ORIGINAL RESEARCH

Automated urine analyser vs manual microscope examination for urinalysis comparison

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ABSTRACT
Aim: To compare the microscopic parameters for analyzing urine using automated and light microscopy and to establish the concordance between manual microscopy and automated urine sediment analyser, considering the increasing use of the later in medical laboratories compared to manual analysis.**Material and Methods:** The present study was done in the haematology section of Department of Pathology, MMIMSR, Mullana on all urine samples received in the laboratory during the period of 1 year from September 2022 to October 2023. It was an observational and correlation study done on randomly selected 509 urine samples.The study focused solely on microscopic parameters in urine analysis, including red blood cell (rbc), white blood cells (wbc), epithelial cells, casts, crystals and bacteria/fungus). All urine samples of 15 ml or more were included and those less than 15ml or containing preservatives were excluded from the study. Urine samples were taken into two tubes; one for automated analysis using the UF-4000 analyser and the other for manual microscopic examination, and the results were compared. **Result:** The average age of patients upon presentation was 44.18 years, with female predominance (57%) and male: female of 1:1.37. Majority of the patients (87.82%) came for a routine health check-up. On comparing the Sysmex 4000 and manual microscopy, we found that the Sysmex 4000 detected wbc, rbc and epithelial cells more accurately than manual microscopy. However, manual microscopy performs better in analysing the types of casts, crystals, and microorganisms (bacteria/fungus).Even though, the Sysmex 4000 provided the data on these microscopic parameters, but itcould not identify the type of cast or crystal, and categorize microorganism. **Conclusion**: We concluded that the red flag functionality was one of the advantages of Sysmex 4000 automated urine analyser. Although it gave specific counts for parameters such as RBCs, WBCs and epithelial cells, in contrast to manual microscopy reporting "full field" for large numbers. However, it only indicated the presence and number of casts, crystals, and bacteria but could not specify their type and morphology, where manual microscopy was required. The Sysmex 4000 took less time as compared to manual microscopy while analysing the urine samples.
Keywords: Urine, Sysmex 4000, Urine Analyser, Manual Microscopy

INTRODUCTION

Urinalysis is one of the most commonly performed tests in the clinical laboratory. However, manual microscopic urinary sediment examination is labour-intensive, time-consuming, and lacks standardization in high-volume laboratories.[1]An accurate urine sediment analysis is a good indicator of the status of the renal and genitourinary system.[2] 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, toxaemia of pregnancy or to the adverse effects of drugs, non-infectious post-renal disease, in pregnant women and patients with diabetes mellitus or metabolic states who may have proteinuria, glycosuria, ketosis or acidosis.[1]

After complete blood count and serum chemistry, urinalysis is the third most common in vitro diagnostic screening test used in clinical practice.

Even though manual analysis techniques are standardized, handling a large number of samples can be laborious.

The methodology of urine particle analysis started with the introduction of automated microscopes and flow cytometry devices inspired by blood cell counting. [2] These analysers 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 present particle dimensions.[1]The image-based analysis systems automatically scan the formed elements of flowing urine and displays 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. [1] However, laboratories who have made the transition from manual microscopic method to automatic systems still have some concerns about the concordance of the results. [2]

We assessed the agreement between urine sediment analysers that are automated and manual microscopy.

AIMS AND OBJECTIVES
- To compare the microscopic parameters for analysing urine using automated urine analyser and light microscopy.

MATERIAL AND METHOD
The present study was done in the Hematology section of Department of Pathology, MMIMSR, Mullana on all urine samples received in the laboratory during the period of 1 year from September 2022 to October 2023. It was an observational and correlation study done on randomly selected 509 urine samples.

The study focused solely on microscopic parameters in urine analysis, including (rbc, wbc epithelial cells, casts, crystals and bacteria/fungus).No ethical issues involved.

Inclusion criteria
Random urine samples with volumes of 15 ml or greater were selected for the study.

Exclusion criteria
- Samples with volumes less than 15 ml, contamination, or spilling out of the container were excluded from the study.
- Samples with preservatives (such as those from 24-hour urine collections) were also excluded from the study.

