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**Clinical Research**

# Comparison of Urine Microscopy Results Obtained From Semi-Automatic Camera System versus Automatic Urine Analyzer

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## Abstract

*Nowadays, it is quite common in medical laboratories to use devices with a fixed slide system under the microscope objective for microscopic examination of urine, which eliminates the hassle of preparing slides between slides for microscopic examination of urine and standardizes the sediment volume.*
***Objective:*** *In our study, we compared the microscopic analysis results obtained with the Sysmex UF-100 device, one of the fully automated systems, and the DiaSys R/S 2003 device with a fixed slide (OSA) system. We aimed to identify the parameters that can be used interchangeably and give results that support each other, to reveal inconsistent results, if any, and to determine the method that we can obtain more quantitatively consistent results.*
***Method:*** *The data obtained from the analysis results for both systems were analyzed into the SPSS 22.0 program. Descriptive values were expressed as number (n), mean (Mean), minimum (min), maximum (max), median standard deviation (SD). Continuous variables were compared with the Wilcoxon Signed Ranks test because they did not fit the normal distribution. $p< 0.001$ was considered statistically significant.*
***Findings:*** *When UF-100 was compared with OSA, the analysis results for erythrocyte, leucocyte, epithelial cell parameters were not compatible with each other. We obtained, the results of manual microscopy with OSA together with the UF-100 device are highly consistent.*
***Conclusion:*** *It has been concluded that it would be more appropriate to perform microscopic examination between the slides for the evaluation of structures such as casts.*

***Key words****: urine analysis, manual microscopy, DiaSys R/S 2003, UF-100*

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## Introduction

Urine is considered a liquid tissue biopsy specimen of the urinary system. It is an economical material that provides detailed information about renal function. For this reason, nowadays routine urine analysis is one of the most requested tests in laboratories (1). The variability of routine urine analysis results depending on the person performing the procedure has led to the need for standardization in this field. In recent years, strips, strip readers, semi-automatic and fully automatic devices that evaluate microscopic shaped elements in urine have been developed to ensure standardization in this field. In addition, devices with a fixed slide system under the microscope objective have been developed for microscopic examination of urine, which eliminates the difficulty of preparing slide-to-lamel preparations and standardizes the sediment volume (2).

In this study, we aimed to examine the consistency and correlation of the results of microscopic analyses performed on the Sysmex UF-100, one of the standard and accepted automated systems, and the DiaSys R/S 2003 system with a fixed slide, Optical Slide Assembly (OSA) system.

## Material and Methods

This study was prospectively planned to perform microscopic analysis of urine samples collected in the morning from outpatients and outpatients in Haydarpaşa Numune Hospital Biochemistry and Clinical Biochemistry laboratories within two hours by two different methods, regardless of age and gender.

Although manual analysis in urine microscopy seems to be disadvantageous in terms of human experience and user differences, in this study, the data obtained from OSA, which seems to be more standardised than manual microscopy, was taken as the basis for comparison.

### Collection of Specimens

341 urine samples were collected from the patients. The number of erythrocytes, leukocytes, epithelial cells counted in the high power field (HPF) and the presence or absence of casts, crystals and yeast cells were evaluated and compared in the Sysmex UF-100 device ( Fig. 1) and DiaSys R/S 2003 (OSA) (Fig 2.) device in the urine samples.

