RESEARCH ARTICLE

Concordance and Validity of Urinalysis Using Sysmex UX-2000, Cobas ᵤ411 and Manual Microscopic Examination

Anak Agung Wiradewi Lestari[1*], Ni Komang Ayu Parmawati[2], Anak Agung Ngurah Subawa[3], Nyoman Mahartini[4]

Department of Clinical Pathology, Faculty of Medicine, Udayana University.

*Corresponding Author: Anak Agung Wiradewi Lestari

Abstract

Objective: Urinalysis is important in screening, diagnosing, and monitoring the progress of certain diseases. However, the procedure takes time to finish, special skill, and lack of standardization in certain labs. This study evaluates the concordance between manual microscopic examination results to Sysmex UX-2000, and urinalysis chemical results between Sysmex UX-2000 and Cobas U411 (Roche Diagnostic), and the validity of urine sediment results between Sysmex UX-2000 and manual microscopic examination. Methods: Eighty urine samples were analyzed using Sysmex UX-2000, Cobas u411, and manual microscopic examination. Concordance level was measured using kappa coefficient. Then we calculated the validity of Sysmex UX-2000 in sediment tests to manual microscopic examination. Results: Chemical urinalysis of Sysmex UX-2000 possesses good and very good concordance level to Cobas u411 (*weighted κ 0.65-0.91). Urine sediment tests of Sysmes UX-2000 possesses good concordance to manual microscopic examination (**weighted κ 0.76-0.77), with 86-100% sensitivity and 73-78% specificity. Conclusion: Sysmex UX-2000 has good concordance level to Cobas u411 for chemical tests urinalysis, and also good concordance and validity between Sysmex UX-2000 to microscopic examination for erythrocyte and leucocyte sediment tests.

Keywords: *Analytical System, Renal Diseases, Quality Assurance and Control.*

Introduction

Urinalysis is a laboratory test that is important to enforce various diagnoses. Multiple metabolism end products are excreted through urine. This test is useful for the clinician to detect kidney diseases, urinary tract infections, and multiple non-kidney-related metabolic disorders. Urinalysis is the third most common ordered lab test by clinicians [1-3]. In Clinical Pathology Laboratory of Sanglah Hospital, a urinalysis test is conducted within two steps. First, a chemical analysis using semi-automatic Cobasu 411 urine analyzer (Roche Diagnostics), and then followed by manual urine sediment examination using a microscope.

Both manual and semi-automatic chemical urine analysis are dependent to contact time between test strip to sample and to the time of reading the change of color from reagent pads. And while manual urine sediment examinations are extremely important, it is also very operator-dependent, so that its inter-operator variability is unavoidable. [4,5] Currently, we are using Sysmex UX-2000 which is fully-automated urine analyzer, and able to provide urine chemical and sediment information within 7.5 minutes turn-around time.[5,6] Using a fully-automated urine analyzer may increase efficiency and reduce time and workforce employed to urine specimens.[7] Several studies have shown that Sysmex UX-2000 possess good validity to manual microscopic examination. Laiwejpithaya et al reported that the weighted-kappa to urine erythrocyte and leucocyte were 0.803 and 0.721 respectively [8].

Sanchez-Mora reported that the number of 0.573 and 0.819 respectively. [9] However an automated urine analyzer cannot fully replace manual examination. Some sediment examinations like dysmorphic cells, bacteria, yeasts, casts, and crystals must be analyzed

by our fully-trained staffs to ensure valid results. This study aims to find out the concordance between manual microscopic examination results to Sysmex UX-2000 and urinalysis chemical results between Sysmex UX-2000 and Cobas U411 (Roche Diagnostic), and the validity of urine sediment results between Sysmex UX-2000 and manual microscopic examination.

Material and Methods

This is a cross-sectional study conducted in Clinical Pathology Laboratory of Sanglah Hospital between Januarys to March 2017. This study was approved by Ethical Research Committee of Sanglah General Hospital and Udayana University. All participants have provided written consent to be included in this study. Eighty urine samples were taken from in-patient and out-patient departments by consecutive sampling. Samples were collected in transparent, disposable containers without preservatives. Urinalysis tests were completed within two hours after the urine sample was taken.

The urine sample with a volume of less than 15 mL is excluded from this study. Urine samples were tested by UX-2000 for urinalysis, Cobas U411 for chemical urine tests, and manual microscope Olympus CX21 for urine sediment examination, consecutively. For microscopic examination, 15 mL of urine sample is centrifuged in conical plastic tubes at 1500 rpm for 5 minutes. Supernatant was then disposed of until 1 mL is left for resuspension using a pipette. Then 20 μL was withdrawn and dropped on a glass slide, covered using 22x20 mm cover-glass. The slide was then put under microscope Olympus CX21.