The collected samples were placed in wide-mouth sterile transparent containers, ensuring no risk of spillage, and were analysed within an hour of collection, by both automated urine analyzer and manual microscopy.

RESULTS
A total of 509 cases were analysed demographically, clinically and results of urine automated analyser (Sysmex 4000) and manual microscopy were compared. As per the protocol relevant demographic details, clinical data and microscopic as well as automated findings were noted and results were analysed.

The age of the patients was widely dispersed ranging from 4 years to 90 years. Majority of patients were in the age group of 21-40 years (40.28%), followed by 41-60 years range with 33.60% cases. The mean age was 44.18±17.92years, with a median age of 44 years.

Graph 1: Distribution of patients as per age
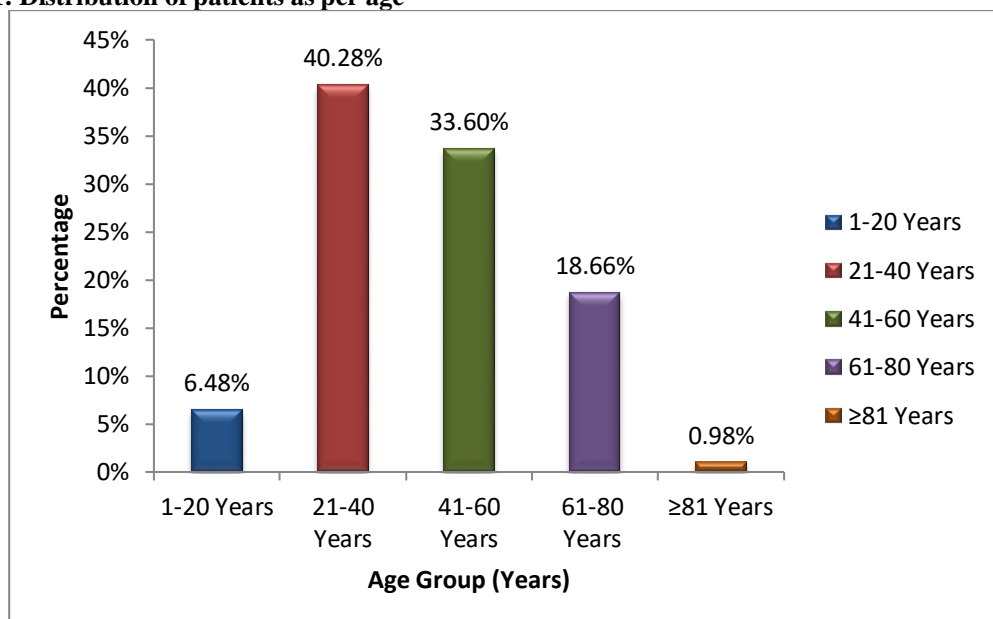
Majority of patients were females (57.96%) with Male: female ratio of 1:1.37.

Graph2: Distribution of patients as per Gender
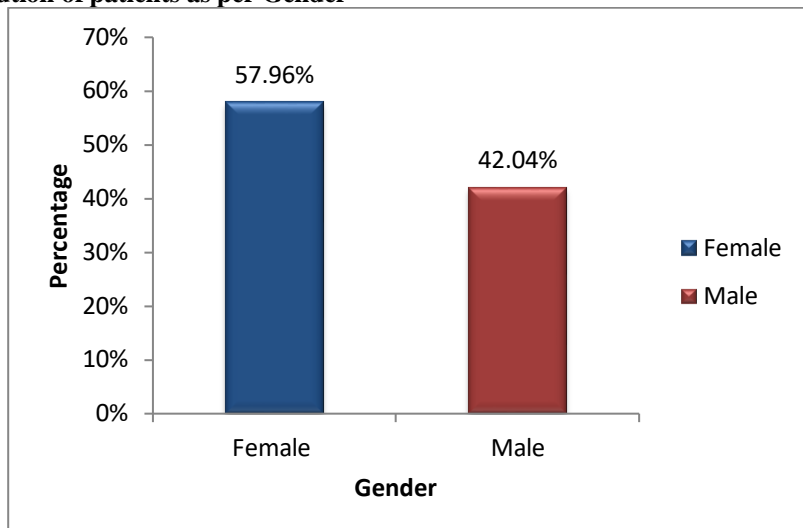
Majority of the cases came for regular health checkup (87.82%). 11.59% cases presented with burning micturition often accompanied by fever in 10.81% cases, followed by abdominal pain (0.98%), pedal edema was noted in one case only.

