Clinica Chimica Acta 484 (2018) 171–178

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Brief reports

Evaluation of the new Sysmex UF-5000 fluorescence flow cytometry analyser for ruling out bacterial urinary tract infection and for prediction of Gram negative bacteria in urine cultures

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ARTICLE INFO

Keywords:
Bacteriuria screening
Flow cytometer
UF-5000
Urinalysis
Urinary tract infection (UTI)
Urine culture

ABSTRACT

Background: We evaluated the new flow cytometer UF-5000 with a blue semiconductant laser as a screening tool for ruling out urine samples negative for UTI and its ability to predict Gram negatives in culture.

Methods: Flow cytometry and microbiological analysis were performed on 2719 urine samples, sent to our microbiology laboratory with a request for urine culture.

Results: UF-5000 showed a very good performance in the screening process. Carryover and cross-contamination was negligible. 797 samples were culture positive at a cut-off of $\geq 10^5$CFU/mL. ROC curve analysis for BACT count demonstrated AUC between 0.973, on 2714 samples, 0.959, on 1516 female samples, and 0.988 on 1198 male samples, respectively. At the cut-off of BACT ≥58/μL AND/OR YLC ≥150/μL, SE was 99.4%, SP 78.2%, PPV 65.4% and NPV 99.7%; false negatives were 0.6%, avoiding unnecessary cultures in 55.5% of specimens. "Gram Neg?" flag predicted Gram negatives in culture with a SE of 81.6% and SP of 93.3%.

Conclusion: The new Sysmex UF-5000 showed high diagnostic accuracy in UTI-screening with a very low rate of false negatives. The instrument is capable of predicting Gram negatives with a good SE and a high agreement with the culture, even if this performance needs further evaluation.

1. Introduction

It is well known that urinary tract infections (UTIs) are among the most frequent infections in both hospitalized and outpatients. As a result, urine specimens constitute a significant proportion of routine microbiology laboratory workload. A diverse spectrum of pathogens, Gram negatives, Gram positives and yeasts, with high predominance of members of the family *Enterobacteriaceae*, in particular *Escherichia coli*, are responsible for these infections [1]. Therefore their antibiotic treatment should be as targeted as possible to ensure optimal treatment, prevention of resistances and, last but not least, cost efficacy [2]. Traditional culture remains the "gold standard" for the diagnostic evaluation of patients suspected of having a UTI: it allows to identify the etiological agents, to estimate the concentration of isolated microorganisms and to offer susceptibility testing for targeting the optimal antibiotic therapy [3]. However this method is laborious, time-consuming and expensive. Moreover, up to 80% of urine samples submitted to the laboratory turn out to be negative.

As a prompt laboratory diagnosis is not available, clinicians usually initiate empirical antibiotic treatments without supportive laboratory evidence, which leades to antibiotics over-prescription and increased risks for resistencies.

During the last 20 years fully automated instruments for particle urinalysis, including bacteria, leucocyte, yeast, erythrocyte and epithelial cell counting have been developed to rule out negative samples before processing them in culture with high efficiency in specimens handling, thus avoiding unnecessary culture tests and saving costs for patients and laboratories. Rapid screening for UTI also helps to reduce the turnaround time (TAT) and negative results can be reported at the day of sample collection [4–7]. Furthermore, the clinical decision could take advantage from the rapid prediction of the type of microorganism before the culture results are available, thus allowing to go for a more specific antibiotic treatment.

Many studies and a recent meta-analysis showed that the

Abbreviations: AUC, area under the curve; BACT, bacteria; B_FSC, bacteria forward scatter; B_FLH, bacteria fluorescent light intensity; Ch, analytical channel; CFU, colony-forming unit; CI, confidence interval; CHRO, CHROMagar™ Orientation; CNA, colistin-nalidixic acid; LIS, laboratory information system; NPV, negative predictive value; PPV, positive predictive value; RBC, red blood cell; ROC, Receiver Operating Characteristic; SE, sensitivity; SP, specificity; SFL, side fluorescence light; SSC, side scatter light; TAT, turn around time; UTI, urinary tract infection; WBC, white blood cell; YLC, yeast-like cell

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https://doi.org/10.1016/j.cca.2018.05.047
Received 15 March 2018; Received in revised form 18 May 2018; Accepted 23 May 2018
Available online 24 May 2018
0009-8981/

parameters bacteria and WBCs could be detected with high sensitivity (SE) by automated urine sediment analyzers and that both are good measures for UTI screening [8]. Among these analyzers, flow cytometers of the UF-Series are widespread in many clinical microbiology laboratories as they have proved performances in line with the EUG guidelines. With the UF-1000i, the second generation of UF-analysers by Sysmex, the analytical quality has been improved due to a dedicated analytical channel for bacteria [9–16].

Having this in mind, we aimed to verify the performance of the new UF-5000 fluorescence flow cytometry analyser as a method to screen out urinary tract infection, also evaluating the ability of the newly introduced Bact Info flag to differentiate Gram negative bacteria in UTI-samples.

2. Materials and methods

2.1. Study protocol and samples

This study was undertaken to evaluate the analytical performance and the diagnostic accuracy to screen out for UTI of the new fully automated UF-5000 fluorescence flow cytometry analyser, in comparison with quantitative standard culture, and to verify the instrumental ability of the new Bact Info flag to differentiate Gram negative bacteria in UTI suspected samples.

The study, conducted according to the Declaration of Helsinki and deemed exempt from the Regional Friuli-Venezia Giulia Ethics Committee was executed from 06 April to 12 June 2017.

