Investigating the recheck rules for urine analysis in children

Y.M. He*, S.W. Yao*, Y.J. Huang, B.S. Liang and H.Y. Liu

Clinical Laboratory, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

*These authors contributed equally to this study.
Corresponding author: H.Y. Liu
E-mail: haiyingliud@163.com

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ABSTRACT. The aim of this study was to establish recheck rules of urinalysis in children by investigating the concordance rate of the results obtained using the LabUMat urine dry chemistry analyzer (referred to as dry chemistry) and the UriSed tangible composition analyzer with that of the microscopic examination. First, 1040 urine samples from children (mean age 6.5 years) were analyzed using LabUMat and UriSed analyzers, and subsequently subjected to microscopic examination. The missed detection rate was evaluated and recheck rules were established to avoid missed diagnoses of abnormal renal function. Finally, clinical validations of the recheck rules were performed on 200 additional specimens. Among the samples used to investigate the recheck rules, the samples with positive microscopic examination results accounted for 58.65% of the total, while the samples with negative results accounted for 41.35%. Of the positive samples, a major portion (>50%) were RBC positive. The samples that were WBC positive and CAST positive accounted for 23.08 and 7.69%, respectively. The concordance rate was 87.5% and the missed detection rate was 2.9%. For the validation of the recheck rules in 200 urine samples, the concordance rate was 87.5% and the missed detection rate was 2.4%. When the detection of

occult blood, WBC, and protein by dry chemistry, and the detection of RBC, WBC, and CAST by the UriSed analyzer are inconsistent, or the differences between them greater than 2 levels, recheck by microscopic examination is suggested.

Key words: Automatic urine analyzer; Microscopic examination; Recheck rules

INTRODUCTION

Since urine specimens are easy to obtain, preserve, and are non-invasive, urine samples are used for routine clinical testing. However, as the number of laboratory specimens increased, the traditional manual microscopic examination methods for urine sediments were unable to meet the current clinical demands. In line with advancements in medical test technology, automatic analyzers used to detect urine-formed elements are considered important for routine urine testing, because these tests are rapid and less labor intensive. As such, automated urine analyzers have become increasingly popular among the healthcare professionals. However, due to urine characteristics including complexity, diversity, propensity to change in children (Gu, 2009), and technological limitations, current clinical laboratory automated urine analyzers cannot completely replace microscopic examinations (Li et al., 2007). Recheck guidelines for urinalysis in children are rarely reported. Therefore, due to the changing urine characteristics in children, there is a need to explore suitable recheck criteria for the use of automated urine analysis.

MATERIAL AND METHODS

Sample source

In total, 1040 urine samples (640 males and 400 females) from outpatients and inpatients (without regard to clinical department) were random collected. Patient age ranged from 1 month to 13 years with an average age of 6.5 years. Another 200 urine samples were randomly collected for investigation of recheck rules.

Instruments and materials

Instruments and materials included the LabUMat urine dry chemistry analyzer (referred to as dry chemistry) (77 Elektronika, Budapest, Hungary), UriSed tangible composition analyzer (77 Elektronika), test strips and sediment supporting plate, Japan Olympus Binocular Microscope (Tokyo, Japan), and a standard laboratory centrifuge.

Automated analysis

Each day, 20 to 30 fresh urine specimens were randomly selected. The LabUMat automated urine analyzer was used for testing dry chemical and physical compositions of the urine. Original test results, which included urine dry chemical occult blood (BLD), leukocytes (LEU), protein (PRO), urinary sediment red blood cells (RBC), white blood cells (WBC), and CAST (urinary casts or tubes) were saved. Automated analyses of all samples were completed within 2 h.

Microscopic examination

The samples, which were analyzed by the automated analyzer, were subjected to microscopic examinations carried out by two experienced competent technicians in a double-blind manner. In accordance with the 3rd Edition of National Clinical Laboratory Procedures (Ye et al., 2006), the mean of the two testing results was used as the result of the microscopic examination. Positive standards for microscopic examination of urinary sediment used herein were as follows: RBC > 3/hydroxylysyl pyridinoline (HP); WBC > 5/HP; and CAST > 1/ lysyl pyridinoline (Ye et al., 2006). The levels of the results detected by the three methods are shown in Table 1.

