# Optimal criteria for microscopic review of urinalysis following use of automated urine analyzer

Varanya Khejonnit, Busadee Pratumvinit, Kanit Reesukumal, Suriya Meepanya, Chanutchaya Pattanavin, Preechaya Wongkrajang *

Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol university, Bangkok, Thailand

## ARTICLE INFO

## ABSTRACT

*Background:* The Sysmex UX-2000 is a new, fully automated integrated urine analyzer. This device analyzes all physical and chemical characteristics of urine and sediments in urine on single platform. Because sediment analysis by fluorescent flow cytometry has limited ability to classify some formed elements present in urine (e.g., casts), laboratories should develop criteria for manual microscopic examination of urinalysis following the use of the automated urine analyzer.
*Methods:* 399 urine samples were collected from routine workload. All samples were analyzed on the automated analyzer and were then compared to the results of the manual microscopic method to establish optimal criteria. Another set of 599 samples was then used to validate the optimized criteria. The efficiency of criteria and review rate were calculated. The false-positive and false-negative cases were enumerated and clarified.
*Results:* We can set 11 rules which are related to the parameters categorized by the UX-2000, including cells, casts, crystals, organisms, sperm, and flags. After optimizing the rules, the review rate was 54.1% and the false-negative rate was 2.8%.
*Conclusions:* The combination of both UX-2000 and manual microscopic method obtain the best results. The UX-2000 improves efficiency by reducing the time and labor associated with the specimen analysis process.

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## 1. Introduction

Urinalysis (UA) is one of the most requested routine tests in clinical laboratories and is very useful in screening, diagnosing, and monitoring many diseases. Urinalysis is not limited in scope to diseases directly involving the urinary tract. Other diseases, including liver disease, diabetes mellitus, and muscle breakdown are also evaluated using urinalysis. This test includes physical analysis (color, specific gravity, and turbidity), chemical analysis (glucose, bilirubin, protein, urobilinogen, blood, leukocyte esterase, nitrite, and ketone), and microscopic examination of urine sediments, such as red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals [1,2].

Currently, the urinalysis procedure in our laboratory has 2 steps. The first step involves the physical and chemical analysis of urine by the Urisys 2400 urine analyzer (Roche Diagnostics), which is a fully automatic system for urine test strip analysis. The second step involves conventional manual microscopy for analyzing urine sediments, by which

numerated sediments are analyzed using a semiquantitative method. Although manual analysis procedures are essential [3], this manual method is associated with unavoidable inter-observer variation, is imprecise, is very labor-intensive, and is time consuming [4].

Recently, many manufacturers have developed fully automated, integrated urine analyzers that can analyze all physical, chemical, and sedimentary components of urine on a single platform. These devices can improve workflow efficiency because they are not only faster, but also offer improved test accuracy and precision [5]. However, these instruments cannot totally replace the manual method, but can only help in reducing the number of samples that require laborious manual counting [6].

## 2. Materials and methods

### 2.1. Study samples

This study was performed in the central laboratory of the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, and was exempted from the Siriraj Institutional Review Board. The urine specimens included in this study were leftover and unidentified samples, collected from daily workload including outpatient and inpatient populations, during March, 2014. A clean, without

preservative container was used for urine collection. All samples were completely processed within 2 h of receipt.

### 2.2. Automated urine analyzers

The UX-2000 (Sysmex Corp.) is a fully automated integrated urine analyzer that can evaluate all physical, chemical, and sedimentary properties of urine in one efficient device. The aspirate volume is 2.2 ml of urine, but requires a minimum sample volume of 5.0 ml.

This instrument is made up of 2 analysis components. The chemical (CHM) analysis component analyzes the physical and chemical characteristics of urine. The flow cytometry (FCM) analysis component analyzes urine sedimentary content. The CHM component uses 3 principles for the physical analysis of urine, which are reflectivity for color, light-scattering for determining turbidity, and transmission refractometry for specific gravity. For chemical analysis, the CHM uses a single wavelength reflectance method for blood and a dual-wavelength reflectance method for other tests. The FCM component, which is used for sediment analysis, utilizes a flow cytometer that counts, analyzes, and gates microscopic particles and microorganisms suspended in a stream of fluid. After the staining process, laser light is projected onto the sample flow within the sheath flow in the flow cell, generating a forward scattered light signal, a laterally scattered light signal, and a fluorescent light signal from each urine particle. These light signals are converted into electrical signals, which are detected and used to identify each particle. All of the measurements are presented by the software as a scattergram. The UX-2000 provides an automatic count of the quantifiable components, which are red blood cells (RBC), white blood cells (WBC), hyaline casts (CAST), bacteria (BACT), and epithelial cells (EC). Other non-quantifiable, but potentially pathologic, components are detected and flagged, where the thresholds are defined by the user, such as crystals (XTAL), yeast-like cells (YLC), small round cells (SRC, including renal tubular cells, transitional epithelial cells, and oval fat bodies), spermatozoa (SPERM), mucus, and pathological casts. Flow cytometry cannot differentiate between types of XTAL, YLC, SRC, and pathological casts, so the UX-2000's flagging system will recommend that operators review sediments by manual traditional method [6].

