Evaluation of the Performance of the Automated Urine Sediment Analyzer "Urised" Compared with the Manual Method

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ABSTRACT

Objective: To compare the performance of the Urised, an image-based automated urine sediment analyzer, against the manual method in urine sediment analysis.

Methods: The study was carried out in the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University in November 2008. One hundred and one urine samples were measured by both automated urine sediment analyzer "Urised" and manual method routinely done in the laboratory. Results from the both methods were compared for their correlation or agreement. Precision, linearity, and carry over of the Urised were also determined.

Results: Urised and the conventional manual examination were in good correlations for the following parameters: red blood cell (RBC), white blood cell (WBC), crystals, bacteria, and mucus. Although the squamous epithelial cell (SQE) analysed from both methods showed excellent correlation, they were not statistically significant. For pathologic casts, the statistics showed good agreement between the two methods, but the ability to detect pathologic casts of the Urised was less than the manual method. No yeast was found in any specimen included in this study, therefore this parameter could not be evaluated. Poor agreement between both methods for evaluation of small round cell (SRC) and hyaline casts was noted.

Conclusion: Urised was reliable in measurement of some certain urinary parameters such as RBC, WBC, crystals, bacteria, and mucus. However, the examination by experienced technicians was suggested for SRC, hyaline casts, and pathologic casts.

Keywords: Urine sediment, automated urine sediment analyzer, conventional manual examination

Disclaimer : The objective of our study was to evaluate the "Urised" in order to decide to implement it in our laboratory. The personnel that performed the study were from the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital. The machine and any supplies used in the study as well as technical assistance were provided by the manufacturer just for the tests in the study. Besides the supplies previously mentioned, the authors have not received any financial support from the manufacturer. None have any other conflict of interest involving the manufacturer or any company upon this machine.

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Microscopic urine examination is essential in urine analysis. It helps to identify and to approximate the amount of insoluble particles in the urine, and therefore provides useful information for diagnosis and patient monitoring in renal and urinary tract diseases. Although this method is considered labor-intensive, most laboratories still use the conventional method. Because it is simple to perform and easy to interpret. Moreover manual microscopy holds the unavoidable inter-observer variation,[1,2] which makes the patient monitoring sometimes difficult. Presently, people are campaigned by health promotions, resulting in not only ill-persons that are going to the hospital but also healthy people are for check-up. Thus, the demand of urinalysis has also markedly increased leading to more chance of errors and variations.

Recently, the automated urine sediment analyzer with options of two technologies, which are image-based analysis using a built-in camera to take pictures

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and flow cytometry, have been introduced to medical laboratories in order to overcome those problems.[3,4] The aim of this study is to evaluate the efficiency of the Urised (77 Elektronika, Budapest, Hungary), an image-based analyzer, by comparing its performance with the conventional urine sediment examination.

MATERIALS AND METHODS

This study was carried out in the Central Laboratory of the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University during a 10-day period in November 2008. The urine specimens included in this study were unidentified samples, from both in- and out-patients, sent to the laboratory with the request sheets and must fulfill the analytical protocol. Specimens less than 20 ml were excluded. Specimens were divided into two parts. The first part was analyzed by the automated analyzer. The other part was managed manually via routine urinalysis. Both methods were performed in parallel and completed within 2 hours after specimen collection. To minimize the inter-observer variability, one qualified medical technician examined every urine samples in this study.

For automated urine analysis, 2 ml of each urine sample was poured into a test tube and positioned in the Urised. Subsequently, 0.2 ml of urine from each tube was aspirated into a special cuvette. The cuvettes were centrifuged at 2,000 rpm for 10 seconds to force all urine particles down to the bottom of the cuvettes. Fifteen frames of urine sediments were captured from several points by the built-in camera, and examined for the following parameters: red blood cells (RBC), white blood cells (WBC), squamous epithelial cell (SQE), small round cells (SRC) or non-squamous epithelial cell, bacteria (BAC), yeast (YEA), mucus (MUS), crystals (CRY), hyaline casts (HYA), and pathologic casts (PAT). All images were been evaluated by UriSed Test Software version 3.7. The results were displayed in separated categories.

The manual microscopic method was performed according to the work instruction used in the Central Laboratory.[5] After 10 ml of each urine sample was centrifuged in a conical tube at 2,000 rpm for 5 minutes, 9 ml of supernatant was discarded. After resuspension, 50 µl of urine was pipetted onto a glass slide, covered with a coverslip and examined under a light microscopy. The examination was done by estimation of the urine sediment in 10 fields either in the high power field (HPF) or in the low power field (LPF), depending on the type of particles.

