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Comparison of the Diagnostic Performance of 3 Automated Urine Particle Analyzers with Manual Microscopy

Naomi ISHIBASHI, Ritsuko YOSHIZAWA, Yoko FUJII, Yu AKAISHI, Kanako OBATA, and Kuniko MABUCHI

Department of Clinical Laboratory, Ohara Healthcare Foundation Kurashiki Central Hospital, 1-1-1 Miwa, Kurashiki, Okayama, 710-8602, Japan

We compared the performance of three models of automated urine particle analyzers with that of manual microscopy, the U-SCANNER II (TOYOBO CO., LTD., image processing) currently used in our hospital, the Atellica UAS800 (Siemens Healthcare Diagnostics K.K., image processing), and the UF-5000 (Sysmex Corporation, flow cytometry). Agreement with manual microscopy of the results obtained with the 3 analyzers was reasonably good except in the case of casts, for which microscopic examination by medical technologists and cross-checking with other parameters was needed. Image processing could contribute to improved detection as it enabled observation of clear images by medical technologists. However, it was affected by the level of expertise of the technologist. Flow cytometry was superior in detecting blood cells and bacteria. The time required for analysis varied depending on the levels of different elements present in the sample. We can save medical technologist labor, make the testing more efficient, and ensure good quality testing by adopting logical analysis strategies that exploit the advantageous features of different analyzers.

Key Words Fully automated urine particle analyzer, UF-5000, Atellica UAS800, U-SCANNER II, Urine sediment

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INTRODUCTION

Urine particle analyzers are currently used in many medical facilities to improve the efficiency and quality of urinalysis. JCCLS GP1–P4 mentions that it is advisable to use urine particle analyzers only after properly understanding their characteristic features. In other words, the guideline takes the position that the use of such analyzers is not merely for automation of conventional urine sediment analysis and that these analyzers can provide new information on particles (formed elements) in urine.[1)] Generally, either image processing or flow cytometry is used as the analysis principle in urine particle analyzers. Flow cytometry is claimed to provide highly reproducible quantitative data. It can also provide new indices, such as information estimated from the gram staining of bacterial cells and estimates of special cells based on the difference in nucleic acid content (fluorescence intensity), that manual microscopy of urine sediments cannot provide.[2)] On the other hand, image processing methods have been reported to have the advantage of being able to make almost confirmatory reporting based on observations and editing on the screen without preparing urine sediment samples. It has also been pointed out that they are useful in providing clinicians with image information on particles such as atypical cells and in educational training. Recently we had an opportunity to observe and compare the performance of three analyzers with different measurement principles and features, namely, the U-SCANNER II (TOYOBO CO., LTD., image processing), the Atellica UAS800 (Siemens Healthcare Diagnostics K.K., image processing) and the UF-5000 (Sysmex Corporation, flow cytometry). We report here the agreement of the results obtained using the three analyzers with the results of conventional urine sediment analysis, as an aid for understanding the characteristic features of these analyzers.

MATERIALS AND METHODS

1. Samples analyzed

Urine samples of 347 cases received for urine sediment analysis at our hospital during March 22 to April 17, 2019 were simultaneously analyzed using the three analyzer models and the results compared with those of manual microscopy.

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2. Analyzers

1) U-SCANNER II (hereinafter US II): the analyzer system currently in use
This system analyses by image processing. The urine sample is mixed with a special staining solution and spread on a special plate. 50 or 100 photographs of the sample are taken with a camera having an autofocus function. The images are then analyzed by a computer and classified according to cell type. Almost microscopic-like images are obtained as the images are overlapped with the captured photographs. The images are edited if necessary.[3)]

2) Atellica UAS800 (hereinafter UAS): a system investigated in this study
The system analyses by image processing. The urine sample is dispensed into a dedicated cuvette and centrifuged for 10 seconds at 2,000 rpm. An unstained image of the sample is captured using the dual focus function camera. This image is then identified by comparing with numerous images stored in the system and classified automatically. The images are edited if needed.[4)]