### 2.3. Manual microscopic method

Manual microscopic examination was performed according to the standard procedure used in the central laboratory. After 10 ml of each urine sample was centrifuged in a conical tube at 2000 rpm for 5 min, 9 ml of supernatant was discarded. The remaining urine samples were resuspended and 50 µl of urine was pipetted onto a glass slide, covered with a coverslip (22 mm × 22 mm), and examined under a CX 31 light microscope (Olympus). The examination was performed by the estimation of the urine sediment in at least 10 fields at 400× magnification (high power field, HPF) for cells or crystals and at 100× magnification (low power field, LPF) for casts. The counts were given as an average per HPF or LPF, depending on the types of particles. The positive manual microscopic review was defined, as follows: red blood cells > 2/HPF [2], white blood cells > 5/HPF [2], squamous epithelial cells >5/HPF, bacteria >few [7], yeast like cells (yeast, budding yeast, pseudohyphae) >few [7], small round cells [7], renal tubular epithelial cells >2/HPF, transitional epithelial cells >2/HPF, oval fat bodies >1/HPF, hyaline casts >2/LPF [2], pathological casts >1/LPF [2], sperms >1/HPF, and crystals >1/HPF.

### 2.4. Study design

Optimized set—399 samples were randomly selected from leftover routine urinalysis specimens in order to establish the threshold of each parameter that achieved the highest efficiency. An experienced technician, without knowing the automated results, manually examined every sample.

To optimize the criteria, we adjusted the review conditions of the urine analyzer originally proposed by the manufacturer. We included parameters, such as a cross-check between CHM part and FCM part results, abnormal scattergrams, and a number of particles, such as pathologic casts, SRCs, crystals, yeasts, and sperm to propose the review criteria.

All samples were evaluated for incongruent results between the results from both components of the UX-2000 urine analyzer (CHM and FCM) and manual microscopic examination. The efficiency and review rate of validation set were calculated. The false-positive and false-negative cases were enumerated and categorized.

Validation set—we then applied the criteria previously established for the optimization set to a separate set of 599 samples. The efficiency and review rate of validation set were again calculated.

### 2.5. Statistical analysis

Statistical analysis was performed using PASW ver 18.0 (SPSS Inc) and Excel. True-positive was indicated when criteria were triggered and the manual microscopic review was positive. If criteria were triggered and the manual microscopic review did not show any positive finding, the sample was defined as false-positive. For true-negative, the sample was not triggered by the criteria and the manual microscopic review was negative. If criteria were not triggered, but the manual microscopic review contained a positive finding, the sample was graded as false-negative. Efficiency is defined as the ability of a test to correctly classify the true outcome. Efficiency is calculated, as follows: [8]

$$\text{Efficiency} = (\text{true-positives} + \text{true-negatives})/\text{all cases}.$$

## 3. Results

From 399 samples used in optimizing the criteria, 164 (41.1%) were positive. Among the positive samples, 1 (0.61%) had 5 parameter abnormalities, 3 (1.83%) had 4 parameter abnormalities, 15 (9.15%) had 3 parameter abnormalities, 63 (38.41%) had 2 parameter abnormalities, and 82 (50%) had a 1 parameter abnormality. For microscopic review, we found a total of 270 occurrences from 164 samples: RBC (48), WBC (63), EC (10), bacteria (110), yeasts (2), hyaline casts (5), pathological casts (16), crystals (13), and SRC (3).

### 3.1. Optimization criteria

We set 11 rules that relate to the parameters categorized by the UX-2000. The criteria included RBC, WBC, EC, small round cells, hyaline casts, pathological casts, crystals, bacteria, yeast like cells, sperms, and other flags (Table 1). Although RBC, WBC, and EC can be quantified by urine analyzer, we established criteria for these cells because we found discrepant results between the manual method and the urine analyzer, within specific ranges. For bacteria criteria, we found that a large amount of RBC could affect the scattergram of bacteria. In those cases in which there was a non-quantified particle and hyaline cast, the lowest particle number of the FCM that was found positive by manual microscopic review was then used as the review limit in the criteria. The review rate and efficiency were 54.1% and 81.5%, respectively. The false-positive rate was 15.8% and false-negative rate was only 2.8%. Flags delivered the most false-positive results; whereas, bacteria delivered the most false-negative results.

### 3.2. Validation of the optimized criteria

After establishing all of the rules for the set of optimized criteria, we validated them by using another separate set of samples (n = 599). In the validation set, the false-positive rate was 11.7% and the false-