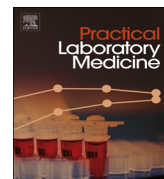


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The comparison of automated urine analyzers with manual microscopic examination for urinalysis automated urine analyzers and manual urinalysis



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ABSTRACT

Objectives: Urinalysis is one of the most commonly performed tests in the clinical laboratory. However, manual microscopic sediment examination is labor-intensive, time-consuming, and lacks standardization in high-volume laboratories. In this study, the concordance of analyses between manual microscopic examination and two different automatic urine sediment analyzers has been evaluated.

Design and methods: 209 urine samples were analyzed by the Iris iQ200 ELITE (Iris Diagnostics, USA), Dirui FUS-200 (DIRUI Industrial Co., China) automatic urine sediment analyzers and by manual microscopic examination. The degree of concordance (Kappa coefficient) and the rates within the same grading were evaluated.

Results: For erythrocytes, leukocytes, epithelial cells, bacteria, crystals and yeasts, the degree of concordance between the two instruments was better than the degree of concordance between the manual microscopic method and the individual devices. There was no concordance between all methods for casts.

Conclusion: The results from the automated analyzers for erythrocytes, leukocytes and epithelial cells were similar to the result of microscopic examination. However, in order to avoid any error or uncertainty, some images (particularly: dysmorphic cells, bacteria, yeasts, casts and crystals) have to be analyzed by manual microscopic examination by trained staff. Therefore, the software programs which are used in automatic urine sediment analyzers need further development to recognize urinary shaped elements more accurately. Automated systems are important in terms of time saving and standardization.

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1. Introduction

An accurate urine sediment analysis is a good indicator of the status of the renal and genitourinary system. General indications for urinalysis are: the possibility of urinary tract infection or urinary stone formation; non-infectious renal disease secondary to systemic diseases such as rheumatic diseases, hypertension, toxemia of pregnancy, or to the adverse effects of drugs; non-infectious post-renal disease; in pregnant women and patients with diabetes mellitus or metabolic

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states who may have proteinuria, glycosuria, ketosis or acidosis/alkalosis [1]. The traditional strategy recommended by the European Urinalysis Guidelines consists of two steps [1]. In the first step, there is a visual inspection and dipstick test. Semi-quantitative dipstick tests are used in this step to exclude the urine samples from further analysis if hemoglobin, leukocyte esterase activity, nitrite and protein are negative. In the second step, if there is erythrocyturia, leukocyturia, bacteriuria or proteinuria, urine samples are subjected to further analysis by microscopy. Because the first step has poor sensitivity and negative predictive value, screening by dipstick alone carries the risk of missing infections and other urinary diseases [2–5].

There are different manual methods for urine sediment examination such as counting in a standardized or non-standardized way under a coverslip or counting of centrifuged or uncentrifuged urine specimens in a chamber. Traditional (non-standardized) urine sediment analysis has been used in many laboratories. However, because of wide uncertainty of results and reduced sensitivity in detecting essential formed elements, the non-standardized sediment procedure was not recommended. Standardized urine sediment examination under a coverslip instead of non-standardized sediment procedure is recommended as a routine visual procedure for kidney-related urine formed elements. A reference method for urine microscopy should provide both correct identification of the different formed elements and quantify them accurately. Currently, no such method exists. In particle counting, bright-field microscopy of unstained preparations is inadequate for detection of bacteria, erythrocytes and hyaline casts. For this reason, either supravital staining or phase-contrast microscopy, or both, is recommended for better examination [1]. However, phase-contrast microscopy is not available in every laboratory.

The potential variables in the microscopic examination of the urine are as follows: the speed and time of centrifugation, the amount of urine remaining in the tube for resuspension, and whether urine is stained. Manual microscopic examination requires well trained and experienced staff and consumes a considerable amount of time. Therefore, automatic urine sediment analyzers for high-volume laboratories were developed in order to provide better standardization, improve the certainty of measurement and save staff time.

The methodology of urine particle analysis started with the introduction of automated microscopes and flow cytometry devices inspired by blood cell counting [1]. These analyzers use two analytical principles for urine sediment analysis, one based on electrical impedance, and the other dependent on image-based analysis systems that sort particles according to preset particle dimensions. It is not currently clear which principle is superior.

The image-based analysis systems automatically scan the formed elements of flowing urine and display the images of formed elements on a screen. Before reporting the results of analysis, the shaped elements must be examined visually by well trained staff who can decide to approve, delete or reclassify them. However, laboratories who have made the transition from manual microscopic method to automatic systems still have some concerns about the concordance of results.

