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ORIGINAL ARTICLE

Clinical Usefulness of BACT Count and BACT-Info Flag of UF-5000 for Screening for Urinary Tract Infection and Prediction of Gram-Negative Bacteria

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SUMMARY

Background: **A rapid and reliable screening test for urinary tract infection (UTI) is needed to reduce the turnaround time and to rule out negative results of urine culture. The aim of this study was to evaluate the performance of BACT count and BACT-Info flag of the UF-5000 for screening for UTI.**
Methods: **A total of 1,063 urine specimens from April to September 2019 were included in this study. We evaluated the diagnostic performance of white blood cell (WBC) count, BACT count, BACT-Info flag, and UTI flag in UF-5000 by comparing with the urine culture results.**
Results: **Of the urine specimens, 16.7% were culture-positive ($\geq 10^5$ CFU/mL) with 15 being yeast positive. A BACT count of > 685.3/µL showed the best diagnostic performance with 93.8% sensitivity and 90.2% specificity. We confirmed that the combination of BACT count (685.3/µL) and BACT-Info flag would be appropriate to use in a clinical laboratory (sensitivity 91.5%, specificity 90.5%). Based on this combination, the sensitivity and specificity of the Gram-negative flag were 95.5% and 94.8%.**
Conclusions: **We recommend the use of a combination of BACT count (685.3/µL) and BACT-Info for UTI diagnosis. This combination is more appropriate for Gram-negative bacteria, and it would be useful for selecting empirical treatment.**
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KEYWORDS

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INTRODUCTION

Urinary tract infection (UTI) is one of the most frequent bacterial infections in hospitalized and community patients [1,2]. Most infections occur in the lower urinary tract, namely the bladder and urethra, but they can spread to the kidneys, which has poor outcomes. A UTI is more frequent in women, and many suffer from recurrent infections after the initial UTI [3]. Many pathogens, including Gram-positive, Gram-negative, and yeast, can cause UTI, of which *Escherichia coli* is the most common [4,5]. An accurate diagnosis of UTI is important for optimal analysis because it can support empirical treatment.

Urine culture is the gold standard for UTI diagnosis. However, this is time consuming and laborious [6]. In addition, most urine cultures yield negative results [7,8]. Thus, there is a need for a rapid and reliable screening test for UTI to reduce the turnaround time and to rule out negative results of urine culture.

An automated urine sediment analyzer including UF-1000i (Sysmex, Kobe, Japan) and Cobas u701 (Roche, Rotkreuz, Switzerland) is commonly used in clinical laboratories and shows good performance for UTI screening [8-12]. Recently, the UF-5000 based on fluorescence flow cytometry has improved with diverse algorithms for bacterial discrimination.

The aims of this study were to evaluate the performance of the UF-5000 including the BACT count and BACT-Info flag for screening for UTI and prediction of Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

A total of 1,063 urine specimens submitted for culture from April to September 2019 were included in this study. For urine culture, 1 µL of well-mixed urine was inoculated quantitatively onto an agar plate and incubated at 37°C for 18 to 24 hours. The growth of pathogens was recorded as the number of colony-forming units per milliliter (CFU/mL). A bacterial count of $\geq 10^5$ CFU/mL was considered proof of a UTI, and $< 10^5$ CFU/mL or no growth was considered a negative result. If two or more pathogens were grown as a total count of $\geq 10^5$ CFU/mL, it was reported as “multiple organisms”. Final identification was done using the VITEK® MS (bio Merieux, Marcy l'Etoile, France).

The urine particle count was analyzed using the residual specimen with the UF-5000 (Sysmex Corporation, Kobe, Japan) within 1 hour after culture. The UF-5000 was operated according to the manufacturer’s instructions. It does not need any preparation step.

The UF-5000 is based on fluorescence flow cytometry and can discriminate 17 reportable parameters in urine. It provides new information of Gram staining based on the difference in the dye intake by light signals of FSC, SFL, and SSH. This information is expressed on BACT-Info flags including Gram-positive, Gram-negative, Gram-pos/neg, and unclassified. Additionally, a new flag of UTI information is reported when both the WBC and BACT counts are > 10/µL.

We focused on two parameters (BACT count and WBC count) and two flags (BACT-Info and UTI). We analyzed the sensitivity and specificity of BACT count and WBC count at different cutoff values. In addition, we evaluated the diagnostic performance of the BACT-Info and UTI flags by comparing them with the urine culture results.

