Comparisons of the Fully Automated Urine Particle Analyzer (UF-1000*i*) to Quantitative Urine Culture and Nitrite Reaction in Urinary Tract Infection Test

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A flow cytometry-based automated urine analyzer, the UF-1000i is a device that can measure the concentrations of red blood cells (RBC), white blood cells (WBC), epithelial cells (EC), casts and bacteria in non-centrifuged urine samples.
In the present study, the results obtained with the UF-1000i were compared with those obtained by conventional quantitative urine culture and nitrite reaction in urine. In addition, we investigated whether the scattergram pattern of the UF-1000i could distinguish between cocci and bacilli.
The results of the UF-1000i and those of conventional quantitative urine culture correlated well, and sensitivity and specificity for bacteriuria by the UF-1000i were 96.7% and 68.1%, respectively. The positive rate for nitrite reaction in bacteriuria (>10^5/mL)which was measured by the UF-1000i was 12.7%, and the large part of detected species was Escherichia coli .
The concordance rates of the scattergram pattern of the UF-1000i for bacilli and cocci were 94.7% and 82.7%, respectively. In bacteriuria (> 10^5/mL), the scattergram pattern could distinguish between cocci and bacilli.

Key Words Fully Automatic Urine Analyzer, UF-1000*i* , Nitrite, Urinary Tract Infection Test

INTRODUCTION

Available tests for urinary tract infection (UTI) include the qualitative tests of Leukocyte esterase, nitrite, urine sediment analysis, Gram staining, and urine culture. Since 2009, our hospital has been using the Sysmex fully automated urine particle analyzer UF-1000*i* (hereinafter UF-1000*i*) concurrently with microscopy, in order to speed up the testing and save on the labor required for urine sediment analysis., the accuracy of the UF-1000*i* bacterial (BACT) analysis has been improved through specific staining of nucleic acids with polymethine dye in an exclusive BACT channel [1]. The BACT count is obtained by irradiating the stained bacteria with a laser beam, measuring the scattered light and fluorescence, and preparing scattergrams from the signals obtained. Basic studies carried out at our hospital showed that the analyzer gave good results, i.e., a within +/- one rank concordance (concordance with accepting +/- one rank difference) of 96.1% with the results of microscopic examination, and linearity up to a count of 1.0×10^8/mL with a coccus (*Staphylococcus aureus*) and a bacillus (*Escherichia coli*) [2]. In the present study we examined the correlation of the UF-1000*i* results with those of other tests like nitrite , etc. of bacteria in urine, and whether it was possible to differentiate the bacteria into bacilli and cocci form the UF-1000*i* scattergram patterns.

ANALYSIS SYSTEMS AND SPECIMENS

1. Analysis systems and reagents

Urine particle analysis: Fully automated urine particle analyzer UF-1000*i* (Sysmex)
Nitrite (Urine test strip analysis): Aution Max AX-4280, Uriflet S (Arkray)
Gram staining: B&M Wako (Wako Pure Chemical

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Industries)
Bacterial identification: VITEK2 (Sysmex bioMeríuex)

2. Specimens

Urine specimens of 871 patients collected during January 5 to May 31, 2010
Specimens prepared by suspending pure cultures of bacteria in a liquid culture medium (used to prepare typical scattergram patterns)

METHODS AND RESULTS

1. Correlation between BACT counts by UF-1000*i* and bacterial counts in quantitative urine culture

Among the patients' urine specimens, the bacterial counts of 138 specimens analyzed by both UF-1000*i* and quantitative urine culture were compared. The sensitivity and specificity of UF-1000*i* analysis, examined taking a bacterial count ≥ 1.0×10^5/mL as positive for bacteria, were respectively 96.7% and 68.1% (***Table 1***). Compared to quantitative urine culture, the BACT counts obtained by UF-1000*i* were higher in the low count range of ≤ 1.0×10^4/mL and lower in the high count range of ≥ 1.0×10^6/mL.

2. UF-1000*i* BACT count and nitrite by urine test strips

Only specimens with UF-1000*i* BACT count ≥ 1.0×10^5/mL and WBC count ≥ 10/μL were taken as bacteriuria, to eliminate the effect of contamination. 12.7% of the specimens with bacteriuria were positive for the nitrite, and 100% of no bacteriuria specimens were negative for it. The percentage of positive specimens increased proportionately with increase in the BACT count (***Table 2***).

Table 1 *Comparison of the results of quantitative urine culture and UF-1000i BACT counts*

UF-1000*i* (/mL) \ Quantitative urine culture (CFU/mL)	10^2	10^3	10^4	10^5	10^6	10^7	≥ 10^8
≥ 10^8							1
10^7				1	2	7	35
10^6		2	3	5	7	8	15
10^5	1	4	5	3	2	1	1
10^4	5	8	6	1	1	1	
10^3	4	8	1				
10^2							

n = 138

A count ≥ 1.0×10^5/mL was designated as positive for bacteria. Specimens that were true positives in both tests are highlighted in yellow and those that were true negatives in gray.

