Original Article

DOI: 10.21276/APALM.2612

Laboratory Diagnosis of Renal Disorders: Automated Urine Sediment Analyzer Compared with Manual Methods

Sharanya K[1] and Prasanna N Kumar[2]*

[1]Department of Pathology, KMCH Institute of Health sciences and research, Coimbatore -641 014, India.
[2]Department of Pathology, PSG institute of Medical sciences and Research, Coimbatore -641 014, India.

ABSTRACT

Background: Urinalysis is one of the earliest methods used to screen and detect patients with kidney diseases. It also helps to monitor and assess the severity of the disease process in already diagnosed patients. Microscopy plays a vital role in routine urinalysis and gives more information when analyzed together with chemical strip tests. Introduction of automation of the conventional methods of urinalysis has reduced the disadvantages of manual methods in terms of accuracy of results and turnaround time.

Method: Aim of the present study is to evaluate the performance of an automatic urinalysis system – FUS-100 (which in cooperates an automatic urine chemistry analyzer H-800) manufactured by Dirui Industrial Co. Ltd., China in patients with renal diseases and compare the results of microscopy with manual microscopic analysis. In addition, our study aims to find out the possibility of safely reducing the number of manual microscopy analyses by cross-interpretation of the results generated by FUS-100 automated urine particle analyzer with manual methods. The urine sediments of five hundred urine samples were examined by these two methods.

Result: Automation of urine analysis decreases the turnaround time and is less labour intensive leading to better accuracy and precision.

Conclusion: Preanalytical errors related to centrifugation and sediment preparation are prevented in automation. Our study tends to suggest that automation of urine microscopy therefore is a more standardized procedure and makes urine microscopy a more objective investigation.

Keywords: *Automation, Renal Disorders, Manual Methods, Urinalysis, Casts, Crystals, Cells*

Introduction

Urinalysis provides information and clues to many diseases and can also be an indication of the condition of a patient's health. In patients with nonspecific symptoms and severe kidney diseases, urinalysis serves as one of the early laboratory investigations to initiate treatment.[1,2]

Routine urinalysis is mainly done by inspection, chemical testing and microscopic examination of the urinary deposits which can have cells, casts, crystals or microorganisms. Traditionally, microscopy has always been done by manual methods. Manual examination of the urinary sediment, even though considered a time tested and standard method is labour intensive and involves considerable time, leading to delays in reporting. Automation of urinalysis has been introduced to reduce the disadvantages of the manual method.

The present study evaluates the results generated by an automated urine analyzer FUS -100, which incorporates an automatic urine chemistry analyzer H-800(manufactured by M/S. Dirui industrial Co.Ltd., China) in patients with renal diseases and compares the results of microscopy obtained by automation with manual microscopic analysis. Simultaneously, control samples from a population without knowing renal disorders were also studied. It was felt that such a study would be useful, if it is designed to find out the possibility of safely reducing the number of manual microscopy analyses by cross-interpretation of the results of the FUS-100 automated urine particle analyzer with manual methods.[1,2,3]

Materials and Methods

This was a prospective observational study, performed on urine samples received in the Clinical Pathology laboratory, Department of Pathology. Routine urinalysis done during a period of one year was analysed. A total of 500 urine samples collected from both outpatients and inpatients were examined within an hour of receipt. Physical and chemical analysis including tests for detecting glucose, ketones, proteins, blood, urobilinogen, pH, specific gravity, haemoglobinuria, myoglobinuria, hemosiderinuria, bile salts and bile pigments were performed on these samples. All the samples were collected exclusive of preservatives. The study included patients with confirmed renal disorders and those suspected to have renal disorders based on abnormal renal function tests. The clinical details and diagnosis related to all the urine samples were obtained from the test request forms.

After receiving the samples, a dipstick chemical strip analyses of the urine samples were performed using the automatic urine chemistry analyzer H-800. Results obtained were used to screen further microscopic urine analysis. An abnormal urine was considered as one in which any of the following parameters tested were out of range - blood, proteins, nitrites, ketones, urobilinogen, bilirubin, WBCs and glucose. The rest of the urine samples were considered as normal controls.

