Comparison of two Automated Urine Analysers (UriScan Super+ YD Diagnostics and Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer) with Routine microscopy

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Abstract

Background: Urinalysis is one of the most commonly performed screening tests in the Clinical laboratory to diagnose and monitor various urological and systemic conditions. Newly developed automated urine analyzers are expected to routinely screen urine to reduce TAT and provide clinicians with prompt clinical information with a lower false-negative rate. The study aimed to evaluate the diagnostic performance of the two Automated Urine analyzers and compare them with microscopy. **Method**: There were 124 randomly selected samples of patients coming to the clinical pathology laboratory were taken for each Automated analyzer and microscopic evaluation. The results of various cells, formed elements and microorganisms were compared between both the automated urine analyzers and microscopy and the degree of concordance was calculated for each parameter. **Results:** The degree of concordance of RBC, WBC and epithelial cells was good between the two automated analyzers; URiSCAN Super+ YD Diagnostics **and** Sysmex UC-3500 -UF 5000 Urine Analyzer and microscopy with $p < 0.001$. The concordance between the Sysmex UC-3500 - UF 5000 Urine Analyzer and manual microscopy was better than between manual microscopy and Uriscan Super + **YD Diagnostics**. There was no concordance between all these methods for either crystals, cast, bacteria and fungi with $p > 0.05$ **Conclusion:** The results from the automated analyzers for RBCs, WBCs and epithelial cells were similar to the result of manual microscopy and the analyzers can be relied upon. However, bacteria, fungi, dysmorphic red cells, casts and crystals need to be analyzed by microscopic examination before giving a final diagnosis.

Introduction

Urinalysis is one of the most commonly performed screening tests in the Clinical Laboratory. It has an important role in evaluating various components and characteristics of urine, thus helping in diagnosing and monitoring nephrological, urological and other systemic conditions.[1,2]The common instances where urinalysis is performed include suspected urinary tract infection, urinary stones, infectious and non-infectious renal disease, systemic diseases such, pregnancy, diabetes mellitus, acidosis or alkalosis.[3]Traditional non-standardized urine sediment analysis was used in many labs initially. However, because of the wide variation in the results and decreased sensitivity in detecting urinary sediments the non-standardized sediment procedure is not recommended to be used now.

Although manual urine analysis procedures are standardized, it is a tedious job if the sample load is larger. Many steps in the manual procedure may cause loss of cells or lysis of cells. Therefore many semi-automated as well as completely automated urine analyzers with inbuilt microscopy like URiSCAN Super+ YD Diagnostics **and**Sysmex UC-3500 -UF 5000 Urine Analyzer respectively have gradually increased in laboratories. These analyzers have an inbuilt software with image-based analysis systems. These images can be stored and viewed later on the workstation screen by the reporting technologist and pathologist. This can reduce and in fact, eliminate the need for manual microscopic examination in most of the samples.

Clinical laboratories that have upgraded themselves from manual microscopic to automated methods have few concerns regarding the accuracy and concordance of results with the manual procedure.[3] Although it helps in saving labour as well as time, before reporting the final results of the analysis, the formed elements must be examined visually by a pathologist or a trained technician who can decide to either approve or reclassify the result.[4]

To the best of our knowledge, there has not been any study from India on the comparison of the two automated urine analyzers URiSCAN Super+ YD Diagnostics **and** Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer with the microscopy method. The study aimed to evaluate the diagnostic performance of urine chemistry and sediment analyzers and assess the concordance between manual microscopy and the two automated urine sediment analyzers URiSCAN Super+ YD Diagnostics **and**Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer.

