

## ORIGINAL ARTICLE

# Evaluating the utility of the A.F. Genital System test for pathogen diagnosis in acute male urethritis

Mehmet Sarier<sup>1,2</sup>  | Nevgun Sepin<sup>3</sup>  | Mestan Emek<sup>4</sup>  | Aysegul Tezcan Germen<sup>5</sup>  | Mustafa Burak Hoscan<sup>6</sup>  | Elcin Konuk<sup>7</sup>  | Hasan Turgut<sup>8</sup> 

<sup>1</sup>Department of Urology, Istinye University, Istanbul, Turkey

<sup>2</sup>Department of Urology, Medical Park Hospital, Antalya, Turkey

<sup>3</sup>Department of Clinical Microbiology and Infectious Disease, Training and Research Hospital Antalya, Turkey

<sup>4</sup>Department of Public Health, Akdeniz University, Antalya, Turkey

<sup>5</sup>Department of Obstetrics and Gynaecology, Medical Park Hospital, Antalya, Turkey

<sup>6</sup>Department of Dialysis, Vocational School of Health Services, Bilim University Antalya, Turkey

<sup>7</sup>Public Health Laboratory, Provincial Directorate of Health, Antalya, Turkey

<sup>8</sup>Faculty of Health Science, Avrasya University, Trabzon, Turkey

## Correspondence

Mehmet Sarier, Medical Park Hospital Department of Urology Muratpaşa, Antalya, 07110 Turkey.  
Email: drsarier@gmail.com

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## Abstract

This study evaluated the effectiveness of the A.F. Genital System (Liofilchem<sup>®</sup>, Italy) in detecting pathogens compared with multiplex real-time polymerase chain reaction (PCR) in men with acute urethritis. Men diagnosed as having acute urethritis between 1 April 2021 and 31 December 2021 were included. Urethral swab samples were obtained for A.F. Genital System and PCR testing in a randomly determined order. The efficacy of the A.F. Genital System was analysed by comparing the results of the two tests. The study included 83 patients (mean age  $34.1 \pm 11.3$  years). A urethritis pathogen was detected in 69 patients (83.1%) by PCR and only 15 patients (18.1%) with the A.F. Genital System. The sensitivity of the A.F. Genital System in detecting acute urethritis pathogens was 21.7% (95% confidence interval [CI]: 13.6–32.8), and the specificity was 100% (95% CI: 78.5–100). Its sensitivity was 20% (95% CI: 7.1–45.2) in the diagnosis of gonococcal urethritis and 19.1% (95% CI: 11.2–30.4) in the diagnosis of non-gonococcal urethritis. PCR detected two or more urethritis pathogens in 9 patients (13.0%), while no polymicrobial infection was detected with the A.F. Genital System. Based on the results of multiplex real-time PCR, the A.F. Genital System had very low sensitivity in the detection of pathogens in acute male urethritis. It should be kept in mind that using this test in patients with acute urethritis may result in a high missed diagnosis rate for urethritis pathogens.

## KEYWORDS

A.F. genital, AF genital system, diagnosis, non-gonococcal, PCR, urethritis

## 1 | INTRODUCTION

Acute male urethritis is inflammation of the urethra in men. It is often caused by sexually transmitted pathogens and poses a serious medical, social and economic burden worldwide. Microscopy of a urethral gram stain smear (GSS) is an inexpensive and easily performed alternative method for differential diagnosis. Not only can GSS diagnose acute urethritis, but it can also reveal the presence of *Neisseria gonorrhoeae* through findings of intracellular Gram-negative diplococci. However, as interpreting GSS requires

experienced personnel, the test is prone to intra- and interobserver errors. (Smith et al., 2003).

Nucleic acid amplification tests such as polymerase chain reaction (PCR) have significantly improved the management of acute urethritis in recent years. Identifying urethritis pathogens by conventional methods is both difficult and time-consuming, whereas PCR tests allow the rapid identification of multiple pathogens in a single sample with high sensitivity and specificity. (Sarier et al., 2017) Today, PCR is the guideline-recommended method for pathogen identification in acute urethritis. (Bonkat et al., 2020; Walensky et al., 2021).