Graph 3: Distribution of patients according to clinical features.
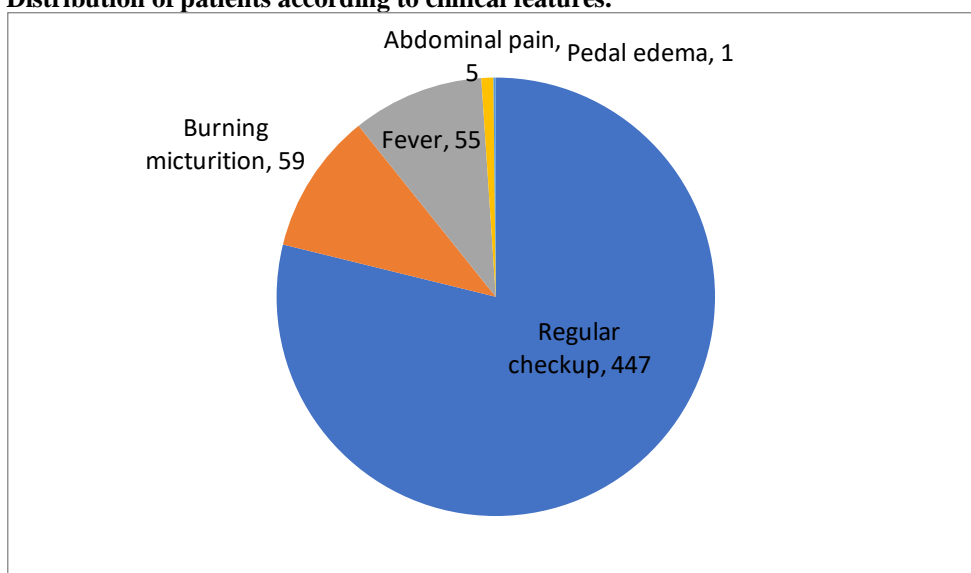
Comparative analysis sysmex 4000 v/s Manual microscopy

Table 4: Comparison of frequency of white blood cells per high-power field (HPF) observed in automated analyser as compared to manual microscopy

WBC	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
≤2-5/hpf	377	74.07%	325	63.85%
>5-10/hpf	64	12.57%	92	18.07%
>10-20/hpf	28	5.50%	50	9.82%
>20/hpf	17	3.34%	19	3.73%
Absent	1	0.20%	1	0.20%
>100/hpf(Sysmex 4000)	22	4.32%	-	-
Full field (manual)	-	-	22	4.32%
Total	509	100%	509	100%
χ^2	378.736			
p value	0.001 (HS)			

Sysmex 4000 reported WBC count of ≤2-5/hpf in 74.07% cases, while on manual microscopic examination we reported 63.85% cases of ≤2-5/hpf. However, for WBC count of >20/hpf or absent results were concordant with the manual microscopic examination. For the WBC count of >100/hpf, Sysmex 4000, reported exact number of cells per high power field, while we reported "WBC Full field" on manual microscopic examination in same number of cases.

Table 5: Displaying the frequency of red blood cells per high-power field (HPF) observed in automated microscopy compared to routine microscopy

RBC	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
≤2/hpf	106	20.83%	89	17.49%
>2-5/hpf	59	11.59%	66	12.97%
>5-10/hpf	37	7.27%	27	5.30%
>10-20/hpf	87	17.09%	43	8.45%
>20/hpf	27	5.30%	32	6.29%
Absent	239	46.96%	237	46.57%
>100/hpf(sysmex 4000)	15	2.95%	-	-
Full Field (manual)	-	-	15	2.95%
Total	509	100%	509	100%
χ^2	989.462			
p value	0.001 (HS)			

Sysmex 4000 reported RBC count of ≤2-5/hpf in 20.83% cases, while on manual microscopic examination we reported 17.49% cases of ≤2-5/hpf. However, for RBC count of >20/hpf Sysmex 4000 reported 5.30% cases, while we reported 6.29% cases on manual microscopy. The results for absent RBCs/hpf were concordant with the manual microscopic examination. For the RBC count of >100/hpf, Sysmex 4000, reported exact number of cells per high power field, while we reported "RBC Full field" on manual microscopic examination in same number of cases. These results imply high sensitivity of Sysmex 4000

Table 6:Displaying the frequency of epithelial cells observed on Sysmex 4000 compared to manual microscopy.