3) UF-5000 (hereinafter UF): a system investigated in this study
The flow cytometry (FCM) method is the principle of measurement of this system. Urine is aspirated and analyzed while passing through two channels. The nucleic acids are specifically stained in the CR channel, where the elements having a nucleic acid are classified. Cell membranes and the cast matrix are stained in the SF channel which classifies the elements without nucleic acid. The stained cells are irradiated with a blue semiconductor laser beam to obtain various types of signal information. This signal information reflects cell size, stainability, nucleic acid content, birefringence, and size and complexity of internal structures. These types of information are combined to classify the elements. The data of elements thus classified can be viewed on scattergrams.[5)]

3. Manual urine sediment analysis

The urine sediment analysis was carried out according the procedure normally followed by our hospital. It is based on "Examination of Urinary Sediment 2010 (JCCLS GP1–P4)".[1)] Microscopic examination was done after staining with the New UriStain (Sysmex Corporation) .

4. Details of analysis

1) Comparison of agreement in analysis results of red blood cells, white blood cells, squamous epithelial cells, bacteria, and hyaline casts
(1) Urine samples of 347 cases received for urine sediment analysis were analyzed using the three different analyzers and the results compared with those of manual microscopy.
(2) Similarly, the results of the 347 specimens obtained with UAS and UF II were compared.
As for the units, the counts of red blood cells, white blood cells and squamous epithelial cells were converted into count/HPF from count/μL using the conversion factors recommended by the manufacturer of each analyzer. In the case of bacteria and casts, they were converted into qualitative measures as shown in ***Table 1***. The results obtained by automatic classification were used in the case of image processing type analyzers.

2) Agreement in red blood cell morphology
The results of 34 samples for which red blood cell morphological analysis was requested along with the urine sediment analysis were compared with the results of manual microscopy. Unstained samples were used for manual microscopy. The UAS analysis was performed by medical technologists making assessments based on images displayed on the system. In the case of the UF, the red blood cell morphology information (RBC-Info.) output by the analyzer was used. As the manual microscopic assessment of red blood cell morphology observations were made without staining, the US II results which were based on stained samples were excluded from the comparison.

Table 1 *Conversion table for qualitative values*

Casts

	UAS · UF	Manual microscopy
1+	1 – 9/LPF	1 – 4/WF
		5 – 9/WF
2+	10 – 29/LPF	10 – 19/WF
3+	30 – 99/LPF	20 – 39/WF
		40 – 59/WF
4+	100 – 999/LPF	1–9/LPF
5+	≥1,000/LPF	≥10/LPF

Bacteria

	UF	UAS	US II
–	0.0 – 149.9/μL	–	******
1+	150.0 – 999.9/μL	+	(1+)
2+	1,000.0 – 9,999.9/μL	++	(2+)
3+	10,000.0 – 9,999,999/μL ++++	+++	(3+)

3) Detection of atypical cells
Samples where atypical cells were found by manual microscopy were evaluated using the three analyzers.
With the US II and the UAS the technologists made assessments based on the images displayed on the systems.
With the UF the cutoff value for the output "Atyp.C" was set at 0.2/μL and samples that gave values higher than this cutoff were taken as positive for atypical cells.

4) Analysis time
10 samples were analyzed one after another and the time from the first sampling up to the output of the results of the 10th sample was taken as the analysis time and used for comparison. Measurements were done both with groups of randomly selected samples and samples selected after excluding turbid urine.

RESULTS

1. Comparison of agreement in analysis results of red blood cells, white blood cells, squamous epithelial cells, bacteria, and hyaline casts

1) Comparison with manual microscopy
(1) Red blood cells
The analysis results for red blood cells are shown in ***Table 2***. The ±1 rank agreement of the results obtained with the three systems with manual microscopy results was 84.1 to 98.0 %, which was broadly satisfactory. When the cutoff value was 5/HPF, the specificity was 78.1 % for US II, 91.8 % for UAS and 93.3 % for UF. Thus, the UAS and the UF, the systems being investigated here, had higher specificity than the US II system currently being used, with fewer cases of misidentification of yeast, bacteria and calcium oxalate crystals as red blood cells. In the circled group, which gave significantly divergent results with the US II, the analyzer misidentified yeast cells, bacteria and calcium oxalate crystals as red blood cells.

