Accuracy of molecular diagnostic techniques in patients with a confirmed urine culture: A systematic review and meta-analysis

Ximena Guzmán Robledo[1,2], Karla Valeria Orejuela Arcila[1], Sergio Hernando Mina Riascos[1,2], Herney Andrés García-Perdomo[1,2]

[1]UROGIV Research Group, School of Medicine, Universidad del Valle, Cali, Colombia; [2]Division of Urology/Uro-oncology, Department of Surgery, School of Medicine, Universidad del Valle, Cali, Colombia

Cite as Guzmán Robledo X, Orejuela Arcila KV, Mina Riascos SH, et al. Accuracy of molecular diagnostic techniques in patients with a confirmed urine culture: A systematic review and meta-analysis. *Can Urol Assoc J* 2022;16(9):E484-9. http://dx.doi.org/10.5489/cuaj.7677

Published online April 11, 2022

Appendix available at *cuaj.ca*

Abstract

Introduction: We aimed to identify the molecular diagnostic techniques available for urinary tract infection (UTI) diagnosis and their accuracy compared to traditional urinary culture.

Methods: A systematic search was performed in MEDLINE (OVID), EMBASE, LILACS, and the Cochrane Central Register of Controlled Trials (CENTRAL). The populations were adult and pediatric patients with confirmed UTI by reference standard urine culture. The index test for the diagnosis of UTI was any molecular diagnostic technique. The primary outcome was the diagnosis of UTI with measures of sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), positive likelihood ratio (LR+), negative likelihood ratio (LR–), diagnostic odds ratio (DOR), and area under the curve (AUC). The operative characteristics were determined, and a meta-analysis was performed. The evaluation of each included study was performed with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.

Results: We identified 1230 studies with the search strategies. Ultimately, 13 studies met the inclusion criteria for qualitative analysis, and seven were included for the meta-analysis. Four molecular techniques were identified; however, it was only possible to synthesize the information from two of them. In multiplex polymerase chain reaction (PCR) meta-analysis, overall sensitivity was 0.80 (95% confidence interval [CI] 0.73–0.86) and specificity was 0.83 (95% CI 0.52–0.95). For the DOR, the overall result was 21 (95% CI 4.8–95). For reverse transcription (RT)-PCR, sensitivity was 0.94 (95% CI 0.73–0.99) and specificity was 0.59 (95% CI 0.063–0.96). For the DOR, the overall result was 23 (95% CI 1.1–467).

Conclusions: Multiplex PCR and RT-PCR are molecular techniques that might be comparable to standard urine culture for UTI diagnosis. Refinement of these new diagnostic tools will avoid unnecessary antimicrobial therapy and the consequent development of drug-resistant resistant pathogens, as well as improve the ability to identify patients at risk and prevent or minimize sequelae derived from the infection.

Introduction

Lower urinary tract symptoms (LUTS) are exceedingly prevalent among adults,[1] and 60–80% of these episodes are related to significant bacteriuria[2] — an indicator of either bacterial colonization or infection of the urinary tract.[3]

Urinary tract infection (UTI) is among the most prevalent infections, affecting near 50% of the population at least once in their lifetime.[4] The initial diagnostic examination begins with clinical symptoms[4] and microbiology techniques to support medical decisions. Urine is cultured on agar plates, and antimicrobial susceptibility testing (AST) is performed.[4] The gold standard is the direct detection of the pathogen itself in clinical samples.[5]

A $\geq 10^5$ CFU/mL threshold has high specificity for a UTI, but sensitivity is only nearly 50%.[1] Nonetheless, it was recently confirmed that low counts of *E. coli* in midstream urine were highly predictive for its presence in the bladder and not caused by contamination.[6] Indeed, lowering the threshold to $\geq 10^3$ increases sensitivity, with minimal reductions in specificity.[1] Despite that, 25–30% of these symptomatic women will have a negative urine culture.[2]

Recent evidence suggests that UTIs are not limited to a superficial luminal infection.[1] Conversely, intracellular bacterial communities (ICB) have been described,[7] complicating the interpretation of the culture-based diagnosis, as bacteria are then undetected by standard urine cultures.[1] Based on these acknowledgments, innovative methods of identification of uropathogens have emerged,[1] increasingly relying on molecular techniques.[5]

To date, there are no systematic reviews on the use of molecular diagnostic techniques in patients with suspected UTIs. We aimed to determine the diagnostic accuracy of novel molecular tools compared to traditional urinary culture to diagnose UTI in patients with LUTS.