For comparing results of both methods, correlation and agreement were determined by statistical analysis using SPSS for Window version 13.0.

To determine the between-run precision of the Urised, commercially assayed control materials from the manufacturer for daily internal quality control were analyzed for RBC and WBC in accordance with the manufacturer's recommendations for 10 days.

For the within-run precision, low and high concentration of the urine particles including RBC, WBC, and bacteria were analyzed 20 times. The precision of each measurement was determined by the *coefficient of variation (CV)*.

To estimate linearity which was the ability of the Urised for counting urine particles in various concentrations of urine samples compared to the actual concentrations, two patient samples were analyzed; one with high WBC counts, another with high bacteria counts. Each specimen was diluted serially with normal pool urine to obtain the fold of 1:1,1:2,1:4,1:8, 1:16, 1:32. The linearity was determined by the *square of coefficient correlation (R^2)*.

Carry over or the contamination across samples was determined by analyzing three patient samples with RBC, WBC, and epithelial cells for three times on the Urised analyzer, followed by the normal urine for three consecutive times. The carry over was calculated using the formula of (N1-N3)/(S3-N3) x100. N1 is the first measure of normal urine while N3 is the third measure of normal urine. S3 is the third measure of the patient sample.

Statistical methods

The data for WBC, RBC, SQE, bacteria, and mucus were categorized as proportional intervals depending on the type of sediments. The correlation between the two methods was determined by *gamma statistics*.

The data for SRC, hyaline casts, pathologic casts, and crystals were categorized as negative or positive. If the crystal was positive, it might be identified according to each urinary crystal specific character. Agreement between two methods was determined by *weighted kappa statistics*.

The *p value* <0.05 was considered statistically significant. The gamma statistics and weighted kappa in a range of 0.5 to 0.75 were defined as good, and more than 0.75 were defined as excellent.

RESULTS

There were 101 urine sediment samples analyzed by both the Urised analyzer and the manual method. The results from both methods were compared. In addition, the precision, linearity, and carry over of the Urised were evaluated.

Comparison of Urised and manual method results

87 samples (86.1%) had their WBC measurements from both methods within the same range. The *gamma statistics* was 0.837 (p <0.001).

90 samples (89.1%) of RBC measured by Urised fell within the same range as the manual method. The *gamma statistic* was 0.918 (p <0.001).

95 samples (94.1%) had their SQE amounts in the same range by both methods. The *gamma statistic* was 0.939 (p = 0.31).

Most urine samples (95 samples) were negative for SRC by both methods. Only one sample was SRC positive, using both methods. The *weighted kappa* was 0.273 (p <0.001).

The bacteria analysis of 61 samples (60.4%) had their results from the two methods within the same range. The *gamma statistic* was 0.834 (p <0.001).

There were 61 samples (60.4%) that yielded the results from both methods for mucus analysis within the same range. The *gamma statistic* was 0.72 (p = 0.001).

For CRY measurement, 99 samples (98.0%) were negative using both methods. One was found positive for calcium oxalate crystals from both methods. The weighted kappa was 0.798 (p <0.001).

Hyaline casts showed poor agreement from both

TABLE 1. Comparison of pair wise results from the Urised and manual method for white blood cell, red blood cell and squamous epithelial cell.

Result	Manual WBC (cells/HPF)						Manual RBC (cells/HPF)						Manual SQE (cells/HPF)					
Urised (cells/HPF)	0-5	5-20	20-50	50-100	>100	Total	0-5	5-20	0-50	50-100	>100	Total	Urised (cells/HPF)	0-5	5-20	20-50	>50	Total
0-5	78	2	0	0	0	80	78	2	0	0	0	78	0-5	94	0	0	0	0
5-20	5	6	0	0	1	12	5	6	0	0	1	5	5-20	1	0	0	0	0
20-50	0	5	1	0	1	7	0	5	1	0	1	0	20-50	2	0	1	0	0
50-100	0	0	0	1	0	1	0	0	0	1	0	0	>50	3	0	0	0	0
>100	0	0	0	0	1	1	0	0	0	0	1	0						
Total	83	13	1	1	3	101	89	7	4	0	1	101	Total	100	0	1	0	101
Gamma				0.837						0.918						0.939		
P value				< 0.001						< 0.001						0.31		

RBC = red blood cell, WBC = white blood cell, SQE = squamous epithelial cell

TABLE 2. Comparison of pair wise results from the Urised and manual method for small round cell, hyaline cast, pathologic cast and crystals.