Due to advances in PCR technology, there are currently four controversial issues in the management of acute male urethritis. (Sarier, 2019a) First, with the widespread use of PCR, the effectiveness of the  $\geq 5$  PMNL/HPF (polymorphonuclear leukocytes per high-power field) cut-off value in GSS, especially in the diagnosis of non-gonococcal urethritis, has been questioned. Therefore, the Centers for Disease Control and Prevention (CDC) reduced the GSS threshold for the diagnosis of non-gonococcal urethritis to  $\geq 2$  PMNL/HPF for the first time in the 2015 Sexually Transmitted Disease (STD) Treatment Guidelines. (Workowski et al., 2015) Second, the use of PCR has highlighted the presence of polymicrobial infection in urethritis. (Sarier, 2019b) Third, the need to update the classification of non-gonococcal urethritis (NGU) pathogens has arisen. (Sarier & Kukul, 2019) And fourth, it is being more frequently recommended to avoid 'classical' empiric treatment approaches in acute urethritis. (Bartoletti et al., 2019) However, the main obstacles to the widespread use of PCR testing are its high cost and the need for appropriate laboratory conditions. Therefore, there is an ongoing search for a low-cost, easy-to-use test that aims to assay a single sample for multiple pathogens, with no specific laboratory requirements and high diagnostic accuracy.

One of the tests developed for this purpose is the A.F. Genital System (Liofilchem<sup>®</sup>, Italy), a 24-well plastic tray that is easy to use and provides a quick, presumptive identification of urogenital pathogens (from vaginal/urethral swabs and seminal fluid). (Savini et al., 2013) However, literature data on the effectiveness of this test in acute urethritis are lacking. The aim of this study was to determine the effectiveness of the A.F. Genital System in detecting pathogens in men with acute urethritis compared with the results of multiplex real-time PCR (rt-PCR).

## 2 | METHODS

### 2.1 | Study design and patients

Men who presented to the urology outpatient clinic of Antalya Medical Park Hospital with complaints of acute urethritis between 1 February 2021 and 31 December 2021 were evaluated in this study. Patients with positive leukocyte esterase test (Combur Test<sup>®</sup>, Roche) in the first urine sample after physical examination and/or  $\geq 2$  PMNL/HPF on microscopy of a urethral gram stain smear (Sarier et al., 2018) were included in the study. Urethral swab samples for both A.F. Genital System and multiplex rt-PCR were obtained from each patient in a randomly determined order. All samples were collected by the same clinician. Swab samples for A.F. Genital System test were studied immediately according to the manufacturer's recommendations. Swab samples obtained for multiplex rt-PCR test were resuspended in 2 ml sterile phosphate-buffered solution and stored at  $-80^{\circ}\text{C}$  until analysis. The patients' demographic information and results from both tests were collected. The effectiveness of the A.F. Genital System in detecting pathogens was calculated according to the results of multiplex rt-PCR by comparing the results of the two tests.

### 2.2 | A. F. Genital System (Liofilchem<sup>®</sup>, Italy)

Urethral swab samples were immersed in 3 ml sterile saline solution bottle provided by the manufacturer. After incubating for 5 min, 0.2 ml of the resulting solution was placed in each well of the test plate. The plate was incubated at  $36 \pm 1^{\circ}\text{C}$  for 24 h. The results were interpreted based on evaluation of colour change and microscopic examination of the test wells according to the manufacturer's instructions. Growth of *Ureaplasma urealyticum* ( $10^3$ ,  $10^4$  and  $\geq 10^5$  colony-forming units [cfu/ml]) and *Mycoplasma hominis* ( $10^4$ ,  $\geq 10^5$  cfu/ml) was calculated semi-quantitatively according to the colour change in the wells. For both of these pathogens, growth was considered positive at a density of  $10^4$  cfu/ml or higher. The presence of *Trichomonas vaginalis*, *Escherichia coli*, *Proteus* spp., *Pseudomonas* spp., *Gardnerella vaginalis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *N. gonorrhoeae*, *Streptococcus agalactiae* and *Candida* spp. was also investigated in the A.F. Genital System (Figure 1). (Savini et al., 2013).