**Figure 1.** Sysmex UF-100 device

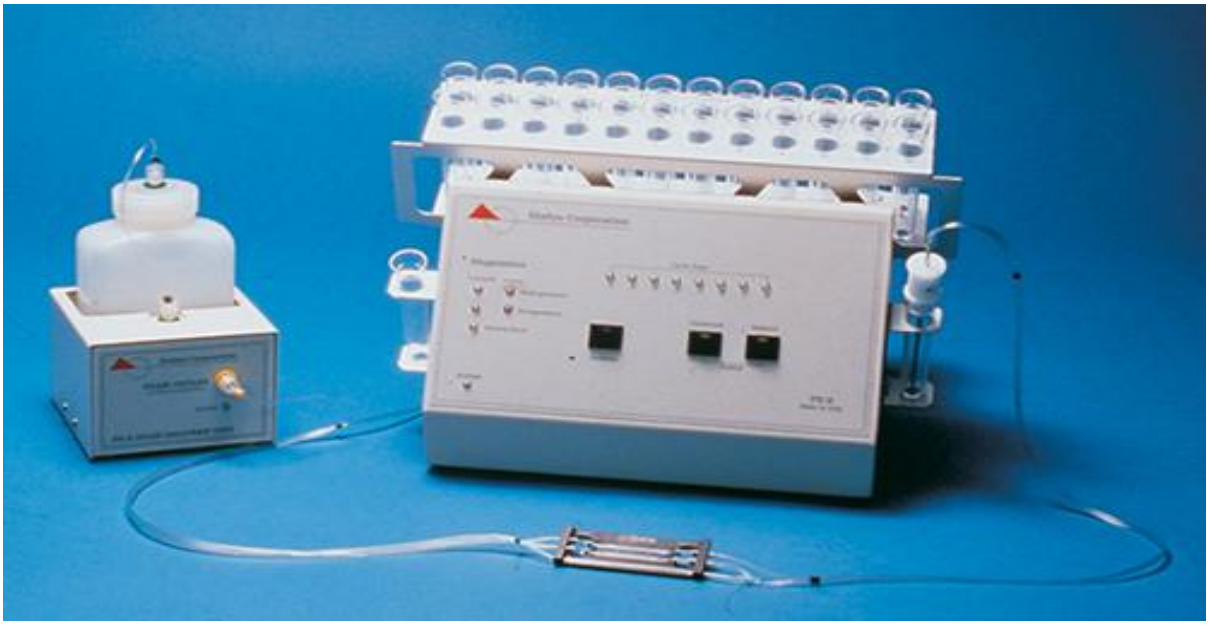

**Figure 2**. DiaSys R/S 2003, Optical Slide Assembly (OSA)

In this study, urine sample volume was determined as 10 ml and appropriate plastic conical tip tubes were used. The urine-filled tubes were then analyzed in the Sysmex UF-100 device without centrifugation. The missing volume was completed and the urine-filled tubes were centrifuged at 400 g for 5 minutes. After centrifugation, the supernatant portion of the urine sample was carefully removed from the sediment

portion and examined microscopically in the chamber connected to the DiaSys R/S 2003 device with 0.5 ml sediment volume. Data were analyzed into the SPSS 22.0 program for statistical analyses. Continuous variables were compared with the Wicoxan Signed Ranks test because they did not fit the normal distribution. $p < 0.001$ was considered statistically significant.

The Sysmex UF-100 instrument (Sysmex TOA Medical Electronics, Europe; GmbH, Hamburg, Germany) is a fully automated urine analysis system. The UF-100 uses a combination of flow cytometry technology and impedance detection to identify and count structures present in urine (3). The structures in the urine pass through the Flow Cell with the 'hydrodynamic focusing' method. During this passage, they are exposed to the laser beam and stained. The change in the scattered beam is measured by staining and impedance (voltage change) and is displayed as a numerical value on the results screen. For each fragment, the change in scattered beam, staining and impedance are converted into electrical signals and the analysis is completed. Fragment distributions are displayed as 'scattergrams' and 'histograms' (4). The UF-100 can evaluate erythrocytes (RBC), leukocytes (WBC), epithelial cells (EC), casts, bacteria, small round cells (SRC), yeast-like cells (YLC), crystals (X'tal) and sperms. The results of RBC, WBC, EC, CAST and bacteria are given by the device in count/microliter and count/HPF (5, 6).

**UF-100 and DiaSys R/S 2003 Devices Analysis**

The UF-100 instrument has the ability to evaluate analysis results as positive or negative. Positive samples receive a 'review', prompting the user to compare the result with a microscope (7). When samples were observed under UF-100, it is determined as positive if the presence of casts, crystals and yeast cells. After the first observation under UF-100, the samples were examined under OSA as double check and the results were determined after both analysis. If both analysis show positive results we interpreted the test as positive for each parameters. All the statistical analysis were obtained according to that evaluation.

The UF-100 instrument can be connected to test strip readers, allowing chemical and microscopic analysis of urine to be obtained in a single study and in a single report format (4). The DiaSys R/S 2003 is a two-part instrument that does not require a special place in the laboratory. The first part is a fixed slide made of high quality optical glass. The device is known as Optical Slide Assembly, commonly abbreviated OSA. There is a capillary space between the OSA that allows the flow of fluid.

