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Comparison of sysmex UF-5000 flow cytometer and fuchs-rosenthal chamber urine sediment analysis

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Abstract

Urine analysis is a routine test performed in clinical practice. Urine sediment analysis is a part of urinalysis that provides precious information to laboratory professionals. Manual review is an application which is time-consuming, as it is the gold standard for analysis. In this study, it was aimed to compare urine sediment analysis performance of the Sysmex UF-5000 flow cytometer with the manual Fuchs-Rosenthal counting chamber. From outpatient clinics, a total of 127 fresh urine samples were analyzed. Sysmex UF-5000 fluorescence flow cytometer was used for urine analysis and Fuchs-Rosenthal counting chamber was used for urine sediment analysis. Two methods were compared using Pearson correlation coefficient (r), Passing-Bablok regression analysis and Bland-Altman plot. CLSI Statis-Pro software version 3.0, Microsoft Excel 2010 and Analyse-it software version 3.80 (Analyse-it Software, Ltd., Leeds, UK) were used. A good correlation was observed between automated and manual white blood cell (WBC) counts in 71 urine samples (r = 0.988; y = 1.162x + 0.489; n = 127). The UF-5000 showed a significant proportional overestimation with the Passing-Bablok regression (95% CI slope: 1.110 to 1.226). Correlation between the counting chamber and UF-5000 was observed in 77 samples for red blood cell (RBC) counts (r=0.996; y=1.1x+0.75). This study showed that flow cytometry urinalysis is a promising area compared to the manual reference method. Urine analyzer automation is commonly used in clinical laboratories all over the world and is effective in reducing report time and workload.

Keywords: Flow cytometer, urine analysis, Fuchs-Rosenthal chamber, UF-5000

Introduction

Urine analysis is a commonly ordered routine test in clinical practice and aids in diagnosing and screening of urinary tract diseases. Urine sediment analysis gives health-care professionals precious information. For the analysis, manual examination is a gold standard method but it requires time and perfect knowledge which can also be affected by the variability of observers. Examination of urine for its cellular constituents under microscope is affected by analytical and preanalytical processes like centrifugation and interpretation of different structures of urine sediment [1]. Therefore manual analysis of urine particles is difficult and is not cost effective due to either lack of trained people or the existence of a high number of samples. With the increasing use of automation in laboratories the whole process of urine analysis is easier and faster.

Lately, fully integrated and automated urine analyzers have developed. They are able to analyze all chemical and physical components of urine so can increase the efficiency of workflow with fast and accurate test results [2].

A reference procedure for measurement is required to evaluate the reliableness of measurement results. Supernatant removal with centrifugation is a tool for urine specimen concentration in urine sediment preparation; on the other hand can be an error source, so may not be a proper application for a reference measurement. Most reliably, counting the particles in uncentrifuged native urine can be done by a chamber counting method. The errors of the chamber method are based on uneven cell distribution in the chamber, observer recognition and technique [3]. For urinary sediment analysis, manual microscopic detection is a reference method. Samples of urine are examined manually under a microscope; however, inspection of each sample brings difficulties with increased turnaround times (TAT), high number of samples and experienced technician deficiency. Thus, a combined strategy of using automated routine urinalysis technology and manual microscopy is an achievable method. [4]. This combination of

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microscopic examination and automated routine urinalysis can be applied to abnormal urine samples; so the samples are reinspected and validated by manual microscopy [5].

This study aimed to evaluate the performance of Sysmex UF-5000 flow cytometer in comparison with the Fuchs-Rosenthal chamber in terms of urine sediment analysis of red blood cells (RBC) and white blood cells (WBC).

Materials and Methods

Patient selection and collection of urine samples

First morning samples of urine were collected from the outpatient clinics and sent to the laboratory within two hours after they were taken. This study was held in a tertiary hospital in October 2018. Individuals between 18-65 years of age admitted to the outpatient clinic in the hospital are included in this study. European urinalysis guidelines are used in this study for the collection, transport and analysis of urine samples [6].