(2) White blood cells
The results of analysis of white blood cells are shown in ***Table 3***. The ±1 rank agreement with manual microscopy was broadly satisfactory for the results obtained with the three systems, being in the range 86.7 to 96.3 %. The data marked with ***** in the Table are of gut epithelial cells or virus infected cells misidentified as white blood cells, which were seen with all the analyzer models. With the US II, in the groups surrounded by triangles, which showed very different results, the analyzer misidentified red blood cells as white blood cells. In the circled group, red blood cells and tubular epithelial cells were misidentified as white blood cells.

(3) Squamous epithelial cells
The results of analysis of squamous epithelial cells are given in ***Table 4***. The ±1 rank agreement was good with all three analyzer models, being 95.9 to 99.4 %.

(4) Bacteria
The results of analysis of bacteria are given in ***Table 5***. The ±1 rank agreement was good with all three analyzer models, being 93.9 to 97.7 %. Amorphous salts and artifacts were misidentified as bacteria in the circled groups in the Table. Such misidentifications were more frequent with the image processing analyzers, the US II and the UAS.

(5) Hyaline casts
The results of analysis for hyaline casts are shown in ***Table 6***. It was impossible to do ranking within +1 and +3 agreement with the US II so it was excluded from the correlation analysis. There was ±1 rank agreement of 83.8 % for the UAS and 87.9 % for the UF with manual microscopy. However, as these showed very low sensitivity of 21.2 % and 46.3 % respectively, the microscopic examination of hyaline casts by a technologist seemed to be required.

2) Comparison between the two investigated systems
Tables 7 to 11 compare the analysis results obtained using the UAS and the UF. There was good correlation between them regarding the red blood cells, white blood cells and squamous epithelial cells. However, as in the comparison with manual microscopy, the UAS in some cases misidentified amorphous salts and artifacts as bacteria (***Table 10***). In the analysis of hyaline casts (***Table 11***), 3 cases out of 5 that showed divergent results with the UF (1+) and the UAS (4+), while manual microscopy detected a large number of hyaline casts (4+). In 3 divergent cases of the UF (2+) and the UAS (−) manual microscopy gave (4+) in 1 case and (3+) in 2 cases.

2. Red blood cell morphology

The results of red blood cell morphology analysis are shown in ***Table 12***. At our hospital, we provide a comment with the analysis results only when the red blood cell count is in the range 10–49/HPF and if typical dysmorphic red blood cells are 1–4/HPF, even if the dysmorphic blood cell count is not within 5–9/HPF. Excluding these results, the agreement was 93.5 % for the UAS and 96.8 % for the UF.

3. Detection of atypical cells

The results of detecting atypical cells are shown in ***Table 13***. Of the 9 cases found by manual microscopy to have atypical cells, 5 could be speculated by the US II and 4 by the UAS from their images. However, the precondition for this was that they had to be present in the images. On the other hand, with the UF, when the cutoff value of Atyp.C was 0.2/μL, 8 cases showed results above the cutoff. Confirmation through microscopy was needed for final assessment of atypical cells with all three analyzers.

Table 2 *Correlation of red blood cell analysis results between manual microscopy and the analyzers*

n = 347

US II (/HPF) \ Manual microscopy (/HPF)	<1	1–4	5–9	10–19	20–29	30–49	50–99	100<
100<	1							6
50–99		1				3	4	1
30–49	1	3		1		5	2	2
20–29	3	1	1	1	1	3	1	1
10–19	3	10	4	8	3			
5–9	17	19	17	2	1			
1–4	46	128	3	6	1			
<1	36		1					