RESULTS

Urine culture

Of the total 1,063 urine specimens, 16.7% (n = 177) were bacterial culture-positive except for 15 that were yeast positive (Table 1). Multiple organisms were observed in three specimens. The most common pathogen was *Escherichia coli* (51.4%, n = 91), followed by *Enterococcus faecium* (12.4%, n = 22), *Pseudomonas aeruginosa* (6.8%, n = 12), *Enterococcus faecalis* (5.6%, n = 10), *Klebsiella pneumoniae* (5.6%, n = 10), and others (18.1%, n = 32).

Comparison of BACT-Info and UTI flags with urine culture

Of the total of 1,063 urine specimens, the BACT-Info flag was positive for 384. The concordance rate between culture and the BACT-Info flag was 78.8% (Table 2). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the BACT-Info flag were 94.9%, 75.6%, 43.8%, and 98.7%, respectively. Nine false-negative results of BACT-Info flag were seven *Enterococcus* species (BACT count 306.3 - 30,556.4, median 1,849.3), one *P. aeruginosa* (BACT count 92.1), and one *K. pneumoniae* (BACT count 14.9).

We compared the BACT Info flag with Gram staining of the culture (Table 3). Of 45 Gram-positive bacteria in the urine culture, the numbers of Gram-positive, Gram-negative, Gram-pos/neg, and negative of the BACT-Info flag were 34, 2, 2, and 7 specimens, respectively. For 129 Gram-negative bacteria, 92 samples were Gram-negative in the BACT-Info flag; however, 2, 33, and 2 were Gram-positive, Gram-pos/neg, and negative, respectively. Among 886 culture-negative results, 216 showed false-positive results, including 147 Gram-positive, 38 Gram-negative, and 31 Gram-pos/neg in the BACT-Info flag. Among 68 Gram-pos/neg results in the BACT-Info flag, only two specimens were real multiple organisms, whereas 33 and 31 were Gram-negative and Gram-negative in urine culture. In addition, 22 of 33 Gram-negative organisms were *E. coli*. Two of the three samples showing multiple organisms in urine culture revealed concordant results of BACT-Info flag as Gram-pos/neg; however, the other specimen was Gram-negative in the BACT-Info flag. We calculated the diagnostic sensitivity and specificity of BACT-Info flag based

Table 1. Microorganisms from 177 culture-positive samples.

Microorganism (≥ 10^5 CFU/mL)	No.	%
Escherichia coli	91	51.4
Enterococcus faecium	22	12.4
Pseudomonas aeruginosa	12	6.8
Enterococcus faecalis	10	5.6
Klebsiella pneumoniae	10	5.6
Acinetobacter baumannii complex	6	3.4
Corynebacterium striatum	6	3.4
Enterobacter cloacae	4	2.3
Staphylococcus aureus	2	1.1
Citrobacter koseri	1	0.6
Klebsiella species	1	0.6
Pasteurella bettyae	1	0.6
Proteus mirabilis	1	0.6
Salmonella species	1	0.6
Serratia marcescens	1	0.6
Staphylococcus epidermidis	1	0.6
Staphylococcus haemolyticus	1	0.6
Staphylococcus warneri	1	0.6
Streptococcus anginosus	1	0.6
Streptococcus mitis(oralis)	1	0.6
Enterococcus faecium/Pseudomonas aeruginosa	3	1.7

Table 2. Overall concordance between urine culture and BACT-Info flag of UF-5000.

BACT-Info flag	Culture with growth of ≥ 10^5 CFU/mL		
	Positive	Negative	Total
Positive	168	216	384
Negative	9	670	679
Total	177	886	1,063

on Gram staining of the culture results. The sensitivity and specificity of the Gram-negative flag were 97.0% and 92.2%, whereas the sensitivity and specificity of the Gram-positive flag were 79.2% and 79.0%.

The UTI flag was not effective for UTI diagnosis because the concordance rate with culture was only 58.2%. The sensitivity, specificity, PPV, and NPV of the UTI flag were 92.7%, 51.4%, 27.6%, and 97.2%, respectively. We tried to establish UTI criteria by determining the best cutoff values of BACT and WBC counts (Figure 1, Table 4). The optimal cutoff values of BACT and WBC counts were > 685.3/µL (area under the curve [AUC] = 0.968) and > 40.7/µL (AUC = 0.826), respectively.

Optimal conditions for UTI diagnosis using UF-5000

We tried to determine the optimal conditions for UTI diagnosis using two parameters (WBC count and BACT count) and one flag (BACT-Info) (Table 5). Among these, a BACT count of > 685.3/µL showed the best performance for UTI diagnosis with 93.8% sensitivity and 90.2% specificity.

We confirmed that the combination of BACT count (685.3/µL) and BACT-Info flag would be appropriate to use in a clinical laboratory because this provides Gram-staining information without a decrease of diagnostic performance. The sensitivity and specificity of this combination were 91.5% and 90.5%, respectively. Based on this combination, the sensitivity and specificity of the Gram-negative flag were 95.5% and 94.8% even though those of the Gram-positive flag were 72.9% and 90.1%.

