Comparisons of the Bact Scattergram Pattern by Fully Automated Integrated Urine Analyzer UX-2000 and Microscopic Examination Results Using Gram Stain

Yumi YASUTAKE[*1], Miki HIGUCHI[*1], Shintaro ODA[*1], Yuki TAMURA[*1], Teizou SHIMADU[*1], Hideyuki KOBAYASHI[*2] and Eiji HANAOKA[*1]

[*1] *Saiseikai Yahata General Hospital, 5-9-27 Harunomachi, Yahatahigashi-ku, Kitakyushu, Fukuoka 805-0050, Japan*

[*2] *Scientific Research Division, Scientific Affairs, Sysmex Corporation, 1-3-2, Murotani, Nishi-ku, Kobe-shi, Hyogo 651-2241, Japan*

UX-2000 (Sysmex) is fully automated integrated urine analyzer, combining the automated urine cell analyzer and the urine chemistry (urine test strips) analyzer.
One of the major advantages of this instrument is quantitative bacteria analysis by the specifically optimized detection unit for the bacteria.
Here, we have examined the relation between bacteria scattergram pattern by UX-2000 and microscopic examination results using gram stain then we found that location of dots on the scattergram of UX-2000 tends to be lower part when gram negative bacteria is measured.
By analyzing the shape of the cluster of dots on the scattergram (bact cluster) with measuring the slope and width, the bact cluster with broad shape was found in the case of the mixture of gram positive and negative bacteria. Accordingly it was suggested to be possible to make an assumption to discriminate between gram positive and negative bacteria by using bact cluster analysis.

Key Words UX-2000, Bact Scattergram, Gram Stain, Broad Shaped Bact Cluster

BACKGROUND AND PURPOSE OF THE STUDY

Urinary tract infection (UTI) is a condition where pathogens like bacteria enter the urinary tract and cause infection. Tests available for detecting UTI include the urine test strip analysis of nitrite and Leukocyte esterase, urine sediment analysis, and Gram staining and urine culture for bacterial testing. Among these, urine sediment analysis has advantages over other tests as the increase in white blood cells, epithelial cells and bacteria in urine can be rapidly verified visually. However, visual urine sediment analysis involves the risk of missing the bacteria when a large amount of amorphous crystals are present. The fully automated, integrated urine analyzer UX-2000 of Sysmex (hereinafter UX-2000) is capable of urine test strip analysis and quantitative flow cytometric analysis (FCM) of particles in the urine. In this analysis, the principle measurement is flow cytometry that uses a red semiconductor laser (wavelength λ = 635 nm) to quantitatively assay red blood cells, white blood cells, casts, epithelial cells and bacteria [1]. Thus, the counts of white blood cells and bacteria, needed for diagnosing UTI, are instantly obtained (***Fig. 1***).

In particular, the UX-2000 carries out an advanced quantitative assay of bacteria through an independent channel for bacteria (BACT channel) by specifically staining the nucleic acids of bacteria with a fluorescent polymethine dye, and can provide graphic displays on the BACT scattergrams [2] (***Figs. 2*** and ***3***). In the present study we had an opportunity to examine the possibility of estimating whether the UTI-causing bacterium is a Gram-positive coccus or a Gram-negative bacillus, etc. from the angle of the dot distribution pattern with the x-axis of the scattergram produced by the BACT channel. The results are reported here.