n = 347

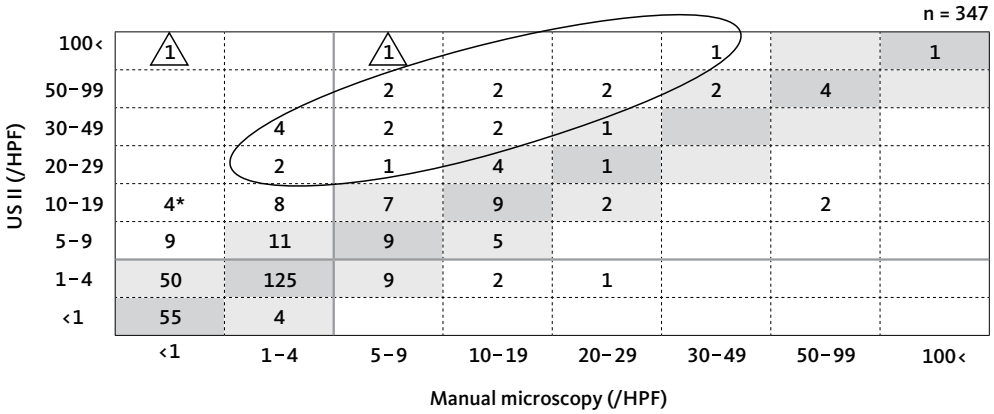
UAS (/HPF) \ Manual microscopy (/HPF)	<1	1–4	5–9	10–19	20–29	30–49	50–99	100<
100<						3	6	9
50–99					1	2	1	1
30–49				2	1	4		
20–29				4	1	1		
10–19	2	13	14	8	3	1		
5–9		7	3	2				
1–4	44	89	7	1				
0–1	61	53	2	1				

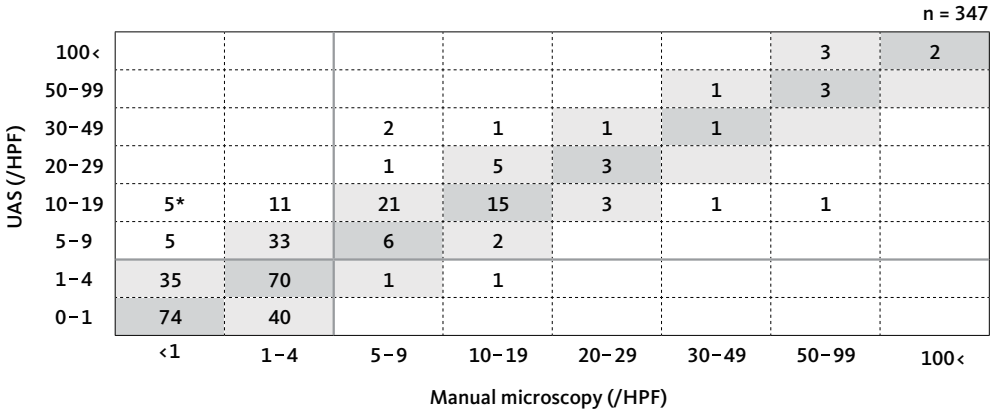
n = 347

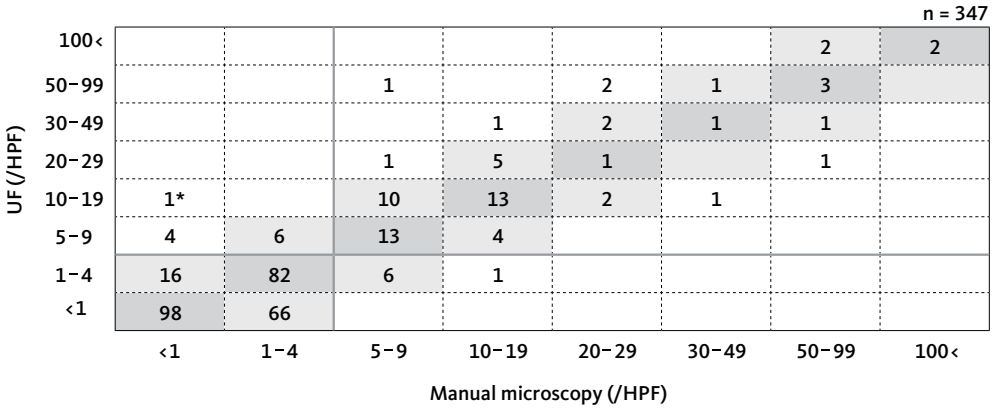
UF (/HPF) \ Manual microscopy (/HPF)	<1	1–4	5–9	10–19	20–29	30–49	50–99	100<
100<							4	8
50–99						8	3	2
30–49				1		2		
20–29		1		3	5	1		
10–19	1	1	5	8	1			
5–9	1	14	11	5				
1–4	22	64	9	1				
<1	83	82	1					