Result	Manual SRC			Manual HYA			Manual PAT			Manual CRY				
Urised	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	Urised	Negative	Positive Uric	Positive Caox	Positive Total
Negative	95	5	100	89	6	95	96	1	97	Negative	99	1	0	100
Positive	0	1	1	1	5	6	3	1	4	Caox	0	0	1	1
Total	95	6	101	90	11	101	99	2	101	Total	99	1	1	101
Kappa (Weighted)		0.273			0.463			0.622			0.798			
P value		< 0.001			< 0.001			< 0.001			< 0.001			

SRC = small round cell, HYA = hyaline cast, PAT = pathologic cast, CRY = crystals

TABLE 3. Comparison of pairwise results from the Urised and manual method for bacteria and mucus.

Result	Manual BAC						Manual MUC					
Urised (cells/HPF)	Neg	1+	2+	3+	4+	Total	Urised	Neg	Few	1+	2+	Total
0-10	42	4	0	0	1	47	0	65	1	2	0	68
10-20	24	17	1	0	1	43	Few	15	5	2	0	22
50-100	0	1	0	0	4	5	1+	3	3	1	1	8
100-200	0	0	2	1	2	5	2+	3	0	0	0	3
>200	0	0	0	0	1	1						
Total	66	22	3	1	9	101	Total	86	9	5	1	101
Gamma			0.834						0.72			
P value			< 0.001						0.001			

BAC = bacteria, MUC = mucus

methods in 94 samples (93.1%), because 7 samples showed contrasting results. The weighted kappa was 0.463 (p <0.001).

For pathologic casts, there was good agreement between the two methods of 97 samples (96.0%). The weighted kappa statistic was 0.662 (p<0.001). No yeast was found in any sample included in this study. The data of each parameter was summarized in Table 1, 2, and 3.

Precision

Within-run precision of the Urised for measurement of low and high levels of RBC counts were 48.7% and 24.4%, of WBC were 46.9% and 19%, and of bacteria were 35% and 12.9% respectively.

The between-run precision of low level and high levels of RBC QC counts were 35.5% and 48.9% respectively. For WBC, the CV of level I (low level) QC material was 27.4% and that of level II (high level) material was 45.9%. The data were shown in Table 4.

Carry over

Three patient samples were assessed to establish the carry over of the Urised analyzer for RBC, WBC, and epithelial cells. The carry over was 0% in all 3 parameters. These data were shown in Table 5.

Linearity

Linearity assessment was done by analysing 6 dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:32) of two urine samples using the Urised analyzer. One sample was high WBC count. Another was high bacteria count. For WBC analysis in 6 dilutions, the number ranges from 251.2 in undiluted urine to 5.72 cells / HPF in 1:32

TABLE 4. Within-run and between-run precision summary for the Urised.

Component	Categories (cells/HPF)	Within-run precision Mean	SD	CV (%)	Analyze	QC sample	Between-run precision No. of sample	Mean	SD	CV (%)
RBC	0-5	3.9	1.9	48.7	RBC	Level I	10	3.9	1.4	35.5
	>100	108.1	80.7	24.4		Level II	10	13.3	6.5	48.9
WBC	0-5	1.2	44.7	46.9						
	50-100	95.2	0.9	19.0	WBC	Level I	10	15.2	4.2	27.4
BAC	10-20	13.1	5.3	35.0		Level II	10	72.6	33.3	45.9
	50-100	95.3	62.2	12.9						

RBC = red blood cell, WBC = white blood cell, BAC = bacteria RBC level I = low RBC count, RBC level II = high RBC count WBC level I = low WBC count, WBC level II = high WBC count

dilution. This resulted in a *square of coefficient correlation* (R^2) of 0.995. For bacteria analysis in 6 dilutions, the number ranges from 183.4 in undiluted urine to 13.9 cells / HPF in 1:32 dilution. This resulted in R^2 of 0.893. The results were shown in Fig 1.

DISCUSSION

Manual urine sediment analysis is considered labor-intensive, time-consuming, high interobserver variable and exhibits low reproducibility.[1,2] Nowadays, automated urine sediment analyzers are available in the market[6]. A number of previous studies evaluated the performance of those analyzers.[3,4,7,8,9]

In this study, the Urised performance demonstrated good correlation with the manual method in the evaluation of RBC, WBC, mucus, bacteria, and crystals. For pathologic casts, three urine samples were found negative by the manual method, but positive by the Urised. One sample was positive with the manual examination, but negative with the Urised. The review of storage pictures combined with the re-evaluation using conventional examination by an experienced technician showed that all these samples were wrong analysis by the Urised. Therefore, the false positive or miss for pathologic casts in some urine samples might occur although the *weighted kappa statistics* yielded greater than 0.5. For SRC and hyaline casts, Urised yielded poor agreement with manual results. It could detect only one SRC-positive sample, whereas the conventional examination detected 6 samples. From this it could be concluded that the capacity of Urised to detect SRC was lower than that of manual examination. Like SRC, up to 11 urine samples were detected positive for hyaline casts by manual examination compared to only 6 positives by the Urised. Moreover, the *p value* of SQE was not statistically significant. This is probably due to the rarely found SQE in urine samples in this study, resulting in difficult statistical evaluation.

