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The Comparison of Automated Urine Analyzers with a Manual Microscopic Examination for Urinalysis-Experience at Tertiary Care Hospital

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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 Iris iQ® 200 automatic urine analyzers has been evaluated.* ***Design and methods:*** *250 urine samples were analyzed by the Iris iQ® 200 and by manual microscopic examination. The degree of concordance (Kappa coefficient) and the rates within the same grading were evaluated.* ***Results:*** *In our study manual method and automated analyzer (Iris iQ® 200) show agreement for almost all parameters studied, but concordance for leukocyte and bacteria is very good. For erythrocyte it is moderate. For epithelial cells, cast and crystal concordance is good. Even for mucous and yeast, there is an agreement between the automated analyzer and manual method.* ***Conclusion:*** *We conclude that automated urine sediment analysis is sufficiently precise and improves the workflow in a routine laboratory significantly. However, to avoid any error or uncertainty, some images (particularly: dysmorphic cells, bacteria, yeasts, casts, and crystals) have to be analyzed by manual microscopic examination. Therefore, the software programs which are used in automatic urine sediment analyzers need further development to recognize urinary-shaped elements more accurately.*

Keywords: Automation, Urine analysis, Urine particles, Urine microscopy

INTRODUCTION

Urinalysis is the third major *in vitro* diagnostic screening test in clinical practice, only behind Serum chemistry and complete blood count [1]. It is one of the most common non-invasive tests for assessing urinary tract and kidney disease. A correct urinalysis result offers a direct indication of the state of the patient's renal and genitourinary system and monitoring of other body systems. Although manual analysis procedures are standardized it is a tedious job if the sample load is more. There are many steps in manual procedure which may cause loss of cell or lysis of cells. The study aimed to evaluate the concordance between manual microscopy and the Iris iQ® 200 automatic urine sediment analyzers.

The Iris iQ200 Urine Microscopy Analyzer is a second-generation automated urine analyzer developed by Iris Diagnostics Inc. (Chatsworth, California, USA), the pioneer of automated urine microscopic instruments since the 1980s [2,3].

The iQ200 analyzer (Iris Diagnostics, Chatsworth, CA, USA) uses laminar flow digital imaging technology as identification software classifies and quantifies the cells and particles in uncentrifuged urine using a single, laminar flow of the specimen through the lens of a charged coupling device video camera. The hundreds of digital camera captures are evaluated by identification software, and refined neural network algorithm APR (Automated Particle Recognition system) uses particle size, shape, contrast, and texture as features to enhance its classification of detected particles [2,4]. Images are stored and can be viewed on the workstation screen, thereby eliminating the need for manual microscopy in most cases [5].

After the classification by the instrument, the operator can reclassify or correct the obtained images in the correct categories. Various studies have reported a strong correlation between iQ200 output and manual cell counts for erythrocytes, leukocytes, and epithelial cells [5].

The objective of the present study was to compare the iQ® 200 microscopic analyzers (Iris Diagnostics, Chatsworth, CA, USA), with manual urine microscopy, using similar parameters (cells or particles per LPF (Lower-Power Field) or HPF (High-Power Field)) for evaluation.

MATERIALS AND METHODS

Inclusion Criteria

We studied 251 freshly collected urine samples (both from outpatient department and in patient department) at central chemical laboratory of tertiary care hospital, Smt. Kashibai Navle Medical College, Narhe, Pune

Only midstream urine samples with 15 ml or more than 15 ml volume samples are selected for study

Exclusion Criteria

Samples less than 15 ml volume, contaminated and spilling out of container are excluded from study

Samples with preservative (24 hour urine collection samples) are excluded from study

Samples are collected in wide mouth sterile transparent containers with no spillage risk and analysed within 1 hr of collection. Primary collected samples are then divided in to two different conical and transparent tubes. In first tube, 3 ml of sample for automated analyser (Iris iQ® 200) is poured.10 ml sample is taken in 2nd test tube and centrifuged for 5 min at 1500 rpm (400 g). Supernant is decanted until 0.5 ml of sample is remained at the bottom of 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 (Lynx by Lawrence and mayo model LM52180). Manual evaluation of urine formed elements was performed by two pathologist, independently using the same microscope slide. During the examination, at least 10 different microscopic fields were scanned at magnifications of 100x and 400x (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 both fields (LPF and HPF) 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. 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. Bacteria, yeast, casts and crystal were classified as negative or positive. Erythrocytes, leukocytes and epithelial cells were classified as semi-quantitatively (0-5 cell/HPF, 6-10 cell/HPF, 11-15 cell/HPF, 16-20 cell/HPF). The semi-quantitative elements were also classified as positive or negative, positive results being those exceeding the cut-off values, defined as 5/HPF for leukocytes, erythrocytes and epithelial cells.