DISCUSSION

Many urine cultures are requested in clinical laboratories to rule out UTI, and a substantial portion of them show negative results. Even though a few urine tests such as dipsticks or manual microscopic assays are used for UTI screening, they lack sensitivity and produce ambiguous results [13,14].

Recently, several automated urine analyzers that are image-based or use flow cytometry have been widely used to screen rapidly to rule out UTI and to reduce the number of urine cultures [15-17]. The Sysmex UF series based on flow cytometric analysis allows automated counting and classifies cellular particles stained with fluorochromes using a laser beam. There are many reports about the usefulness of the UF-100 that previously launched for screening of UTI [7,18]. However, it has been concluded that the UF-100 system was not useful to screen for UTI because of its low sensitivity [19]. The UF-1000i, a third-generation cytometer system, showed good performance with high sensitivity [11,20]. Kadkhoda et al. [11] confirmed that the sensitivity for Gram-negative and Gram-positive organisms were 99.2% and 85.0%, respectively. Pieretii et al. [9] reported that the UF-1000i is acceptable as a screening test for UTI, and it could reduce the number of bacterial cultures by 43%. However, this analyzer cannot discriminate between Gram-negative and Gram-positive organisms, which is important in prescribing antibiotics in the face of UTI-positive specimens.

In this study, we evaluated the clinical performance of UF-5000, a new fully automated urine analyzer for screening for UTI. This device improved the bacterial count compared with the previous UF-1000i system and offers new flags of UTI and BACT-Info. We confirmed that the sensitivity and specificity of the BACT-Info

Table 3. Comparison BACT-Info flag of UF-5000 with Gram staining of cultures.

BACT-Info flag	Urine culture results (n)				
	Gram-positive	Gram-negative	Multiple *	Negative	Total
Gram-positive	34	2		147	183
Gram-negative	2	92	1	38	133
Gram-pos/neg	2	33	2	31	68
Negative	7	2		670	679
Total	45	129	3	886	1,063

*** Multiple - Multiple organisms including Gram-positive and Gram-negative bacteria.**

Table 4. Diagnostic performance of BACT count and WBC count of UF-5000 at different cutoff values.

UF-5000 parameters		Cutoff value (units/μL)				
BACT count	> 685.3	> 100	> 500	> 1,000	> 5,000	> 10,000
Sensitivity (%)	93.8	98.9	94.4	89.8	75.1	44.1
Specificity (%)	90.2	69.5	89.3	92.8	97.4	98.4
WBC count	> 40.7	> 10	> 50	> 100	> 500	> 1,000
Sensitivity (%)	83.6	92.7	79.1	65.5	27.1	19.8
Specificity (%)	72.9	49.7	75.4	82.5	93.3	97.0

Table 5. Diagnostic performance of WBC count, BACT count, BACT-Info flag, and their combinations.

UF-5000	Growth of ≥ 10^5 CFU/mL (n = 1,063)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance (%)
One parameter only					
WBC count 10	92.7	49.7	26.9	97.1	56.8
WBC count 40.7	83.6	72.9	38.1	95.7	74.7
BACT count 10	100.0	22.2	20.4	100.0	35.2
BACT count 100	98.9	69.5	39.3	99.7	74.4
BACT count 685.3	93.8	90.2	65.6	98.6	90.8
BACT-Info flag	94.9	75.6	43.8	98.7	78.8
WBC count and BACT count					
WBC 10 and BACT 10	92.7	51.4	27.6	97.2	58.2
WBC 10 and BACT 100	92.7	75.6	43.2	98.1	78.5
WBC 10 and BACT 685.3	89.8	91.4	67.7	91.4	91.2
WBC count and BACT-Info flag					
WBC 10 and BACT -Info	91.0	81.0	48.9	97.8	82.7
WBC 40.7 and BACT-Info	82.5	85.7	53.5	96.1	85.1
WBC count or BACT-Info flag					
WBC 10 or BACT-Info	96.6	44.2	25.7	98.5	53.0
WBC 40.7 or BACT-Info	96.1	62.9	34.1	98.8	68.4
BACT count and BACT-Info flag					
BACT 100 and BACT-Info	94.9	75.6	43.8	98.7	78.8
BACT 685.3 and BACT-Info	91.5	90.4	65.6	98.2	90.6
BACT count or BACT-Info flag					
BACT 100 or BACT-Info	98.9	69.5	39.3	99.7	74.4
BACT 685.3 or BACT-Info	97.2	75.4	44.1	99.3	79.0

