About the Usefulness of the Urinary Tract Infection Screening Using UX-2000

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The UX-2000 is a fully automated integrated urine analyzer, combining the automated urine cell analyzer and the urine chemistry (urine test strips) analyzer. The UX-2000 quantitative bacterial count, for the rapid assumption of UTI, and bacteria scattergram pattern, for the assumption of the bacterial species were compared to with the microbiological examination reference method.
In the quantitative measurement performance study, it was found that the agreement ratio between UX (cut off = 1.0 × 10^5/mL) and quantitative microbiological examination was 89%. Sensitivity, specificity and agreement of the UX for the diagnostics of UTI were 94%, 85% and 89% respectively.
In the comparison study between the bacteria scattergram pattern and bacteria species, 62% of agreement was obtained between UX and gram positive coccus. In the case of gram negative rods, the agreement was 87% and in mixed rod and coccus samples, the agreement was 73%.
Accordingly, it was suggested that the quantitative bacterial count by the UX could be available as a screening method to determine the necessity for microbiological examination and the scattergram pattern could be available as a rough estimation of the bacterial species.

Key Words UX-2000, Scattergram Pattern, UTI

INTRODUCTION

Urinary tract infection (UTI) is an infection that doctors often come across irrespective of their diagnostic field. Apart from the clinical symptoms and conventional routine urinalysis, identification of the causal microorganisms and their drug susceptibility is crucial for definite diagnosis of UTI and its treatment. In reality, however, the diagnosis is often based on conventional routine urinalysis alone and the treatment is started before the causal organism is identified [1]. The Sysmex fully automated integrated urine analyzer UX-2000 (UX-2000) uses flow cytometry (FCM) and is capable of quantitatively assaying urine formed elements like red blood cells, white blood cells, bacteria, epithelial cells, casts, etc. in about one minute. Reportedly, it can also estimate the approximate classification of the bacteria (Gram-positive or Gram-negative) from the bacterial scattergram pattern [2,3]. We have focused on this aspect and examined the reliability of the bacteriacount and estimation of the approximate classification of bacteria by the UX-2000 in rapid diagnosis of UTI, by comparing the results with those of microbiological tests.

ADVANTAGEOUS FEATURES OF UX-2000

The UX-2000 is an automated integrated urine analyzer that can perform both urine test strip analysis and FCM particle analysis on urine (***Fig. 1***). A function that cross-checks whether there is a mismatch between the results of test strip analysis and particle analysis, and a reflex test function that automatically goes for FCM when there is abnormality in the test strip analysis results. Both are based on integrated stored data and are major features of the UX-2000. The screen displaying the measurement results is shown in ***Fig. 2***.
Bacteria are specifically stained, analyzed and the

Note: This article is translated and republished from Sysmex Journal Web Vol.13 Suppl.2 2012.