The examination was done under 10 counting area. They were zoomed at 100x (low power field /LPF) for cylinders, and at 400x (high power field/HPF) for leucocytes, erythrocytes, epithelial cells, and bacteria.[2,4,10] Results were recorded as mean cells numbers per LPF or HPF. Positive results were defined as the following: for erythrocyte >3/HPF, leucocytes >6/HPF, squamous cells present/HPF, and bacteria present/HPF.[4, 11] All microscopic examination were examined by one practiced operator.

Sysmex UX-2000 requires a minimum of 5.0 mL urine sample, with a 2.2 mL aspiration

volume. UX-2000 combines flow cytometry and fluorescence coloring technique for sediment analysis, and light reflectance to read the strip color in chemical analysis. UX-2000 is capable of reporting erythrocyte (RBC), leucocyte (WBC), epithelial cells (EC), cylinders (CASTS), bacteria (BACT), and flag parameters include crystals (XTAL), yeasts (YLC), pathologic cylinders, spermatozoa (SPERM), small round cells, transitional epithelial, and oval fat bodies. Results were presented in μL/HPF or μL/LPF, scatter gram, and histogram.

Erythrocyte and leucocyte analysis are presented in a mean number of cells per HPF. Flow cytometry technique unable to differentiate crystals, yeasts, small round cells, and pathologic cylinders, so its flagging system will recommend follow-up review using manual microscopic. [4-6] Parameters like specific gravity, color, and turbidity were measured by CHM (Chemistry) unit using an internal refractometer. For chemical analysis, UX-2000 has two strip options, Meditape II 9U and Meditape II 10 K.

Meditape II 9U has 9 basic parameters like occult blood (BLD), leucocyte esterase (LEU), nitrite (NIT), glucose (GLU), protein (PRO), urobilinogen (URO), bilirubin (BIL), ketone (KET), and pH. While Meditape II 10K is added with protein/creatinine ratio (PCR) and albumin/creatinine ratio (ACR) [6]. Cobas U411 is a semi-automated dipstick test using light reflectance to read its strip test. For chemical analysis it uses Strip Combur Test® M with 10 basic parameters like specific gravity (SG, occult blood (BLD), leucocyte esterase (LEU), nitrite (NIT), glucose (GLU), protein (PRO), urobilinogen (URO), bilirubin (BIL), ketone (KET), and pH [12].

Data were statistically analyzed using Med Calc v17 and Microsoft Excel 2010 software. Chemical and sediment urine results were classified semi-quantitatively. The positive result is defined when the result obtained exceeded each reference values. We used kappa coefficient to see the inter-method concordance. A score of 0-0.21 is poor, 0.21-0.40 is fair, 0.40-0.60 is moderate, 0.61-0.80 is good, and 0.81-1.00 is very good [13,14]. The concordance rate is considered at a similar level when the difference is ±1 from the most accurate results.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each method are also calculated.

Results

Concordance test between Sysmex UX-2000 and Cobas u411 to 80 subjects using weighted kappa coefficient showed very good agreement (***weighted к = 0.84-0.91) for RBC, WBC, NIT, GLU, and KET. While for PRO, URO, and BIL the weighted kappa showed good agreement (****weighted к = 0.65-0.73), as shown in Table 1.

Table 1: Concordance test for chemical urine results between Sysmex UX-2000 and Cobas u411

Test Strip Parameters	Concordance (%)	Kappa (95%CI)
Leucocytes	100.00	0.910 (0.893-0.942)
Erythrocytes	88.80	0.918 (0.893-0.942)
Nitrites	98.80	0.918 (0.893-0.942)
Protein	97.50	0.718 (0.611-0.824)
Glucose	98.80	0.844 (0.778-0.911)
Ketone	98.80	0.867 (0.891-0.916)
Urobilinogen	97.50	0.659 (0.300-1000)
Bilirubin	100.00	0.737 (0.586-0.888)

Table 2: Concordance test results of urine sediment between Sysmex UX 2000 and manual microscopic examination

Parameters	Concordance %	Kappa (95% CI)
Erythrocytes	81.25	0.77 (0.666-0.878)
Leucocyte	77.50	0.76 (0.675-0.843)
Epithelial cells	41.30	0.38 (0.129-0.640)
Bacteria	47.50	0.11 (0.289-0.200)

The results of the concordance test for erythrocyte and leucocyte were 81.25% and 77.50% respectively, as shown in Table 2.

