laboratory at the Microbiology Department, Atma Jaya Hospital, North Jakarta, Indonesia. Clinical specimens were inoculated in agar plates containing the following media (all from Oxoid Ltd., Hampshire, UK): mycoplasma agar base (CM0401), urea agar base (CM0053B), yeast extract solution (LP0021B), urea 40% solution (SR0020K), horse serum (SR0035C), and mycoplasma supplement-G (SR0059). The inoculated plates were incubated at 35 ± 2°C for 48 hours and monitored for up to 14 days under microaerophilic conditions using a candle jar. Finally, Gram staining was performed to identify characteristic mycoplasma colonies (*M. hominis* and *U. urealyticum*) by microscopic examination.

**Detection of *M. hominis* and *U. urealyticum by PCR.** Clinical specimens (vaginal and urine samples) in cotton swabs (cotton was removed from the lid) were added to 400 μL of phosphate-buffered saline, vortexed, and centrifuged. Next, DNA extraction was performed using the QIAamp DNA Mini Kit column (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Purified DNA from these specimens was eluted in 150 μL of double distilled water and stored at −20°C until further use. This served as the template DNA for PCR.

PCR was performed according to the protocol of Sleha et al. with slight modifications.\(^6\) Briefly, the reaction mix comprised 2× HotStarTaq\(^R\) Master Mix (Qiagen) (2.5 units of HotStarTaq\(^R\) DNA polymerase, 1× PCR buffer containing 1.5 mM MgCl\(_2\), and 0.2 mM of each deoxyribonucleotide triphosphate), 2 μM of each oligonucleotide/primer (10× concentrated), 1 μL of template DNA (added last), and double-distilled water to 25 μL. The
primer sequences used in this study were as follows. *M. hominis*: forward primer 5'-GGAAGATATGTAACAAAAGAAGGTGCTG-3', reverse primer 5'-TTTATCTTCTGGGCCTAAATGATATCTTCG-3'; *U. urealyticum*: forward primer 5'-GTGACCAGTCCTATCCAAG-3', reverse primer 5'-TAAGCCGTTTACACCTCAA-3'. These primer pairs were specific to the gap (144 bp) and urease (225 bp) genes of *M. hominis* and *U. urealyticum*, respectively. The cycling conditions for the PCR were as follows: initial denaturation at 95°C for 15 minutes, denaturation at 94°C for 1 minutes, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. The amplified fragments were analyzed on 1.5% (w/v) agarose gels containing ethidium bromide and visualized using a UV-transilluminator. A no-template reaction served as the negative control while genomic DNA from *M. hominis* ATCC 15488 and *U. urealyticum* ATCC 27813 were used as positive controls.

**Sample size calculation**

The sample size, 'n' was calculated using the formula: 

\[ n = \frac{Z^2 \times p \times q}{d^2} \]

In this equation, 'Z' represents the conversion of the area under the normal curve at a given confidence level (when the accuracy interval applied was 95%); 'p' is the estimated proportion of patients positive for mycoplasma and was assumed to be 20% or 0.2 (based on prior studies); 'q' equals 1-p (0.8); and 'd' is the degree of precision desired (±10% or 0.1).

Thus,

\[ n = \frac{1.96^2 \times 0.2 \times 0.8}{0.1^2} = 61.5 \]

Sixty-two participants were required based on this calculation.

**Results and discussion**

Eighty-eight clinical specimens were examined in this study. Detection of *M. hominis* and *U. urealyticum* in these specimens using the AF Genital System®, culture methods, and PCR methods is shown in Tables 1, 2, 3, and 4.

The prevalence (positive detection rate) of *M. hominis* and *U. urealyticum* in vaginal swabs was 20.5% (18/88 specimens) and 21.6% (19/88 specimens), respectively, using the AF Genital System®. The prevalence (positive detection rate) of *M. hominis* and *U. urealyticum* in vaginal swabs was 6.8% (6/88 specimens) and 10.2% (9/88 specimens) using PCR.

**Table 1. Results of *M. hominis* detection using the AF Genital System® and PCR.**

| PCR                      | POSITIVE | NEGATIVE | TOTAL |
|--------------------------|----------|----------|-------|
| **AF Genital System®**   |          |          |       |
| POSITIVE                 | 4        | 14       | 18    |
| NEGATIVE                 | 2        | 68       | 70    |
| TOTAL                    | 6        | 82       | 88    |

Diagnostic parameters of the AF Genital System® versus PCR in detecting *M. hominis*

|                          |       |
|--------------------------|-------|
| Sensitivity              | 66.6% |
| Specificity              | 82.9% |
| PPV                      | 22.2% |
| NPV                      | 97.1% |

PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value.
Table 2. Results of *M. hominis* detection using the AF Genital System® and culture methods.

| CULTURE | POSITIVE | NEGATIVE | TOTAL |
|---------|----------|----------|-------|
| AF Genital System® | | | |
| POSITIVE | 8 | 10 | 18 |
| NEGATIVE | 6 | 64 | 70 |
| TOTAL | 14 | 74 | 88 |

