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Establishment and development of the personalized criteria for microscopic review following multiple automated routine urinalysis systems

Juan Du, Jia Xu, Fei Wang, Ye Guo, Feng Zhang, Wei Wu, Yujuan Zhang, Qian Chen, Yue Li, Wei Cui *

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

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ABSTRACT

Background: This study intends to develop the microscopic review criteria for automated urine chemistry analyzer and integrated urine chemistry and formed-element analyzer.
Methods: A total of 1058 samples were analyzed using chemistry analyzer (Siemens Atlas) for proteins (PRO), blood (BLD) and WBC. Cast, RBC and WBC were analyzed using 4 different instruments, IRIS IQ200, AVE-766, US 2026, and Sysmex UF-1000i. A phase-contrast microscopy was used as reference to evaluate false-negative rate (FNR) and review rate (RR).
Results: The optimized review criteria for Atlas were either PRO 2+, or BLD 2+, or WBC 2+, or specimen from nephrology department. FNR was 4.65% and RR was 40.41%. The optimized criteria for integrated Atlas and IQ200, or AVE-766 or US 2026 or UF-1000i were either BLD ≥ 2+ (>2+ for females), or RBC count ≥ 2 times reference range, or different WBC results between chemistry and formed-element analysis, or PRO ≥ 2+ or CAST > the reference range. One additional rule for integrated Atlas and UF-1000i was different results between BLD and RBC counts. FNR was 1.94%, 2.03%, 1.74%, 1.65%, and RR was 41.09%, 40.12%, 44.86%, 50.39%, respectively.
Conclusions: These specific review criteria ensured a low missed-diagnosis rate and a reasonable workload in practice.

1. Introduction

Routine urinalysis is a commonly used laboratory examination in clinical practice. In recent years, with the continuous advancement of automated urinalysis technology, an increasing number of parameters are detected by routine urinalysis. They include physical parameters (pH values, density, electrical conductivity, and urine osmolality), chemical parameters (urine glucose, urine ketone, and urine protein), and formed-element urinalysis parameters (various types of cells, crystals, casts, pathogens, etc.) [1,2]. Among these parameters, the formed-element parameters are very important and possess significant diagnostic value for some diseases [3].

Currently, standardized manual microscopic detection is still the reference method for urinary sediment analysis [4]. In principle, each urine sample should be manually examined under a microscope; however, in clinical practice, it is difficult to manually inspect every sample due to the large number of samples requiring testing, the short turnaround times (TAT) required, and a lack of technicians. Therefore, a strategy combining primary screening using automated routine urinalysis technology with microscopic review has become a practical and feasible method [5]. That is, a combination of automated routine urinalysis and microscopic re-examination is employed to screen for otherwise undetectable abnormal urine samples that are then re-inspected and validated by manual microscopy.

Due to differences in the sensitivity, specificity and clinical performance, each type of instrument should have personalized review criteria. Moreover, even when review criteria have been specifically developed for the same type of instrument, those criteria should ideally be validated and confirmed to meet the standards of a specific laboratory and clinical requirement. The purpose of this study was to develop microscopic review criteria for the use of an automated urinary dry-chemistry analyzer and for the combined use of an automated dry-chemistry analyzer with 4 different types of urinary formed-element analyzers.

2. Study subjects and methods

2.1. Study subjects

2.1.1. Specimen sources

We collected 1424 fresh urine specimens from outpatients and Health examination center who received routine physical examinations at the Peking Union Medical College Hospital from June to October 2013.

* Corresponding author at: Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China. Tel./fax: +86 10 69159713.
E-mail address: cuiw@pumch.cn (W. Cui).

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This study was approved by the Ethics Committee of Peking Union Medical College Hospital. Among them, 1058 urine specimens, including 411 from males with an average (minimum, maximum) age of 43.78 (1, 86) y and 647 from females with an average (minimum, maximum) age of 42.41 (1, 91) y, were used for the establishment of review criteria for the automated routine urinalysis. These specimens were obtained from >40 clinical departments, including the Departments of Urology, Nephrology, Rheumatology, Endocrinology, Gastroenterology, Obstetrics and Gynecology, Neurology, Otorhinolaryngology, Stomatology, and Dermatology. A total of 366 urine specimens from Health examination center, including 217 from males with an average (minimum, maximum) age of 42.97 (4, 81) y and 149 from females with an average (minimum, maximum) age of 43.39 (18, 74) y, were used to determine the reference ranges for the IQ200, AVE766, and US2026 formed-element urinalysis analyzers and for the UF-1000i urinary sediment analyzer. A Health examination center was defined as follows: one who, within the latest six months, was not taking any medication; did not have a fever and any disease of the urinary system; had no systemic diseases such as hypertension, diabetes mellitus, hepatitis, or rheumatism; did not have a family history of urinary system disease; did not have a history of urinary tract surgery or urinary tract calculus; did not have edema; was negative for BLD, GLU, WBC, PRO, and NIT in urinary dry-chemistry analysis and had urinary creatinine and urea levels within the reference range; and did not have any noticeable abnormality in an abdominal ultrasound examination. The female specimens were collected from patients who were not menstruating. Two tubes of clean-catch random urine specimens were collected from 30 to 60 randomly selected patients per day; 10 ml was collected in each tube, and the samples were immediately examined (within <10 min). Tube 1 was used for automated routine urinalysis, and an appropriate amount of urine specimen was added into a FastRead quantitative counting chamber for non-centrifuged urine formed-element counting. After centrifugation, the urinary sediment in Tube 2 was subjected to manual microscopy under a phase-contrast microscope.