Table 2 *UF-1000i BACT count and Nitrite (NIT)*

	BACT count	NIT (+)	NIT (-)	NIT positive specimens (%)
Bacteriuria	≥ 10^7	21	28	42.9
	10^6	27	163	14.2
	10^5	19	265	6.7
No bacteriuria	10^4	0	225	0.0
	10^3	0	123	0.0

n = 871

3. Relation between the bacterial species detected and Nitrite

Bacterial identification tests were carried out with some of the specimens examined in Section 2 above and the relationship between the bacterial species detected and positive response in Nitrite was examined. The percentage of specimens found positive in Nitrite was high with bacilli, and among the bacilli the percentage of NIT positives was highest at 70% with *E. coli*. Among the cocci, only *Staphylococcus* species were positive in Nitrite (***Fig. 1***).

4. Differentiation between bacilli and cocci based on UF-1000*i* scattergram patterns

It has been reported that in relation to the shape of the cells, bacilli are plotted in BACT scattergrams along a low angle of approximately 10-20° from the x-axis whereas cocci tend to appear at a higher angle of 30-45°[3]. Therefore, with 127 patients' urine specimens having a BACT count ≥ 1.0 × 10^5/mL, we designated scattergram patterns with an angle ≥ 30° as cocci and < 30° as bacilli, and examined the concordance of the results with those obtained by microbiological identification of the bacteria species. Examples of scattergram patterns of a bacillus (*E. coli*) and a coccus (*S. aureus*) are shown in ***Fig. 2***. The y-axis is the forward-scattered light intensity, which reflects the size of the particles, and the x-axis is the lateral fluorescent light intensity, which represents the level of staining of the particles. Of the 75 specimens that showed distribution patterns with an angle < 30° from the x-axis of the scattergram, 71 (94.7%) yielded bacilli on microbiological identification and 4 (5.3%) yielded cocci (***Table 3***). The cocci were *Enterococcus* species in two specimens, *Streptococcus* species in one, and an unidentified coccus in one. Of the 52 specimens that

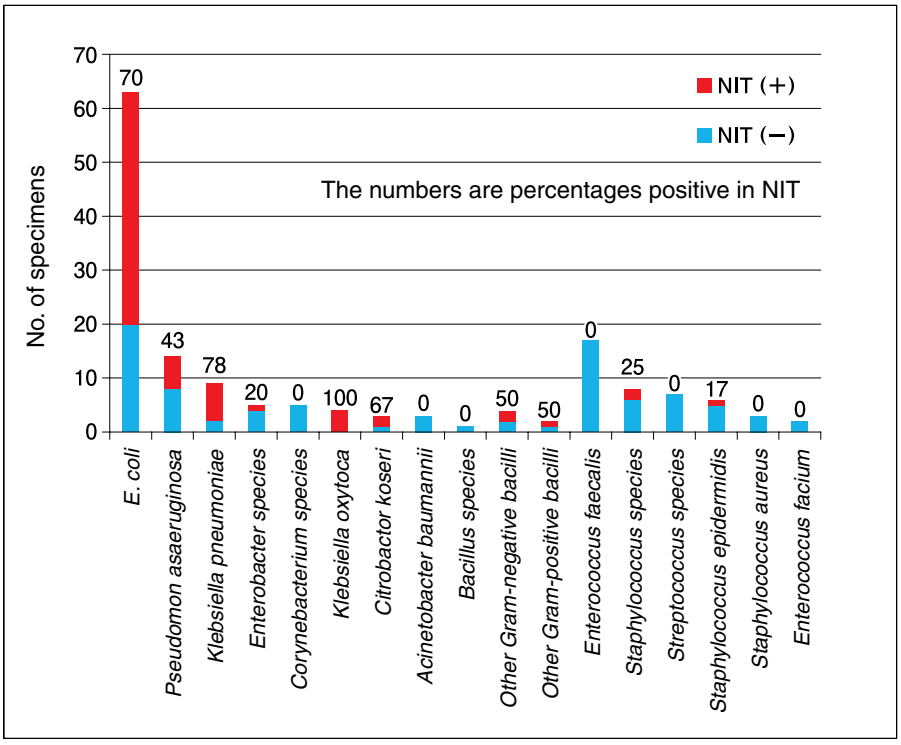
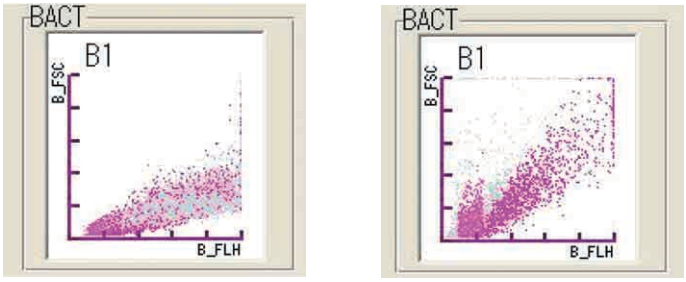