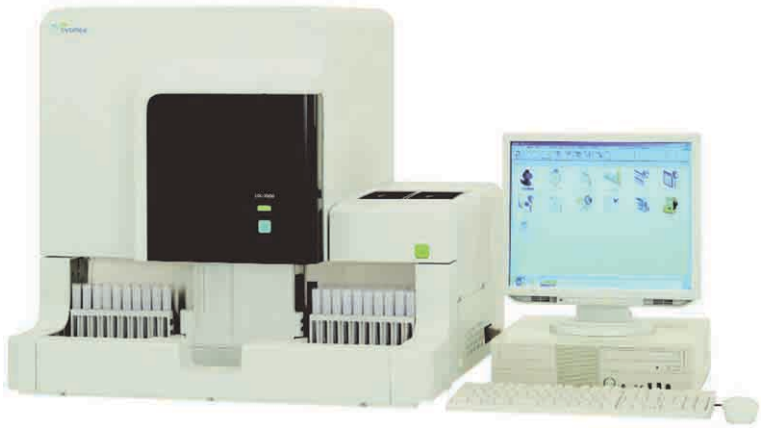


Fig. 1 *The fully automated integrated urine analyzer UX-2000*

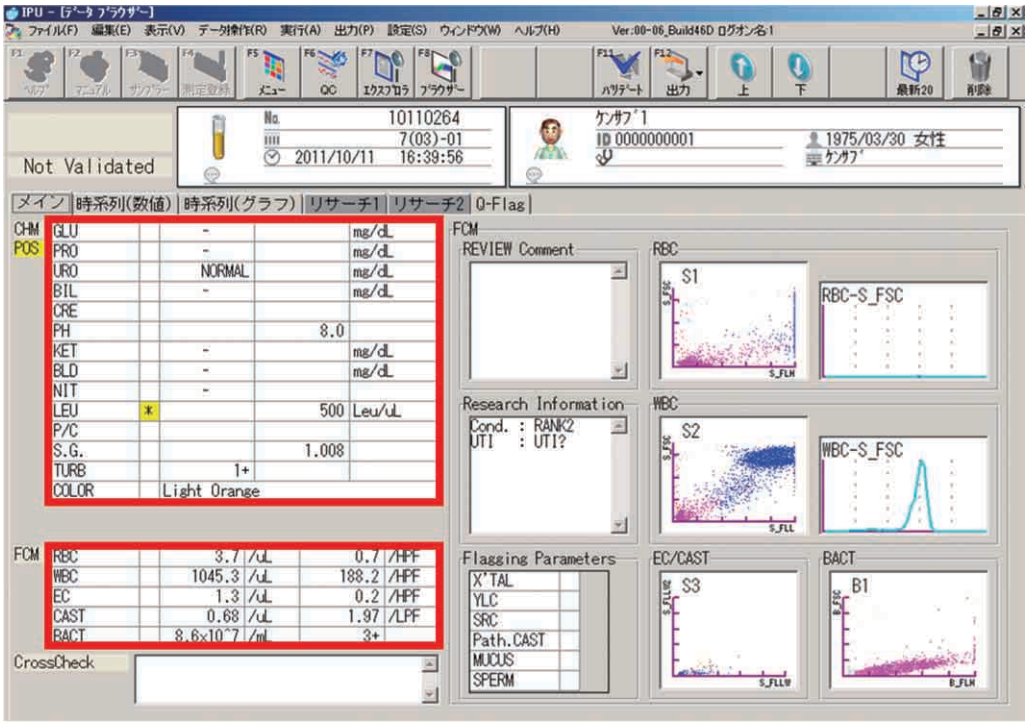


Fig. 2 *A screen showing the results of analysis by UX-2000*

quantitative results are displayed as well as the scattergrams (***Fig. 3***). The forward scattered light intensity, which is the y-axis of the scattergrams, reflects the size of the particles, bacterial aggregates, etc. The fluorescence signal intensity, the x-axis, reflects the level of staining of bacterial cell nuclei. A bacterial aggregate is sometimes displayed as a single dot in the bacterial scattergrams. Gram-positive bacteria divide irregularly and multiply along 2 or 3 planes;therefore they are positioned in an area of the scattergram as highly stained and relatively large. As a result, they are plotted above the 30° angle line from the x-axis, which passes through the origin on the scattergram (***Fig. 4-(1)***). On the other hand, Gram-negative bacteria divide once after reaching a certain size then multiply and become independent entities. Thus, their level of staining remains more or less constant and they are seen on the scattergrams as an area that contains plots of a smaller size and a more lightly stained area compared to cocci. Eventually, these dots are distributed below the 30° angle line from the x-axis, which passes through the origin on the scattergram (***Fig. 4-(2)***). It is said that the scattergram pattern changes according to the type of cell multiplication and the morphology of the bacteria, and that it is possible to some extent to estimate the type of bacteria (Gram-positive or Gram-negative) from them [2,3].

MATERIALS AND METHODS

225 urine specimens sent to us for bacterial testing during October through December 2011 were used in this study. Bacteria counts obtained through FCM by UX-2000 and the results of quantitative urine culture were compared and the concordance between the two methods when the cutoff count for bacteria positive specimens was set at 1.0×10^5/mL was examined. Further, in relation to UTI, we calculated the probability of correct UTI diagnosis under 3 conditions, cutoffs of bacteria count (1) 1.0×10^4/mL, (2) 1.0×10^5/mL and (3) 1.0×10^6/mL, all with a white blood cell count ≥ 10/μL, by the UX-2000 analyzer.

For estimating the type of bacteria, 93 specimens that definitely had bacteria identified bacteria as present by Gram stain, were chosen from 102 specimens found to have bacteria count ≥ 1.0×10^5/mL by UX-2000. The UX-2000 scattergrams of these specimens were classified into (1) High Angle Pattern (characteristic of cocci), (2) Low Angle Pattern (characteristic of bacilli), and (3) Broad Pattern (characteristic of the presence of multiple bacterial types) and the concordance with the results of microscopic examination of Gram stained specimens and the one with the results of bacterial identification by quantitative urine culture was examined.