For performing automated microscopic analysis (FUS-100), uncentrifuged urine samples were placed on the sample rack provided with the analyzer. The FUS100 aspirates 0.95 mLof urine and at the end of one minute, the artificial intelligence identification software within the analyzer classifies the particles into twelve categories (Red blood cells, white blood cells, white blood cell clumps, hyaline casts, pathological casts, squamous epithelial cells, non-squamous epithelial cells,bacteria,yeast, crystals,sperm, mucus) present in the given sample by capturing each of their images. Using this classification the software calculates the visible component concentration by taking into account the number of images and the scanned urine volume. All visible component images of a given urine sample are shown on the monitor in separated gridsand presented in the form of numbers/low power or high power field. FUS Focus, FUS positive control, and FUS negative control were run according to the manufacturer's instructions were run every day before testing the urine samples.

The samples apportioned for manual microscopy were gently mixed and centrifuged in a centrifuge test tube at 1500rpm for five minutes and the supernatant was discarded. A drop from the remaining sediment was placed onto a microscope glass slide and covered by means of a cover slip. Microscopic examination of the sediment was done initially under low power (10X) to identify casts and then high power (40X) was used for erythrocytes, leukocytes, crystals, epithelial cells, bacteria, yeast and other significant findings. An average of ten fields was taken and the results were calculated semi-quantitatively and expressed as a range (Table-1).

Among the randomly examined samples with renal function abnormalities, the microscopic sediments quantified from the manual method was compared with that of the automated analyzer.

Results

The following are the results for the various constituents of the urine sediment examined through automation and by manual microscopy.

The area under the curve (AUC) and sensitivity for detecting RBCs by the manual method were 0.8648 and 82%, respectively. By automated method using FUS-100 these values were 0.8763 and 86.5% respectively, which are higher than the values obtained by manual method (Table 2). The changes noticed were seen to be statistically significant, suggesting that FUS-100 analyzer is more efficient than the manual method at detecting RBCs when compared to the manual method. However, the specificity and PPV for detecting RBCs through manual method was found to be higher (Table 2). The AUC for WBCs was registered as 0.6568 by FUS-100 while with manual method was found to be 0.6524 (Table 2). This result reflects that the accuracy of both the methods had comparable results in detecting WBCs, though the result of FUS-100 is marginally higher. The Sensitivity for detecting WBCs was once again marginally increased with the investigations performed by FUS-100 (50.5%), as against the manual method (48%) (Table 2). However, we found a higher specificity for WBCs (80.28%) with the manual method. Finally, the PPV for detecting WBCs by manual method is higher (63%) than with FUS-100 analyzer which is 62%.

In the people without any renal abnormality, nearly 92.7% were detected to have epithelial cells (within a range of 0 to 5) by automated microscopy whereas only 84.7% were detected to have them by manual method. The specificity of manual method in recognizing epithelial cells was 98% and positive predictive value was 70%. However FUS-100 had a better negative predictive value which was 59 % (Table 2).

Among the study population, 482(96.4%) cases detected by FUS-100, 283(94.3%) cases detected positive for bacteria were patients who did not have any renal abnormalities, whereas in the manual method such cases constituted only 265 (88.3%) (Table 3). This shows that automated microscopy is more efficient in detecting bacteria when compared to the manual method whereas,yeasts were detected with equal accuracy by both the methods. However, since simultaneous cultures were not done in any of the cases studied, it is not possible to determine if these bacteria are contaminants or clinically significant entities.

Among the 200 patients with renal abnormalities, the sediments contained a variety of crystals like Ammonium Biurate, Amorphous urates, calcium carbonate, uric acid, etc. Among these, some of the crystals were identified by both the methods, while a few others were identified only by one of the methods. The details of distribution of these deposits are tabulated (Table 4). Additionally, the FUS-100 analyzer classified some of the amorphous urates and calcium carbonates as RBCs, which were reclassified while reviewing the analyzed images.

In addition to the entities mentioned above, FUS-100 identified a total of 74 cases to have hyaline casts out of which only 35 cases truly had the casts. Out of the 35 true positive cases, 3 cases were seen in patients with renal abnormalities in the range of 6-10. However, the manual method was able to detect 29 cases of hyaline casts out of which 26 were in the range of 0 to 5 and the other 3 cases in the range of 6 to 10. Waxy casts, noted in a patient by manual microscopy, was not detected by the automated analysis (Table 4). However, FUS-100 categorizes most of the detected casts and crystals as "unclassified" making it essential for the operator to review the images on the screen before finalizing the reports from the analyzer.