Epithelial Cell	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
0-5/hpf	368	72.30%	356	69.94%
6-10/hpf	41	8.06%	48	9.43%
11-20/hpf	8	1.57%	12	2.36%
>20/hpf	3	0.59%	4	0.79%
Absent	88	17.29%	88	17.29%
>100/hpf (sysmex 4000)	1	0.20%	-	-
Numerous (manual)	-	-	1	0.20%
Total	509	100%	509	100%
χ^2	657.779			
p value	0.001 (HS)			

Sysmex 4000 reported 0-5 epithelial cells /hpf in 72.30% cases, 6-10/hpf in 8.06% 11-20/hpf in 1.57%, >20/hpf in 0.59% and absent on 17.29% cases, which was almost comparable to manual microscopic examination of urine for epithelial cells. However, for the epithelial cell count of >100/hpf, Sysmex 4000, reported exact number of cells per high power field, while we reported "Numerous" on manual microscopic examination in same number of cases. These results imply high sensitivity of Sysmex 4000

Table 7: Displaying the frequency of crystals observed in automated microscopy compared to routine microscopy.

Crystal	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
0-10/hpf	30	5.89%	31	6.09%
>10/hpf	3	0.59%	7	1.38%
Absent	476	93.52%	471	92.53%
Total	509	100%	509	100%
p value	0.001 (HS)			

Results were almost comparable in crystal count of 0-10/hpf and absent, while there was a difference in the counts reported in the range of >10/hpf by Sysmex 4000 and manual microscopic examination. These results implies that Sysmex 4000 was highly specific while counting crystal count per hpf.
But Sysmex 4000 was not able to specify the type and morphology of crystals, hence manual microscopy was necessary to report the same.

Table-8: Comparison of the presence of casts per high-power field (HPF) between automated and manual microscopy.

Cast	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
0-5/hpf	23	4.52%	23	4.52%
6-10/hpf	3	0.59%	3	0.59%
>10/hpf	1	0.20%	1	0.20%
Absent	482	94.70%	481	94.50%
Total	509	100%	509	100%
p value	0.001 (HS)			

Results were concordant in cast count of 0-5/hpf, 6-10/hpf, >10/hpf and absent, by Sysmex 4000 and manual microscopic examination. These results implies that Sysmex 4000 was highly specific while counting casts count per hpfBut Sysmex 4000 was not able to accurately specify the type and morphology of casts and was giving hyaline cast as the only type of cast, hence manual microscopy was necessary to report the same.

Table9 - Displaying the frequency of others (Bacteria/fungus) observed through automated microscopy compared to routine microscopy

Other	Sysmex-4000		Manual	
	Patients	Percentage	Patients	Percentage
Absent	487	95.68%	486	95.48%
<100/hpf	17	3.33%	16	3.14%
≥100/hpf	5	0.98%	7	1.37%
Total	509	100%	509	100%
p value	0.001 (HS)			

Results were almost comparable while counting microorganisms (bacteria/fungus) by Sysmex 4000 and manual microscopic examination. These results implies that Sysmex 4000 was highly specific while counting microorganism count per hpf.

But Sysmex 4000 was not able to specify the type of microorganism and only reported them as bacteria, hence manual microscopy was necessary to report the type of microorganism whether it was bacteria or fungi.

Table10- Displaying the frequency of different concordances on Sysmex 4000 compared to manual microscopy.