Table 1. Results of the three detection methods for each level.

Level	RBC or BLD			WBC or LEU		
	Urised (number/μL)	Dry chemistry	Microscope (number/μL)	UriSed (number/μL)	Dry chemistry	Microscope (number/μL)
Level 1	0.00-5.00	-	0.00-3.00	0.00-9.00	-	0.00-5.00
Level 2	5.01-40.00	1+	3.01-10.00	9.01-45.00	1+	5.01-15.00
Level 3	40.01-80.00	2+	10.01-20.00	45.01-100.00	2+	15.01-30.00
Level 4	80.01-150.00	3+	20.01-40.00	100.01-150.00	3+	30.01-50.00
Level 5	>150	4+	>40.00	>150	4+	>50.00

RESULTS

Quality control of artificial microscopic detection

The criterion of microscopic examination was compared by a chief technician and competent technicians in clinical tests every week and the concordance was greater than 95%.

Assessment of the automated urine analyzer

Based on microscopic examination standards for RBC, WBC, and CAST, the sensitivity, specificity, and concordance rate detected by the LabUMat automated urine analyzer were assessed and are shown in Table 2. Among the samples used to investigate the recheck rules, the samples with positive microscopic examination results accounted for 58.65% of the total, while the samples with negative results accounted for 41.35%. Of the positive samples, a major portion (>50%) were RBC positive. The samples that were WBC positive and CAST positive accounted for 23.08 and 7.69%, respectively. The concordance rate was 87.5% and the missed detection rate was 2.9%. The microscopy recheck criteria of urine for children were as follows: if the results of the BLD, LEU, PRO by dry chemical analyzer, and the RBC, WBC, and CAST by UriSed sediment analyzer were inconsistent, or if there were two or more levels of difference between the results, they should be rechecked. Additionally, when the instruments prompt a review of the pictures, they should be rechecked. Complete recheck rules for urine analysis are shown in Table 3.

Validation of recheck rules

About 200 urine samples were randomly selected to be verified; the compliance rate was 87.5% and the missing rate was 2.4%.

Table 2. Sensitivity, specificity, and concordance rate of RBC, WBC, and CAST detected using an automated urine analyzer.

	Threshold value	Sensitivity (%)	Specificity (%)	Coincidence rate (%)
RBC	5.00	94.23	84.62	96.15
WBC	9.00	73.08	98.72	92.31
CAST	2.00	75.00	90.63	89.42

Table 3. Automated urine analyzer recheck criteria.

Rule number	LabUMat automatic analyzer recheck criteria	Number of cases
1	LabUMat dry chemical BLD positive, UriSed RBC positive	400
2	LabUMat dry chemical BLD positive, UriSed RBC positive	60
3	LabUMat dry chemical BLD negative, UriSed RBC positive	10
4	LabUMat dry chemical LEU negative, UriSed WBC positive	110
5	LabUMat dry chemical BLD positive, UriSed RBC negative	130
6	LabUMat dry chemical LEU positive, UriSed WBC negative	30
7	LabUMat dry chemical PRO positive, UriSed CAST positive	110
8	LabUMat dry chemical PRO positive, UriSed CAST negative	170
9	LabUMat dry chemical PRO negative, UriSed CAST positive	90
10	LEU, BLD, PRO and corresponding the WBC, RBC, CAST a corresponding order of magnitude difference level 2 above	350
11	Picture prompt review instrument	120

DISCUSSION

There are many limitations of existing tangible component analysis technology of urine from children including: asymptomatic proteinuria or hematuria, and low molecular weight proteinuria not forming tubes in the urine due to child hyperactivity; child urine secretions are fewer than those of adults; and child urine sample complexity, diversity, and propensity to change *in vitro*. The pathological changes of occult nephritis in children are not the same as adults, and can be relatively mild, especially in simple hematuria, which may be related to the young age of children and hence the early stage of disease. Therefore, it is necessary to set recheck rules of child urinalysis to avoid false negatives and missed diagnoses of child urinary system diseases and reduce illness delay, which would aid in improving work quality and efficiency, as well as meet clinical needs.