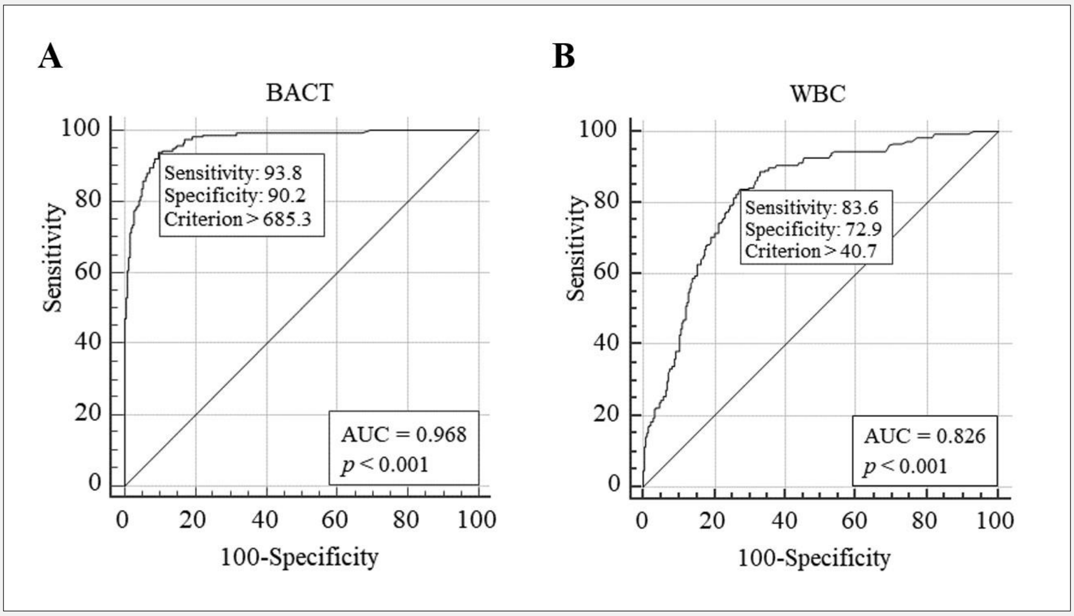

Figure 1. ROC curves of BACT count (A) and WBC count (B) in UF-5000.

flag were 94.9% and 75.6%, respectively. In contrast, the sensitivity and specificity of the UTI flag were lower at 92.7% and 51.4%, respectively. Both flags showed a sufficient sensitivity; however, the specificity was too low to use this device for UTI diagnosis.

There were many differences in BACT-Info flag results according to Gram staining in this study. The Gram-negative flag showed a better sensitivity (97.0%) than the Gram-positive flag (79.2%). This is similar to the previous report by Kim et al. [21], as 91.7% and 81.3% of the sensitivity of Gram-negative and Gram-positive bacteria, respectively. Ren et al. [22] reported that 67.7% of specimens provided the correct results for BACT-Info (the rate of Gram-negative versus Gram-positive strains was 48:15). The concordance rates of “Gram-negative” and “Gram-positive” UF-5000 BACT-Info flags were 62.5% and 59.1%, respectively, in the report of Enko et al. [23]. Consequently, we concluded that the Gram-negative flag is vastly superior to the Gram-positive flag.

We determined the best cutoff value of BACT count to be > 685.3/µL and the diagnostic performance (sensitivity 93.8%, specificity 90.2%) was better than those of WBC count, BACT Info flag, and UTI flag. In the report of Enko et al. [23], the sensitivity (92.1%) and specificity (85.4%) were lower than our results even though the cutoff value is lower at 135/µL. De Rosa et al. [24] determined the optimal BACT count to be 58/µL. Their specificity was low (78.2%), although the sensitivity (99.4%) was higher than ours, which was achieved by lowering the cutoff value. If we adopt the cutoff value of the BACT count as 100/µL, the sensitivity and specificity were 98.9% and 69.5%, respectively. However, we determined that a BACT count of 685.3/µL is the optimal value, showing high sensitivity and specificity in the diagnosis of UTI.

We tried to find the optimal conditions for UTI diagnosis by combining WBC count, BACT count, and BACT-Info. The use of a single parameter, BACT count (685.3/µL), was most valuable for UTI diagnosis under various conditions (Table 5). However, we can get the information of Gram staining when we add the BACT-Info flag without much difference in the diagnostic performance. So, we recommend the use of a combination of BACT count (685.3/µL) and BACT-Info for UTI diagnosis. Moreover, this combination is more appropriate for Gram-negative bacteria with higher sensitivity (95.5%) and specificity (94.8%). It would be useful for selecting empirical treatment.

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Declaration of Interest:
No potential conflicts of interest relevant to this article were reported.

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