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

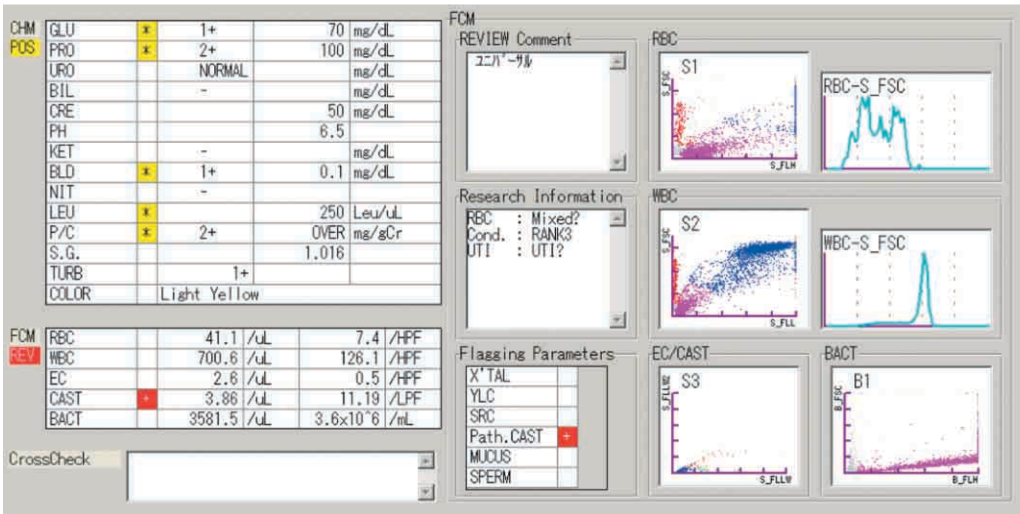

Fig. 1 Main screen of UX-2000

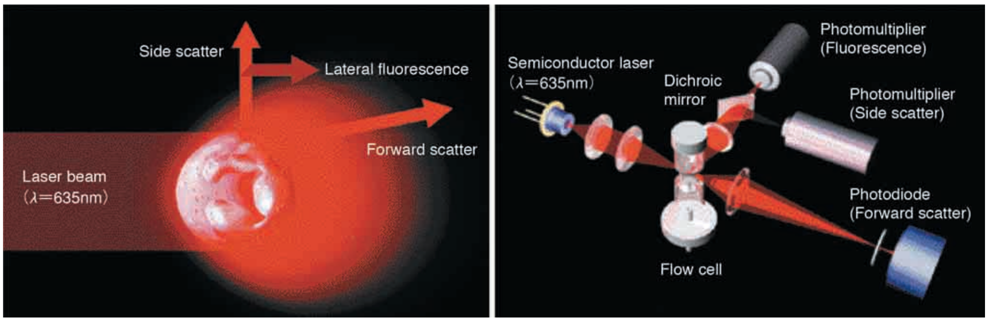

Fig. 2 Flow cytometry that uses a red semiconductor laser

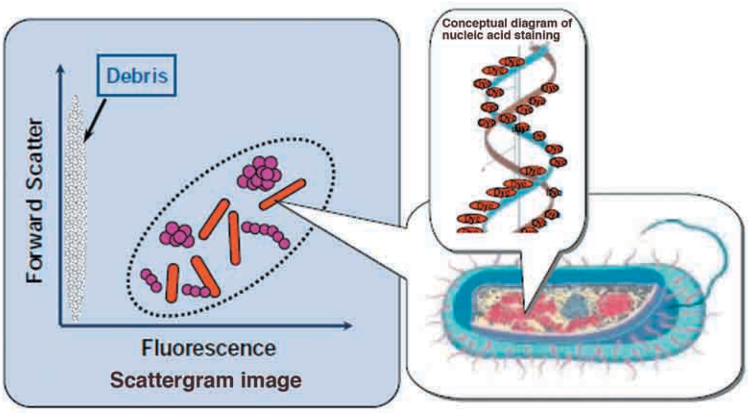

Fig. 3 An illustration of bacterial staining and the scattergram[3)]

MATERIALS AND METHODS

Urine specimens of outpatients and hospitalized patients submitted for testing during the 79-day period from September 1 through November 18, 2011 were analyzed by UX-2000. Specimens with confirmed presence of bacteria had patient identification removed and were randomly selected for Gram staining (Feyber G Nissui, Nissui Pharmaceutical) and also cultured to identify the bacteria species. The BACT scattergrams generated by UX-2000 were classified into 3 groups according to the distribution patterns as per the criteria suggested by Muratani [3] and Ozawa et al. [4]. Taking the angle of 30° from the x-axis as the threshold, scattergrams with dots distributed at 30° or higher were classified as "High Angle Pattern", those with dots distributed at an angle lower than 30° as "Low Angle Pattern", and those with the dots distributed both above and below the 30° angle "Broad Pattern". The results were then compared with those of Gram staining and urine culture.