Samples were first analyzed under the microscope visually by using Fuchs and Rosenthal counting chamber then they were applied to Sysmex UF-5000 automated urine analyzer.

This is accepted as the reference method for determination of WBC and RBC in uncentrifuged urine samples. Fuchs-Rosenthal counting chamber using a 10X eyepiece set and 40X lens set with 400X total was used with a phase contrast microscope. Uncentrifuged samples examined by two expert pathologists on manual microscopy without knowing the results from the urine analyser. All specimens examined via manual microscopy.

The UF-5000 is an automated urine analyser manufactured by Sysmex (Japan). The technique is based on the technology of fluorescence flow cytometry. Depolarized side scattered light was added in this analyzer to better evaluate red blood cells versus crystals and increase the sensitivity of crystals. Comparison between the Fuchs–Rosenthal chamber and UF-5000 count in terms of WBC and RBC were evaluated using Pearson's correlation coefficient (r), Passing–Bablok regression analysis and Bland–Altman bias plot in 127 urine samples [7,8].

Ethical approval of the study

The study was approved prior to the initiation, by the decision of the Clinical Ethics Committee (03/26/2018, 2018-06/01). Informed consents of the participants were obtained according to the principles of the Helsinki Declaration.

Statistical analysis

Statistical analysis was performed using CLSI Statis-Pro software version 3.0, Microsoft Excel 2010 and Analyse-it software version 3.80 (Analyse-it Software, Ltd., Leeds, UK).

Results

Comparison between the Fuchs–Rosenthal chamber and UF-5000 count was evaluated using Pearson's correlation coefficient (r), Passing–Bablok regression analysis, and Bland–Altman bias plot in 127 urine samples.

The intercept and slope of Passing–Bablok regression with 95% CI (confidence interval) were calculated for if statistical significance is proportional.

WBC counts changed from 0 to 427.9 × 106 /L; RBC counts from 0 to 258×106 /L in the counting chamber. A good agreement was observed between automated and manual WBC counts in 71 urine samples (r = 0.988; y = 1.162x + 0.489; n = 127) (Figure 1). The mean bias was 8.51×106 /L (95% CI: −29.46 to 46.48) (Table 1). 71 of the 127 urine samples (60.6%) gave results of manual WBC count <20 × 106 /L. However, the UF-5000 showed proportional overestimation which is significant with Passing–Bablok regression (95% CI slope: 1.110 to 1.226). Bland–Altman plot showed a statistically significant bias of 8.51 × 106 /L (95% CI: 5.108 to 11.912 × 106 /L) (Figure 2). Correlation between UF-5000 and the Fuchs–Rosenthal chamber WBC counts demonstrated in Figure 3.

For 77 samples, a good agreement between UF-5000 and the Fuchs Rosenthal counting chamber for RBC was observed (r = 0.966; y = 1.1x + 0.75) (Figure 4). RBC counts with UF-5000 were slightly higher than the counting chamber. The mean bias was 4.47×106 / L (95% CI: −19.39 to 28.32) (Table 1) (Figure 5). Correlation between Fuchs–Rosenthal chamber and UF-5000 RBC counts demonstrated in Figure 6.

Table 1. The comparison of WBC and RBC counts obtained with UF-5000 and the reference counting chamber

		r value	Passing-Bablok regression		Bland-Altman difference plot	
			Slope (95% CI)	Intercept (95% CI)	Mean Bias	95% Limits of agreement (mean bias ±1.96 SD)
WBC	All Sample (n=127)	0.988	1.162 (1.110 to1.226)	0.4890 (-0.1069 to 1.0000)	8.51 (5.108 to 11.912)	-29.46 to 46.48'
	WBC <20 x 10^6 /L (n= 71)				1.08 (0.564 to 1.602)	-3.21 to 5.38'
RBC	All Sample (n=126)	0.966	1.1 (1.038 to 1.180)	0.75 (- 0.1 to 1.25)	4.47 (2.321 to 6.614)	-19.39 to 28.32'
	RBC <20 x 10^6 /L (n=77)				0.89 (0.019 to 1.758)	-6.62 to 8.40'