	US II	UAS	UF
Complete agreement (%)	59.1	50.7	53.0
Agreement within ±1 rank (%)	84.1	92.5	98.0
Sensitivity (%)	85.9	85.9	85.9
Specificity (%)	78.1	91.8	93.3

Table 3 *Correlation of white blood cell analysis results of manual microscopy and analyzers*

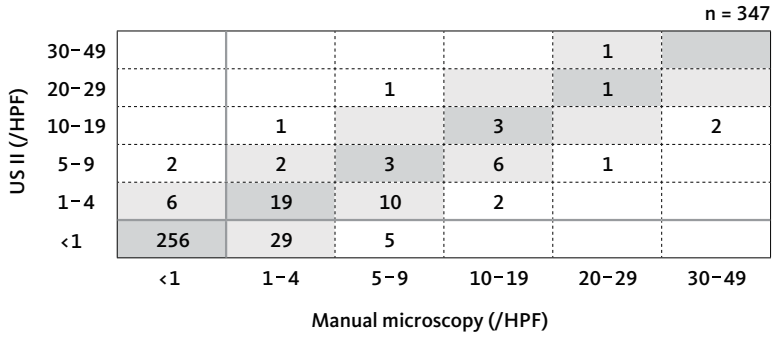


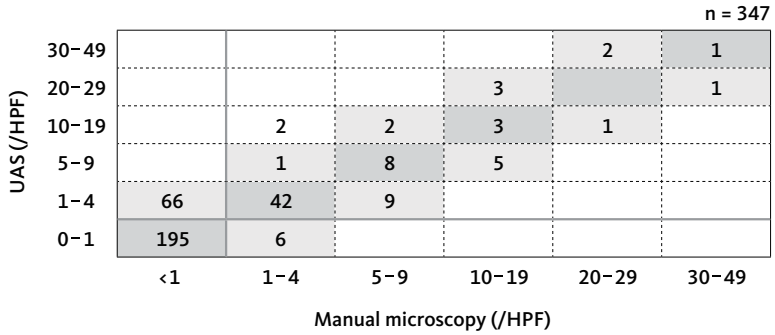


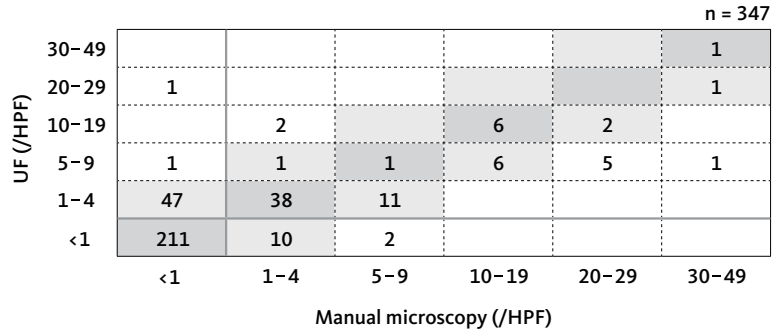


	US II	UAS	UF
Complete agreement (%)	58.9	50.1	61.4
Agreement within ±1 rank (%)	86.7	91.9	96.3
Sensitivity (%)	83.7	97.3	90.5
Specificity (%)	85.7	80.2	96.0

Table 4 *Correlation of squamous epithelial cell analysis results of manual microscopy and analyzers*