Methods

This systematic review was performed according to the recommendations of the Cochrane Collaboration and following the PRISMA-P statement.

Eligibility criteria

We included clinical trials, cohort, case-control, and cross-sectional studies providing data on accuracy for diagnosis of UTI in adult and pediatric patients.

The index test for the diagnosis of UTI was any molecular diagnostic technique. The reference standard was urine culture.

Studies were included if they fulfilled the following criteria: 1) reference standard for UTI represented by urine culture performed before/after any molecular diagnostic technique; 2) availability of many nondiagnostic urine cultures; and 3) availability of many diagnostic urine cultures classified as true positives (TPs), false positives (FPs), false negatives (FNs), and true negatives (TNs) either as group totals or by case-by-case enumeration of diagnoses.

The primary outcome was the diagnosis of UTI with measures of sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR), and area under the curve (AUC).

For all outcomes, no time specification was necessary.

We excluded studies that did not meet the criteria described above, those not related to UTI, non-related to a diagnostic method, flow-cytometry studies, animal studies, non-bacterial urinary infections, genitourinary tuberculosis infection, and neurogenic patients. Also, we excluded articles that could not be found or whose full-text were not available.

Information sources

A systematic search of studies was performed in the following databases from inception to December 1, 2021: MEDLINE (OVID), EMBASE, LILACS, and the Cochrane Central Register of Controlled Trials (CENTRAL) (Appendix; available at *cuaj.ca*). We scanned references from relevant articles identified through the search, conferences, thesis databases, Open Grey, Google scholar, and *clinicaltrials.gov* to ensure literature saturation. There were no setting or language restrictions.

Study selection and data collection

Two reviewers (XG, KO) independently evaluated the systematically searched titles and abstracts. They scanned full-texts of relevant studies, applied prespecified inclusion and exclusion criteria, and extracted the data. Disagreements were resolved by consensus between two reviewers; where disagreement could not be solved, a third reviewer (HG) was consulted.

Using a standardized form, two trained reviewers independently extracted the following information from each article: study design, year of publication, geographic location, authors names, title, number of patients included, timing, variables, interventions, outcomes, and association measures.

Risk of bias

We used the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS2) tool to assess the risk of bias in these studies.

Data analysis/synthesis of results

The statistical analysis was performed using R and Review Manager 5.3 (RevMan® 5.3). For the outcomes, information about sensitivity, specificity, likelihood ratios, and DORs was reported based on concordance between molecular diagnostic techniques results and urinary cultures. The results are displayed in forest plots of the estimated effects of the included studies with a 95% confidence interval (95% CI), and we pooled the information with a random effect meta-analysis according to the heterogeneity expected.

Publication bias

An evaluation was conducted to identify reporting or publication bias using the funnel plot.

Sensitivity analysis

We performed sensitivity analysis extracting weighted studies and running the estimated effect to find differences.

Analysis by subgroup

A subgroup analysis was performed for the polymerase chain reaction (PCR) and multiplex-PCR.

Results

Selection of studies

In the initial search, a total of 1230 studies were found. After the initial filter by full-text, 13 studies met the inclusion criteria and seven were included for the meta-analysis (Figure 1).[8-20]

Characteristics of included studies

The studies were published between 2010 and 2020. Seven studies were performed in the U.S., six in Europe, and one in Japan.