The OSA is a 81.92 mm x 27.94 mm slide with tubings on the short sides that allow fluid to enter. One tubing starts from the short side of the slide and ends with the aspirator tip, while the other tubing extends between the slide and the device. The end that ends with the aspirator tip allows the sediment to be aspirated. The instrument is automatically washed and cleaned between these two aspiration systems. This prevents sample-to-sample contamination (8)

An equal volume of 210 microliters of urine sediment sample is aspirated each time. The total sample volume in the slide is 5 microliters and is divided into 100 small squares in the middle section, the volume of this section is 1 microliter. The volume of a single small square is 0. 01 microliter. The depth of field is 0. 127mm. Counting is done in the area of 100 small squares (9).

The second part of the device consists of a fixed unit. On this unit there is a holder system that allows the tubes to be placed. Behind the unit are reservoirs containing the fluids used for cleaning. Microscopic studies performed with this device have been found to comply with NCCLS and CLIA criteria and have been approved by the FDA (10,11).

**Statistical Analysis**

I All statistical analysis were run using SPSS 2022. All data were represented as mean ± standard deviation (SD). Statistical differences between OSA and UF-100 were determined via McNemar statistical test, Chi-Square Analysis for crystals, yeasts and casts.

Statistical comparison between OSA and UF-100 for RBC, WBC, EC was performed with Wicoxan Signed Ranks statistical test. As a result of statistical evaluation, a significant difference of $p<0.001$ was found between the UF-100 device and OSA (Table 1).

**Table 1:** Results for RBC, WBC, EC counted in UF100

| | **OSA (mean±sd)** | **UF100 (mean±sd)** | **p** |
|---|---|---|---|
| **RBC** | 6,33±13,23 | 30,92±229,43 | <0,001 |
| **WBC** | 5,32±10,17 | 23,61±162,16 | <0,001 |
| **EC** | 2±2,84 | 3,88±9,9 | <0,001 |

**Table 2:** Results for cast, crystal, yeast cell

| | OSA | | UF100 | | p Value |
|---|---|---|---|---|---|
| | Negatıve | Positive | Negative | Positive | |
| **Cast** | **311** | **30** | **302** | **39** | **<0,151** |
| **Crystal** | **282** | **59** | **235** | **106** | **<0,001** |
| **Yeast Cell** | **334** | **7** | **324** | **17** | **<0,05** |

**Results**

When analyzing for casts, crystals and yeast cells on the UF 100 device, it was recorded as positive or negative, and a comparison was carried on. The results were recorded as positive if an observation was recorded for casts, crystals and yeast cells, and negative if no observation was recorded on the OSA device .

When samples were observed under UF-100, it is determined as positive if the presence of casts, crystals and yeast cells. After the first observation under UF-100, the samples were examined under OSA as double check and the results were determined after both analysis. If both analysis show positive results we interpreted the test as positive for each parameters.

**Results for Casts:** 311 urine samples were negative in UF-100, 30 urine samples were positive in OSA and 39 urine samples were positive in UF-100 under microscopic examination. The P value was 0. 151, indicating that there was no significant difference between the two devices. In addition, the sensitivity of the UF-100 device for cast was 63%, specificity 94%, positive predictive value 49% and negative predictive value 96%. The sensitivity of OSA for cast is 61.7%, the specificity was 100%, positive predictive value was 100% and negative predictive value was 90.3%.

**Results for crystal:** 282 urine samples were negative for crystals in OSA, 235 urine samples were negative for crystals in UF-100, 59 urine samples were positive in OSA and 106 were positive in UF-100. $p<0.001$, indicating a significant difference between the two devices. The sensitivity of the UF-100 device for crystal was 75%, specificity 83%, positive predictive value 94% and negative predictive value 99%. The crystal sensitivity of OSA was 49.1% specificity 55.6%, positive predictive value 56.8% and negative predictive value 94.9%.

**Results for yeast cells:** Yeast cells were found negative in 334 urine samples in OSA, negative in 324 urine samples in UF-100 device, positive in 7 urine samples in OSA and positive in 17 samples in UF-100 device. p<0. 05 was obtained and a significant difference between the two devices was determined. The sensitivity of the UF-100 device for yeast cells was 71%, specificity 96%, positive predictive value 42% and negative predictive value 99%. The sensitivity of OSA for yeast cells was 61.2%, specificity 96.4%. The positive predictive value was 36.8% and the negative predictive value was 99.4%.