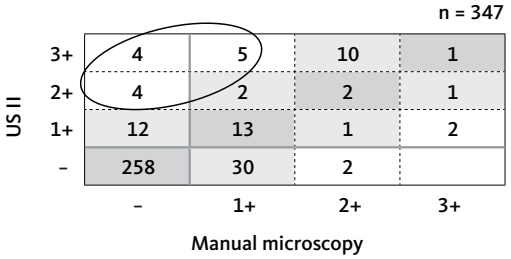


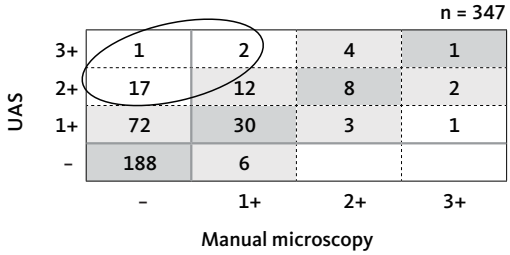


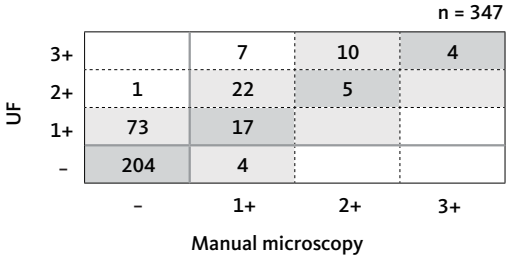


	US II	UAS	UF
Complete agreement (%)	81.3	71.8	74.1
Agreement within ±1 rank (%)	95.9	99.4	96.5
Sensitivity (%)	60.4	83.0	86.0
Specificity (%)	98.0	74.7	80.8

Table 5 *Correlation of bacteria analysis results of manual microscopy and analyzers*

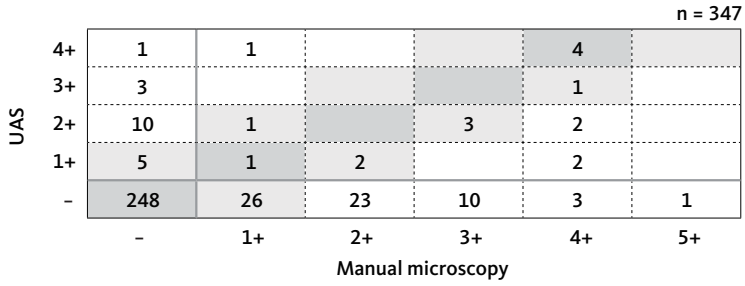


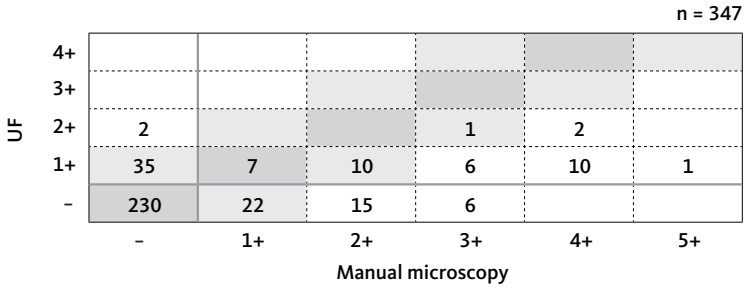




	US II	UAS	UF
Complete agreement (%)	79.0	65.4	66.3
Agreement within ±1 rank (%)	95.1	93.9	97.7
Sensitivity (%)	53.6	91.3	94.2
Specificity (%)	92.8	67.6	73.4

Table 6 *Correlation of hyaline cast analysis results of manual microscopy and analyzers*





	UAS	UF
Complete agreement (%)	72.9	68.3
Agreement within ±1 rank (%)	83.8	87.9
Sensitivity (%)	21.2	46.3
Specificity (%)	92.9	86.1

Table 7 *Comparison of red blood cell analysis results obtained by the two investigated analyzer models*

n = 347

UAS (/HPF) \ UF (/HPF)	0–1	1–4	5–9	10–19	20–29	30–49	50–99	100 <
100 <							6	12
50–99					1		4	
30–49				1	2	3	1	
20–29				2	3		1	
10–19		8	18	10	4		1	
5–9	1	6	4	1				
1–4	65	69	6	1				
0–1	100	13	3	1				