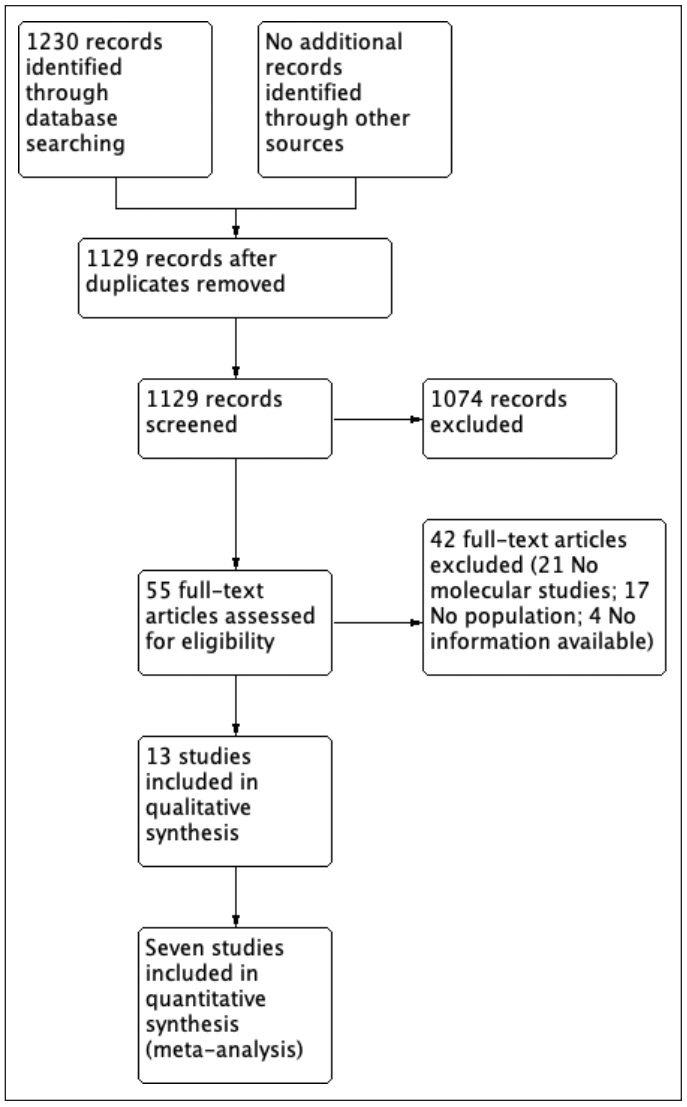

Figure 1. Flowchart of selected studies.

The total number of participants was 3995, with an average number of participants per a study of 285 (10–958). The age range was between <28 days and 104 years, and most individuals were women (64%).

The definition for a positive urine culture varied between $<10^3$ CFU/ML to $\geq 10^5$ CFU/ML; the most used molecular technique was qPCR (43%), and the top frequent germ isolated irrespective of the method was *E. coli* (Supplementary Table 1; available at *cuaj.ca*).

Risk of bias assessment

All the included studies were evaluated with a low risk of bias concerning the reference standard, mainly regarding flow and timing; however, most of the studies had an unclear risk about the index test because they did not specify the threshold used for the diagnosis or its standard test execution. As for the selection of patients, two studies had a high risk of bias, one because it included both symptomatic and asymptomatic patients and the other because of the exclusion of patients with fever and acute pyelonephritis (Figures 2A, 2B).

Results of the individual studies

Diagnosis of UTI with RT-PCR

Regarding this molecular technique, we pooled five studies.[9,12,15,17,18]

The bivariate random-effects model and estimation of summary receiver operating characteristic (SROC) curves indicated that the overall sensitivity was 0.94 (95% CI 0.73–0.99) and that the overall specificity was 0.59 (95% CI 0.063–0.96). For the DOR, the overall result was 23 (95% CI 1.1–467) (Figure 3A, Table 1).

Diagnosis of UTI with multiplex PCR

Regarding multiplex PCR, we included two studies.[8,20]

The bivariate random-effects model and estimation of SROC curves indicated that the overall sensitivity was 0.80 (95% CI 0.73–0.86) and that the overall specificity was 0.83 (95% CI 0.52–0.95). For the DOR, the overall result was 21 (95% CI 4.8–95) (Figure 3B, Table 1).

Multiple techniques

The remaining studies had other diagnostic methods, including MALDI-TOF MS mass spectrometry, next-generation DNA sequencing (NGS) technology-based on bacterial 16S rRNA amplicon sequencing analysis (a panel of recombinase polymerase amplification assays and narrow-angle forward laser light scattering technology).[10,11,13,14,16,19]

Three studies evaluated MALDI-TOF MS mass spectrometry with a sample size of 1378 and had variable sensitivity ranging from 67–92% and specificity above 70–100%. Only the two studies performed in Spain reported the characteristics of the population, all adults and primarily female; the facility was variable.