### 2.3 | Multiplex real-time PCR

Multiplex rt-PCR tests were performed with the PREP-NA PLUS and PREP-GS PLUS kits on an Elite Prime<sup>®</sup> rt-PCR device (DNA-Technology<sup>®</sup>, Moscow, Russia) and interpreted according to the manufacturer's recommendations. Findings of *N. gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *T. vaginalis* and human simplex virus types 1–2 were considered absolute positive. In addition, the microbial load of *U. urealyticum*, *G. vaginalis*, *Candida* spp., *M. hominis*, *Enterococcus* spp./Enterobacteriaceae spp., *Ureaplasma parvum*, *Corynebacterium* spp., *Streptococcus* spp., *Staphylococcus* spp. and *Lactobacillus* spp. was quantitatively measured. A microbial load of  $10^4$  or higher for opportunistic pathogens was considered positive, as per the manufacturer's recommendation.

### 2.4 | Ethical approval and statistical analysis

This study was approved by the ethics committee of the Antalya Medical Park Hospital Complex (approval no: 011/2018), and written informed consent was obtained from all patients. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

Statistical analysis was performed using OpenEpi<sup>®</sup> version 3.01 (Atlanta, GA, USA) statistics software. Age was expressed as mean  $\pm$  standard deviation (minimum-maximum); other descriptive data were presented as frequency and percentage. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of the A.F. Genital System were calculated within a 95% confidence interval (CI).

## 3 | RESULTS

The study included 83 patients with a mean age of  $34.1 \pm 11.3$  (18–55) years. A urethritis pathogen was detected in 69 patients (83.1%)

FIGURE 1 A.F. Genital System tray



by multiplex rt-PCR and only 15 patients (18.1%) with the A.F. Genital System. The sensitivity of the A.F. Genital System in detecting acute urethritis pathogens was 21.7% (95% CI: 13.6–32.8), and the specificity was 100% (95% CI: 78.5–100).

*N. gonorrhoeae* was detected by multiplex rt-PCR detected in 15 patients and by the A.F. Genital System in 3 patients. The sensitivity of the A.F. Genital System was 20% (95% CI: 7.1–45.2) in the diagnosis of gonococcal urethritis. Non-gonococcal pathogens were detected in 63 patients by rt-PCR and in only 12 patients with the A.F. Genital System. The sensitivity of the A.F. Genital System was 19.1% (95% CI: 11.2–30.4) in the diagnosis of non-gonococcal pathogens (Table 1).

Of the non-gonococcal pathogens included in the multiplex rt-PCR test and A.F. Genital System, *G. vaginalis* was detected in 10 patients by rt-PCR, while the A.F. Genital System detected only one of these patients. *Enterococcus/Enterobacteriaceae* spp. were

detected in 8 patients with rt-PCR, while the A.F. Genital System detected *E. faecalis* in 3 of these patients. *M. hominis* was detected in 4 patients by rt-PCR and 1 patient with the A.F. Genital System. *U. urealyticum* was detected in 8 patients on rt-PCR and in 7 patients in the A.F. Genital System. However, only 4 of the 7 patients with *U. urealyticum* detected in the A.F. Genital System were among those detected by multiplex rt-PCR. *M. genitalium*, which is not included in the A.F. Genital System, was detected in 9 patients by rt-PCR, whereas the A.F. Genital System results indicated *U. urealyticum* in 3 of these patients. The distribution of the pathogens detected in both tests is shown in Table 2.

When evaluated in terms of polymicrobial urethritis, multiplex rt-PCR detected two or more urethritis pathogens in 9 patients (13.0%), while no polymicrobial infection was detected in any patient with the A.F. Genital System.

TABLE 1 Diagnostic utility of the A.F. Genital System in acute urethritis

Parameter	Acute urethritis		Gonococcal urethritis		Non-gonococcal urethritis	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Sensitivity	21.7%	(13.6–32.8)	20%	(7.1–45.2)	19.7%	(11.2–30.4)
Specificity	100%	(78.5–100)	100%	(94.6–100)	100%	(83.9–100)
Positive predictive value	100%	(79.6–100)	100%	(43.8–100)	100%	(75.7–100)
Negative predictive value	20.6%	(12.7–31.6)	85%	(75.6–91.2)	28.2%	(19.1–39.5)
Diagnostic accuracy	34.9%	(25.6–45.7)	85.5%	(76.4–91.5)	38.5%	(28.8–49.3)

Abbreviation: CI, Confidence Interval.