Trypticase soy agar medium with 5% sheep blood and MacConkey agar medium (Sysmex bioMeríuex) were used for culturing, and VITEK and VITEK 2 (Sysmex bioMeríuex) were used for identification of the bacteria.

RESULTS

A total of 178 specimens, 55 from males and 123 from females, were tested. Specimens from which bacterial species could not be identified by urine culture were excluded from these, and the remaining 160 specimens were studied.

Among the bacteria identified by urine culture, Gram-negative bacilli were found in 69.2% and Gram-positive cocci in 29.8 % of the specimens. A few Gram-positive bacilli were also detected (***Fig. 4***). More than one type of bacteria was detected in 38 specimens by culturing or Gram staining or both. *Escherichia coli (E. coli)*, a Gram-negative bacillus, was the most frequently identified bacterium. The next most commonly detected species were the Gram-positive cocci of *Enterococcus* spp. Firstly, we shall show (***Fig. 5***) the scattergrams of some bacteria that had been pure-cultured after they were identified. There were 7 Gram-negative bacteria and 4 Gram-positive bacteria. As can be seen from ***Fig. 5***, the Gram-negative bacteria showed the Low Angle Pattern. The Gram-positive bacteria had varied scattergram patterns. Among these, *Enterococcus* spp. showed patterns similar to those of Gram-negative bacteria.

