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Verification and Application Evaluation of Intelligent Audit Rules for The UN9000 Urine Analysis System

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Abstract: *Objective:* To apply and verify the application of intelligent audit rules for urine analysis by Cui *et al*. *Method:* A total of 1139 urine samples of hospitalized patients in Tai'an Central Hospital from September 2021 to November 2021 were randomly selected, and all samples were manually microscopic examined after the detection of the UN9000 urine analysis line. The intelligent audit rules (including the microscopic review rules and manual verification rules) were validated based on the manual microscopic examination and manual audit, and the rules were adjusted to apply to our laboratory. The laboratory turnaround time (TAT) before and after the application of intelligent audit rules was compared. *Result:* The microscopic review rate of intelligent rules was 25.63% (292/1139), the true positive rate, false positive rate, true negative rate, and false negative rate were 27.66% (315/1139), 6.49% (74/1139), 62.34% (710/1139) and 3.51% (40/1139), respectively. The approval consistency rate of manual verification rules was 84.92% (727/856), the approval inconsistency rate was 0% (0/856), the interception consistency rate was 12.61% (108/856), and the interception inconsistency rate was 0% (0/856). *Conclusion:* The intelligence audit rules for urine analysis by Cui *et al*. have good clinical applicability in our laboratory.

Keywords: Urinalysis; Manual verification rules; Intelligent verification; TAT

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1. Introduction

Routine urine analysis is of great significance for screening kidney and urinary tract diseases, and its efficiency and accuracy are important indicators for evaluating laboratory quality [1]. In recent years, with the continuous progress of automatic urine analysis technology, the automatic urine analyzer can quickly provide several physical parameters, chemical parameters, and typed component parameters [2]. As manual microscopy is the standard method for the analysis of urinary-formed elements, the combination of automatic urine analyzer preliminary screening and manual microscopy is still the most commonly used method for routine urine

analysis [3]. Due to the different abilities of inspectors and the mental fatigue caused by reviewing a large number of reports, the review of urine test results lacks standardization, hence the need for an automatic review system is crucial. The complex composition of urine requires the comprehensive judgment of a large number of parameters, making it difficult to establish standardized rules for urine analysis, hence the application of automatic review of urine analysis is rarely reported. In 2020, by utilizing the Sysmex UC - 3500 Urine Automatic Chemical Analyzer and Sysmex UF - 5000 Urine Sediment Component Analyzer as the detection system, Cui *et al*. systematically verified and improved the urinalysis intelligent audit rules according to the latest requirements in WS/T616-2018 Automatic Audit of Quantitative Test Results in Clinical Laboratories, providing an important scientific basis for the automatic review of routine urinalysis for laboratories using the same detection system [4]. This study applied and adjusted the latest intelligent audit rules in our laboratory and verified their applicability.

2. Materials and methods

2.1. Materials

A total of 1139 urine samples were randomly collected from inpatients in Tai'an Central Hospital from September 2021 to November 2021, including 579 males and 560 females aged 1–97 years old. The median age for males and females were 61 years and 59 years respectively, covering more than 30 clinical departments such as urology, pediatrics, gynecology, endocrinology, digestive medicine, general surgery, cardiology, neurology, and the emergency department. The urine specimens were collected according to the National Guide to Clinical Laboratory Procedures (4th Edition) [5], where the volume of individual samples was 10 mL and was subjected to analysis within 2 h.

2.2. Instruments and reagents

The *UN9000* (UF5000 + UC3500) automatic urine analysis system (Sysmex Corporation, Japan) and its supporting reagents, calibrators, and quality control materials were used in this study. The BX43 general optical microscopes (Olympus Optical Industries Co., Ltd., Japan) were also used in this study.

2.3. Methods

2.3.1. Validation of reference range of urine sediment

Sixty urine samples were collected from healthy subjects undergoing physical examination to verify the reference range of urine sediment components of the UF5000 analyzer. Subjects included 28 males and 42 females aged 11–89, with a median age of 45.