Multiplex real-time PCR: 78 pathogens in 69 patients		A.F. Genital System: 15 pathogens in 15 patients	
Pathogens	n (%)	Pathogens	n (%)
<i>Chlamydia trachomatis</i>	20 (25.6)		
<i>Neisseria gonorrhoeae</i>	15 (19.2)	<i>Neisseria gonorrhoeae</i>	3 (20.0)
<i>Gardnerella vaginalis</i>	10 (12.8)	<i>Gardnerella vaginalis</i>	1 (6.7)
<i>Mycoplasma genitalium</i>	9 (11.5)		
<i>Ureaplasma urealyticum</i>	8 (10.2)	<i>Ureaplasma urealyticum</i>	7 (46.6)
Enterobacteriaceae/Enterococcus spp.	8 (10.2)	<i>Enterococcus faecalis</i>	3 (20.0)
<i>Mycoplasma hominis</i>	4 (5.1)	<i>Mycoplasma hominis</i>	1 (6.7)
<i>Trichomonas vaginalis</i>	3 (3.8)		
<i>Candida</i> spp.	1 (3.8)		

Abbreviation: Spp., Species.

TABLE 2 Distribution of pathogens detected in the A.F. Genital System and multiplex real-time PCR

## 4 | DISCUSSION

Acute urethritis is the most common sexually transmitted disease in men. Around 62 million new cases of gonococcal urethritis and 89 million new cases of non-gonococcal urethritis are reported worldwide annually, and this number is increasing every year. (Sarier, 2019b) One of the reasons for this increase is believed to be inappropriate or incomplete treatment approaches. (Rossignol et al., 2019) Empirical therapy based on microscopy of a urethral GSS can still be found in guidelines on the treatment of acute urethritis. (Aravinda et al., 2021) However, empirical treatment approaches are known to be an important factor in the development of resistant strains. Antibiotic treatment that does not address undetected pathogens that may be present in urethritis can promote the selection of antibiotic-resistant strains. (Hahn et al., 2021) Of these, antibiotic resistance in *M. genitalium* in particular and also *N. gonorrhoeae* has become a global problem. (Derbie et al., 2020; Fernández-Huerta et al., 2020) We believe that targeting the causative pathogen must be adopted as a therapeutic approach in acute urethritis. However, the cost-effectiveness of this approach remains a subject of debate. For these reasons, research continues on the introduction of practical, inexpensive, rapid and accurate diagnostic methods that will

guide both diagnosis and treatment. In this study, we investigated whether the A.F. Genital System may be an alternative test that meets these criteria.

The A.F. Genital System is an easy test to perform. Adequate sampling, especially when obtaining urethral swabs, may be important when using the A.F. Genital System. Acute urethritis in men has three major symptoms: urethral discharge, urethral itching and dysuria. Urethral discharge is the most important symptom of urethral inflammation. (Bachmann et al., 2015) However, these symptoms may be mild or absent in cases of urethritis with low inflammation. In a recent study, the presence of visible urethral discharge was observed in only 32.3% of patients. (Sarier et al., 2020) This suggests that the A.F. Genital System should be avoided if an adequate sample cannot be obtained, especially patients with low-inflammation acute urethritis. In order to better understand the diagnostic significance of the amount of discharge in the A.F. Genital System, we believe that its effectiveness should also be evaluated in patients with acute vaginitis. Discharge is much more pronounced in vaginal infections than urethral infections. Therefore, the A.F. Genital System may have greater diagnostic value in these cases. As literature data on this subject are scarce, further comparative studies are needed. (Simon et al., 2015).

The inability of the A.F. Genital System to assess for *C. trachomatis* and *M. genitalium* is an important limitation of the test. *C. trachomatis* is the cardinal pathogen in non-gonococcal urethritis, responsible for approximately 25% to 50% of cases. (Pond et al., 2014; van der Veer et al., 2016) However, *M. genitalium* has been detected at an increasing rate in recent years due to the widespread use of PCR, and its prevalence in acute urethritis varies between 6% and 16.7% according to age and region. (Libois et al., 2018; Sarier, 2019b) Unlike *U. urealyticum* and *M. hominis*, other members of the Mycoplasmataceae family, *M. genitalium* is not opportunistic and is considered an absolute pathogen of sexually transmitted disease according to the latest guidelines. (Bonkat et al., 2020) Resistance is an important problem in *M. Genitalium*, and as mentioned above, failure to detect acute urethritis cases caused by *M. genitalium* using the A.F. Genital System will increase the development of antibiotic resistance due to incomplete treatment. If using the A.F. Genital System to identify pathogens of acute urethritis, additional testing for *C. trachomatis* and *M. genitalium* should be performed in these patients.