Materials and methods

There were 124 randomly selected samples of patients in the clinical pathology laboratory of a tertiary Care hospital. The study was performed in compliance with the ethical guidelines. The samples were collected, transported, and prepared for urinalysis in concordance with European Urinalysis Guidelines. [4] Around 30 ml of midstream clean catch urine samples were taken in clean containers and transported to the Clinical laboratory. Three transparent glass tubes of 10 ml each were taken and a urine sample was poured into each tube for microscopy and automated analysis by the two analyzers respectively and was examined in a period of 60 minutes. The first tube was centrifuged for 10 min at 1500 RPM and one drop of sediment was placed on the microscope slide and 10 different microscopic fields were scanned at magnifications Low power and High powe respectively. The results were calculated by taking the average of the formed elements and reporting them as Cells/ HPF. The result was analysed by two pathologists and if any discrepancy found in the result, the analysis was repeated to resolve the discrepancy. Another 10 ml sample was run in each semi-automated URiSCAN Super+ YD Diagnostics **and** Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer respectively. The results from the instruments were obtained as the average of formed elements per LPF and HPF. The analytical principle of the analyzers uses flow cytometry cell digital imaging and identification using an artificial intelligence technique. Finally, the result from both auto analyzers was compared for urine chemistry between the analyzers and the result of formed elements of urine from the Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer was compared with manual microscopy. The results of various cells formed elements and microorganisms were compared between both the automated urine analyzers and microscopy.

Statistical Analysis: Degree of concordance was used to interpret the strength of agreement and kappa value was observed. A comparison between the two groups was conducted utilizing Cohen's kappa ($\varkappa$) analysis to see the concordance between all three values. The statistical analyses were checked using SPSS version 24, developed by IBM Co. in Armonk, NY, USA. Results with p-values below 0.05 were considered to be statistically significant.

Result:

The degree of concordance of RBC, WBC and epithelial cells was good between both Automated analyzers (Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer and Super+ **YD Diagnostic Urine** analyzer) and routine microscopy with p <0.001. The concordance for formed elements and cells between the Sysmex UC-3500 -UF 5000 Urine Chemistry Analyzer and the manual microscopy was better than the degree of concordance between the manual microscopic method and the Super+**YD Diagnostic Urine** analyzer. **(Table 1, Table 2).** The agreement of the Sysmex UC-3500 -UF 5000 with manual microscopy was almost good ($\varkappa > 0.6$) for erythrocytes and leucocytes and moderate ($\varkappa = 0.41$-0.60) for squamous epithelial cells. There was moderate agreement ($\varkappa = 0.41$-0.60) for WBC, RBCs and squamous epithelial cells for URiSCAN Super+ **YD Diagnostics** analyzer and microscopy. The agreement of both the Analyzers was very good for sugar, ketone bodies and protein (0.81-1.00). There was no concordance observed between all the methods for crystals, cast, bacteria and fungi with p >0.05. Both the automated analyzers showed similar results to the manual microscopic examination for red cells, white blood cells and epithelial cells.**(Fig 1,2,3,4)**

However, bacteria, fungi, dysmorphic cells, casts and crystals need to be analyzed by manual microscopy for a definite diagnosis. There is a need for further upgradation of the software programs for accurate results and better concordance with microscopy.

Discussion

A proper standardised method of urine microscopy provides both correct identification of the different formed elements and quantifies them with accuracy and precision. However, no such method exists in the real world. Urine microscopy is a simple method to analyse cells and sediments in the urine and is considered the "gold standard" technique.[5] For particle counting, bright-field microscopy of unstained preparations although performed regularly is not always adequate for the detection of RBCs, bacteria, fungi and hyaline casts. Therefore, there is a need for supravital staining phase-contrast microscopy, or both for better examination of these urine sediments and organisms. However, phase-contrast microscopy is not readily available in all the labs. There are other preanalytical variables which affect the quality of microscopic examination like the amount of urine remaining in the tube for resuspension, speed and time of centrifugation and whether the urine is either stained or not.