Statistical Analysis: All the diagnostic test evaluation analyses sensitivity, specificity, positive predictive values and negative predictive values for FUS-100 automated urine sediment analyzer and manual microscopic method were done using Medcalc statistical software & STATA statistical software package release 11. Simple calculations like percentages, proportions and mean values were derived. A type I error of 0.05 was considered in all analyses.

Table 1: Semi- quantitative range classification of urine particles used for this study.

Parameters	Ranges				
Erythrocyte/ HPF	0 – 5	6 – 10	11 – 20	21 – 30	> 30
Leukocyte/ HPF	0 – 5	6 – 10	11–20	21-30	>30
Epithelial cells/ HPF	0 – 5	6 – 10	11-20	21-30	>30
Casts/LPF	0 -5		5-10		>10
Crystals/ HPF	Positive		Negative		

Abbreviations: HPF, High power field; LPF, Low power field.

Table 2: Diagnostic accuracy of urine sediment analysis between the automated and manual methods.

	RBC		WBC		Epithelial Cells	
	Automated	**Manual**	**Automated**	**Manual**	**Automated**	**Manual**
AUC	0.8763	0.8648	0.6568	0.6524	0.5141	0.5124
SE	0.0159	0.0167	0.0218	0.0216	0.0119	0.0076
95% CI	0.8441 to 0.9038	0.8312 to 0.8937	0.6130 to 0.6986	0.6083 to 0.6946	0.4681 to 0.5598	0.4648 to 0.5597
Z	23.7	21.9	7.21	7.04	1.19	1.63
P Value	<0.01	<0.01	<0.01	<0.01	>0.05	>0.05
Sensitivity	86.5	82	50.5	48	8.08	3.66
95% CI	81.0 - 90.9	76.0 - 87.1	43.4 - 57.6	40.9 - 55.2	4.7 - 12.8	1.5 - 7.4
Specificity	83.61	84.48	79.25	80.28	94.6	98.82
95% CI	78.9 - 87.6	79.8 - 88.5	74.2 - 83.7	75.2 - 84.7	91.3 - 96.9	96.6 - 99.8
PPV	78	79	62	63	52	70
95% CI	71.9 - 83.2	72.3 - 83.9	54.4 - 69.8	54.6 - 70.4	33.1 - 69.8	32.8 - 94.1
NPV	90	87	70	69	59	58
95% CI	86.1 - 93.5	82.7 - 90.9	64.9 - 75.1	63.8 - 74.0	54.4 - 63.7	52.9 - 62.4

Abbreviations: AUC, area under the curve; SE, standard error; CI, confidence interval; PPV, Positive Predictive Value; NPV ,Negative Predictive Value.

Table 3: Distribution of cells other than RBCs and WBCs in the study population

Cells Other than RBCs and WBCs	Automated			Manual		
	Normal renal function	Abnormal renal function	Total	Normal renal function	Abnormal renal function	Total
Urine without other particles	16	0	16	34	0	34
Bacteria	283	199	482	265	199	464
Yeast	1	1	2	1	1	2
Total	**300**	**200**	**500**	**300**	**200**	**500**

Table 4: Details of crystals and casts identified in the study population.

Casts/crystals	Automation			Manual		
	Normal renal function	Abnormal renal function	Total	Normal renal function	Abnormal renal function	Total
Nil	267	159	426	273	158	431
Ammonium Biurate	0	6	6	0	6	6
Amorphous Urates	0	1	1	0	1	1
Calcium Carbonate	0	2	2	0	2	2
Cholesterol crystals	0	1	1	0	1	1
Calcium Oxalate	1	18	19	1	18	19
Uric acid crystals	0	4	4	0	4	4
Sulfa crystals	0	1	1	0	1	1
Triple phosphate crystals	0	4	4	0	4	4
Hyaline casts	32	3	35	26	3	29
Granular casts	0	1	1	0	1	1
Waxy casts	0	0	0	0	1	1
Total	**300**	**200**	**500**	**300**	**200**	**500**

Discussion

Urinalysis is one of the earliest methods used to screen and detect patients with kidney diseases. In many laboratories, urinalysis is restricted to chemical strip analysis, which in fact, is a cost-effective and widely accepts screening method. However, microscopy plays a vital role in routine urinalysis and gives more information when analyzed together with chemical strip tests.[4] Better results can be obtained only with the use of standardized techniques for this procedure.