The use of the automated analyzers (Iris iQ200 ELITE and Dirui FUS-200) has gradually increased in medical laboratories. These analyzers are both image-based analysis systems and there has not been any published study comparing the two instruments. We evaluated the concordance between manual microscopy and the two automatic urine sediment analyzers.

2. Materials and methods

209 randomly selected patients who attended the laboratory of our hospital were studied. All patients provided signed written consent prior to enrollment into the study, and the study was performed in compliance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

The collection, transport, preparation of specimens and urinalysis was performed according to European Urinalysis Guidelines [1]. Mid-stream samples (30 mL) were collected into primary containers which had no risk of spillage, transported in the primary containers and transferred to secondary containers (three different conical tubes) in the laboratory. The secondary tubes were translucent to allow a clear view of the sample. 10 mL urine sample was added to each tube. Each sample was examined within one hour by the three methods. The first tube was centrifuged for 5 min at 1500 rpm (400 g) for manual microscopic examination. The supernatant was decanted until 0.5 mL urine remained at the bottom of the tube. The sediment was resuspended, and then one drop of sediment was placed on a microscope slide, covered with a cover slip and examined by light microscope. Evaluation of urine formed elements was performed by one biochemistry specialist and one biologist, independently using the same microscope slide. During the examination, at least 10 different microscopic fields were scanned at magnifications of $\times 100$ and $\times 400$ (per low power field; LPF and per high-power field; HPF). The results were calculated by averaging the formed elements and reported as cells or particles in a field. If there was an inconsistency between the results of the two evaluators, the analysis was repeated with another sample in order to resolve the discrepancy.

The evaluation of urine formed elements in the other two (uncentrifuged) tubes was performed on the Iris iQ200 ELITE (Iris Diagnostics, Chatsworth, CA, USA) and Dirui FUS-200 (DIRUI Industrial Co., Changchun, China) automatic urine sediment analyzers. The results from the instruments were obtained as average of formed elements per LPF and HPF. The analytical principle of the Dirui FUS-200 analyzer is flow cell digital imaging and identification using an artificial intelligence technique. The analytical principle of the Iris iQ200 ELITE analyzer is Digital Flow Morphology technology using the Auto-Particle Recognition (APR) software. As the urine passes through the flow cell, urine is illuminated by a special light source, and the images are recorded by a digital camera placed into the eyepiece of the microscope and transmitted to the computer. The software classifies these images and displays them on the screen for the operator. The operator accepts, changes

or deletes these sediment images.

To determine the coefficients of variation for between run imprecision, we used the results of positive controls (Dirui FUS-200 positive control and Iris iQ200 positive control) instead of the native urine samples (for stability reasons) over 20 days. Within-run imprecision was determined using two different pooled urine specimens with various concentrations of erythrocytes, leukocytes and epithelial cells tested on 20 runs with each analyzer. The results were expressed as the number of particules/HPF.

Statistical analyses were performed by SPSS Statistics 20.0 (Statistical Package for Social Sciences version 20.0, IBM Corporation, Armonk, NY, USA) and Excel 2007 (Microsoft, Seattle, WA, USA) programs. Erythrocytes, leukocytes and epithelial cells were classified semi-quantitatively (0–5, 6–10, 11–20, > 20 cell/HPF). Bacteria, yeast, casts and crystal were classified as negative or positive. The semi-quantitative elements were also classified as positive or negative, positive results being those exceeding the cutoff values, defined as 5/HPF for leukocytes, erythrocytes and epithelial cells.

Cohen's kappa coefficient was calculated for concordance between the methods [6]. Values for Cohen's kappa coefficient of 0–0.21, 0.21–0.40, 0.40–0.60, 0.61–0.80 and 0.81–1.00 are characterized as poor, fair, moderate, good, and very good agreement, respectively [7]. Concordance rates within the same grade were calculated. The analytical sensitivity, specificity and positive and negative predictive value for the automated analyzers relative to manual microscopic examination were assessed.

3. Results

The within-run imprecision for erythrocytes, leukocytes and epithelial cells (CV%, mean, \pm SD of pooled urine specimens) of the FUS-200 and Iris iQ200 was 4.9% (73.3 ± 3.6 particles/HPF) and 5.0% (161.5 ± 8.1 particles/HPF), respectively. The between-run imprecision (CV%, mean \pm SD of positive control) of the FUS-200 and Iris iQ200 was 5.0% (178.4 ± 9.9 particles/HPF) and 5.9% (177.2 ± 10.5 particles/HPF), respectively.