Only one study assessed NGS technology. No cutoff value for UTI diagnosis by standard urine culture was reported, and only 10 patients participated. Most of them had upper and complicated UTI (80% and 70%, respectively). The overall sensitivity was 100%; however, specificity could not be calculated since there were no TNs.

Also, only one study reviewed narrow-angle forward laser light scattering technology in a pediatric population. It included 439 patients and presented a sensitivity of 96% with a specificity of 71%; no socio-demographic characteristics were recorded (Table 1).

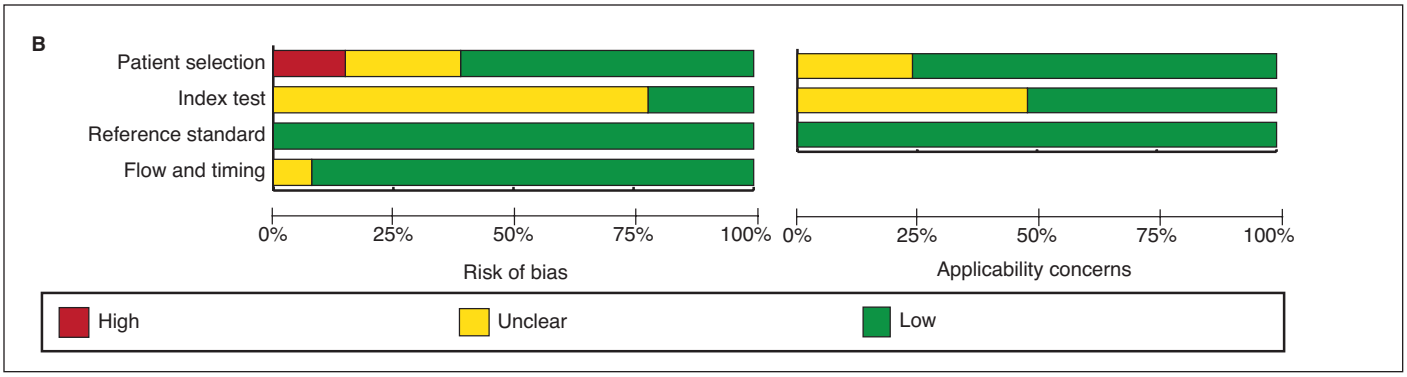

Figure 2. Risk of bias assessment **(A)** within studies, and **(B)** across studies.

almost one-third of symptomatic patients have negative test results.[1,2,4]

Even if urine culture remains the benchmark, there are several disadvantages, as it carries essential contamination rates and high thresholds, which may miss relevant infections.[9]

Since no etiology for LUTS is found in patients with standard negative urine cultures, treatment of such patients is targeted at symptom management based on classifications as syndromic entities and diagnoses of exclusion (such as overactive bladder and interstitial cystitis/bladder pain syndrome) according to their primary complaint. This type of treatment strategy could affect patients' quality of life due to its low complete recovery rate.[1]

Additionally, the time needed to obtain a definitive result in traditional culture tests is usually ≥24 hours, so prolonged turnaround times obligate the physician to initiate aggressive empiric antibiotic before (or with no) pathogen identification. Each hour of delay in antimicrobial administration in patients with septic shock is associated with a mean 8% decrease in survival rate.[20]

The most significant benefit of molecular diagnostic techniques is faster pathogen identification, which allows an earlier selective antimicrobial therapy;[8] however, PCR methods limit microorganism detection because pathogens that are not included in the settings panel will be restricted.

Discussion

Summary of the primary outcomes

Both multiplex PCR and reverse transcription (RT)-PCR showed high overall sensitivity, specificity, and DOR.