RESULTS

The pairwise agreement within the same grade for erythrocytes, leukocytes, and epithelial cells is shown in Table 1, Table 2, and Table 3. The best concordance between methods was leukocyte counting. Compared to the manual method, the Iris iQ® 200 detected fewer leukocyte and epithelial cells in the 6-10 cells/HPF range.

The evaluation is based on clinical positive results (Table 4). It is clear that manual methods and automated analyzers show agreement for almost all parameters studied, but concordance for leukocytes and bacteria is very good. For erythrocyte it is moderate. For epithelial cells, cast and crystal concordance is good. Even for mucous and yeast, there is an agreement between the automated analyzer and manual method.

The sensitivity, specificity, positive and negative predictive values were obtained using established criteria and given in Table 5. The specificity of Iris iQ® 200 is better than sensitivity.

Table 1 Erythrocyte, leukocyte, and epithelial cell counts by manual microscopy

	Cells/HPF by manual microscopy				
Parameter	0-5	6-10	11-15	16-20	>20
Erythrocyte	235	11	1	4	0
Leukocyte	229	4	3	14	1
Epithelial cell	250	1	0	0	0

Table 2 Erythrocyte, Leukocyte and Epithelial cell counts by automated urine analyzer Iris iQ200

	Cells/HPF by automated urine analyzer (Iris iQ200)				
Parameter	0-5	6-10	11-15	16-20	>20
Erythrocyte	207	15	3	15	11
Leukocyte	215	9	0	8	19
Epithelial cell	247	3	0	1	0

Table 3 Comparison (numbers of samples in each category) between manual microscopy and Iris iQ200

	Manual																
Iris iQ200	**Erythrocyte Cells/HPF**	0-5	6-10	11-15	16-20	>20	**Leucocytes Cells/HPF**	0-5	6-10	11-15	16-20	>20	**Epithelial cell/HPF**	0-5	6-10	11-15	16-20	>20
	0-5	207	0	0	0	0	0-5	215	0	0	0	0	0-5	247	0	0	0	0
	6-10	10	5	0	0	0	6-10	8	1	0	0	0	06-Oct	3	0	0	0	0
	11-15	2	0	1	0	0	11-15	0	1	0	0	0	Nov-15	0	0	0	0	0
	16-20	13	0	0	2	0	16-20	3	0	1	2	1	16-20	0	1	0	0	0
	>20	3	6	0	2	0	>20	3	2	2	12	0	>20	0	0	0	0	0

Table 4 Degree of concordance between methods for clinically positive results

Sr. No	Parameters	Iris iQ200 *vs.* Manual		
		Kappa value	95% CI	p-value
1	**Erythrocyte**	0.55	0.42-0.65	<0.001
2	**Leukocyte**	0.87	0.83-0.90	<0.001
3	**Bacteria**	0.97	0.96-0.98	<0.001
4	**Epithelial cell**	0.63	0.53-0.71	<0.001
5	**Crystals**	0.68	0.59-0.75	<0.001
6	**Cast**	0.67	0.57-0.74	<0.001
7	**Mucous**	0.64	0.54-0.72	<0.001
8	**Yeast**	0.61	0.50-0.70	<0.001

Table 5 Sensitivity, Specificity, and predictive values for automatic analyzer compared to manual microscopy

Sr. No.	Variables	Iris iQ200 *vs.* Manual microscope			
		Sensitivity	Specificity	PPV	NPV
1	**Erythrocytes**	88.05	100	100	63.64
2	**Leukocytes**	93.89	100	100	38.89
3	**Bacteria**	100	100	100	0
4	**Epithelial cell**	98.8	100	100	75

5	**Crystals**	100	100	100	57.15
6	**Cast**	100	100	100	0
7	**Mucous**	93.82	100	100	65.22
8	**Yeast**	96.29	100	100	52.94

DISCUSSION

Indian laboratories recently started adapting automated urine analyzers for routine microscopic examination purposes. To our knowledge, this is the first study in India that has compared Iris iQ200 and the manual microscopic method.

In this study, we use certain parameters which are related to microscopic examination only, like erythrocytes, leukocytes, bacteria, yeast, etc. These are the parameters that we use to help clinicians in routine lab work. In our study, we have not considered biochemical analysis.

For clinically positive results the concordance between manual method and automated instrument ranged from good to very good for leukocytes, bacteria and moderate for erythrocytes. For other parameters it is good.

Erythrocytes, Leukocytes, Epithelia Cells

Chein, et al. showed the correlation between Iris iQ® 200 and manual microscopy was good for erythrocytes and leukocytes [6]. The cell counts of Iris iQ® 200 were higher than the manual method at higher erythrocytes and leukocyte counts.