Manual microscopy of the urine may be affected by preanalytical variables such as the speed and time for centrifugation, sediment preparation and interpretation all of which may lead to imprecise and inaccurate results. For a reliable urine microscopy report, the examiner should be skilled to recognize the constituents of the urine sediment correctly and possess knowledge about the clinical importance of detecting substances in urinary sediments. With the introduction of automation in all spheres of clinical laboratory practice, it is no wonder that in the recent past, companies have come out with equipments that automate urine microscopy. [5,6]

A number of instruments offering automated urine analyses are now available. These include the IRIS iQ200, the UF1000i, sediMAX and more recently the DIRUI FUS -100 Series urine sediment analyzer.[7] However, there are only a few studies that have compared the results from automated urine analyzers and microscopic methods in parallel.

This study made use of fresh samples of urine, examined within an hour of collection. However, in reality, when there is a delay in transporting urine samples, they are processed after refrigeration. The reliability of results for such samples derived from the FUS analyzerhas not been studied and is unknown.

Manual examination of urine requires the sample to be centrifuged for five minutes, after which it is examined. It is thus advantageous that the FUS-100 uses urine samples for analysis which are not centrifuged and reduces the turnaround time.

In our study, we found that the sensitivity for RBCs was better in FUS-100 while the specificity for detecting erythrocytes was higher in the manual method. This may be because of the fact that FUS-100 classifies calcium crystals, yeast and sometimes amorphous urates as RBCs. Hence, this will require reclassification. Literature shows similar studies which have made a comparison of FUS-100 with UriSed and manual methods. These studies reveal higher sensitivity for detection and discrimination of RBCs with FUS-100 than with UriSed and manual method.[5,7]

Yuksel et al reported that FUS-100 shows high sensitivity (73%) and high negative predictive value for RBCs (95%) when compared with manual microscopy. Similar to their results, in our study we found that the sensitivity (86.5%) negative predictive value (90%) and also AUC (0.8763) were higher for RBCs with FUS-100 which indicates minimal false negative results even though the possibility of false positive results are more when compared with manual microscopy.[5,7] The automated analyzers iQ200 and UF-100 recognize dysmorphic RBCs in their routine urinalysis and this has been substantiated through studies. [7,8] However this recognition is not possible with the FUS-100.

Leukocytes are routinely noticed in normal urine (upto 5 cells/HPF) and also in interstitial nephritis and proliferative glomerulonephritis.

In our study, we have shown that FUS-100 has a higher sensitivity for RBCsand WBCs in comparison with conventional manual methods. This may be due to the fact that the analyzer recognizes epithelial cells, RBCs and crystals as WBCs. We have been able to detect squamous epithelial cells in the range of 0 to 5 cells/HPF more frequently with FUS-100 (99%) when compared with manual method (95.5%). In the case of the iQ200, David et al in their study state that cell counts for erythrocytes, WBCs and epithelial cells correlate well with manual counts using counting chambers. They also found out that the AUC did not alter even after correcting the captured images for erythrocytes, WBCs and squamous epithelial cells. He concluded that manual review can be greatly reduced when the interpretation of automated analyzer is combined with chemical strip test.

Increased numbers of hyaline casts in urine indicate pathological conditions like acute glomerulonephritis and other renal diseases with proteinuria. We found that the FUS-100 classifies 14.8% (74 cases) of particles as hyaline casts out of which only 7% (35 cases) were true hyaline casts as evidenced by microscopy. The stored images which were captured by the in-built imaging system were also reviewed and the findings were in agreement with what was seen in the manual microscopy.

The falsely elevated hyaline cast count (14.8%) generated by the FUS-100 is due to the inclusion of folded squamous epithelial cells. This was also seen with other analyzers like the sediMAX, iQ200 and UriSed, built imaging system. Granular casts are usually associated with tubulointerstitial disease.[5,7,8] We found that FUS-100 recognized granular casts in patient suspected to have renal abnormalities as did the manual method.