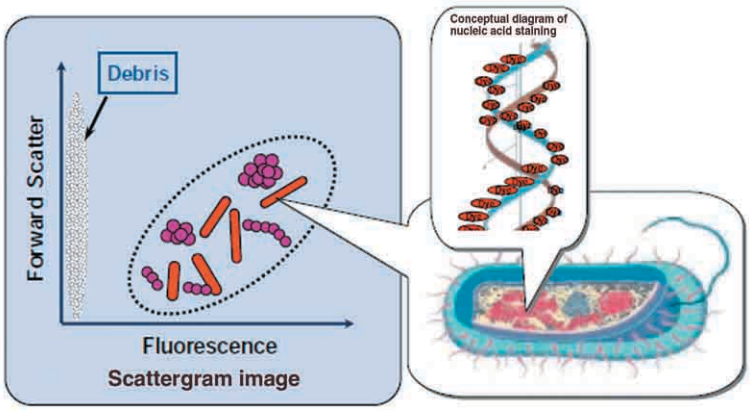
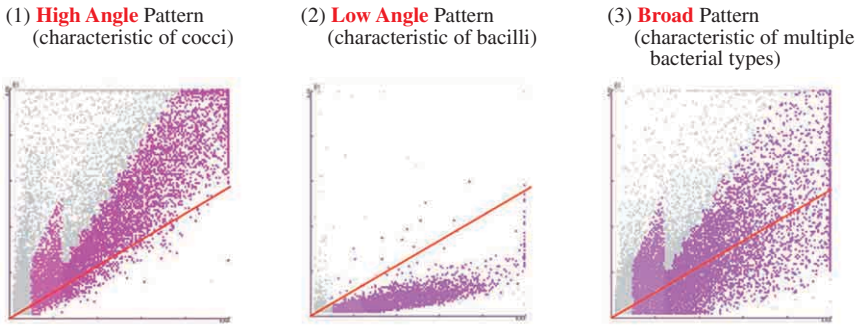


Fig. 3 *Conceptual diagram of bacterial assay by UX-2000*



The morphology of bacteria was estimated from the UX-2000 scattergram patterns by classifying them into (1) High Angle Pattern (cocci), (2) Low Angle Pattern (bacilli) or (3) Broad Pattern (more than one type of bacteria) using the 30° angle line that forms angle with the x-axis which passes through the origin as the reference line.

Fig. 4 *Scattergram patterns*

RESULLTS

1. Correlation in bacteria counts

The correlation between bacteria counts by UX-2000 and quantitative urine culture is shown in ***Fig. 5***. There was relatively good correlation, with a correlation coefficient of 0.855 and a regression line with a slope of 0.37, for the log-log plot. With the cutoff value of 1.0×10^5/mL for bacteria positive status, the concordance for bacteria positives and negatives was 89% (199/225). There were no cases (0%) that were bacteria positive in urine culture and negative in UX-2000 analysis. The 26/225 (11%) specimens that showed discrepancy were specimens that were negative in urine culture and positive by UX-2000. The breakdown of urine culture results of these 26 specimens was 9 negative (7 had prior antibacterial treatment), 9 with small number of normal inhabitants as urine bacteria, 6 with confirmed bacterial populations of 10^3-10^4 CFU/mL, and 2 with confirmed fungi.

2. The probability of accurate diagnosis of UTI

83 UTI and 142 non-UTI specimens were used for this analysis. The diagnosis of UTI was based on microbiological test results, patient records and assessment by an infectious disease specialist. The probability of accurate diagnosis was calculated for 3 cutoff values, UX-2000 bacteria counts of (1) 1.0×10^4/mL, (2) 1.0×10^5/mL and (3) 1.0×10^6/mL, all with a white blood cell count ≥ 10/μL, by the UX-2000 analyzer. As a result the sensitivity was found to be 96%, specificity 54% and concordance 68% for the category with bacteria count ≥ 1.0×10^4/mL. These were respectively 94%, 85% and 89% for the category of ≥ 1.0×10^5/mL, and 72%, 97% and 88% for the category of ≥ 1.0×10^6/mL. As the specificity, sensitivity and concordance were all satisfactory for the ≥ 1.0×10^5/mL category, it was decided to use a bacterial count of ≥ 1.0×10^5/mL as the criterion for diagnosing UTI using UX-2000 data (***Table 1***).