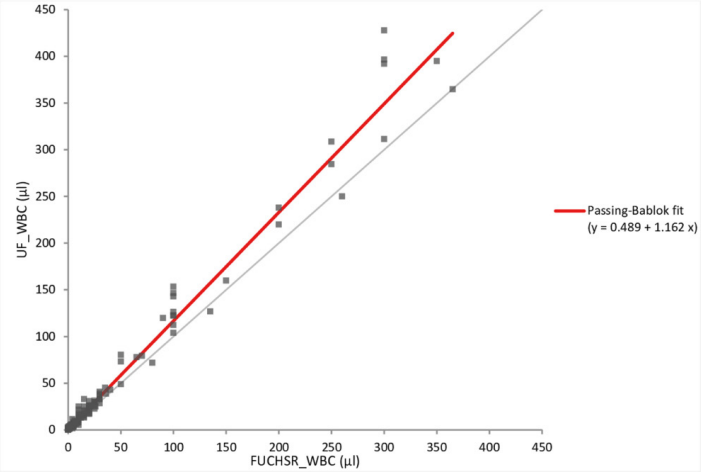

Figure 1. Agreement between automated and manual WBC counts in 71 urine samples in Passing-Bablok regression

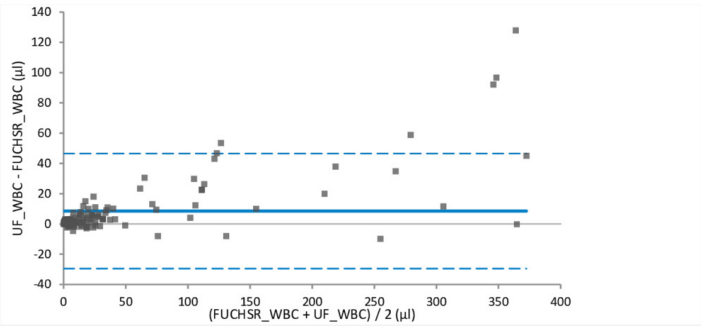

Figure 2. Agreement between automated and manual WBC counts in 71 urine samples in Bland Altman Plot

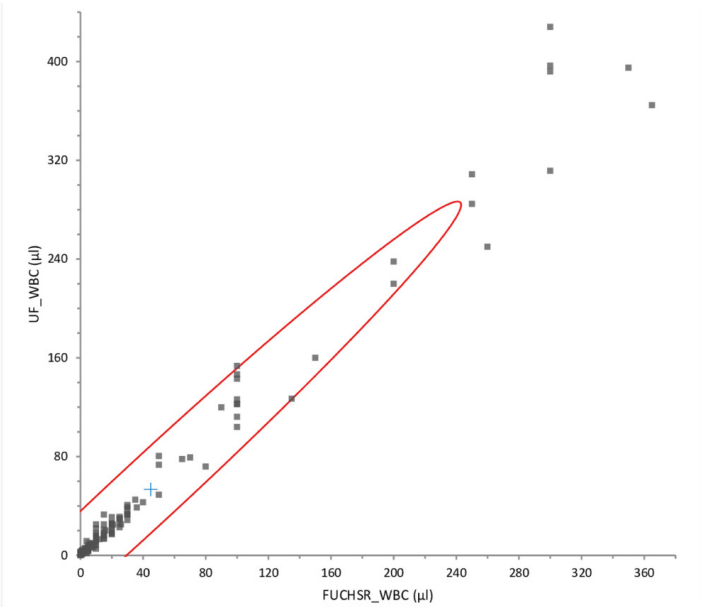

Figure 3. Correlation between automated and manual WBC counts in 71 urine samples