Diagnostic parameters of the AF Genital System® versus culture methods in detecting *M. hominis*

| Parameter | Value |
|-----------|-------|
| Sensitivity | 57.1% |
| Specificity | 86.5% |
| PPV | 44.4% |
| NPV | 91.4% |

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

Table 3. Results of *U. urealyticum* detection using the AF Genital System® and PCR.

| PCR | POSITIVE | NEGATIVE | TOTAL |
|-----|----------|----------|-------|
| AF Genital System® | | | |
| POSITIVE | 5 | 14 | 19 |
| NEGATIVE | 4 | 65 | 69 |
| TOTAL | 9 | 79 | 88 |

Diagnostic parameters of the AF Genital System® versus PCR in detecting *U. urealyticum*

| Parameter | Value |
|-----------|-------|
| Sensitivity | 55.5% |
| Specificity | 82.3% |
| PPV | 26.3% |
| NPV | 94.2% |

PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value.

Table 4. Results of *U. urealyticum* detection using the AF Genital System® and culture methods.

| CULTURE | POSITIVE | NEGATIVE | TOTAL |
|---------|----------|----------|-------|
| AF Genital System® | | | |
| POSITIVE | 7 | 12 | 19 |
| NEGATIVE | 2 | 67 | 69 |
| TOTAL | 9 | 79 | 88 |

Diagnostic parameters of the AF Genital System® versus culture methods in detecting *U. urealyticum*

| Parameter | Value |
|-----------|-------|
| Sensitivity | 77.8% |
| Specificity | 84.8% |
| PPV | 36.8% |
| NPV | 97.1% |

PPV, positive predictive value; NPV, negative predictive value.
specimens), respectively, using PCR (Table 1 and Table 3). The prevalence (positive detection rate) of *M. hominis* and *U. urealyticum* in vaginal swabs was 15.9% (14/88 specimens) and 10.2% (9/88 specimens), respectively, using culture methods (Table 2 and Table 4). These findings differed from the results of a comparative study by Amirmozafari et al. These authors reported that a higher prevalence of genital mycoplasmas was detected by PCR compared with culture methods, while a few other studies reported lower prevalence.12–14

Compared with PCR and culture methods, respectively, the sensitivity of the AF Genital System® in detecting *M. hominis* was 66.6% (Table 1) and 57% (Table 2) and the sensitivity of the AF Genital System® in detecting *U. urealyticum* was 55.5% (Table 3) and 77.8% (Table 4). Compared with PCR and culture methods, respectively, the specificity of the AF Genital System® in detecting *M. hominis* was reasonably good at 82.9% and 86.5% (Table 1 and 2) while the specificity of the AF Genital System® in detecting *U. urealyticum* was 82.3% and 84.8% (Table 3 and 4). Furthermore, compared with PCR and culture methods, respectively, the positive predictive value (PPV) of the AF Genital System® for detection of *M. hominis* was 22.3% and 44.4% (Table 1 and 2) and the PPV of the AF Genital System® for detection of *U. urealyticum* was 26.3% and 36.8% (Table 3 and 4). Compared with PCR and culture methods, respectively, the negative predictive value (NPV) of the AF Genital System® for detection of *M. hominis* was 97.1% and 91.4% (Table 1 and 2) and the NPV of the AF Genital System® for detection of *U. urealyticum* was 94.2% and 97.1% (Table 3 and 4). These findings contrast with the observations by Redelinghuys et al., who reported a PPV and NPV of 97.1% and 28.6%, respectively, for detection of genital mycoplasmas using a similar assay.17

Although the sensitivity of the AF Genital System® in detecting *M. hominis* and *U. urealyticum* in vaginal swab specimens was below that of PCR (under 80%) (Table 1 and 3), specificity was reasonably good at 82% to 83% (Table 1 and 3). The high NPV of the AF Genital System® in detecting *M. hominis* and *U. urealyticum* indicates that the system is quite reliable in ruling out infection for patients whose specimens lack these bacteria. Thus, mycoplasma can be ruled out as a possible cause of genital infection in these patients with abnormal vaginal discharge. In developing countries like Indonesia, where limited data are available on mycoplasma-related genital infections in the female population, this method is recommended for routine diagnosis, especially when no other causes for vaginal discharge are identified.6 Moreover, the AF Genital System® has the advantages of being safe, fast, and less expensive for routine microbiological investigations of mycoplasma compared with other methods; this is a particular advantage in developing countries like Indonesia. Although many other studies have examined similar diagnostic techniques, to the best of our knowledge this is the first report on detection of genital mycoplasmas (*M. hominis* and *U. urealyticum*) using the AF Genital System®.12–14,16,17 However, this observational study was performed using a limited number of clinical specimens, and the small sample size may have affected the statistical significance of the results. Therefore, further studies of a greater larger of samples from other populations should be conducted, including in patients (male and female) with infertility disorders. Such studies are necessary to establish the diagnostic value of the AF Genital System® versus other standard techniques.
Conclusion
For patients in whom no other causes for vaginal discharge can be identified, specimen examination using the AF Genital System® is recommended. Our findings support the use of the AF Genital System® for identification of mycoplasma-associated diseases in developing countries like Indonesia with scarce resources and limited facilities.