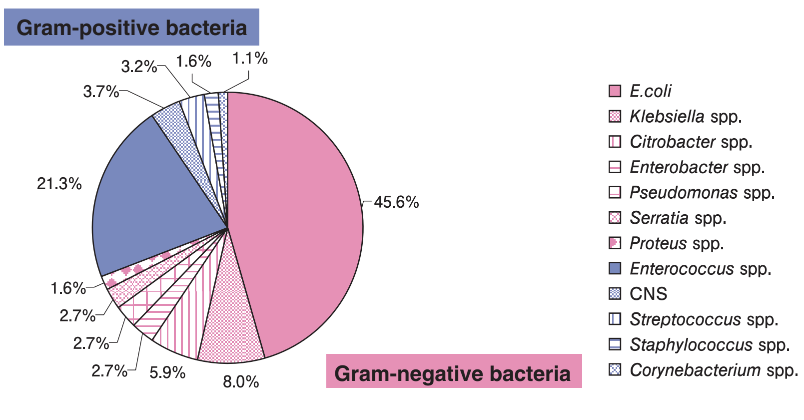

Fig. 4 *Bacterial species identified by urine culture*

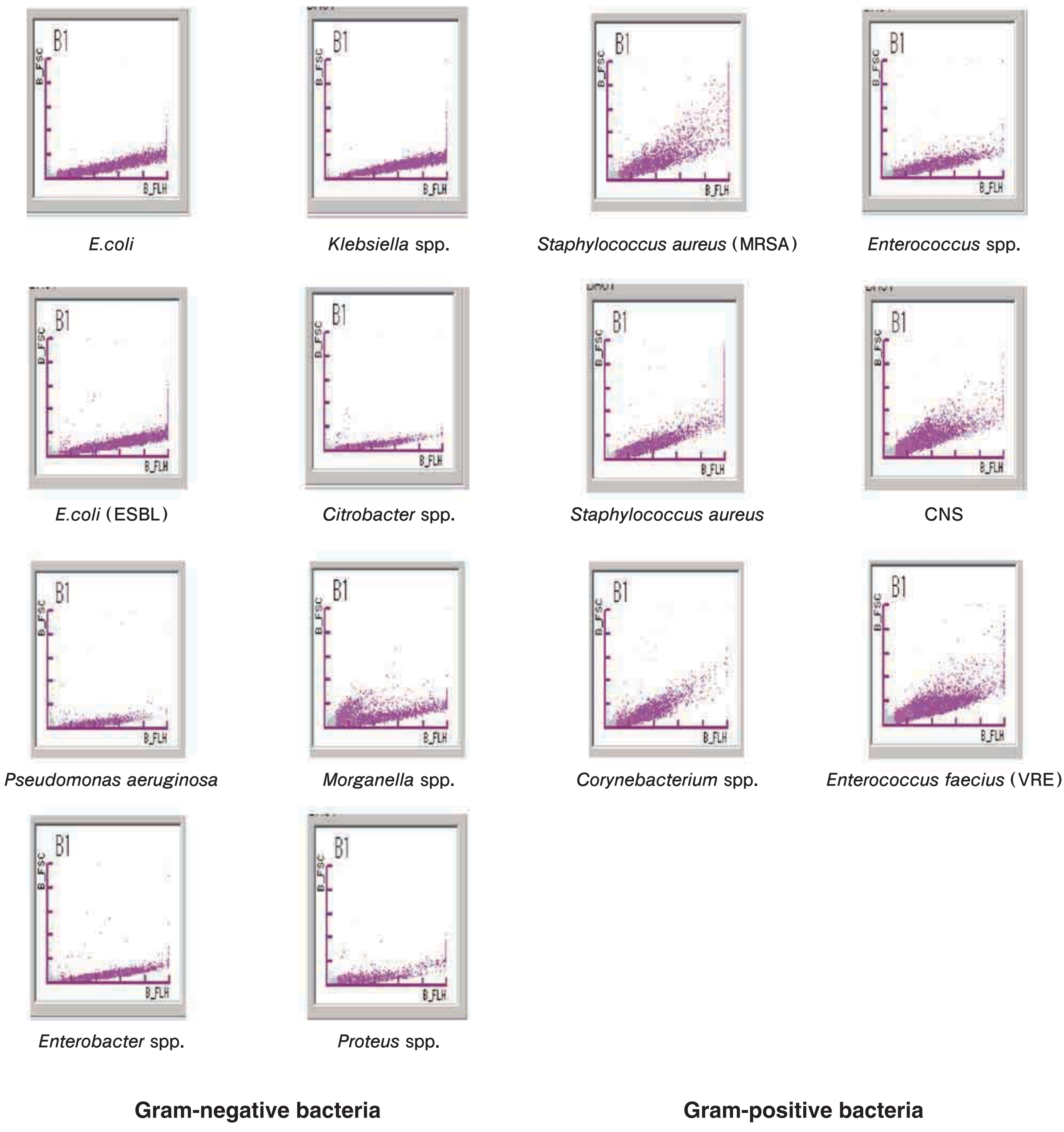

Fig. 5 *Scattergrams of different bacteria pure-cultured after identification*

In the next step, the angles of the scattergram distribution patterns of urine specimens were examined and compared with results of Gram staining. Identification of the bacteria was also done simultaneously. Of the 160 specimens, the scattergrams of 140 that revealed a single type of bacterium in Gram staining were classified according to the distribution pattern of the dots, taking an angle of 30° as the threshold angle, and 122 specimens that showed the presence of a single type of bacterium both in Gram staining and urine culture were also similarly classified (***Tables 1*** and ***2***, ***Fig. 6***). The results showed that most of the bacterial species that had the Low Angle Pattern (< 30°) of the scattergram were Gram-negative bacteria whereas those that showed the High Angle Pattern (≥ 30°) had both Gram-negative and Gram-positive bacteria in equal measure. However, a majority of specimens with the Gram-positive bacteria had the High Angle Pattern, and those that showed the Low Angle Pattern all had *Enterococcus* spp.

Table 1 *Specimens that showed a single type of bacterium in Gram-staining classified according to the angle*

	< 30°	≥ 30°	Total
Gram-positive bacteria	6	30	36
Gram-negative bacteria	71	33	104
Total	77	63	140

Table 2 *Specimens that showed a single type of bacterium both in Gram-staining and urine culture classified according to the angle*

	< 30°	≥ 30°	Total
Gram-positive bacteria	5	25	30
Gram-negative bacteria	65	27	92
Total	70	52	122