Agreement within ±1 rank (%) 94.8
Complete agreement (%) 59.1

Table 8 *Comparison of white blood cell analysis results obtained by the two investigated analyzer models*

n = 347

UAS (/HPF) \ UF (/HPF)	0–1	1–4	5–9	10–19	20–29	30–49	50–99	100 <
100 <							1	4
50–99						1	3	
30–49			1		1	1	2	
20–29			1	4	2	1	1	
10–19	1	12	15	22	5	2		
5–9	5	33	7	1				
1–4	55	50	2					
0–1	103	10	1					

Agreement within ±1 rank (%) 93.1
Complete agreement (%) 55.6

Table 9 *Comparison of squamous epithelial cell analysis results obtained by the two investigated analyzer models*

n = 347

UAS (/HPF) \ UF (/HPF)	< 1	1–4	5–9	10–19	20–29	30–49
30–49				2		1
20–29			1	2	1	
10–19			3	5		
5–9		5	8	1		
1–4	56	59	1		1	
< 1	167	32	2			

Agreement within ±1 rank (%) 98.3
Complete agreement (%) 69.5

4. Analysis time

The time needed for the analysis is compared in ***Table 14***. In a group of randomly selected specimens, the analysis time for 10 samples was 14 minutes and 45 seconds with the USII, 5 minutes and 15 seconds with the UAS, and 22 minutes and 40 seconds with the UF. The corresponding analysis times were respectively 15 minutes and 30 seconds, 5 minutes and 40 seconds, and 11 minutes and 20 seconds for groups of samples without turbid urine. Thus, the analysis was fastest with the UAS. A significant difference in analysis time was seen with the UF in the randomly selected sample group. This is believed to be because 9 out of the 10 cases were bacteriuria positive samples which required longer automated washing.

DISCUSSION

All three analyzers showed broadly satisfactory agreement with the results of manual microscopy. Especially in the analysis of the red blood cells, both the image processing and FCM methods showed quite a few instances of misidentification of yeast-like fungi and calcium oxalate crystals as red blood cells,[6] but the UAS and the UF, the systems studied here, showed many fewer such instances, thus demonstrating improved performance. The sensitivity for detection of bacteria was low for the US II and UAS analyzers, which use image analysis for the measurement and misidentified amorphous salts and artifacts as bacteria. The UF showed a sensitivity of 94.2 %. It appeared that the use of a reagent that specifically stained nucleic acids and the blue semiconductor laser has improved the detection accuracy of microelements. In the analysis of casts, the performance of all three analyzers was hardly satisfactory. As for the identification of pathological casts type, the US II can be automated classification, while the UF and the UAS cannot. With the UAS, medical technologists can make this assessment by viewing the images. However, the UF has a higher sensitivity for cast detection compared to the two other models. Therefore, pathological casts can be identified by setting suitable review rules and carrying out manual microscopy when needed. It is necessary to cross-check with other parameters and carry out manual microscopy as needed to identify casts with any of these analyzers. The UAS cannot provide much information on atypical cells as unstained images are examined. Therefore, detection of atypical cells largely depends on expertise of the analyst. The UF Atyp.C reflects cell size, nucleic acid content, and size information including complexity of internal structure. It is possible to improve the atypical cell detection rate by comprehensively considering information that is different from what is captured visually and combining it with microscopy. In fact, at cutoff value of 0.2/μL, the detection rate of samples positive for atypical cells was higher with the UF than with the other analyzers. Therefore Atyp.C data from the UF appears to be useful as reference information for use with microscopy. For red blood cell