The pairwise agreement within the same grade for erythrocyte, leukocyte and epithelial cells is shown shaded Tables 1 and 2. The best concordance between methods was in the erythrocyte counting. Compared to the manual method, the instruments detected fewer leukocyte and epithelial cells in the 6–10 cells/HPF range. Agreement between the Iris iQ200 and the manual method was higher than the agreement between the FUS-200 and the manual method for erythrocyte and leukocyte, and this was reversed for epithelial cells (Table 3).

The evaluation is based on clinical positive results (Table 4). It is clear that manual method and automated analyzers show agreement for erythrocytes and leukocytes, but the concordance between the two instruments is much better.

For epithelial cells, the concordance between the manual method and instruments was moderate, but the concordance between the two instruments was much better.

For bacteria and crystals, the concordance between the manual method and the instruments was again moderate, and the concordance between the two instruments was much better regarding bacteria and crystals. For yeast, the concordance between instruments and the manual method was fair, and the concordance between two devices was moderate. The count of casts showed no concordance between all methods.

Table 1

Comparison (numbers of samples in each category) between manual microscopy, FUS-200 and Iris iQ200. The shaded numbers indicate samples with the same grade agreement.

Particules	Erythrocytes (cells/HPF)	Manual microscopy													
		Erythrocytes (cells/HPF)				Leukocytes (cells/HPF)				Epithelial cells (cells/HPF)					
		0-5	6-10	11-20	>20	0-5	6-10	11-20	>20	0-5	6-10	11-20	>20		
FUS-200	0-5	167	8	1	0	0-5	110	26	3	1	0-5	145	20	1	0
	6-10	7	1	4	1	6-10	4	12	6	1	6-10	7	15	1	0
	11-20	0	0	4	2	11-20	0	2	9	4	11-20	3	1	8	1
	>20	2	3	1	8	>20	1	2	4	24	>20	0	0	2	5
Iris iQ200	0-5	172	2	2	0	0-5	108	19	2	0	0-5	153	29	2	0
	6-10	7	4	0	1	6-10	4	15	2	1	6-10	2	7	4	0
	11-20	1	1	6	2	11-20	2	4	11	0	11-20	0	0	5	4
	>20	0	0	0	11	>20	1	4	7	29	>20	0	0	1	2

Table 2

Comparison (numbers of samples in each category) between the two automated analyzers. The shaded numbers indicate samples with the same grade agreement.

		Iris iQ200											
		Erythrocyte (cells/HPF)				Leukocyte (cells/HPF)				Epithelial cell (cells/HPF)			
FUS-200		0-5	6-10	11-20	>20	0-5	6-10	11-20	>20	0-5	6-10	11-20	>20
	0-5	173	3	0	0	125	12	3	0	164	2	0	0
	6-10	4	3	5	1	4	10	7	2	15	8	0	0
	11-20	0	0	3	3	0	0	6	9	5	3	5	0
	>20	3	1	0	10	0	0	1	30	0	0	4	3

Table 3

Agreement of urinalysis results between the FUS-200, Iris iQ200 and manual microscopy represented as the rate(%) with the same grade.

	Erythrocytes	Leukocytes	Epithelial cells
FUS-200 vs Manual	86.1	74.1	82.7
Iris iQ200 vs Manual	92.3	77.9	79.9
FUS-200 vs Iris iQ200	90.4	81.8	86.1

Table 4

Degree of concordance between methods for clinically positive results.

	FUS-200 vs Manual		Iris iQ200 vs Manual		FUS-200 vs Iris iQ200	
	Kappa (95% CI)	p Value	Kappa (95% CI)	p value	Kappa (95% CI)	p Value
Erythrocytes	0.68 (0.54–0.82) ^c	< 0.001	0.77 (0.65–0.90) ^c	< 0.001	0.81 (0.70–0.92) ^d	< 0.001
Leukocytes	0.65 (0.55–0.76) ^c	< 0.001	0.73 (0.63–0.82) ^c	< 0.001	0.81 (0.72–0.89) ^d	< 0.001
Epithelial cells	0.59 (0.46–0.72) ^b	< 0.001	0.50 (0.36–0.64) ^b	< 0.001	0.62 (0.48–0.76) ^c	< 0.001
Bacteria	0.47 (0.33–0.62) ^b	< 0.001	0.52 (0.38–0.67) ^b	< 0.001	0.82 (0.70–0.93) ^d	< 0.001
Crystals	0.54 (0.37–0.71) ^b	< 0.001	0.57 (0.40–0.74) ^b	< 0.001	0.69 (0.49–0.88) ^c	< 0.001
Yeast	0.17 (–0.14–0.47)	0.001	0.19 (–0.13–0.52)	< 0.001	0.67 (0.05–1.00) ^c	< 0.001
Casts	0.13 (–0.06–0.32)	0.051 ^a	0.10 (–0.13–0.32)	0.135 ^a	0.04 (–0.10–0.18)	0.45 ^a

^a $p > 0.05$, Kappa is not significant. There is no agreement between methods.