Contrast with literature

At present, urine culture continues to be the gold standard for the diagnosis of UTI; however, although specificity is relatively high, sensitivity remains a pitfall since

Furthermore, PCR also detects important or dead pathogens, as well as DNA fragments from degraded pathogens, unlike urine cultures, which exclusively detect viable and reproductive organisms.[8] Interpretation of positive PCR findings in the absence of clinical UTI signs is unclear, but it might suggest a passed or subclinical infection.[8]

In the context of increasing clinical symptoms, ongoing indisposition, and risk of ascending infections or urosepsis, initially choosing an inappropriate antimicrobial contributes to certain levels of morbidity and mortality if UTI is undiagnosed or untreated.[3,4] In addition, the problem of increased resistance reduces the chance of efficient prophylaxis and treatment.[9]

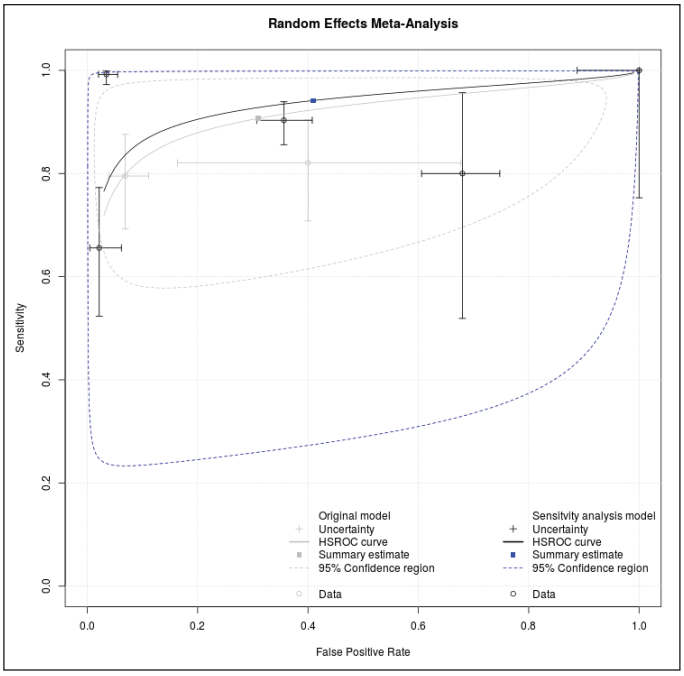

Figure 3A. Receiver operating characteristic (ROC) curve for diagnosis of urinary tract infection with reverse transcription polymerase chain reaction (RT-PCR).

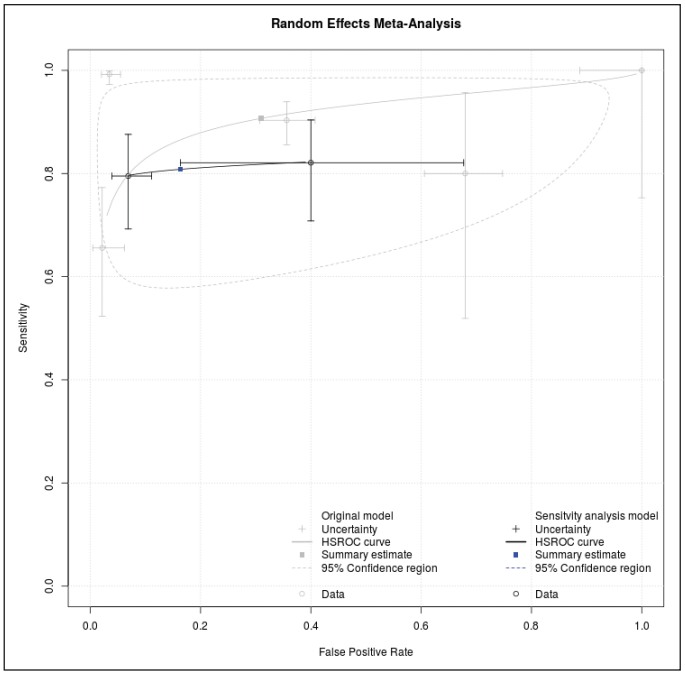

Figure 3B. Receiver operating characteristic (ROC) curve for diagnosis of urinary tract infection with multiplex polymerase chain reaction (PCR).

A significant limiting factor of molecular techniques is that microbiological cultures are still the only way to obtain an antibiogram with sensitivity tests necessary for ongoing treatment.[8,20,21] Therefore antibiotic therapy triggered by PCR results alone might be insufficient due to undetected antibiotic resistance.[8,20]

The investigation has led to the search for innovative methods of identifying uropathogens, aiming to improve diagnostic performance that will impact the understanding of the physiopathology and control of intracellular bacterial communities.