According to FD Ince, et al. agreement between the automatic analyzer and manual machine is good for leukocyte and epithelial cell [3].

In the manual method, there may be cellular lysis and loss of cell due to processing and handling of the sample at many steps such as centrifugation, suspension, resuspension. Various studies have shown there is an agreement between the manual method and Iris iQ200 for erythrocyte count, leukocytes, and epithelial cells [2,4,7].

In our study also there is a higher agreement in leukocytes than epithelial cells and erythrocyte.

The automated instrument does not count damaged leukocytes but may count distorted and disrupted cells as an artifact. In a study done by Shayanfar, et al. Iris iQ200 counts fewer erythrocytes if abnormal erythrocytes such as ghost and dysmorphic cells are present in some cases falsely high erythrocyte count may occur due to misclassification of yeasts [8]. Similar false-positive results have been reported by Wah, et al. therefore urine samples from patients suffering from kidney disorders must be analyzed by manual microscopy [4].

In our study machine detects a higher number of erythrocytes in the 16-20 erythrocyte/HPF range.

Dewulf, et al. 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 [9]. They speculated that the poor specificity and positive predictive value for erythrocytes (24% and 42%, respectively) were due to the insensitivity of the manual method used for comparison.

In our study, the negative predictive value for erythrocyte & leukocyte is 63% and 38% respectively. In our study, we found a moderate amount of agreement for erythrocyte and 100% specificity.

Bacteria

There are some problems in the analysis of microorganisms and a possible reason for this is the limited ability of classification software [8]. Chien, et al. found bacteria in most samples by microscopic examination in comparison to the Iris iQ200 [6]. FD İnce, et al. found bacteria in more samples by manual microscopy in comparison to the instruments [3]. In our study, there is very good agreement between automated machines and manual microscopy noted. Studies mentioned above recommended manual microscopy if bacteria presence is noted in urine by machine.

Crystals

Some false-positive results were observed due to evaluation of dysmorphic erythrocyte as crystal by Iris iQ200 study done by FD İnce, concordance between manual and automated machine was moderate [3]. They stated that automated instrument detects fewer samples in comparison with manual method. In our study, the automated machine was unable

to classify some common crystals and mentioned them as unclassified crystals. In 7 out of 250 numbers of cases, the machine gave a higher number of crystals. Careful manual microscopic re-inspection is recommended for the classification and confirmation of crystals in many other studies [3,10,11].

Yeast Cell

According to Chien, et al. yeast cell/crystals were not key elements for basic particle analysis and could be eliminated by adjusting the corresponding thresholds in Iris iQ200 reports [6]. It also stated that Iris iQ® 200 had a high false-positive rate for yeast cells. In a study done by FD İnce, there is a fair agreement for yeast cell analysis between Iris iQ200 and the manual microscopic method [3]. In our study yeast, cells have a good agreement and confirmed by manual microscopy.

Still, some studies suggest review the store images or confirm the results by manual microscopy, and to some extent, we agree with the same [8,12].

Casts

Detection of the cast by the automated system was found difficult for some study groups [2,12]. In our study, for analysis, we consider all casts in a single group and found moderate agreement with manual methods. FD İnce, et al. in their study found that there is poor agreement between automated machine and manual microscopy method [3]. Shayanfar, et al. stated that Iris iQ® 200 was good in detecting casts but unable to distinguish the type of cast [8]. Both (FD İnce, et al. and Shayanfar, et al.) studies recommended manual microscopic examination in presence of casts.

In our study also we found that automated machine is unable to classify the type of cast at certain instances but the agreement is good between machine and manual microscopy.

Limitation of Study

The primary limitation is the low number of positive/abnormal samples. We have not received a urine sample from a patient suspected or diagnosed with urinary tract malignancy during our study period. Thus we are not able to comment on the differentiation of atypical cells or malignant cells from epithelial cells. We did not perform linearity, accuracy, or carry-over studies. We have not included the biochemical parameters of the automated machine in our study.

The automated urine analyzers count both live and dead bacterial particles giving higher particle counts. This is a limitation of all automated urine analyzers should be compared with culture to differentiate contaminated sample from actual infection [3]. However, this was not within the scope of our study.

CONCLUSION

In India due to a high number of urine samples each time manual microscopic method is not possible so automatic urine analysis is gaining importance nowadays. Automated systems are important in terms of time-saving and standardization.

An automatic analyzer can analyze a large number of samples in a short period and reduce the turnaround time. However, to avoid any error or uncertainty, in pathological cases, some images (particularly: dysmorphic cells, bacteria, yeasts, casts, and crystals) have to be analyzed by manual microscopic examination. Therefore, the software programs which are used in automatic urine sediment analyzers need further development to recognize urinary-shaped elements more accurately.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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