2.3.2. Automated routine urinalysis

Each day, approximately 20–50 fresh midstream urine specimens submitted by the ward were randomly selected for analysis, and each specimen was subjected to the UN9000 automatic urine analysis system within 2 h after collection. The original reports of the tests were saved and backed up.

2.3.3. Microscopic examination of urinary sediment

The two staff members involved in urinary sediment microscopy should have more than 5 years of working experience and passed the morphological assessment of urinary sediment. There were 50 pictures of urinary sediment in this evaluation, with a full score of 100, and those who scored above 90 were qualified. Double-blind manual microscopic examination was performed on all 20–50 specimens after UN9000 testing, regarding

the National Clinical Laboratory Practice (4th edition). Each urine specimen was centrifuged at 400 × g for 5 min. The supernatant was discarded, and 0.2 mL of precipitate was collected and mixed. One drop of the precipitate was applied onto the slide, covered with an 18 mm × 18 mm coverslip, and left to stand. The slide was first observed with a low-power microscope (10 × 10) and then carefully observed with a high-power microscope (10 × 40). The cells in 10 high-power fields (HPF) were counted to determine the average, and the casts in 20 low-power fields (LPF) were counted to obtain the mean value. The microscopic examination included: (1) red blood cell (RBC) count, white blood cell (WBC) count, epithelial cell (EC) count, and cast (CAST) count; (2) other indicators including yeast (YLC), sperm, crystals (XTAL), bacteria (BACT) and mucus; (3) the nature and quantity of CASTs; (4) the type and number of ECs. The positive criteria for the microscopic examination of urinary sediment that were used in this study were [5,6]: (1) WBC: 3/HPF (male), 5/HPF (female); (2) RBC: 3/HPF; (3) CAST: transparent casts 1/LPF or pathological casts ≥ 1/LPF; (4) EC: 4/HPF (male), 9/HPF (female). All specimen microscopy results were entered into an Excel sheet for statistical analysis.

2.3.4. Verification of intelligent audit rules in our laboratory

The intelligent audit rules for urinalysis established by Cui *et al*. were integrated into the laboratory information system. The intelligent audit rules included microscopic review rules (1–8) and manual verification rules (9–19). When the microscopic review rules are triggered, the urine specimen must be manually microscopically examined; when the manual audit rules are triggered, the staff should make a comprehensive judgment by combining the specimen situation with the patient's clinical diagnosis and other information [4]. For specimens violating the microscopic review rules (1–8), the manual microscopy results were used as the standard for statistical analysis of the true positive rate, false positive rate, true negative rate, false negative rate, and retest rate. For specimens that do not trigger the microscopic review rules but violate the manual verification rules, approval consistency rate, approval inconsistency rate, interception consistency rate, and interception inconsistency rate were statistically analyzed using the manual microscopy results as the standard.

2.4. Statistical methods

Statistical analysis was performed using the Laboman UriAccess 3.0 and the Stata 15.0 software with the criteria as follows:

(1) False positive rate = Number of specimens determined to be positive by the review rules and negative by manual microscopy/Total number of specimens for rule validation × 100%

(2) True positive rate = Number of specimens determined to be positive both by the review rules and manual microscopy/Total number of specimens for rule validation × 100%

(3) True negative rate = Number of specimens determined to be negative both by the review rules and manual microscopy/Total number of specimens for rule validation × 100%

(4) False negative rate = Number of specimens determined to be negative by the review rules and positive by manual microscopy/Total number of specimens for rule validation × 100%

(5) Retest rate = Number of specimens triggering review rules/ Total number of specimens for rule validation × 100%

(6) Interception consistency rate = Number of specimens intercepted by both manual verification rules and manual review/Number of specimens that did not trigger the retest rule × 100%

(7) Interception inconsistency rate = Number of specimens intercepted by manual verification rules and passed by manual review/Number of specimens that did not trigger the retest rule × 100%

(8) Approval consistency rate = Number of specimens passed by both manual verification rules and manual review/Number of specimens that did not trigger the retest rule × 100%

(9) Approval inconsistency rate = Number of specimens passed by manual verification rules and intercepted by a manual review/Number of specimens that did not trigger the retest rule×100%