Author contributions
ET and LHM: Conceptualization, visualization, supervision, methodology, validation, and data curation. SJ: Formal analysis, data curation, original draft preparation, review, and editing. All authors contributed significantly to the study and have approved the final manuscript.

Funding
The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: The authors thank the School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia for providing financial support for this study (funding number: 07/PHK-PKPD-sekr/03-2013).

Data availability
All relevant data are present in the manuscript.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

ORCID iD
Enty Tjoa https://orcid.org/0000-0001-7187-4280

References
1. Horner P, Donders G, Cusini M, et al. Should we be testing for urogenital Mycoplasma hominis, Ureaplasma parvum and Ureaplasma urealyticum in men and women? - a position statement from the European STI Guidelines Editorial Board. J Eur Acad Dermatol Venereol 2018; 32: 1845–1851. https://doi.org/10.1111/jdv.15146
2. Ljubin-Sternak S and Meštrović T. Chlamydia trachomatis and genital mycoplasmas: Pathogens with an impact on human reproductive health. J Pathog 2014; 2014: 183167. https://dx.doi.org/10.1155%2F2014%2F183167
3. Otgonjargala B, Becker K, Batbaatar G, et al. Effect of Mycoplasma hominis and cytomegalovirus infection on pregnancy outcome: A prospective study of 200 Mongolian women and their newborns. PLoS One 2017; 12: e0173283. https://dx.doi.org/10.1371/journal.pone.0173283
4. Doroftei B, Ilie OD, Armeanu T, et al. The prevalence of Ureaplasma urealyticum and Mycoplasma hominis infections in infertile patients in the Northeast Region of Romania. Medicina (Kaunas) 2021; 57: 211. https://doi.org/10.3390/medicina57030211
5. Moridi K, Hemmaty M, Azimian A, et al. Epidemiology of genital infections caused by Mycoplasma hominis, M. genitalium and Ureaplasma urealyticum in Iran; a systematic review and meta-analysis study (2000–2019). BMC Public Health 2020; 20: 1020. https://doi.org/10.1186/s12889-020-08962-5
6. Sleha R, Boštíková V, Hampl R, et al. Prevalence of Mycoplasma hominis and Ureaplasma urealyticum in women undergoing an initial infertility evaluation. Epidemiol Mikrobiol Imunol 2016; 65: 232–237.
7. Horner PJ and Martin DH. Mycoplasma genitalium infection in men. J Infect Dis 2017; 216: S396–S405. https://doi.org/10.1093/infdis/jix145
8. Grześko J, Elias M, Manowiec M, et al. Genital mycoplasmas – morbidity and a potential influence on human fertility. Med Wieku Rozwoj 2006; 10: 985–992.
9. Feriyawati L, Anggraini DR and Nasution TA. Co-infection of human papillomavirus with Mycoplasma hominis/Ureaplasma urealyticum among female sex workers in Medan, Indonesia. Open Access Maced J Med Sci 2019; 7: 3425–3428. https://doi.org/10.3889/oamjms.2019.438
10. Baczynska A, Svenstrup HF, Fedder J, et al. Development of real-time PCR for detection of Mycoplasma hominis. *BMC Microbiol* 2004; 4: 35. https://doi.org/10.1186/1471-2180-4-35

11. Cunningham SA, Mandrekar JN, Rosenblatt JE, et al. Rapid PCR detection of *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum*. *Int J Bacteriol* 2013; 2013: 168742. https://doi.org/10.1155/2013/168742

12. Abele-Horn M, Wolff C, Dressel P, et al. Polymerase chain reaction versus culture for detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the urogenital tract of adults and the respiratory tract of newborns. *Eur J Clin Microbiol Infect Dis* 1996; 15: 595–598. https://doi.org/10.1007/bf01709369

13. Stellrecht KA, Woron AM, Mishrik NG, et al. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol* 2004; 42: 1528–1533. https://doi.org/10.1128/jcm.42.4.1528-1533.2004

14. Amirmozafari N, Mirnejad R, Kazemi B, et al. Comparison of polymerase chain reaction and culture for detection of genital mycoplasma in clinical samples from patients with genital infections. *Saudi Med J* 2009; 30: 1401–1405.

15. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573–577. https://doi.org/10.7326/0003-4819-147-8-200710160-00010

16. Tibaldi C, Cappello N, Latino MA, et al. Vaginal and endocervical microorganisms in symptomatic and asymptomatic non-pregnant females: Risk factors and rates of occurrence. *Clin Microbiol Infect* 2009; 15: 670–679. https://doi.org/10.1111/j.1469-0691.2009.02842.x

17. Redelinghuys MJ, Ehlers MM, Dreyer AW, et al. Comparison of the new Mycofast Revolution assay with a molecular assay for the detection of genital mycoplasmas from clinical specimens. *BMC Infect Dis* 2013; 13: 453. https://doi.org/10.1186/1471-2334-13-453