^b Moderate agreement.

^c Good agreement.

^d Very good agreement.

Table 5

Sensitivity, specificity and predictive values for automatic analyzers compared to manual microscopy.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
FUS-200				
Erythrocyte	72.7	94.9	72.7	94.9
Leukocyte	68.1	95.7	92.8	78.6
Epithelial cell	61.1	93.7	76.7	87.3
Iris iQ200				
Erythrocyte	75.8	97.7	86.2	95.6
Leukocyte	77.7	93.9	91.2	83.7
Epithelial cell	42.6	92	92	83.2

Analytical sensitivity, specificity, and positive and negative predictive value were assessed relative to manual microscopic results (Table 5). The specificity of the automated instruments was found to be better than their sensitivity.

4. Discussion

Recently, FUS-200 and Iris iQ200 analyzers have been adopted for use in medical laboratories for microscopic examination of urine. To our knowledge, this is the first study which has compared the FUS-200 with the Iris iQ200. Both instruments employ similar technology.

In our study, we classified the cellular elements to compare manual microscopic examination with automatic analyzer according to the clinical decision limit.

For clinically positive results, the concordance between manual method and the two instruments ranged from good to moderate for erythrocytes, leukocytes, epithelial cells, bacteria and crystals. The concordance between the manual method and the instruments was fair for yeast. There was no concordance between all methods for casts. Generally, the concordance between the two instruments was better than between instruments and the manual method for all cells.

4.1. Erythrocytes, leukocytes and epithelial cells

Chein et al. [8] showed that the correlation between the Iris iQ200 and manual microscopy was good for erythrocytes and leukocytes. Even though there was no significant difference between the Iris iQ200 and the manual method, the cell counts of the Iris iQ200 were higher than the manual method at high erythrocyte and leukocyte counts.

In the manual method, many steps such as centrifugation, decantation and re-suspension may lead to cellular lysis and loss. Various studies have reported a good correlation between the Iris iQ200 and manual cell counts for erythrocytes, leukocytes and epithelial cells [9–11], however, the Iris iQ200 found fewer cells than manual methods [10].

Budak et al. found that leukocyte counting by instruments is higher than by the manual method [12]. The Iris iQ200 automatically resuspends particles by injecting a bolus of air into each sample before testing. This might dissociate cellular clumps, resulting in cell counts that are higher than those recorded by manual counting methods.

Akgün et al. found that the erythrocyte and leukocyte counting of Iris iQ200 was more successful than manual methods, and the epithelial cell counting was less successful. This is because the Iris iQ200 does not count deformed epithelial cells [13]. According to our classification, the agreement between the manual method and two instruments for leukocytes and epithelial cells was good. But the instruments detected lower cell counts in the 6–10 cells/HPF range of the manual method. The relative agreement rate between the methods was of erythrocyte > leukocyte > epithelial cells. The concordances between the Iris iQ200 and manual method for erythrocytes and leukocytes were higher than those between the FUS-200 and manual method, but lower for epithelial cells.

In the study of Shayanfar et al. [14], the detection sensitivities of the Iris iQ200 for erythrocytes and leukocytes (70% and 76%, respectively) were found to be similar to our study. Another study determined the sensitivities of the Iris iQ200 for erythrocytes and leukocytes as 75.8% and 85.5%, respectively [15]. In our study, the Iris iQ200 demonstrated good diagnostic sensitivity and specificity for all elements except epithelial cells.

The automated instruments take photographs of the morphological structures. They do not count damaged leukocytes but may count distorted and disrupted cells as artefacts. Shayanfar et al. state that the Iris iQ200 counts fewer erythrocytes if abnormal erythrocytes such as ghosts and dysmorphic cells are present [14]. In some cases, falsely high erythrocyte counts may occur due to misclassification of yeasts. Similar false positive results have been reported by Wah et al. [10]. Therefore, urine samples from patients suffering from kidney disorders must be analyzed by manual microscopy [16].

In contrast to our study, Dewulf et al. [17] found sensitivities of the Iris iQ200 for erythrocyte and leukocyte to be 95% and 100%, respectively; the negative predictive values were 93% and 100%, respectively. They speculated that the poor specificity and positive predictive value for erythrocytes (24% and 42%, respectively) were due to insensitivity of the manual method used for comparison [17].