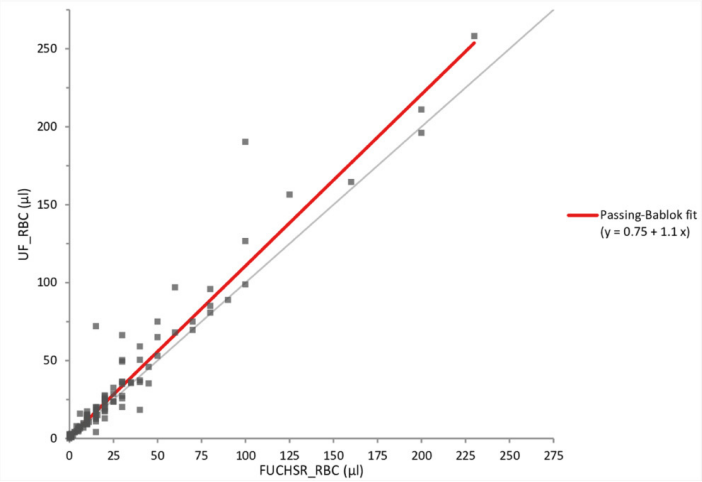

Figure 4. Agreement between automated and manual RBC counts in 77 urine samples in Passing-Bablok regression

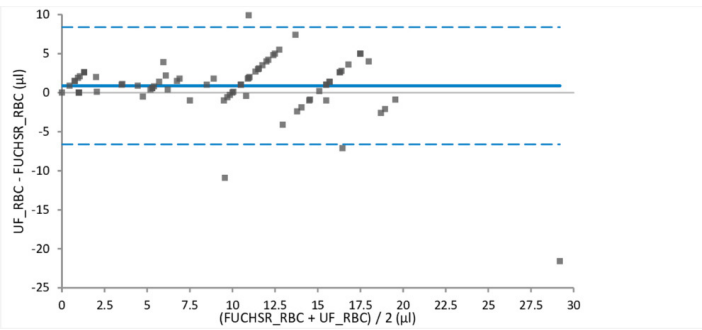

Figure 5. Agreement between automated and manual RBC counts in 77 urine samples in Bland Altman Plot

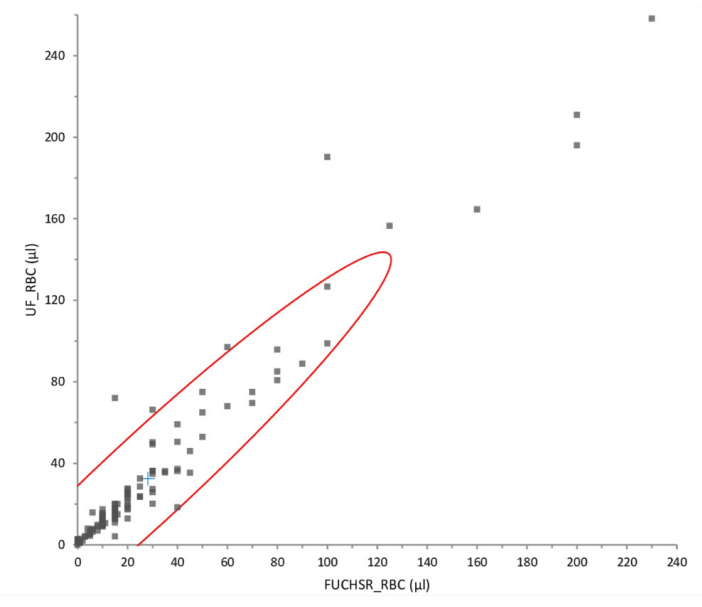

Figure 6. Correlation between automated and manual RBC counts in 77 urine samples

Discussion

Urinalysis is a commonly ordered test by physicians however there is no reference method for urine sediment examination [9]. RBC and WBC are frequently examined in a clinical sense Our results show a good correlation between flow cytometry and Fuchs-Rosenthal chamber. They are consistent with findings of other studies [10,11].