The microscopic examination performed manually is a labour-intensive and time-consuming procedure with manual subjective variations and false negative results. Additionally, it demands skilled personnel to ensure precise outcomes and proper interpretation. To address these challenges, there is the emergence of automated urine analyzers designed to streamline the urine analysis process. [6,7]

Automated urine analysis started with the use of inbuilt microscopes and flow cytometry inspired by CBC analyzers. These have been developed for high-workload laboratories to provide standardization and precision of results as well as save time. By adopting strategic analysis methods that leverage the unique strengths of various analyzers, valuable benefits can be realized. These encompass not only staff labour savings and enhanced testing efficiency but also a heightened standard of testing quality.[11]

These automated urine analyzers are based on two analytical principles for urine sediment analysis, one based on electrical impedance, and the other on image-based analysis that sorts particles according to preset particle dimensions. It is not known which principle is better than the other. Automated urine sediment analyzers like UF-1000i urine analyzer from Sysmex, Kobe, Japan works on Flow cytometry whereas Cobas 6500 urine analyzer from Roche Diagnostics Mannheim, Germany functions on the assessment of digitalized images capture of formed elements in urine[8,9] with great accuracy in detecting Red cells white cells as well as epithelial cells .[10]

There could be cell destruction in the Manual microscopic examination during processing and handling of the sample .FD Ince et al., concluded that there is a good agreement between the automated analyzer and manual microscopy for WBCs and epithelial cells· [11] good agreement has been noticed between the manual method and Iris iQ200 for red cells, white cells and epithelial cels.[12,13] This was similar to the observation made in our study.

However, the difficulty with automated Analyzer is that it does not count damaged WBCs but it may count distorted and lysed cells as an artefact. Shayanfar et al., in their study, concluded that Iris iQ200 counts fewer RBcs if there are presence of dysmorphic cells and ghost cells in cases of hemolytic disorder or glomerulonephritis.[14] Similar false-positive results have also been observed by Wah et al.[12] Therefore samples from the urine of patients suffering from renal disorders, like glomerulonephritis need to be reconfirmed by manual microscopic evaluation.[12]

In cases of hematuria in glomerulonephritis, hematuria, RBCs travel both the glomerular filtration barrier and the convoluted tubules which can cause changes in RBC morphology.[15] If the red cell gets lysed or becomes dysmorphic in the process, it might not be identified through microscopic examination. The automated urine sediment analyzers excel at identifying normal-shaped RBCs, but they might not perform as well when it comes to detecting RBCs with abnormal morphologies.[16,17]

There are some discrepancies also noted in the analysis of microorganisms and a possible reason could be

the inefficiency of the software installed in classifying them.[14] Falsely high RBC count may occur due to misclassification of yeasts.[14] Chien et al., found differences in the presence and count of bacteria in most samples by microscopy when compared with another urine analyzer Iris iQ200.[6]Similarly, FD İnce et al., noticed the presence of bacteria in more number of cases by manual microscopy when compared to the Automated analyzers.[8]We also did not get any concordance in microorganisms, crystals and casts similar to these studies.

The accuracy of Urine particle flow cytometers is better than microscopy and is beneficial in saving time as well as human manpower with better accuracy. [18 The] initial urine flow cytometer in the market was UF-100 (Sysmex, Kobe, Japan), which helped in identifying squamous epithelial cells, transitional epithelial, tubular cells, red cells, white cells, bacteria, fungus, hyaline casts, crystals, and spermatozoa, using argon laser flow cytometry. It showed acceptable linearity over clinically working ranges, and consistently and significantly less imprecision was detected compared to manual microscopic evaluation with a negligible carry-over. UFCs have been compared with chamber counts, quantitative urine microscopy tests, test strips, sediment counts, bacterial culture, and others and others showed similar results .[18-20] This technology is useful in diagnosing and monitoring urinary tract infections and other non-infectious renal disease.[9]

The newer variant of Sysmex analyzers(UF-5000 and UF-4000) works on the principle of forward scatter light ,side scatter light , side fluorescent light, and depolarized side scattered light .The newer DSS technique is a unique technology which helps in detecting crystals and differentiating them from red cells.[28]This can significantly reduce the need for manual microscopy without affecting the quality of the test .[9]

Flow cytometry has demonstrated its superiority in detecting both blood cells and bacteria and is dependent upon the concentrations of different elements within the sample. The utilization of image processing is promising in enhancing detection capabilities, granting Lab technologists/pathologist a chance to visualise distinct images.