According to the European urinalysis guidelines, the parameters that should be counted most accurately are RBC, WBC, and bacteria.[7] As shown in this study, the capability of the Urised for measurement of RBC, WBC, crystals, bacteria, and mucus were acceptable.

Considering the reproducibility of the Urised, within-run precision of high and low levels of WBC, RBC and bacteria ranged from 12.9% for high level of bacteria to 48.7% for low level of RBC. Although, the precision of the manual method in this study was not evaluated, Tzu-I Chien et al, reported that the CV of the manual method ranged from 12.5% to 43.9%.[7] Thus, it should be implied that the within run precision of the Urised in this study was similar to the manual method. For between-run precision, the CV were 27.4% for a low level of WBC count and 48.9% for a high level of WBC count.

TABLE 5. Carry over analysis of the Urised analyzer.

Run No	RBC (cells/ul)	WBC (cells/ul)	SQE (cells/ul)
S1	45	13.7	39.9
S2	45	12.0	39.0
S3	45	11.6	40.5
N1	0.1	0	0
N2	0	0	0
N3	0	0	0
Carry over %*	0	0	0

RBC = red blood cell, WBC = white blood cell, SQE = squamous epithelial cell

* Carry over % = (N1-N3)/(S3-N3) x100

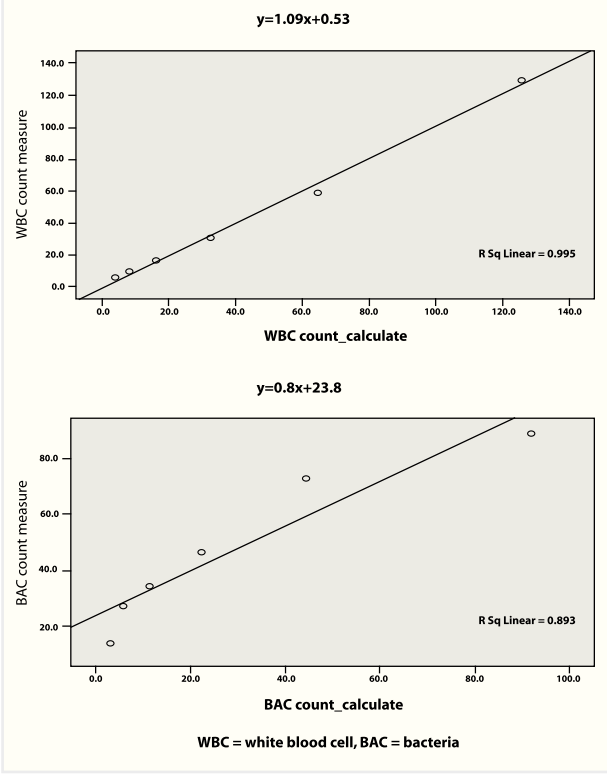


Fig 1. Linearity of counting white blood cell and bacteria with the Urised analyzer.

Linearity was shown in *a square of coefficient correlation* (R^2). R^2 of urine samples with high WBC count showed excellent linearity (R^2 greater than 0.98). The R^2 of urine samples with high bacteria count was acceptable. No carry over that determined contamination across samples was detected.

However, some limitations were considered in this study. The sample size might be too small. In some parameters like SQE and crystals, almost all samples were negative, and therefore unable to give a large enough variety of urine sediments for complete comparison. Moreover, no specimen tested positive for yeast, so the Urised performance could not be evaluated.

In summary, the Urised was considered reliable in the measurement of RBC, WBC, crystals, bacteria, and mucus. However, the detection of SRC, hyaline casts, and pathologic casts, should be further evaluated with the assistance of a technologist. Urised eliminates certain steps in sample preparation, and also provides achieved images for re-evaluation. Review of the stored pictures or manual re-examination is required before finalizing the report. Therefore, if the Urised analyzer is used in the laboratory, it is better applied in the section of routine urine check-up to reduce workload, to increase speed of throughput and to improve turn around time.

The Urised analyzer could not properly replace the well-trained technologists, especially for abnormal urinalysis. It rather helps on reducing human error in many steps of urine preparation. It provides more efficient reports by integration to the laboratory information system. Furthermore this automated urine analyzer can be connected to a reagent strip chemistry analyzer to provide a fully integrated chemical and microscopic analysis of urine.

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