Table 10 *Comparison of bacteria analysis results obtained by the two investigated analyzer models*

n = 347

UAS \ UF	–	1+	2+	3+	4+
4+					
3+		1		6	1
2+	12	6	10	11	
1+	44	42	17	3	
–	152	41	1		

Agreement within ±1 rank (%) 95.1
Complete agreement (%) 60.5

Table 11 *Comparison of hyaline cast analysis results obtained by the two investigated analyzer models*

n = 347

UAS \ UF	–	1+	2+
4+		5	1
3+	1	3	
2+	5	10	1
1+	7	3	
–	260	48	3

Agreement within ±1 rank (%) 94.8
Complete agreement (%) 76.0

Table 12 *Correlation of red blood cell morphology analysis results of manual microscopy and the analyzers*

n = 34

		Manual microscopy: Non Glomerular	Manual microscopy: Glomerular	Manual microscopy: Comment
UAS	Impossible		2	1
	Glomerular		22	1
	Non Glomerular	7		1

With the UAS, the medical technologist makes an assessment from the images.
Agreement 85.2 %
93.5 % (Those reported with comment were excluded.)

n = 34

		Manual microscopy: Non Glomerular	Manual microscopy: Glomerular	Manual microscopy: Comment
UF	Dys		6	1
	Mixed	1	18	2
	Iso	6		

With UF, the red blood cell information output by the analyzer was used.
Agreement 88.2 %
96.8 % (Those reported with comment were excluded.)

Note:
Dys: Dysmorphic? (Dysmorphic: Estimated to be of glomerular origin)
Mixed: Mixed? (Mixed type)
Iso: Isomorphic? (Isomorphic: Estimated to be of non-glomerular origin)

Table 13 *Detection of atypical cells*

n = 9

	US II	UAS	UF
Number of atypical cells detected	5	4	8
Detection (%)	55.6	44.4	88.9

Table 14 *Analysis time*

Randomly selected groups

	US II	UAS	UF
For analysis of 10 samples	14 min and 45 sec	5 min and 15 sec	22 min and 40 sec
Time taken per sample	1 min and 26 sec	32 sec	2 min and 16 sec

Groups without turbid samples

	US II	UAS	UF
For analysis of 10 samples	15 min and 30 sec	5 min and 40 sec	11 min and 20 sec
Time taken per sample	1 min and 33 sec	34 sec	1 min and 8 sec

morphology, it is indicated that both the UAS and UF provide information that can be used clinically. With the UAS, medical technologists must verify the image. The UF, on the other hand, carries out automated assessment. This makes the analysis efficient and reduces variation in analysis results from one analyst to another, besides providing useful reference information for manual microscopy. The US II and UAS, the image processing analyzers, take almost no time for washing, as dedicated disposable cassettes are used. On the other hand, with the FCM based UF, a longer than usual washing time is required when there is a high concentration of red blood cells, white blood cells or bacteria, which makes the analysis time longer. Therefore, pre-analysis sorting of sample is essential for reducing the analysis time.

Clear imaging is an essential requirement for image processing. In the UAS, the cuvette is centrifuged within the analyzer to concentrate the particles. This results in very clear images. However, when the sample has a high concentration of particles, there is overlapping of the elements making the classification difficult. In some cases, it becomes necessary to edit the images. So, efficient use of the analyzer depends on the expertise of the analyst. Therefore, appropriate protocols for application of the analyzer must be devised. The FCM based UF on the other hand is not affected much by the number of particles present in the sample, as the particles and cells that pass through the flow cell are analyzed one at a time and the analyzer is particularly good in detection of blood cells and bacteria. Since the editing operation by the technologist is unnecessary, results are not affected by their skill, and even the technologist who does not usually perform urinalysis but has to cover for nights or holidays can report results with confidence. Nevertheless, the sorting of samples before analysis becomes necessary with the UF as highly concentrated samples require longer washing. It would be possible to save medical technologist time and labor, make testing efficient, and ensure high quality results if clinical labs exploit the advantageous features of each analyzer and employ logical testing strategies suited for individual medical facilities.

CONCLUSION

The correlation between results of manual microscopy and the results obtained with the three analyzer models studied here was generally good, but both the UAS and the UF, which were the systems investigated here, showed better performance than the currently used US II. It is believed that reduction of labor and improved efficiency and quality of the test results can be achieved by exploiting the advantageous features of these image processing and flow cytometry analyzers. The US II will soon complete ten years of use at our hospital. We also have high hopes for the newly released USCANNER premio.

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