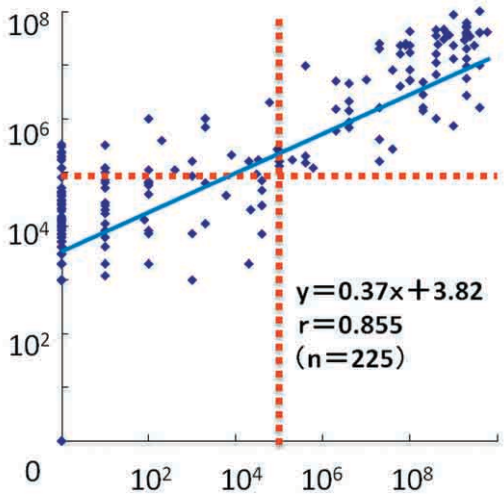


Fig. 5 *Correlation between the bacterial counts obtained by UX-2000 and by urine culture*

Table 1 *Probability of correct diagnosis of UTI*

Cutoff	Sensitivity	Specificity	Concordance
≥ 10^4	96%	54%	68%
≥ 10^5	**94%**	**85%**	**89%**
≥ 10^6	72%	97%	88%

The values given in the above Table were calculated by grouping **83** UTI cases and **142** non-UTI cases according to the following criteria: Bacteria counts ≥ 10^4/mL, ≥ 10^5/mL and ≥ 10^6/mL, all with a WBC count ≥ 10/μL, by UX-2000.

3. Concordance in estimation of type of bacteria

For estimating the type of bacteria, the scattergram patterns of specimens that had UX-2000 bacteria count ≥ 1.0×10^5/mL and were positive for bacteria in microscopy after Gram staining were classified as shown in ***Fig. 4*** and the result compared with the results of microscopy after Gram staining. Of the 26 specimens that had the High Angle Pattern (***Fig. 6***), 16 (62%) were found to have cocci by the microscopic examination. The frequency of occurrence was in the decreasing order *Staphylococcus* in 13 specimens, *Streptococcus* in 3, *Enterococcus* in 2, *Escherichia* in 2, *Pseudomonas* in 2.

Of the 45 specimens that had the Low Angle Pattern (***Fig. 7***), 39 (87%) were identified to have bacilli. The frequency of occurrence was in the decreasing order of *Escherichia* in 24 specimens, *Pseudomonas* in 6, *Klebsiella* in 5, *Citrobacter* in 4, *Serratia* in 2. Among the 22 specimens that showed the Broad Pattern (***Fig. 8***), 16 (73%) were identified to have more than one type of bacteria. 15 specimens had mixtures of Gram-positive cocci and Gram-negative bacilli. Among the specimens that had single type of bacteria, the frequency of occurrence was *Escherichia* in 3 and *Klebsiella* alone in 2.

Concordance in detection of cocci: 62% (16/26) (Comparison with findings of Gram staining)

Staphylococcus: 13 strains ***Enterococcus***: 2 strains ***Streptococcus***: 1 strain (all single type of bacteria)

No concordance: 38% (10/26) *Gram staining showed bacteria other than cocci

Bacterium identified	Results of urine culture (bacteria count: CFU/mL)	Major Gram staining findings
Escherichia 2 strains	(1) Bacteria count 4×10^9, with a very small number of ***Staphylococcus*** (2) Bacteria count 1×10^8, single	Coccus-like bacillus, filamentation
Pseudomonas 2 strains	Bacteria count 2×10^2 and 1×10^5, both single	
Providencia 1 strain	Bacteria count 2×10^7, single	GPC^+ smear
Morganella 1 strain	Bacteria count 4×10^2, with a small number of ***Streptococcus***	
Corynebacterium 1 strain	Present with a small number of ***Streptococcus***	
Bacteria-negative in urine culture: 3 specimens		1 GPC^+, 2 GPR^+

Fig. 6 *Breakdown of 26 specimens that showed High Angle Pattern scattergrams*

Concordance in detection of bacilli: 87% (39/45) (Comparison with findings of Gram staining)

Escherichia: 24 strains ***Klebsiella***: 5 strains ***Citrobacter***: 4 strains
Serratia: 2 strains ***Pseudomonas***: 6 strains ***Acinetobacter***: 1 strain (includes specimens having mixtures)

No concordance: 13% (6/45) *Gram staining showed bacteria other than bacilli

Bacterium identified	Results of urine culture (bacteria count: CFU/mL)	Major Gram staining findings
Enterococcus 2 strains	(1) Bacteria count 2×10^8, with 2×10^9 ***Klebsiella*** (2) Bacteria count 1×10^8, single	
Streptococcus 1 strain	Bacteria count 1×10^9, single	Chains of cocci
Staphylococcus 2 strains	(1) Bacteria count 3×10^4, with 2×10^4 ***Acinetobacter*** (2) Bacteria count 6×10^4, with a small number of ***Corynebacteriu***	
Bacteria-negative in urine culture: 1 specimen		1 GNR^+