Sr. No.	Variables	Sysmex-4000 vs Manual					
		Sensitivity	Specificity	PPV	NPV	Accuracy	DOR
1	WBC	100	100	100	100	100	1.02030
2	RBC	99.26	100	100	99.16	99.61	1.02038
3	Epithelial Cell	99.76	100	100	98.86	99.80	1.02033
4	Crystal	87.50	99.79	97.22	98.94	98.82	1.02181
5	Cast	96.43	100	100	99.79	99.80	1.02069
6	Other (Bacteria)	91.30	99.79	95.45	99.59	99.41	1.02131

The above table shows concordance of results of Sysmex 4000 with manual microscopic examination, showing high sensitivity, specificity, positive predictive value, negative predictive value and accuracy, and a diagnostic odds ratio of almost 1.

Figure 1:Sysmex urine analyser 4000 installed at the central laboratory of MMIMSR, Mullana, Ambala Cantonment
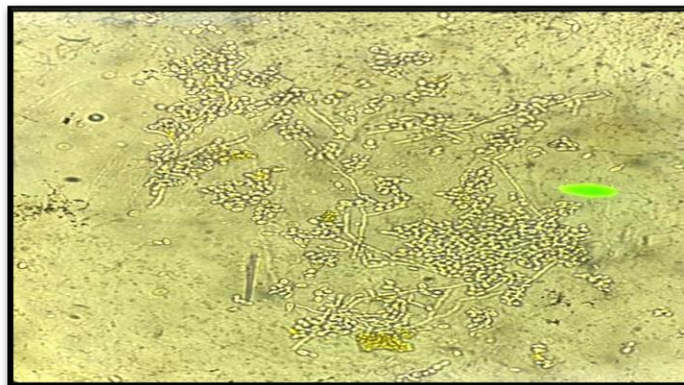

Figure 2: Bacterial organism observed in urine sediment under routine microscopy at 40x magnification
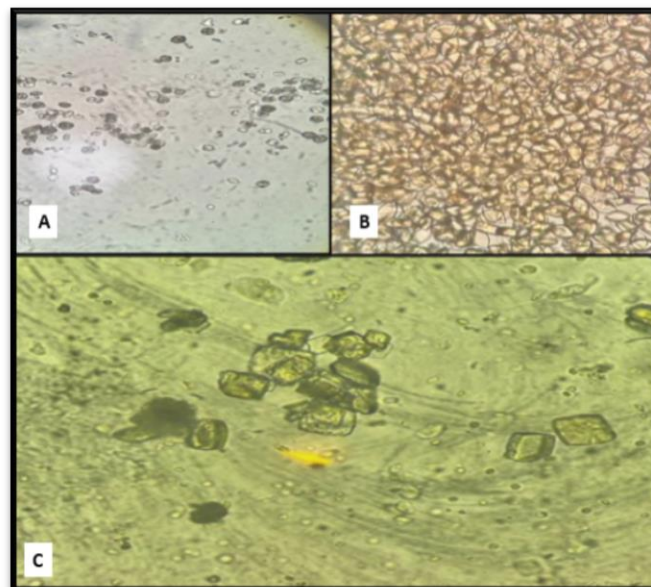

Figure 3: Uric acid crystals observed in urine sediment under routine microscopy at 40x magnification. A, B,C; 40x magnification, uric acid crystals.
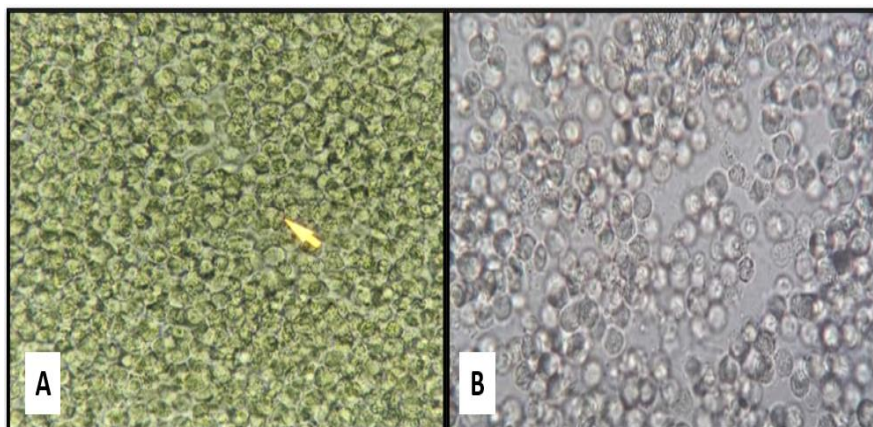
Figure 4: Pus cells (Full field)observed in urine sediment under routine microscopy at 40x magnification.