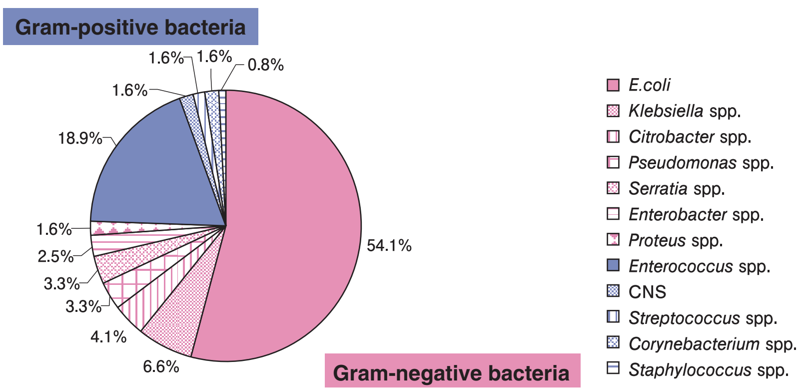

Fig. 6 *Bacterial species detected in specimens that showed a single type of bacterium in both Gram-staining and urine culture*

Bacterial species found in 38 specimens that showed more than one type of bacteria in either Gram staining or urine culture, or the both are shown in the pie chart (***Fig. 7***). There was no difference between frequency of Gram-positive and Gram-negative bacteria in the specimens that had more than one type of bacteria. In the next step, the specimens having more than one type were classified into those with and without Gram-positive bacteria, and the scattergrams of each group were examined using 30° as the dividing angle. A high proportion (96%) of the specimens having more than one type of bacteria that included a Gram-positive bacterium had distribution patterns with ≥ 30° angle (***Table 3***). Also, the specimens having more than one type of bacteria that included both Gram-positive and Gram-negative bacteria showed the Broad Pattern, i.e., with a low angle cluster and a cluster exceeding the 30° angle, which suggested the presence of two or more types of bacteria. When the species in such specimens were identified, they were indeed found to contain both Gram-positive and Gram-negative bacteria.

Table 3 *Specimens with more than one type of bacteria, classified according to the scattergram angle*

	< 30°	≥ 30°	Total
Gram-positive bacteria detected	7	27 (96.4%)	34
Gram-negative bacteria not detected	3 (30.0%)	1	4
Total	10	28	38

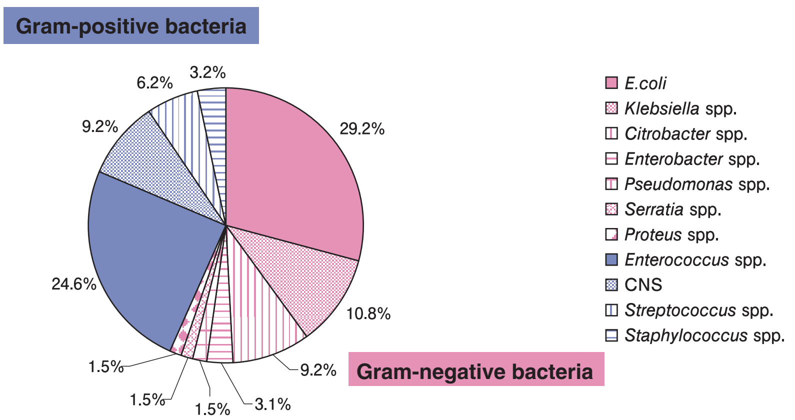

Fig. 7 *Bacterial species detected in specimens that showed more than one type of bacteria in Gram staining or urine culture, or the both.*

Examination of the scattergrams of the 160 specimens revealed that 110 specimens showed the Low Angle Pattern, and most of them had Gram-negative bacilli. The Broad Pattern was seen in 47 specimens. The High Angle Pattern was seen in only 3 specimens, and they had *Corynebacterium* spp. or *Staphylococcus* spp. Of the 47 specimens that showed the Broad Pattern 23 showed more than one bacterial species in either Gram staining or urine culture, or the both. The simultaneous presence of both Gram-positive and Gram-negative bacteria was seen in 15 specimens, and 3 specimens had two or more Gram-positive bacteria.