Sysmex launched another variant UF-1000i which works on bacteria forward scatter (FSC) and fluorescent light scatter (FLH) and differentiates between gram-positive and gram-negative bacteria which causes urinary infections.[29] It can reduce the frequency of samples needing culture, thereby causing ease in diagnosis time, and reducing the time and costs of each test [23,24] This new technology has been found to reduce the number of culture by 28–60%.[25,26] This faster technique will substantially reduce the need for unnecessary empirical antibiotic therapy. However, a drawback of this autoanalyzer compared to urine culture is that it counts both living and dead bacteria yielding a higher bacterial count.

Enko et al. conducted a comparative study on 195 urine samples between the UF-5000 (Sysmex , Kobe, Japan) working on UFC and the cobas® u 701 (Roche Diagnostics, Rotkreuz, Switzerland) urine analyzers which work on manual phase-contrast microscopy. The level of agreement between the UF-5000 and manual microscopy was nearly perfect ($\varkappa > 0.8$) for tubular epithelial cells, red blood cells, white blood cells, hyaline casts, bacteria, and yeast. Cobas® u 701 analyzers ashowed substantial agreement ($\varkappa$ = 0.61-0.80) for white blood cells, moderate agreement ($\varkappa$ = 0.41-0.60) for hyaline casts, and fair agreement ($\varkappa$ = 0.21-0.40) for RBCs, squamous cells, tubular epithelial casts, bacteria, and yeast. UF-5000 analyzer demonstrated a sensitivity ranging from 98.5% for red blood cells to 83.3% for casts. On the other hand, the cobas® u 701 analyzer had a sensitivity ranging from 83.0% for white blood cells to 31.6% for yeast cells. Thus they concluded that the UF-5000 analyzer had a stronger agreement with manual microscopy compared to cobas® u 701 modules and recommended the Sysmex urine analyzers can be used with a reliable result. However, urine samples for cast and crystals should be confirmed manually.[27]

Tantisaranon et al analyzed in 2021 and examined 100 routine urine samples to compare three different analyzers– Cobas 6500, UN3000-111b, and iRICELL 3000. They found there was a good correlation between the three urine analyzers with an overall concordance of more than 80%. The level of agreement between manual analysis and the three instruments for sediment analysis varied. It was very good to good for erythrocytes, leucocytes, and epithelial cells, and moderate for bacteria. Fair to good agreements were found between manual microscopy and the 3 instruments for cast detection. They concluded that automated urine

analyzers could serve as effective tools for initial urine testing. However, they also emphasized the necessity for manual microscopic analysis to accurately classify urine sediments, particularly in cases involving pathological specimens. The results were similar to our study.[28]

The iQ200 analyzer (Iris Diagnostics CA, USA) works on the principle of laminar flow digital imaging technology. It classifies and quantifies cells and analyzes each particle based on its distinctive attributes, such as shape, contrast, and texture. The operator has the option to review and reclassify the generated images into their appropriate categories.[29-30] FD İnce et al ., found that there was a fair agreement for yeast cell analysis between Iris iQ200 and the manual microscopic method.[11] Chien et al., postulated that Yeast cells and crystals are not key elements for particle analysis and could be removed by adjusting the corresponding thresholds in Iris iQ200 reports.[6]

There is a strong correlation between the iQ200 output and manual microscopic cell counts for epithelial cells, red cells and white blood cells. However, it did not accurately count damaged WBCs and tended to undercount RBCs when abnormal RBCs like ghost cells and dysmorphic cells were present.[31] Some issues arise during the analysis of microorganisms.[32]It is challenging to classify as cocci "bacteria" although better results have been achieved for some rod-shaped forms .[32]It can also detect malignant or atypical urothelial cells, with a sensitivity rate of 87.5% for identification. Shayanfar et al. observed that although casts were detected by Iris iQ® 200 the instrument was not able to differentiate between various casts.[14] There were other studies which observed that the automated machine was unable to detect the cast by automated Analyzers.[12] Therefore they recommended that manual microscopic examination needs to be done for cases where urinary cast is suspected. [11,14]

There is a wide variation noted in the sensitivity and specificity of the obtained results by different analyzers. These could be due to variations in the clinical conditions of the patient populations enrolled in these different studies. These could also be attributed to the difference in definitions used to classify UTIs. Therefore, we conclude that the applicability of flow cytometry strongly depends on population characteristics to screen various cases.[33,34]

To streamline the laboratory work, automated analyzers need to be successfully installed. In addition to mechanical integration, the development of expert systems needs to be enabled and integrated to successfully and accurately identify cases which need manual review, improving the overall quality of the results.[35]

Limitations: The results may differ from other studies as the data collected has a smaller sample size.

