

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

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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%).

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Keywords

AF Genital System[®], culture method, *Mycoplasma hominis*, *Ureaplasma urealyticum*, genital mycoplasma, polymerase chain reaction, diagnostics, sensitivity, specificity

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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).¹ 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.^{2–6} 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.^{7,8} Data regarding the prevalence of *M. hominis* and *U. urealyticum* among Indonesian women with abnormal vaginal discharge are lacking,⁹ potentially because of limited availability of diagnostics for these infections. Routine diagnosis largely relies on culture methods, polymerase chain reaction (PCR), and rapid tests.^{10–14} Conventional culture methods for microbiological detection are time-consuming, laborious, cumbersome, and less sensitive while PCR-based methods are costly and require considerable expertise.^{10–13} 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>; LIOFILC HEM[®] 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.¹⁵

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 staining³⁻⁵ and analysis using the AF Genital System[®], culture methods,³⁻⁵ and PCR⁶ 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[®]

The AF Genital System[®] 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[®] 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[®]. The inoculated wells were then overlaid with a drop of Vaseline oil and incubated at $36 \pm 1^\circ\text{C}$ 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 $35 \pm 2^\circ\text{C}$ for 48 hours and monitored for up to 14 days under micro-aerophilic 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 \times HotStarTaq[®] Master Mix (Qiagen) (2.5 units of HotStarTaq[®] DNA polymerase, 1 \times PCR buffer containing 1.5 mM MgCl_2 , and 0.2 mM of each deoxyribonucleotide triphosphate), 2 μM of each oligonucleotide/primer (10 \times 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'-TTTATCTTCTGGCGTAA TGATATCTTCG-3'; *U. urealyticum*: forward primer-5'-GTGACC GTCCTATCCAAG-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, 'Zα' 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

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 <i>M. hominis</i>			
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 <i>M. hominis</i>			
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 <i>U. urealyticum</i>			
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 <i>U. urealyticum</i>			
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.^{12-14,16}

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.

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Data availability

All relevant data are present in the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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