Table 3: Disconcordance rate erythrocyte results of urine sediment between Sysmex UX 2000 and manual microscopic examination

UX-2000	Manual Microscopic						
≥ 100	0	0	1	1	4	0	1
51 - 99	0	0	0	0	1	0	0
26 - 50	1	0	0	2	2	0	0
11 - 25	1	0	2	1	0	0	0
6 - 10	7	3	1	0	0	0	0
3 - 5	8	4	1	0	0	0	0
0 - 2	38	1	0	0	0	0	0
/ LPB	0 - 2	3 - 5	6 -10	11 - 25	26 - 50	51 - 99	≥ 100
Total Samples	80						
Number of disconcordance	15						
Disconcordance rate (%)	18.75						

Table 4: Disconcordance rate leucocyte results of urine sediment between Sysmex UX 2000 and manual microscopic examination

UX-2000	Manual Microscopic						
≥ 100	0	0	1	2	5	0	1
51 - 99	0	0	1	1	0	0	0
26 - 50	0	1	1	0	2	0	0
11 - 25	1	3	3	3	0	0	0
6 - 10	2	5	1	1	0	0	0
3 - 5	5	6	0	0	0	0	0
0 - 2	31	4	0	0	0	0	0
/ LPB	0 - 2	3 - 5	6 - 10	11 - 25	26 - 50	51 - 99	≥ 100
Total Samples	80						
Number of disconcordance	18						
Disconcordance rate (%)	22.5						

The results of dis concordance test for erythrocyte and leucocyte were 18.75% and 22.50% respectively, as shown in Table 3 and 4.The validity of Sysmex UX-2000 to manual microscopic examination shown in Table 5.

Table 5: The validity of urine sediment tests of Sysmex UX-2000 compared to manual microscopic examination

PARAMETER	SENSITIVITY (%)	SPECIFICITY (%)	NPP (%)	NPN (%)
Erythrocytes	86.96	73.68	57.14	93.33
Leucocyte	100.00	78.69	59.38	100.00
Epithelial cells	N/A*	82.50	N/A*	100.00
Bacteria	41.10	100.00	100.00	14.00

Discussion

Concordance test between Sysmex UX-2000 and manual microscopic examination method showed good concordance rate, and weighted kappa coefficient showed good agreement. These results are similar to reports by Laiwejpithaya and Sanchez-Mora [8, 9]. The discrepancy between results for erythrocyte and leucocyte sediments between Sysmex UX-2000 and manual microscopic examination methods (18.75% and 22.50%) may be caused by differences between the two methods. While centrifuge and decantation is an inseparable step in manual microscopic exam method, they also increase the chances of cell lysis that reduce the original number of cells [1, 2, 7, 10, 16].

Laiwejpithaya reported that the concordance rate of epithelial cells was 98.56% with weighted kappa of 0.45 (moderate agreement) [8].These differences may be caused again due to method-related. In manual microscopic method, we only reported squamous and round epithelial cells. Cylinder epithelial cells were reported as positive or negative, and the types of cylinder cells were noted. In Sysmex UX-2000 epithelial cells were counted from all types of epithelial cells. The cylinders cells were counted as all types of cylinder cells. Centrifuge and decantation also may play a role in these variables [10, 15, 16].

The concordance rate for bacteria sediments between Sysmex UX-2000 and manual microscopic was 0.11 (poor agreement). This result may be caused by the differences between categorical values of each method. In microscopic method, the bacteria sediments were reported as positive or negative without calculating the numbers of bacteria, while in Sysmex UX-2000 bacteria was reported as the calculated number of bacteria. In the validity test, epithelial cells and bacteria possess high specificity (>82%) and lower sensitivity. These results were also reported by both Laiwejpithaya and Wesarachkitti [7].

Conclusion

The results of chemical urinalysis by both Sysmex UX-2000 and Cobas u411 possess good and very good agreement (*weighted κ = 0.65-0.91). For urine sediment results, Sysmex UX-2000 and manual microscopic method showed good agreement for erythrocyte and leucocyte parameters (**weighted κ = 0.76-0.77), with 86-100% sensitivity and 73-78% specificity. Standardization is required in urinalysis to enhance the quality of manual microscopic method. The use of a fully-automated urine analyzer together with manual microscopic method may increase efficiency and throughput, and decrease workforce and time needed to process the urine specimens.

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