Conclusion: Manual microscopy although commonly used requires well-trained and experienced staff and is time-consuming, especially in a high throughput lab. We observed that the UF-5000 analyzer exhibited stronger diagnostic agreement with manual phase-contrast microscopy compared to the modules. Agreement with manual microscopy of the results obtained with the Sysmex analyzers was reasonably good except in the case of crystals, casts, and bacteria. Automated urine analyzers can be used as a reliable option for urine sediment analysis. However, the urine samples should still be confirmed through manual microscopy, especially in cases of bacteria, fungus, cast and crystals. This can save staff labour, make the testing more efficient, and ensure better quality testing by adopting logical analysis strategies that exploit the advantageous features of different analyzers.

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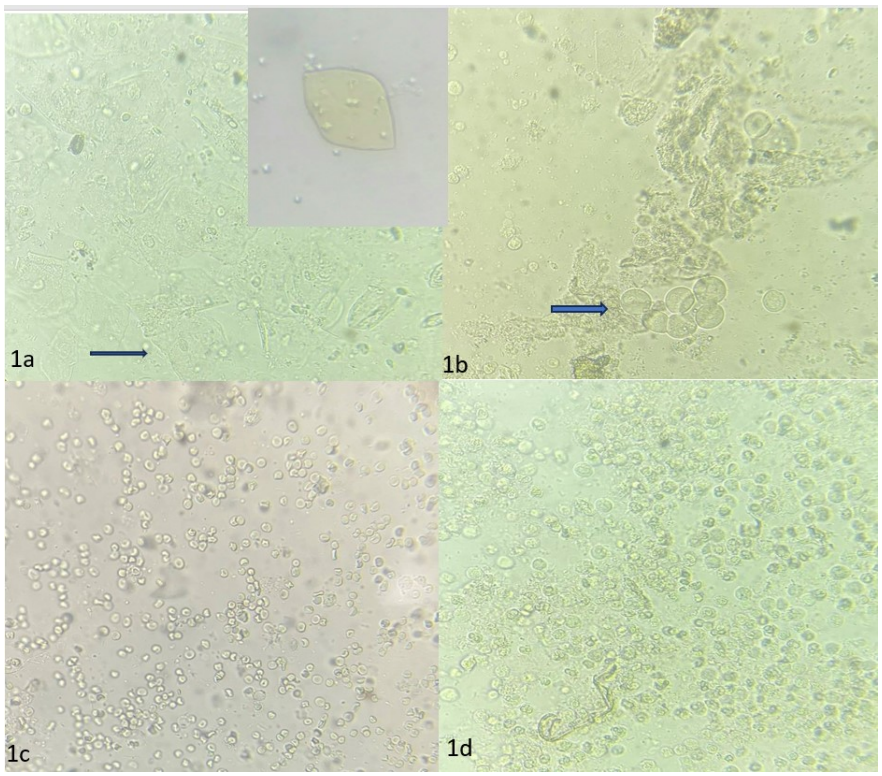
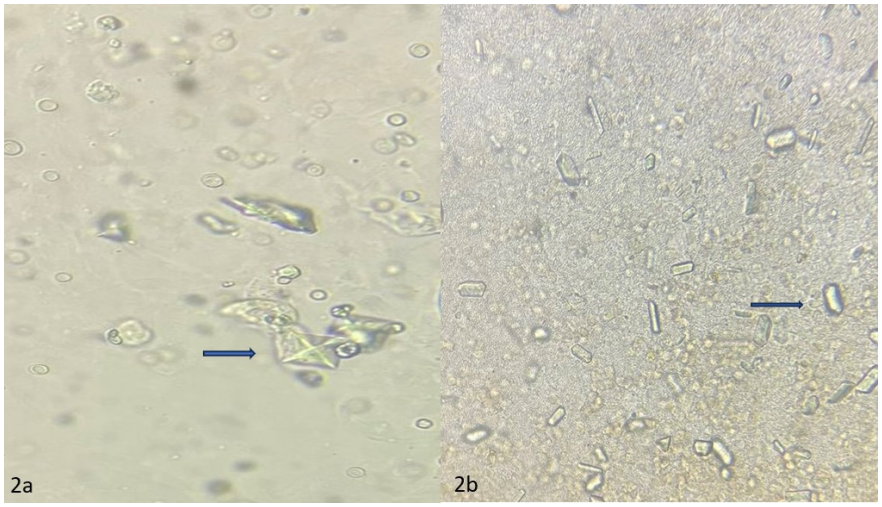
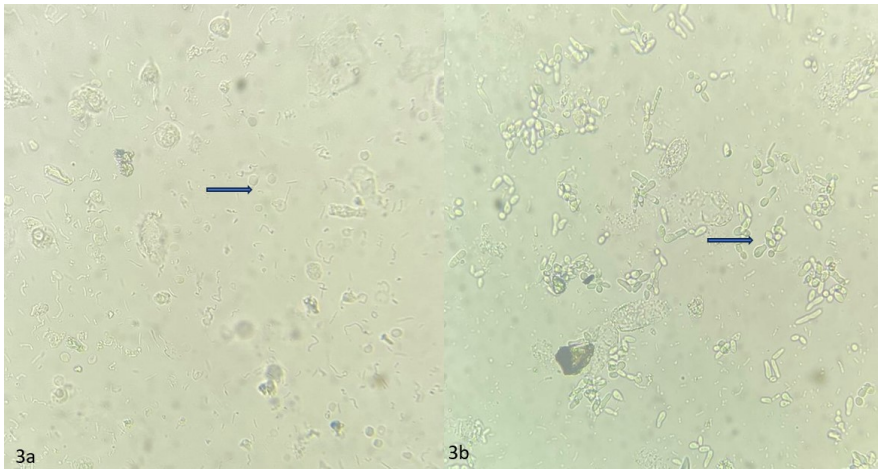
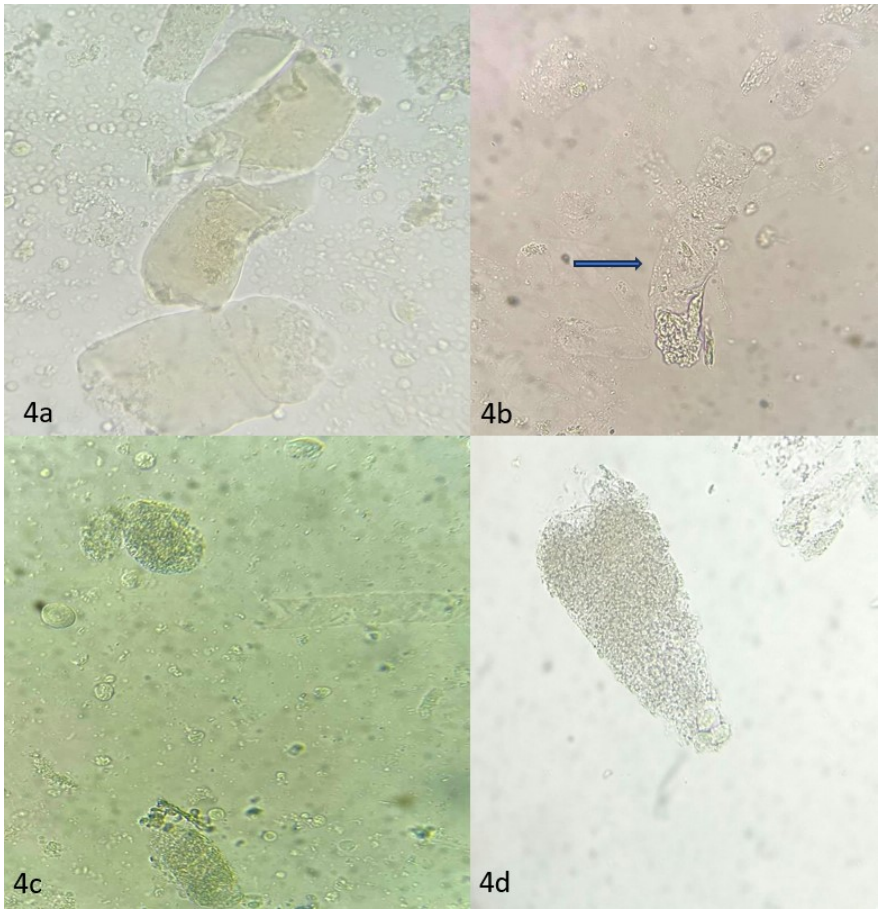
Preechaborisutkul K, Lomsomboon P, et al. Urine sediment examination: a comparison between the manual method and the iQ200 automated urine microscopy analyzer. Clin Chim Acta. 2005;358(1-2):167-174. doi:10.1016/j.cccn.2005.02.021 31. Kocer D, Sarıguzel FM, Karakukcu C. Cutoff values for bacteria and leukocytes for urine sediment analyzer FUS200 in culture-positive urinary-tract infections. Scand J Clin Lab Investig. 