Screening tests should be easy to use, cost-effective, and amenable to point-of-care testing. They must deliver pathogen-positive or pathogen-negative results in minutes and antimicrobial susceptibility testing within a few hours of sample collection. This efficiency would obviate the initiation of empirical antibiotics without pathogens and facilitate pathogen-specific antibiotic selection.

Even if the PCR methods cannot yet replace the traditional microbiological urine culture, PCR detection of resistant genes as surrogate parameters for antibiotic resistance is achievable.[8] It can then supplement urine culture and reduce the time needed to decrease clinical symptoms and resistant pathogens.[8,20,21]

The greatest obstacle to implementing PCR technology into clinical practice is financial feasibility. Although costs associated with PCR testing are significantly higher than current diagnostic methods, savings could result from a faster and more accurate diagnosis, including decreasing the length of hospitalization and preserving hospital resources.[15,21]

To date, there are no systematic reviews nor meta-analyses on the use of molecular diagnostic techniques in patients with suspected UTI with which to compare our results.

Table 1. Diagnosis of UTI with RT-PCR

	RT-PCR			Multiplex PCR		
Parameter	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
Sensitivity	0.941	0.731	0.990	0.808	0.734	0.866
Specificity	0.591	0.063	0.969	0.837	0.527	0.959
False positive rate	0.409	0.031	0.937	0.163	0.041	0.473
Diagnostic odds ratio	23 222	1152	467 969	21 625	4888	95 680
Likelihood ratio positive	2300	0.387	13 661	4957	1403	17 515
Likelihood ratio negative	0.099	0.017	0.562	0.229	0.158	0.333

RT-PCR: reverse transcription polymerase chain reaction; UTI: urinary tract infection.

Strengths and limitations

One of the significant limitations of our analysis was the unclear risk of bias regarding the index tests. The FP rate is another important limitation of molecular diagnostic techniques. The test can be repeated to overcome the FP issue. In fact, in some cases, a test is performed three times, and the patient is declared positive only if two out of the three tests are positive. Accordingly, we might not consider these molecular tests confirmatory but a screening test.

Although promising, molecular diagnostic techniques cannot replace standard diagnostic methods. Currently, they serve as adjuvants to traditional urine cultures, considering the scarce published literature. Consequently, our results must be interpreted cautiously.

Conclusions

Multiplex PCR and RT-PCR are molecular techniques comparable to standard urine culture for the diagnosis of UTI. Refinement of these new diagnostic tools will avoid unnecessary antimicrobial therapy and development of drug-resistant pathogens. It may also improve our ability to identify patients at risk so as to prevent or minimize sequelae derived from infection.

Competing interests: The authors do not report any competing personal or financial interests related to this work.

This paper has been peer-reviewed.