Fig. 1 *Bacterial species detected and Nitrite (NIT) of the specimens*



E. coli *S. aureus*

Fig. 2 *Examples of scattergram patterns*

showed distribution patterns with an angle ≥ 30° from the x-axis, 43 (82.7%) specimens yielded cocci on microbiological identification, and bacilli were found in 9 (17.3%) specimens. Among the specimens yielding bacilli, 7 showed a broad spread of the angle of scattergram distribution that started from a low angle, and 2 of them yielded *Gardenerella vaginalis* (a small bacillus). There were 5 specimens wherein the type of bacteria could not be identified from the scattergrams due to a low BACT count of only approximately 1.0×10^5/mL. Among these 5 specimens, in fact 2 had cocci and 3 had both bacilli and cocci. Rough differentiation into bacillus and coccus from the scattergrams was possible. However, the distribution pattern was variable particularly with *Enterococcus* species due to the variable shape of the bacteria, and some specimens showed a bacillus like pattern. Among the cocci, *Neisseria gonorrhoeae* is said to show a particularly low angle distribution pattern. This coccus showed a low angle distribution pattern of about 20? from the x-axis in the study conducted at our hospital also.

DISCUSSION

BACT analysis can be carried out rapidly by a simple operation of UF-1000*i*. In the range of bacterial counts above of a certain value, the type of bacterium, i.e., whether coccus or bacillus, could be identified from the scattergram pattern. Regarding the correlation between the bacterial counts determined by quantitative urine culture and the BACT count determined by UF-1000*i*, some specimens gave false positive results with UF-1000*i* in the low bacterial count range. However, almost all the specimens that gave such apparently false positive results, compared to the results of urine culture, had revealed bacteria when examined through microscopy. In the present study, we did not consider whether the subjects had been under treatment with antibacterial agents. This result may have been caused as a result of the bacteria not growing well or dying during the quantitative culture due to the effect of antibiotics administered to the patients. The reason for the UF-1000*i* showing relatively low BACT counts in the high bacterial count range could be that bacterial aggregates that passed through the flow cell were counted as single bacteria. This tendency was more pronounced with cocci. In specimens with bacteriuria, the higher the bacterial count the higher was the percentage of nitrite positives. However, the overall percentage of positives was low at 12.7% because of differences between bacterial species in their nitrate reducing capacity [4] and reasons such as the requirement that the urine has to stay in the bladder for at least 4 hours to elicit a positive nitrite [5]. However, those specimens that were positive in the nitrite test were all assessed as having bacteriuria by UF-1000*i*, and there were no false positives. The bacillus/coccus differentiation based on the scattergram patterns was moderately satisfactory. However, such differentiation was difficult with low bacterial count samples for which culture tests, etc. also will have to be done concurrently. When the angle of the scattergram pattern is around 30°, there is the possibility of the simultaneous presence of both bacilli and cocci, and the differentiation is difficult. The results of the present study under Section 3 (Relationship between the bacterial species detected and the nitrite) and Section 4 (Differentiation between bacilli and cocci based on UF-1000*i* scattergram patterns) suggest the presence of bacilli when the nitrite is positive. When it is negative, both bacilli and cocci, such as *Enterococcus*, could be present. Currently, with UF-1000*i*, we have to estimate from visual inspection of the scattergram patterns whether the bacteria present are bacilli or cocci. We hope that one day the machine itself would be able to make this differentiation.

CONCLUSION

In the present study we could rapidly enumerate bacteria and differentiate between bacilli and cocci in urine using UF-1000*i*. Thus, the results indicate the possibility of UF-1000*i* being useful in testing for UTI, together with other tests like the nitrite test, urine sediment analysis and urine culture tests.

Table 3 *Bacillus/coccus differentiation from scattergram patterns*

Scattergram pattern (angular degree)		Bacilli	Cocci
		No. of specimens (Percentage)	
< 30	75 (100)	71 (94.7)	4 (5.3)
> 30	52 (100)	9 (17.3)	43 (82.7)

The type of bacteria could not be identified from the scattergrams in 5 specimens.
Of these, 2 had cocci and 3 had both bacilli and cocci.

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