2.1.2. Instruments and reagents

The instruments and reagents used in this study included an Atlas dry-chemistry analyzer (Siemens) and its related reagents, an IQ200 automated urinary formed-element analyzer (IRIS) and its related reagents/quality-control samples, an AVE-766 urinary formed-element analyzer (Ave Science& Technology Company Ltd.,) and its related reagents, a US 2026 automated formed-element analyzer (Chongqing Tianhai Medical Equipment Co.) and its related reagents, a UF-1000i automated urinary sediment analyzer (Sysmex) and its related reagents/quality-control samples, FastRead-10 quantitative urinary sediment counting chambers (VESTEC), and a phase-contrast microscope (BX41, Olympus Optical Co., Ltd.).

Urinary dry chemistry analyzer is mainly based on the measurement of light reflection [6]. The mechanism underlying image recognition for urine sediment analysis (also called machine-vision technology) is based on digital imaging principles, such as IQ200, AVE766, and US2026 analyzers, which are based on capturing images from planar flow of urine particles with a CCD (charged coupling device) camera [7]. For UF-1000i, a flow cytometer that counts, separates and analyzes microscopic particles suspended in a fluid stream. It also performs simultaneous, physico-chemical, multi-parametric analyses on single cells flowing through a detection system, in order to obtain adequate classification of urinary particles. The measured parameters are converted into electric signals, and the signal analysis enables classification and quantitation of each particle accordingly [8].

2.2. Methods

2.2.1. Automated routine urinalysis

We conducted chemical analysis and formed-element analysis for each fresh urine specimen from each patient using an Atlas dry-chemistry analyzer and 4 types of automated urinary formed-element analyzers. All the original detection reports were copied to a backup archive.

2.2.2. Microscopic detection of urinary formed elements

An appropriate amount of urine specimen from Tube 1 was pipetted into a FastRead quantitative counting chamber to count the urinary formed elements in non-centrifuged urine. The numbers of red blood cells (RBC) (cells/μl), white blood cells (WBC) (cells/μl), and casts (CAST) (casts/μl) were recorded, as were the types of urinary casts and crystals. The urine specimen from Tube 2 was centrifuged at 400 *g* for 5 min, and an accurately measured volume of 0.5 ml of urinary sediment was retained after removing the supernatant using a disposable pipette. After being mixed evenly, the sediment was placed into the FastRead-10 quantitative counting chamber for counting, and the following procedure was conducted. First, the entire specimen was observed microscopically under low power (10 × 10); next, the specimen was carefully observed under high power (10 × 40). The number of cells was counted in at least 10 high-power fields (HPF), and the observation of casts was performed in at least 20 low-power fields (LPF). Microscopic detection was double-blinded and was conducted by two experienced professional technicians following the same standards for each specimen. The average value obtained by the two technicians was used as the final result. The results obtained from microscopic detection include the number of RBC (the lowest to the highest, per HPF) and WBC (the lowest to the highest, per HPF), the number and type of urinary casts (the lowest to the highest, per LPF), and the type of crystals.

2.2.3. Positive criteria for urinary formed-element microscopic detection and automated routine urinalysis

Manual microscopy was conducted with each specimen by 2 experienced technicians in a double-blinded fashion. The average value obtained by the 2 technicians was used as the final result for analysis. Microscopic examination involved the quantitative detection of RBC, WBC, and CAST and the qualitative detection of other elements, such as crystals, sperm, mucus, and bacteria. The positive criteria were "RBC > 3/HPF, WBC > 5/HPF, and CAST ≥ 1/lPF" [9].

The reference ranges for urinary dry-chemistry analysis were provided by the manufacture, and the positive criterion was that the RBC, WBC and PRO values all exceeded the upper limits. The reference ranges of the 4 types of automated urinary formed-element analyzers were determined in this study, and the positive criterion was that the RBC, WBC and CAST values all exceeded the upper limits of reference range.

2.2.4. Design and evaluation of different review strategies

Using the microscopic detection of formed elements in centrifuged urine as a reference, Laboman UriAccess Ver. 3.0 software (Sysmex Company) was used to analyze the results of BLD, WBC, and PRO obtained from the dry-chemistry urinalysis and to analyze the counting results of RBC, WBC, and CAST from the formed-element analyzers. Internationally accepted review criteria were used to evaluate the measured parameters [10–13]. Based on the true-positive rate, the false-positive rate, the true-negative rate, the false-negative rate (i.e., missed diagnosis rate), and the review rate, optimal review criteria were determined. A true positive was defined as when the result of instrument hits the review criteria, and the smear microscopy confirmed the sample as positive; a false positive was defined as when the result of instrument hits the review criteria, but the smear microscopy indicated a negative result; a true negative was defined as when the result of instrument did not hit the review criteria, and the smear microscopy confirmed sample as negative; and a false negative was defined as when the result of instrument did not hit the review criteria, but the smear microscopy indicated that the sample was positive. The results were calculated using the following formula: true-positive rate = the number of true-