The LabUMat automatic tangible component analyzer is a new automated urine analyzer with the following benefits: it provides a clear panoramic view of urine samples through microscope imaging technology; it utilizes neural network control technology; and it has intelligent automatic recognition functions, which together provides results that are intuitive and visible. Additional tangible components that cannot be accurately identified by the instrument can be identified and amended manually to obtain accurate and reliable results.

In the present study, dry chemistry was used to detect BLD, LEU, PRO, as well as tangible components such as RBC, WBC, and CAST via an automated urine analyzer. These were detected in accordance with rules for urine sediment microscopic examination, which was used as the reference method. With these guidelines, the missing rate was 2.9%, which meets the clinically acceptable false-negative rate of less than 5% (Barnes et al. 2005). Of the 61 false-negative samples, most were from children with nephrotic syndrome, purpura kidney disease, or lupus erythematosus, whereas few were from children with influenza, hemangiomas, or peritonitis. More specifically, 3 to 10 RBC false-negative cases were found in one HP, and microscopic examination revealed that majority were due to the RBC profile

or crystallization; 5 to 15 WBC false-negative cases were found in one HP, and microscopic examination found that most of them were caused by WBC deformation, too many epithelial cells, or other components; CAST false-negative cases were primarily from patients with fever, diarrhea and vomiting, or peritonitis. Recheck results were in positive critical value. There were few false negatives due to the amorphous effect, which cannot be recognized by the instrument. In false-positive samples, many were misidentified as pathological tubes due to cloudy urine, amorphous crystals, bacteria, or impurities-bacterial-cell adhered by mucus. Those misidentified as RBC were due to calcium oxalate crystals, urate, phosphate, yeast-like fungi, or fat droplets. Interference factors of WBC detection were relatively fewer than those of the RBC, and there was a high concordance rate with the microscopic examination. However, when epithelial cells are present simultaneously with WBC, they can affect each other, causing identification errors in the instrument, i.e., the instrument is unable to differentiate between small round epithelial cells that are similar to WBC in size, or WBC and deformed WBC that are similar to the epithelial cells. Since the LabUMat automatic tangible component analyzer provides a real-time panoramic view, manual correction can be performed via the user interface directly, greatly reducing the rate of re-examination, which can increase quality and improve efficiency.

Automated detection of urine tangible components is important for diagnosis and prognosis of urinary system diseases, especially when the specimen is not suitable for screening or direct microscopic examination (Cong, 2011). Since urine testing was affected by many factors in this study, in the following circumstances, a recheck is highly recommended: if the BLD, LEU, or PRO detected by dry chemistry does not match the RBC, WBC, or CAST detected by UriSed tangible component analyzer, or there are differences that are greater than two levels between them and the instrument prompts review of the pictures; if the specimens of urinary system diseases need re-examination to prevent missed diagnosis; and lastly, if there is a large difference between current and prior results. For severe hematuria, pyuria, or severe crystallized urine, automated detection should be performed after sample dilution to obtain accurate and reliable results.

The missing rate reported herein was slightly different from those previously reported in the literature (Chen et al., 2011; Yang et al., 2012), although it was similar to others (Ma et al., 2011; Li et al., 2014), which may be related to the source of the samples, rule settings, and different instruments. The samples in this study were from children, and pathological changes of occult nephritis in children are relatively mild and are not the same as adults, so clinical symptoms were not obvious. Therefore, laboratories should develop recheck rules that are applicable to their own circumstances, and continue to perform clinical validation and adjustment, which can greatly improve the accuracy of the results and work efficiency.

Conflicts of interest

The authors declare no conflict of interest.

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