DISCUSSION

In spite of the great advancements in modern medicine, infections are still major diseases. In the field of emergency medicine, rapid identification of the causal bacterium can facilitate a speedy start to the treatment. Identification of bacterial species by urine culture still takes more than 48 hours. Because of this, the so-called empiric therapy, which relies largely on the experience of the physician, is often resorted to in the emergent treatment with antibiotics.

In urine sediment analysis, it has not been possible until now to set up very clear-cut criteria for semi-quantitative parameters of bacteria and the considerable variation of results depending on the individual analyst has been an issue.

UX-2000, which has been inducted into our hospital, is an analyzer that carries out urine test strip analysis and FCM in a fully automated process. The analyzer can quantitatively determine the bacterial count and white blood cell count in urine in a short time and confirm the presence of bacteria even in specimens that also contain a large number of amorphous crystals (***Fig. 8***). Furthermore, as was verified in the present study, whether the bacterial species present are Gram-positive or Gram-negative can be estimated fairly well in a short time from the distribution pattern of the BACT scattergram, which suggests its usefulness in emergency situations.

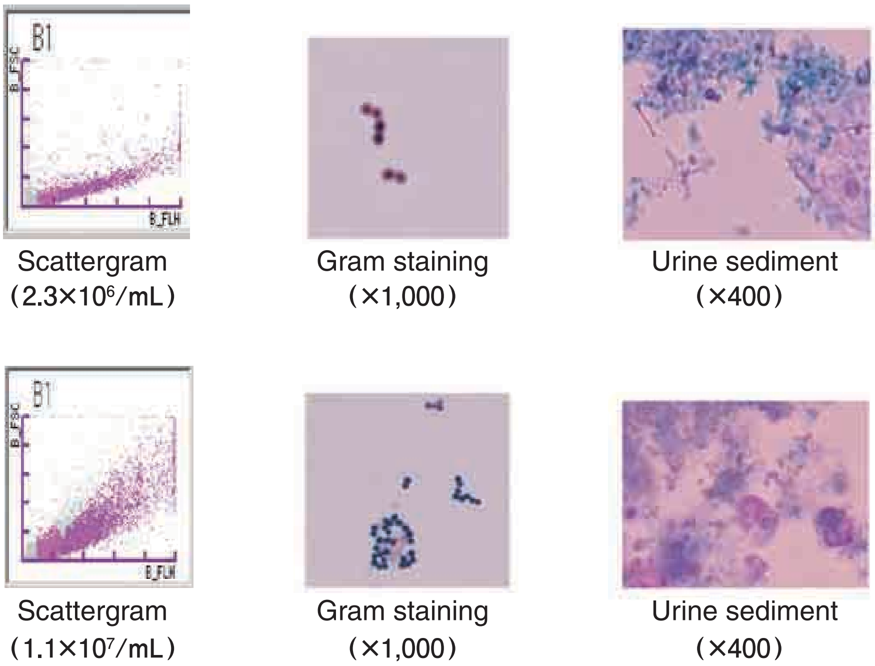

Fig. 8 *Scattergrams and Gram staining of specimens having both bacteria and amorphous crystals*

Among the scattergrams of specimens examined in the present study, the majority of those that revealed only one type of bacteria had the Low Angle Pattern and mostly Gram-negative bacteria only. The only Gram-positive bacteria detected were *Enterococcus* spp. The abscissa of the BACT scattergram is the fluorescence intensity, which reflects information on the nucleic acid content. The ordinate is a measure of the size of the particles as determined by the intensity of forward scattered light, which also reflects size information (***Fig. 9***). In general, a bacillus, because of its characteristic nature, divides into two independent entities, once it achieves certain size by cell growth . Therefore the nucleic acid content of the particles tends to be constant, and the scattergram tends to have a fairly well-defined distribution. On the other hand, staphylococci divide irregularly and multiply along 2 or 3 planes whereas streptococci multiply along one plane. Therefore the nucleic acid content will increase, and their distribution on the scattergram is expected to show wider dispersion[5]. *Enterococcus* spp. is often seen in the form of short chains of 4-8 cells. Therefore, their scattergrams are expected to be similar to those of Gram-negative bacteria (***Fig. 10***).