One of the interesting findings in our study was that 3 of 9 patients found to have *M. genitalium* by multiplex rt-PCR had results indicating *U. urealyticum* in the A.F. Genital System. These results likely occurred because the two microorganisms are in the same family. In this respect, the specificity of the test for subtypes can also be questioned. However, if the aim is to evaluate its effectiveness as a targeted diagnostic test, this should be considered a serious error. In contrast, differentiation of *U. urealyticum* and *U. parvum* in the A.F. Genital System is an important advantage of this test. Based on the current evidence, the role of *U. parvum* in acute urethritis pathogens is controversial. Unlike *U. urealyticum*, the most recent European Urology Association guideline does not list *U. parvum* as a causative pathogen of acute urethritis. (Bonkat et al., 2020) However, because *U. parvum* was not detected by multiplex rt-PCR in any of the patients in our study, we could not evaluate how effective the A.F. Genital System is in distinguishing between these two *Ureaplasma* species.

Identifying opportunistic pathogens is important in acute urethritis. As these microorganisms are found in the normal flora but are considered pathogenic at high microbial loads, the ability of a test to accurately quantify these pathogens will guide the correct approach in diagnosis and treatment. Although these measurements can be made with nucleic acid tests, the A.F. Genital System only evaluates the presence or absence of a few opportunistic pathogens (*G. vaginalis*, *Candida* spp. and *E. faecalis*). This can lead to false-positive results and should be considered another important limitation of the test.

Polymicrobial urethritis is a clinical condition that has become more prominent with the widespread use of multiplex PCR. Prevalence studies have shown that urethritis in which two or more pathogens are detected simultaneously account for up to 16.9% of all urethritis cases. (Sarier, 2019b) In the present study, the frequency of polymicrobial urethritis in multiplex rt-PCR was found to be 13.0%. However, no cases of polymicrobial urethritis were detected by the A.F. Genital System due to its low sensitivity in our

study. The inability of the A.F. Genital System to recognize any of these patients can be considered an important shortcoming.

The main advantages of the A.F. Genital System are that it is economical, easy to perform, and requires no specific laboratory environment. At present, multiplex rt-PCR testing in our hospital costs approximately \$140 (~1200 TL), while the A.F. Genital System costs only \$30 (~260 TL). Therefore, its low cost is clearly advantageous. However, the most important finding in this study is that the A.F. Genital System had very low diagnostic accuracy for acute urethritis pathogens. The importance of this in terms of public health is much greater than the low cost of the test. According to the results of multiplex rt-PCR, approximately 4 out of every 5 patients are misdiagnosed. These patients will continue to sexually transmit these pathogens due to inappropriate treatment. The value of a test is determined by its reliability. In this respect, we believe that it would be wrong to evaluate the A.F. Genital System as cost-effective.

Although we randomized the order of sampling for the two tests in our study, the fact that we did not wait for a certain time between samples to allow the production of new discharge can be considered a limitation, especially in patients without visible urethral discharge. This suggests that sampling may not have been adequate for the A.F. Genital System. However, there are also insufficient data regarding the amount of sample needed for this test.

In summary, in our study the A.F. Genital System had 100% specificity but very low sensitivity (21.7%) when compared with multiplex rt-PCR results in the detection of pathogens causing acute urethritis in men. Although its low cost and easy applicability are an important advantage, it should be kept in mind that using this test in men with acute urethritis will result in a substantial proportion of pathogens being missed. In this regard, the A.F. Genital System requires improvement. Its sensitivity must be increased, and assessment of *C. trachomatis* and *M. genitalium* should also be incorporated. If this can be achieved, it may fill an important gap in the tests used for pathogen diagnosis in acute male urethritis in the future.

## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

## ETHICAL APPROVAL

The study protocol was approved by the local ethics committees.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

## ORCID

Mehmet Sarier  <https://orcid.org/0000-0002-8656-7416>

Nevgun Sepin  <https://orcid.org/0000-0002-2485-6765>

Mestan Emek  <https://orcid.org/0000-0001-6843-4954>

Aysegul Tezcan Germen  <https://orcid.org/0000-0003-1796-9419>

Mustafa Burak Hoscan  <https://orcid.org/0000-0002-7887-9575>

Elcin Konuk  <https://orcid.org/0000-0001-9927-2185>

Hasan Turgut  <https://orcid.org/0000-0001-9793-6734>

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