DISCUSSION

In our research, we observed a significant advancement in Indian laboratories, where machine analysers are now employed for routine microscopic examinations. Our study marks a pioneering initiative in India, as we contrasted the traditional routine microscopic technique with the Sysmex-4000, diverging from the typical usage of the Iris IQ 200. Our findings illustrate that this study emphasizes the reliability and effectiveness of automation compared to the urine microscopic method, presenting a promising pathway for improving laboratory procedures and diagnostic accuracy in India.

In our study, we choose to conduct only microscopic examination, concentrating on components such as rbc, wbc epithelial cells, casts and crystals and others (bacteria/fungus) to compare these with readings of automated urine analyser SYSMEX 4000.

Amongst various studies done all over the world to compare the efficacy of urine automated analyzers with manual microscopic examination, different automated analyzers were used i.e., IRIS IQ 200 ELITE & IRIS FUS 200 by FD Ince et al[1]. The SYSMEX UF 100 and IRIS IQ 200 were more frequently analysed, however we analysed the efficacy of SYSMEX 4000 in our study. We had a sample size of 509 random study subjects, in contrast to other studies with relatively lesser sample size ranging from 167 in study by Linko et al[3] to 436 in the study by Chien et al.[4]

Table 11:Comparison of various cells counts by automated analyser and manual microscopy in our study with the study by FD Ince et al[1] (IRIS IQ 200 & IRIS FUS 200)

Sr. No.	Variables	Sysmex-4000 vs Manual Present study; SYSMEX 4000				Sysmex-4000 vs Manual FD Ince et al[1];IRIS IQ 200			
		Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
1	WBC	100	100	100	100	77.7	93.9	91.2	83.7
2	RBC	99.26	100	100	99.16	75.8	97.7	86.2	95.6
3	Epithelial Cell	99.76	100	100	98.86	42.6	92	92	83.2

Table 12:

Sr. No.	Variables	Sysmex-4000 vs Manual Present study; SYSMEX 4000				Sysmex-4000 vs Manual FD Ince et al[1];IRIS FUS 200			
		Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
1	WBC	100	100	100	100	68.1	95.7	92.8	78.6
2	RBC	99.26	100	100	99.16	72.7	94.9	72.7	94.9
3	Epithelial Cell	99.76	100	100	98.86	61.1	93.7	76.7	87.3

FD Ince et al[1]intheir study compared the results of two automated urine analysers i.e., IRIS IQ 200 and IRIS FUS 200 with manual microscopy, they concluded that IRIS IQ 200 had the Sensitivity, specificity, PPV, NPV of 77.7, 93.9, 91.2 and 83.7 for WBCs respectively, while we had these values as 100 each fo3r WBCs in our study. On comparing these parameters with IRIS FUS 200 they reported Sensitivity, specificity, PPV, NPV of 68.1, 95.7, 92.8 and 78.6. This implies that Sysmex 4000 had the best

Sensitivity, specificity followed by IRIS IQ 200 and IRIS FUS 200.

For RBCs we reported a sensitivity and specificity of 99% and 100% respectively, in contrast to FD Ince et al[1]repoting 75.5 and 97.7 for IRIS IQ 200 and 72.7 and 92.9 for IRIS FUS 200.

For epithelial cells also Sysmex 4000 gave promising results with sensitivity and specificity of 99.76 and 100 respectively, while FD Ince et al[1]repoting the

same as 42.6 and 92 for IRIS IQ 200 and 61.1 and 93.7 for IRIS FUS 200.

Chein *et al*, Akgün *et al* and Budak *et al* also reported a high degree of agreement between the Iris iQ® 200 and manual microscopy. [4,5]

According to Akgün *et al*. found that the erythrocyte and leukocyte counting of Iris iQ200 was more successful than manual methods, but the epithelial cell counting was less successful. This is because the Iris iQ200 does not count deformed epithelial cells[1]. The concordances between the Iris iQ200 and manual method for erythrocytes and leukocytes was higher than those between the FUS-200 and manual method, but lower for epithelial cells.