Among the specimens wherein more than one type of bacteria was detected, those that had Gram-positive bacteria mostly showed the High Angle Pattern of ≥ 30°. This suggests the possibility of estimating the presence of Gram-positive bacteria from the scattergram.

Among the specimens that showed the Broad Pattern and revealed more than one type of bacteria, Gram-positive bacteria were seen at the high frequency of 78.3% (***Table 4*** and ***Fig. 11***). The specimens that showed a broad distribution and had Gram-negative bacteria showed elongated bacterial bodies that suggested the effect of antibiotics (***Fig. 12***).

The results described above suggest that scattergrams with the Low Angle Pattern indicate the presence of Gram-negative bacteria, and possibly Gram-positive bacteria also if more than one type of bacteria is present. The specimens with the Broad Pattern included interesting ones like those having Gram-positive bacteria and those showing the effect of antibiotics. Nevertheless, only 47 specimens showed the Broad Pattern. Therefore, in the future we would like to study more such cases and accumulate data to understand how the presence of Gram-positive bacteria or antibiotic-induced bacterial filaments would impact the scattergrams of patients' urine specimens.

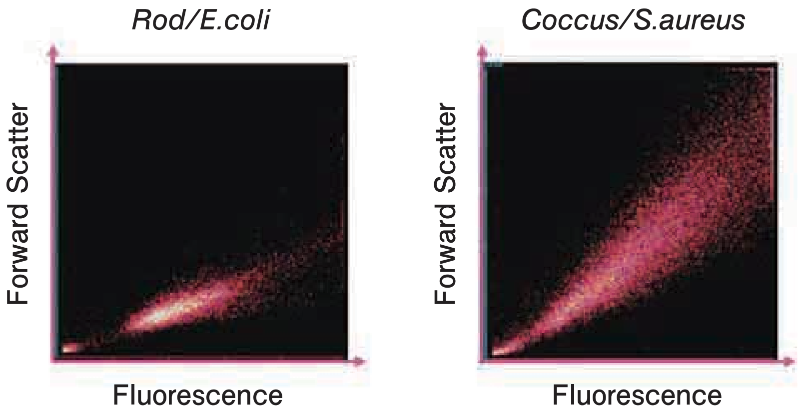

Fig. 9 *Examples where bacteria were detected*

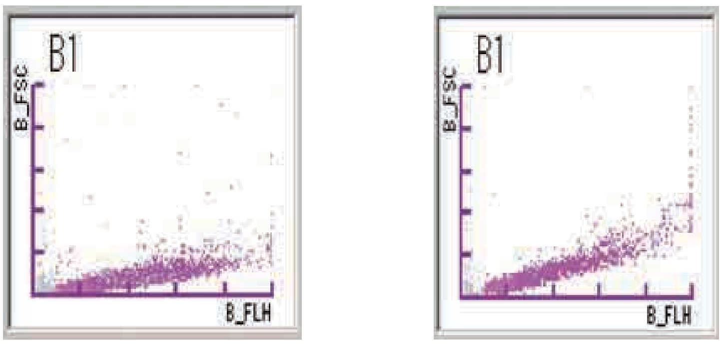

Fig. 10 *Scattergrams of E. coli and Enterococcus*

***Table 4** Gram staining-based classification of specimens that showed the Broad Pattern and had more than one type of bacteria*

Gram staining reaction of the bacterial species	No. of specimens
Gram-positive bacteria + Gram-positive bacteria	3
Gram-positive bacteria + Gram-negative bacteria	15
Gram-negative bacteria + Gram-negative bacteria	5
Total	23