Yüksel et al. [18] found the sensitivity of the Dirui FUS-100 for erythrocytes and leukocytes (73% and 68%, respectively) to be similar to our study. Additionally, the concordance between the FUS-100 and the Urised analyser was found to be better than manual microscopy [18].

4.2. Bacteria

Most of the problems occurred in analysis of microorganisms, and a possible reason for this is the limited ability of classification software [14]. Chein et al. found bacteria in most samples by microscopic examination in comparison to the Iris iQ200 [8]. Some researchers have stated that the images of small cocci detected by the Iris iQ200 were difficult for the medical technologist to classify as “bacteria”, except for some rod forms [12,19]. Other studies suggested that the presence of bacteria should be confirmed by manual microscopy [20,21]. Koçer et al. found low detection sensitivity for the FUS-200 analyzer compared to urine culture as the reference method [21]. Similar to other studies, we found bacteria in more samples by manual microscopy in comparison to the instruments. On the other hand, the automated urine analyzers count both live and dead bacterial particles giving higher particle counts. This is a limitation of all automated urine analyzers compared with culture [22].

4.3. Crystals

Akgün et al. stated that the IrisiQ200 has more positive results than the manual method for crystal [13]. Some false-positive results were observed due to the evaluation of dysmorphic erythrocyte as crystals by the Iris iQ200 [14]. In our study, the concordance between the manual method and analyzers was moderate, and the instruments detected crystals in fewer samples in comparison with the manual method. Careful manual microscopic re-inspection has been recommended [23–25].

4.4. Yeast cells

Some studies found that the Iris iQ200 had a high false positive rate for yeast cell [8,9,19]. It was stated that yeast or crystals were not key elements for basic particle analysis, and could be eliminated by adjusting the corresponding thresholds in Iris iQ200 reports [8]. As we found, the results of yeast cell analysis on Iris iQ200 have been shown to be in fair agreement with manual microscopy [14,19]. Therefore, it is necessary to review the stored images or confirm the results by manual microscopy.

4.5. Casts

The detection of casts by automated systems is difficult [8,12]. In our study, because of the low number of pathological specimens, all varieties of casts were considered in a single group. As stated in other studies, we found that the results of the analyzers for casts were showed poor agreement with manual methods. However, Shayanfar et al found the Iris iQ200 to be generally good at detecting casts, but unable to distinguish the type of cast [14]. In the presence of casts, microscopic examination is recommended [16,26–29].

4.6. Limitations of study

The primary limitation of our study was the low number of pathological samples. Thus, we could not differentiate between squamous and non-squamous epithelial cells; type of crystal and amorphous mass; types of candida and mycelia for yeast. The results of microscopic examination were not supported by urine dipstick results. We did not perform linearity, accuracy or carry-over studies. Our results for the FUS-200 could not be compared with others in the literature, due to the lack of published studies on this instrument.

5. Conclusion

Some studies have indicated that automated analyzers are superior to manual microscopy for detection of urine formed elements, especially in measuring erythrocytes, leukocytes, casts and bacteria [30,31]. Automatic analyzers can rapidly analyze a large number of urine samples with high repeatability. However, especially in pathological cases, confirmation of urinalysis results by manual methods or dipstick testing is recommended because of the limitations of automated analysers [18,32,33]. In addition, proper attention to specimen collection and storage is essential to obtain reliable results and to avoid artefacts.

Well trained staff are also important if the urine elements detected by automatic analyzers are to be evaluated correctly. Some formed elements (particularly dysmorphic cells, bacteria, yeasts, casts and crystals) must be analyzed by a manual method.

It has been suggested that automatic urine analyzers can be used for screening urine samples. When combined with urine chemistry analysis, these analyzers may provide a rapid and accurate screening in routine urine analysis. Automation of urine sediment examination may decrease the inter-individual variation and reduce the manual review rate. The main error-prone steps such as centrifugation and manual preparation are omitted and therefore sample analysis is completed in a very short turnaround time [19,27]. Shayanfar et al. indicated that in a laboratory performing approximately 25,000 urinalyses per year, this strategy reduced the workload by approximately 30% [14].

Automated urinary sediment analysers are indispensable in terms of improved standardization of measurement and more efficient working, but the ability of the software programs to recognize the urine formed elements accurately needs to be improved.

Conflict of interest

The authors did not receive financial or any other support whatsoever in the experimental work or in the preparation of their manuscript.

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