But in our study by Sysmex 4000 concordance was almost similar for erythrocytes, leukocytes and epithelial cells.

In the study by Shayanfar *et al* (2007) the sensitivity of the Iris iQ200 for erythrocytes and leukocytes were 70% and 76%, respectively[6] which was relatively lesser than our study with a sensitivity of 99.26 and 100 respectively for RBCs and WBCs.

Shayanfar *et al* also state that the Iris iQ200 counts fewer erythrocytes if abnormal erythrocytes such as ghosts and dysmorphic cells were present. Therefore, urine samples from patients suffering from kidney disorders must be analyzed by manual microscopy. However, in our study majority of the patients were healthy subjects came for regular health checkup, it was not feasible and justifiable to look for these kind of dysmorphic cells.[6]

O. K.AKIN *et al* determined the sensitivity of the Iris iQ200 for erythrocytes and leukocytes as 75.8% and 85.5%, respectively. In their study, the Iris iQ200 demonstrated good diagnostic sensitivity and specificity for all elements except epithelial cells.[7]

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. However, the specificity and PPV for the same were reportedly less, 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.[8]

Chien *et al* discovered bacteria in most samples by microscopic examination in comparison to the Iris iQ200.[4]

Similarly, FD Ince *et al* also detected bacteria in a greater number of samples using manual microscopy rather than IRIS IQ 200 ELITE & IRIS FUS 200. [1]

In our study, we observed very good agreement between Sysmex 4000 and manual microscopy. But, Sysmex 4000 was not able to report the type of microorganism and was reporting bacteria even to the fungi, thus the importance of manual microscopy cannot be ignored.

The limited capability of classification software may be the cause of several issues with microorganism analysis.

Chien *et al* claimed that by altering the corresponding thresholds in Iris iQ200 reports, yeast cells and crystals could be excluded from basic particle analysis.[4] In addition, they also noted that the Iris iQ® 200 had a high false positive rate for yeast cells.

In a study by FD Ince, the Iris iQ200 and the manual microscopic method agree fairly well for yeast cell analysis. Manual microscopy confirms that the yeast cells in their study show good agreement.

However, some research suggested looking at the store photos or manually checking the results using microscopy, and we agree to this statement. In our study yeast cells were reported as bacteria by Sysmex 4000, and their presence could only be confirmed by manual microscopy.

According to FDInce *et al,* there were some false-positive results from the Iris iQ200 when evaluating dysmorphic erythrocytes as crystals; the concordance between the automated machine and the manual method was moderate. [1]They stated that an automated instrument detects fewer samples than a manual method. Several common crystals were reported as unclassified by the automated system in their study. In 7 out of 250 cases, the machine reported more crystals.[1]

Therefore, a thorough manual microscopic re-inspection is recommended for the classification and confirmation of crystals, which holds true for our study also, as Sysmex 4000 was only able to report the number of crystals per hpf was not able to classify them on the basis of morphology.

According to FD İnce *et al* there was insufficient agreement between the automated machine and the manual microscopy method [1]

Iris iQ® 200 as reported by Shayanfar *et al* was good at detecting casts but could not identify their type. [6]

This is similar to our study where Sysmex 400 was only able to quantify the number of casts per high power field, and could not report the type of cast, for which we had to rely upon manual microscopy.

LIMITATION OF THE STUDY
- There were not enough abnormal samples, which was the main limitation.
- We only examined specific microscopic criteria provided by machine.

CONCLUSION
The concordance of results of Sysmex 4000 with manual microscopic examination, showed high sensitivity, specificity, positive predictive value, negative predictive value and accuracy, and a diagnostic odds ratio of almost 1.

The red flag functionality was one of the advantages of Sysmex 4000 automated urine analyser. Although it gave specific counts for parameters such as rbcs,wbcs and epithelial cells, in contrast to manual microscopy reporting "full field" for large numbers. However, it only indicated the presence and number of casts, crystals, and bacteria but could not specify

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their type and morphology, where manual microscopy was required. The Sysmex 4000 took less time as compared to manual microscopy while analysing the urine samples and was less labour intensive.

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