Waxy casts are usually associated with serious renal pathologies like chronic diseases and amyloidosis. [9,10]

Increased uric acid crystals may be associated with hyperuricosuria and acute nephropathy.[7] In our study, we noticed four cases with uric acid crystals in patients with abnormal renal function. All the four cases were recognized by both automated and manual microscopic methods.

This study reveals that even though the FUS-100 was able to recognize and classify RBCs, WBCs, and squamous epithelial cells, all the crystals and casts other than hyaline casts are categorized as “unclassified” by this analyzer. This therefore requires technical assessment of the components of the urine sediment. This observation was also reported in the study done by Yuksel et al. and by Wah DT et al who recommend that a technician review the captured images by the iQ200 analyzer for all casts, WBC clumps and yeasts for confirmation. All the above studies support our observations with the FUS-100. The inability to identify casts in automated urine microscopy is an intrinsic limitation of this technology.

In order to eliminate the false positive results seen in automated urine microscopy a dedicated and well trained technician is required for the urinalysis workstation to visualize and correct the wrongly labeled cells and to classify the images shown as abnormal casts or “unclassified” before finalizing the results. However, the results that were analysed in this study were unedited results from the analyzer. For the correct identification of unknown particles and pathological casts, the manual method is still required for confirmation.

Conclusion

The FUS-100 automated urine particle analyzer performance is better than the manual microscopy for detecting RBCs, WBCs and squamous epithelial cells when compared with the manual method. Though it recognizes some crystals and casts, it fails to classify them. Therefore, manual urine microscopy must supplement the urine examination, especially in patients with abnormal renal function and abnormal urine chemical analysis.

Conventional microscopy is still required for identifying dysmorphic erythrocytes, yeasts, Trichomonas, oval fat bodies, and for the differentiation of various casts and crystals.

Automation of urine analysis decreases the turnaround time and is less labour intensive leading to better accuracy and precision. Automated urine microscopy combined with dipstick results can be used as a screening procedure for large numbers of urine samples in places with a high workload.

Abbreviations

RBC – Red blood cells
WBC –White blood cells
PPV- Positive predictive Value
NPV- Negative predictive Value
AUC-Area under the curve
HPF- High power field

References

1. McPherson RA, Ben-Ezra J. Basic examination of urine. In: McPherson, Pincus MR. Editors. Henry's Clinical diagnosis and Management by Laboratory methods. 22nd Ed. Ch. 28. Philadelphia: Saunders Elsevier; 2011.
2. Clinical and laboratory standards Institute, Urinalysis: Approved guideline- 3rd Ed. 2009.
3. European urinalysis group. European urinalysis guidelines. Scand J Lab Invest 2000; 60:1-96.
4. Darci RB, John CL. Automated urinalysis in the clinical lab. Medical laboratory observer; 2012.
5. Yuksel H, Kilic E.E, Ekinsi A, Evliyaoglu O. Comparison of fully automated urine sediment analyzers H800-FUS100 and LabumatUrised with manual microscopy. J Clin Lab Anal. 2013; 27:312-316.
6. Coppens A. Speeckaert M, Delanghe J. The pre-analytical challenges of routine urinalysis. Acta Clin Belg. 2010; 65:182-9.
7. Foggazi GB, Verdesca S, Garigali G. Urinalysis: Core curriculum 2008. Am J Kidney Dis. 2008; 51:1052-1067.
8. Budak YU, Huysal K. Comparison of three automated systems for urine chemistry and sediment analysis in routine laboratory practice. Clin Lab. 2011; 57:47-52.
9. Winkel P, Statland BE, Jorgensen K. Urine microscopy, an ill defined method, examined by a multifactorial technique. Clin Chem. 1974; 20:436-439.
10. Brody LH, Sallada JR, Ambaster K. Urinalysis and the urinary sediment. Med Clin North Am. 1971; 55:243-266.

***Corresponding author:**
Dr. Prasanna N Kumar, Professor, Department of Pathology, PSG institute of Medical sciences and Research, Coimbatore - 641 004 INDIA
Email: drpnkumar2001@yahoo.com

Financial or other Competing Interests: None.