2014;74(5):414-417. doi:10.3109/00365513.2014.900189. 32. Poropatich CO, SM, Mendoza SM, Hitlan JJ, Wilkinson DS. Inconsistent Detection of Bacteriuria With the Yellow IRIS Automated Urinalysis Workstation. Lab Med. 1988;19(8):499-501. doi:10.1093/labmed/19.8.499. 33. Martín-Gutiérrez G, Porras-González A, Martín-Pérez C, Lepe JA, Aznar J. Evaluation and optimization of the Sysmex UF1000i system for the screening of urinary tract infection in primary health care elderly patients. Enferm Infecc Microbiol Clin 2015;33(5):320-323.doi:10.1016/j.eimc.2014.07.010. 34.Broeren MA, Bahçeci S, Vader HL, Arents NL. Screening for urinary tract infection with the Sysmex UF-1000i urine flow cytometer. J Clin Microbiol 2011;49(3):1025-1029. doi:10.1128/JCM.01669-10. 35. Jolkkonen S, Paattiniemi EL, Kärpänoja P, Sarkkinen H. Screening of urine samples by flow cytometry reduces the need for culture. J Clin Microbiol 2010;48(9):3117-3121.doi:10.1128/JCM.00617-10.**Legends** 1a.Smears examined show singly scattered epithelial cells in urine sediment smear. (unstained slide, 400x.).The inset shows a squamous epithelial cell in 100X (unstained slide, 400x.) 1b. Smears examined show small clusters of round to oval tubular epithelial cells along with a few pus cells(WBCS) in urine sediment smear. (unstained slide, 400x.) 1c. Smears examined show small singly scattered round to oval cells with an absence of a nucleus (Red blood cells.) (unstained slide, 400x.) 1d. Smears examined show singly scattered and small clusters of round to oval with the presence of granules (WBCS). (unstained slide, 400x.) 2a. Smears examined show the presence of envelope-shaped oxalate crystals marked with a blue arrow; (unstained slide, 400x.) 2b. Smears examined show the presence of coffin lid-shaped triple phosphate crystals marked with blue arrow; (unstained slide, 400x.) 3a. Smears examined show thin rod-shaped bacilli, marked with blue arrow;(unstained slide, 400x.) 3b. Smears examined show fungus with pseudohyphae, marked with blue arrow; (unstained slide, 400x.) 4a. Smears examined show few RBCs with hyaline cast; (unstained slide, 400x.) 4b. Smears examined show few RBCs with hyaline cast; (unstained slide, 400x.) 4c. Smears examined show few scattered RBCs with RBC Cast; (unstained slide, 400x.) 4d. Smears examined show few scattered WBCs with the presence of WBC Cas; (unstained slide, 400x.)









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