Fig. 7 *Breakdown of 45 specimens that showed Low Angle Pattern scattergrams*

Concordance in detection of more than one type of bacteria: 73% (16/22) (Comparison with findings of Gram staining)

Gram-positive cocci + ***Gram-negative bacilli***: 15 specimens
Gram-positive bacteria only: 1 specimen

No concordance: 13% (6/45) *Gram staining showed bacteria other than bacilli

Bacterium identified	Results of urine culture (bacteria count: CFU/mL)	Major Gram staining findings
Escherichia 3 strains	Bacteria count 4×10^9, 2×10^9 and 4×10^6	Bacteria of varied size in all 3 specimens, filamentation in 2 specimens
Klebsiella 2 strains	Bacteria count 2×10^7 and 2×10^6	Bacteria of varied size in 1 specimen, filamentation in 1 specimen
Citrobacter 1 strain	Bacteria count 1×10^8	Bacteria of varied size in 1 specimen

Fig. 8 *Breakdown of 22 specimens that showed Broad Pattern scattergrams*

DISCUSSION

As for the correlation between the bacteria counts obtained by the two methods, the UX-2000 generally gave higher values than quantitative urine culture in the range < 1.0×10^4/mL and lower values in the range ≥ 1.0×10^6/mL. This is believed to be because of the analysis sensitivity 5.0×10^3/mL by UX-2000 in counting bacteria and the range of measurement defined by the upper detection limit of 9.9×10^7/mL. Besides this, when a large number of bacteria such ≥ 1.0×10^6/mL are present, aggregates of bacteria are sometimes counted as one bacterium, which is also a cause of apparently low values given by the analyzer [4]. Concordance was good when the cutoff for bacteria positive status was 1.0×10^5/mL. No specimen (0%) was bacteria positive in quantitative culturing and negative by UX-2000. Thus, there was no false negative case by UX-2000 analysis when the result of quantitative culture was taken as the reference. On the other hand 26 (11%) specimens were positive by UX-2000 although negative in the quantitative culture. This non-concordance could possibly be due to counting of dead bacteria and misidentification of small white blood cell fragments, etc. by the analyzer, and failure of bacterial growth because of prior antibacterial treatment.

Estimation of the type of bacteria from the scattergram pattern had a high concordance with the findings of Gram staining. On the other hand, a detailed examination of micrographs and the results of bacterial identification by culturing of specimens that showed non-concordance between scattergram pattern by UX-2000 and the results of Gram staining revealed the following: Of the 10 specimens that had non-cocci bacteria despite showing the High Angle Pattern, 3 had mixtures of Gram-negative bacilli and Gram-positive cocci, 1 each showed coccus-like bacilli in Gram staining and filamentous *Escherichia*. Out of the 6 specimens that showed the Low Angle Pattern but had non-bacillus bacteria, 3 had Gram-positive cocci intermixed with Gram-negative bacilli, and 1 had *Streptococcus* that showed chains of cocci in Gram staining. Among the specimens that showed the Broad Pattern, 6 were found to have single Gram-negative bacilli and almost all of them showed size variation of the bacterial bodies and filamentation in Gram staining. In this manner, when more than one type of bacteria was present, or the bacterial cells showed morphological variation in Gram staining, their scattergram distribution pattern also changed and the reliability of estimating the bacterial type from the scattergram pattern apparently decreased.

CONCLUSION

The measurement of bacteria count in urine by UX-2000, and estimation of the type of bacteria from the scattergram patterns showed high concordance with results of microbiological tests. Therefore UX-2000 analysis is considered to be useful as a rapid screening test for UTI.

The estimation of bacterial type by UX-2000 had high concordance for bacilli, but microbiological tests are required for confirming the identity of the bacteria species. Even when urine test strip analysis alone are requested, the UX-2000 has a function that makes an automatic assessment from the Leucocyte esterase reaction, nitrite reaction by test strip examination, and turbidity then carries out particle analysis by FCM, we can obtain in real time information that suggests the presence of UTI (***Fig. 3***). Therefore, the bacteria count obtained by UX-2000 can be used as a screening test to decide whether urine culture is required. Besides this, the type of bacteria can be estimated to some extent from the scattergram pattern displayed, there is the possibility of using the information as a guide in the selection of a suitable antibiotic.

The contents of the present paper had been presented at the 23rd Annual Meeting of the Kyushu branch of the Japan Society of Clinical Chemistry.

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