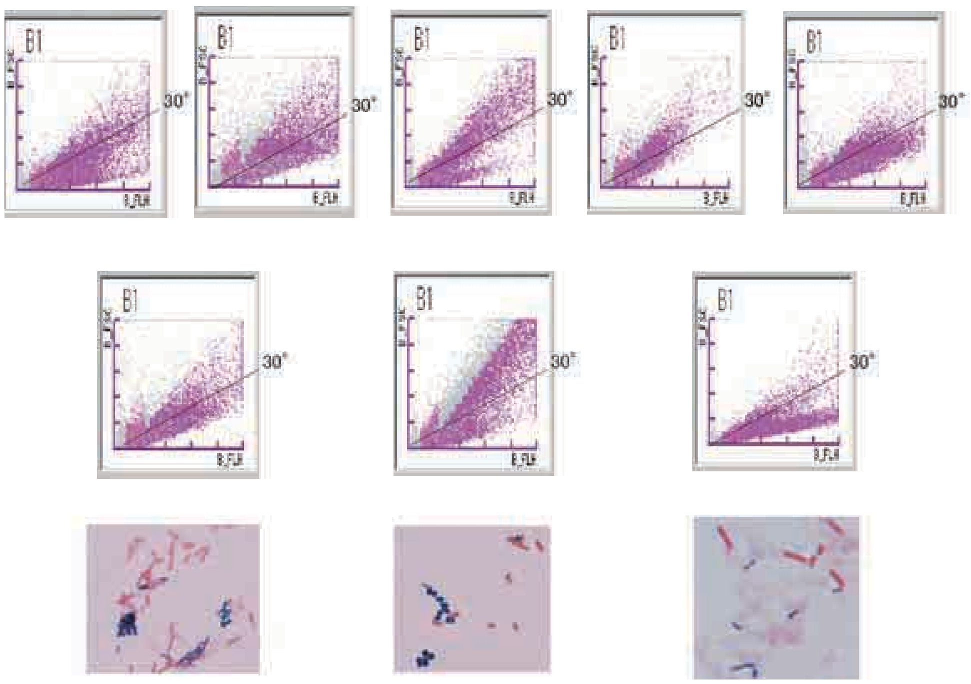

***Fig. 11** Scattergrams of specimens with more than one bacterial species (All were mixtures of Gram-positive and Gram-negative bacteria.)*

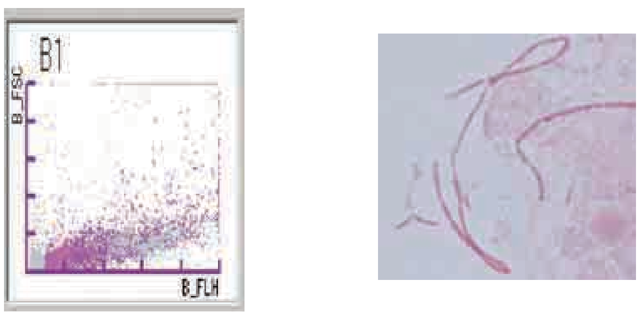

***Fig. 12** Scattergrams and micrographs of Gram stained bacteria affected by antibiotic treatment*

CONCLUSION

The present study examined the feasibility of differentiating Gram-negative from Gram-positive bacteria from the scattergram patterns obtained by UX-2000. Its results show the possibility of early diagnosis of UTI and appropriate use of antibiotics. The category of the Broad Pattern scattergrams needs further study with a larger number of specimens, which is a task for the future.

References

1) *Kajikuri Y et al. Basic Evaluation of the Fully Automated Urine Cell Analyzer UF-1000i. Sysmex J. 2006; 29:54-64 (Japanese)*
2) *Muratani T et al. The Possibility of the Bacterial Class Estimate Using Urine from Patients with the Urinary Tract Infection by the Fully Automated Urine Particle Analyzer UF-1000i. Sysmex J. 2010; 33:87-96 (Japanese)*
3) *Ozawa H. et al. Causative Bacterial Group Prediction by Bacterial Scattergram Using the Fully Automated Urine Particle Analyzer UF-1000i. Sysmex J. 2011; 34, suppl. 1:19-25 (Japanese)*
4) *Okada H et al. The Basic Performance of Bacteria Counting for Diagnosis of Urinary Tract Infection Using the Fully Automated Urine Particle Analyzer UF-1000i. Sysmex J; 2007. 30:95-103 (Japanese)*
5) *Nakagawa H et al. Clinical Usefulness of Urine Formed Element's Information, Obtained from Bacteria Detection by Flow Cytometry Method that Uses the Nucleic Acid Staining. Rinsho Byori. 2009; 57(3): 221-227 (Japanese)*