References

1. Scott VCS, Haake DA, Churchill BM, et al. Intracellular bacterial communities: A potential etiology for chronic lower urinary tract symptoms. *Urology* 2015;86:425-31. https://doi.org/10.1016/j.urology.2015.04.002
2. Heytens S, De Sutter A, Coorevits L, et al. Women with symptoms of a urinary tract infection but a negative urine culture: PCR-based quantification of *Escherichia coli* suggests infection in most cases. *Clin Microbiol Infect* 2017;23:647-52. https://doi.org/10.1016/j.cmi.2017.04.004
3. Kimberly L. Cooper MD GMBM y MPRM. Infections of the urinary tract. *Campbell-Walsh-Wein Urology*. Twelfth Ed. Elsevier Inc. 2021. p. 1129-1201.e14.
4. Davenport M, Mach KE, Shortliffe LMD, et al. New and developing diagnostic technologies for urinary tract infections. *Nat Rev Urol* 2017;14:298-310. https://doi.org/10.1038/nrurol.2017.20
5. Kurkela S, Brown DWG. Molecular diagnostic techniques. *Medicine* 2009;37:535-40. https://doi.org/10.1016/j.mpmed.2009.07.012
6. Hooton TM, Roberts PL, Cox ME, et al. Voided midstream urine culture and acute cystitis in premenopausal women. *N Engl J Med* 2013;369:1883-91. https://doi.org/10.1056/NEJMoa1302186
7. Khandelwal P, Abraham SN, Apodaca G. Cell biology and physiology of the uroepithelium. *Am J Physiol Ren Physiol* 2009;297:F1477-501. https://doi.org/10.1152/ajprenal.00327.2009
8. Lehmann LE, Hauser S, Malinka T, et al. Rapid qualitative urinary tract infection pathogen identification by Septifast® real-time PCR. *PLoS One* 2011;6:1-7. https://doi.org/10.1371/journal.pone.0017146
9. Mcdonald M, Kameh D, Johnson ME, et al. A head-to-head comparative phase 2 study of standard urine culture and sensitivity vs. DNA next-generation infections. *Rev Urol* 2017;19:213-20. https://doi.org/10.3909/riu0780
10. DeMarco ML, Burnham CAD. Diafiltration MALDI-TOF mass spectrometry method for culture-independent detection and identification of pathogens directly from urine specimens. *Am J Clin Pathol* 2014;141:204-12. https://doi.org/10.1309/AJCPQYW3B6JLKILC
11. Ishihara T, Watanabe N, Inoue S, et al. Usefulness of next-generation DNA sequencing for the diagnosis of urinary tract infection. *Drug Discov Ther* 2020;14:42-9. https://doi.org/10.5582/ddt.2020.01000
12. Wojno KJ, Baunoch D, Luke N, et al. Multiplex PCR based urinary tract infection (UTI) analysis compared to traditional urine culture in identifying significant pathogens in symptomatic patients. *Urology* 2020;136:119-26. https://doi.org/10.1016/j.urology.2019.10.018
13. Guzmán-Puche J, Gracia-Ahufinger I, Causse M, et al. Combination of Coral UTI Screen™ system, gram-stain and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for diagnosis of urinary tract infections directly from urine samples. *J Chemother* 2019;31:74-80. https://doi.org/10.1080/1120009X.2019.1573284
14. Raja B, Goux HJ, Marapadaga A, et al. Development of a panel of recombinase polymerase amplification assays for detection of common bacterial urinary tract infection pathogens. *J Appl Microbiol* 2017;123:544-55. https://doi.org/10.1111/jam.13493
15. Felt JR, Yurkovich C, Garshott DM, et al. The utility of real-time quantitative polymerase chain reaction genotype detection in the diagnosis of urinary tract infections in children. *Clin Pediatr (Phila)* 2017;56:912-9. https://doi.org/10.1177/0009922817706144
16. Montgomery S, Roman K, Ngyuen L, et al. Prospective evaluation of light scatter technology paired with matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid diagnosis of urinary tract infections. *J Clin Microbiol* 2017;55:1802-11. https://doi.org/10.1128/JCM.00027-17
17. Tchesnokova V, Avagyan H, Rechkina E, et al. Bacterial clonal diagnostics as a tool for evidence-based empiric antibiotic selection. *PLoS One* 2017;12:1-15. https://doi.org/10.1371/journal.pone.0174132
18. Chapelet G, Corvec S, Montassier E, et al. Rapid detection of amoxicillin-susceptible Escherichia coli in fresh uncultured urine: A new tool to limit the use of broad-spectrum empirical therapy of community-acquired pyelonephritis. *Int J Antimicrob Agents* 2016;47:486-9. https://doi.org/10.1016/j.ijantimicag.2016.04.012
19. Burillo A, Rodríguez-Sánchez B, Ramiro A, et al. Gram-stain plus MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) for a rapid diagnosis of urinary tract infection. *PLoS One* 2014;9:e86915. https://doi.org/10.1371/journal.pone.0086915
20. Lehmann LE, Hauser S, Malinka T, et al. Real-time polymerase chain-reaction detection of pathogens is feasible to supplement the diagnostic sequence for urinary tract infections. *BJU Int* 2010;106:114-20. https://doi.org/10.1111/j.1464-410X.2009.09017.x
21. Cybulski Z, Schmidt K, Grabiec A, et al. Usability application of multiplex polymerase chain reaction in the diagnosis of microorganisms isolated from urine of patients treated in cancer hospital. *Radiol Oncol* 2013;47:296-303. https://doi.org/10.2478/raon-2013-0044

Correspondence: Dr. Herney Andrés García-Perdomo, UROGIV Research Group, School of Medicine, Universidad del Valle, Cali, Colombia; herney.garcia@correounivalle.edu.co