In this study, urine samples were first analyzed by Fuchs Rosenthal chamber for microscopic review which was followed by the use of the UF-5000 automated urine analyzer. Passing Bablok regression and Bland Altman plot for statistical and graphical analysis, which are commonly performed in method comparison studies,

were used. Since components of urine are various and there are limitations of performance in the instrument itself, errors can be seen during the determination of components.

The UF-5000 urine analyzer evaluates morphology of RBC toward size of RBC. This automated urine flow cytometer can easily name the elements of the urine sediment.

UF-5000 enables standardization and has advantages of determining absolute cell numbers per field [12]. Flow cytometry method is used widely by many laboratories. Their performance varies from analyzer to analyzer, most of the time they have successful validation analysis.

Errors also can be seen with the use of Fuchs-Rosenthal chamber due to different techniques, different distribution of the cells in the chamber or recognition of observer; so evaluation of the urine sediment by well trained technicians enables more accurate interpretations in terms of dysmorphic erythrocytes, cylinders or casts [3,13].

As the performance of Sysmex UF-5000 Flow Cytometer in comparison with Fuchs-Rosenthal Chamber method was evaluated, previous researches also appreciated the same and other versions of flow cytometers.

In use and the interpretation of results of Symex UF-5000 Flow Cytometer; Previtali et al. indicated the greatest interest of the analyzer for detecting urine particles which are related to kidney and urinary tract pathologies [14]. Rosa et al. showed the Symex UF-5000 Flow Cytometer's high diagnostic performance among screening of urinary tract infections, detecting gram negative bacterias and urine culture results [15]. Ippoliti et al. suggested that the use of flow cytometers for urine analysis in clinical laboratories can support health care professionals in terms of diagnosis and prevent unnecessary antibiotic use with presence of false-positive results [16]. In use of Sysmex UF-5000 Flow Cytometer for other samples than urine, Seghezzi et al. indicated cerebrospinal fluid analysis showed accurate counts with use of microscopic review in samples with abnormal counts [17].

Performance of other versions of flow cytometers were evaluated in previous research. Shayanfar et al. demonstrate the efficacy of Iris IQ200 microscopy system and Sysmex UF-100 Flow cytometer; in terms of comparison Sysmex UF-100 Flow cytometer showed higher sensitivity than Iris IQ200 microscopy system however with false-positive results in the examination of leukocytes [18]. Wesarachkitti et al. revealed the similar performance of automated urinalysis analyzers Cobas 6500 and UX-2000in urinalysis with advantages toward reduced workload and increased time saving, also suggested use of manual examination in pathological specimens [19]. Lee et al. suggested that while Automated Cobas u 701 Urine Microscopy and UF-1000i have satisfactory results in examination of WBC, RBC and epithelial cells, for other particles in urine sediment still requires visual microscopy [20].

Limitations of this study

There are some limitations in this study. First of all, there were a low number of samples. Validation studies like linearity, accuracy, carry-over and precision are not performed. Determination of red blood cell morphology by means of isomorphic and dysmorphic were not performed. Other components of the urine such as crystals, cylinders were not evaluated.

Conclusion

In conclusion, this study demonstrated that urine analysis with use of flow cytometers and automation in clinical laboratories worldwide can help health-care professionals in practice, it is likely that new analyzers will take more place than the manual visual microscopy in the future. This replacement will reduce the workload as well as TAT and workforce needed in laboratories, also reduce the number of specimens for microscopic evaluation. However examination via microscopy will be applied in some urine specimens to evaluate urine sediment in terms of some elements like casts and crystals.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was approved prior to the initiation, by the decision of the Clinical Ethics Committee (03/26/2018, 2018-06/01).

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