During this period 2719 midstream urine samples were evaluated from 1198 (44.1%) male and 1521 (55.9%) female, aged from 0 and 97 years (median = 66y), submitted to our microbiology laboratory with a specific request for urine culture. Inpatients were 23.9% and outpatients 76.1%. Written and/or oral instructions were provided to collect the specimens in a sterile leakproof container (60 mL) fully equipped for sampling by preservative-free vacuum PET tubes (Vacutest Kima, Arzergrande PD, Italy). Immediately after sample collection, the tubes were filled through the straw of the container and, if analysis was delayed by > 1 h, stored and transported at controlled temperature of 4–8 °C to the laboratory.

Specimens were in the first line excluded from the study because the available sample volume was of < 5 mL or the sample was positive for exclusion criteria as indicated by the manufacturer (abundant mucus, high turbidity, macroscopic pyuria and hematuria) to prevent both instrumental failures and interferences during the measurement. All specimens passing the criteria were analyzed with the Sysmex UF-5000 in accordance with the manufacturer's recommendations immediately after inoculation of the culture at the same day of collection and within one hour after the arrival in the laboratory. None of the specimens analyzed were excluded from evaluation.

Gram staining of centrifuged specimens was conducted in case of discrepancies between the results of the UF-5000 Bact Info flag and the culture method. The samples were stored at 4–8 °C until culture analysis and Gram staining procedure was performed and both results were available.

2.2. Sysmex UF-5000

The UF-5000, the third generation of fully automated flowcytometry analysers for the particle analysis in urine, has recently been lauched by Sysmex Corporation (Kobe, Japan). The analyser can discriminate and count 17 diagnostic parameters of cells and formed elements in urine and offers an integrated body fluid mode (BF), available on the instrument with a switch, that can classify and count seven diagnostic parameters. The system employs fluorescence flow cytometry technology, using a new blue semi-conductor laser at 488 nm wavelength, hydrodynamic focusing in two different analysis chambers, surface (SFch) and core (CRch). Particles are stained by specific fluorochromes for nucleic acids and for surface structures, then sent through the laser beam. Counting and classification is based on signals of forward scattered light (FSC), side scattered light (SSC), side fluorescent light (SFL) and the new, additional depolarized side scattered light (DSS). The pattern of individual light signals is translated by specific algorithms into individual 'fingerprints' allowing counting, identification and classification into the particle categories.

Compared to the previous cytometers of the UF-Series, technological innovations aimed to improve the SE and the specificity (SP) for some elements of urinary sediment, particularly for the determination of bacteria. Further on the performance for yeast detection seems to be better in UF-5000 [17]. The light signals of FSC, SFL and SSH differ for Gram negatives and Gram positives due to a different dye intake by the cell wall structures. On this basis UF-5000 provides information on the Gram morphology of bacteria displayed as the Bact Info flag. The "Gram Neg?" flag can be considered particularly interesting for rapid identification of Gram negative microorganisms causative for UTI.

The Sysmex UF-5000 has a maximum theoretical throughput declared of 105 samples/h, requiring a minimum volume of 2.0 mL of uncentrifuged, native urine sample in an automated mode, or 0.6 mL in a STAT mode available for both urine and BF mode. In automatic and STAT mode, the aspiration volume is 0.45 mL for both urine and BF.

2.3. Microbiological analysis

On all the samples, a standard quantitative urine culture was performed inoculating 1-μL of well-mixed urine specimen by a calibrated loop both on a nonselective chromogenic agar plate (CHRO CHROMagar Orientation, Kima Arzergrande, PD, Italy) supporting the growth of UTI pathogens and on a selective colistin-nalidixic acid agar plate with 5% sheep blood (CNA Columbia agar, Kima Arzergrande, PD, Italy). CHRO was used as a quantitative reference; CNA enables isolation and preliminary identification of Gram positive bacteria and allows to discriminate contaminants from uropathogenic species easier. The plates were incubated aerobically at 35–37 °C for 18–24 h and examined for significant bacteriuria. The results were expressed as the number of colony forming unit per milliliter (CFU/mL). For the purposes of this study, cultures that presented microorganisms usually causative of UTI with growth of 10^5 CFU/mL or more were considered positive and these microorganisms were identified by using an automated instrument (Vitek 2; bioMérieux) or by conventional biochemical methods which are used in our laboratory. If more than two organisms were grown in culture, although UTI is unlikely in these patients, the specimen was considered positive and reported as "mixed flora". In these samples bacteria were not subjected to the identification procedure. A specimen was considered negative if there was no growth or there was $< 10^5$/mL bacterial growth, interpreted as insignificant growth.

2.4. Carryover analysis

Rinsing steps between samples were used in all analyses. In this mode carryover evaluation for bacteria and WBCs was performed by measuring specimens with high values of the two parameters (BACT mean value = 99,359/μL; WBC mean value = 1805/μL) in triplicate, followed by a triplicate of specimens with very low values (blank). This serie was consecutively analyzed three times. The carryover was determined by the formula: Carryover = (blank 1–blank 3)/(high 3–blank 3) for all three runs and mean values were calculated for each parameter. Cross-contamination, ***i.e.*** transfer of cells or particles from one sample tube to the following one, was also evaluated. Four racks were prepared, each one containing a positive sample with high bacteria count $\geq 10^6$ CFU/mL followed by three aliquots of a low bacteria count (negative) sample.

After the racks were run on the analyser, all the tubes were cultured in order to observe if there was a cross-contamination in the negative