## Discussion

Today, routine urine analysis is the most commonly performed analysis in laboratories. Urine analysis has gained an indispensable place among routine laboratory tests because urine sample can be obtained easily, no invasive intervention is required, and it provides important information about renal and extrarenal diseases (6,12). Microscopic analysis of urine is the most time-consuming and labor-intensive part of urine analysis and involves a wide variety of observers (13). Microscopic analysis of urine is still not standardized. In recent years, test strips and strip readers have been developed for rapid and reliable chemical analysis of urine, and some standardization has been achieved in this way. Similarly, microscopic analysis also needs to be standardized. Automatic and semi-automatic systems developed for this purpose have been examined by many researchers through comparison studies (9, 10,14). In our study, more erythrocytes, leukocytes and epithelial cells were seen in the urine samples studied with the UF-100 device than in manual microscopic urine analysis (MSA), in accordance with other studies. Toru Hyoda et al. stated that the UF-100 device can count both lysed and unlysed erythrocytes present in fresh urine that has not been centrifuged, it can determine from which part of the urinary system the erythrocytes originate, and microscopic elements (such as bacteria and crystals) that are equal in size and smaller than erythrocytes can be considered as erythrocytes. Fogazzi et al. In their study of 1943 urine samples, they found that the number of erythrocytes determined by the UF-100 device was higher than expected compared to manual microscopic examination because the erythrocytes were lysed during the centrifugation process required for manual microscopic examination and therefore could not be detected and that this discrepancy was especially among samples containing high amounts of yeast, crystals and bacteria (15).

In our study, we found that there was no significant difference between both devices

for casts. The results were consistent with each other. However, when the studies compatible and incompatible with our study results are evaluated; Fogazzi et al. (2007) reported that the reason for the weak correlation between the UF-100 device and manual microscopy in terms of casts was that mucus was evaluated as false positive casts by the device in urine samples containing high amounts of mucus in the UF-100 device, while Jonathan Ben-Ezra et al. reported in their study that the UF-100 device discovered casts less than cellular elements (15); Brilha et al. explained in their study on 1001 urine samples that the UF-100 device evaluated some shaped elements in urine as casts, which may cause false positive results (16). According to our results; the sensitivity of the UF-100 device for casts was 63%, specificity was 94% and there is no significant difference between the devices for casts.

In our study, we found a significant difference between the UF-100 device and OSA in detecting crystals in urine. Consistent with our study results, Bai et al. reported that the UF-100 device was able to detect half of the crystal-positive manual microscopy specimens in their study of 438 urine specimens (14), while Jonathan Ben-Ezra et al. reported that the UF-100 device did not discover crystals very well but increased sensitivity by warning the user (17). Likewise, in our study, a significant difference was found in the detection of yeast cells when the UF-100 device was compared with OSA. (14) found a concordance between UF-100 and manual microscopy in terms of yeast cells in their study, while Brilha et al., in their study with 1001 urine samples, stated that the UF-100 device is not a good determinant, explaining the reason as the positive interference of yeast cells for erythrocytes, and that using the strip method is more helpful in this case.

In the study, a significant difference was found between the two devices for the presence of yeast cells (P>0,05). The sensitivity of the UF-100 device for yeast cells was 71%, specificity 96%.

In the study, manual microscopic examination was performed with OSA on a DiaSys R/S 2003 device. We considered that one of the reasons for the lower cell counts in the results of the study performed with OSA was the lack of adequate homogenization during the resuspension of the urine sediment, and the other reason was that the shaped elements with different molecular weights in the urine sediment may prevent homogeneous distribution between the chambers.

**Conclusion**

In the light of these considerations, it is seen that there is not yet a reference method for

analyzing urine. It is obvious that there is a need for the use of all automatic, semi-automatic and manual methods and that the routine use of automatic systems will increase in the future with the increase in microscopic discrimination power. In the future, the evaluation of data by artificial intelligence and the use of machine learning will add a new dimension to urine analysis methods.

## Conflict of Interest

There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

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