(10) Interception rate = Number of specimens that triggered the manual verification rule/Total number of specimens for rule validation × 100%

(11) Automated audit pass rate = Number of specimens that did not trigger the intelligent audit rule/Total number of specimens for rule validation × 100%

3. Results

3.1. Validation of the reference range of urine sediment

Based on the normal reference interval of the UF5000 urine analyzer for tangible fractions reported by Cui *et al*, the ratio R-value was calculated as several tests in the reference interval/total number of tests. The R-values for erythrocytes, leukocytes, and tubular and epithelial cells were all greater than 95%, proving that this reference range applied to our laboratory [7,8].

3.2. Results of manual microscopy

According to the positive criteria for the microscopic examination of urinary sediment, there were 387 positive specimens and 752 negative specimens. Positive specimens accounted for 33.98%, of which 36.69% (142/387) were RBC positive, 61.75% (239/387) were WBC positive, 13.95% (54/387) were EC positive, and 9.81% (38/387) were CAST positive.

3.3. Results of intelligent rule validation

Among the 1139 specimens verified by the intelligent audit rules, 283 cases triggered the review, with a retest rate of 24.85% (283/1139); 129 cases triggered the manual verification rules, accounting for 11.32% (129/1139). The true positive rate, false positive rate, true negative rate, and false negative rate of the review rules in the intelligent audit rules were 26.51% (302/1139), 6.49% (74/1139), 62.34% (710/1139), and 4.65% (53/1139) respectively, using manual microscopy as the standard. For the 856 specimens that did not trigger the microscopic review rules, the approval consistency rate of the manual review rule was 84.93% (727/856), the approval inconsistency rate was 2.45% (21/856), the interception consistency rate was 12.62% (108/856), and the interception inconsistency rate was 0% (0/856), using the manual review result as the standard. The triggers for each rule are shown in **Table 1**.

Table 1. Triggering description of intelligent audit rules

Rules	Times	Percentage	Factor	TP	TN	FP	FN	Description
1	54	0.200	R	8	0	46	0	Among the 46 false positive cases, 25 cases were crystalline interference, 2 cases were yeast interference, and the remaining 16 cases were interference by other factors such as amorphous salts.
2	26	0.096	R	3	21	1	1	One false positive case for crystalline interference and 1 false negative case for shadow red blood cells not recognized by the instrument.
3	9	0.033	R	7	0	2	0	Two false positive cases for crystalline interference.
4	16	0.059	W	15	0	0	1	One case of false negative leukocytes was caused by epithelial cell interference.

Table 1 (Continue)

Rules	Times	Percentage	Factor	TP	TN	FP	FN	Description
			SEC	16	0	0	0	
5	11	0.041	W	0	11	0	0	Nine of the 11 cases were women and two were elderly men, and the positive leukocyte esterase probably came from the rupture of germinal tract leukocytes.
6	65	0.241	W	59	0	6	0	Six false positive cases were caused by the interference of epithelial cells or other impurities in the urine. 59 true positives included five cases in which the original result of leukocyte esterase was negative by the instrumental test, and after adding urine manually to the test strip module and extending the reaction time, leukocyte esterase was read as positive by the naked eye.
7	7	0.026	C	2	0	5	0	5 false positive cases were caused by interference with epithelial cells or mucus filaments.
8	58	0.215	C	10	42	6	0	Six false positive cases were caused by mucus filament interference.
9	21	0.078	R	21	0	0	0	All 21 cases were true positive, and there is no mandatory requirement for the classification of anomalous red blood cells in our laboratory.
10	7	0.026	R	6	0	1	0	One false positive case was caused by crystallization interference.
11	7	0.026	R	5	0	2	0	Urine red blood cell count was interfered with crystals in one false-positive and one true-positive specimen.
12	15	0.056	R	2	12	1	0	One false positive case was caused by crystallization interference.
14	7	0.026	W	7	0	0	0	The results of the manual microscopic examination of 7 specimens were consistent with the original results of the instrument.
15	36	0.133	C	0	36	0	0	No tubular type was detected by manual microscopy in 36 specimens, but to exclude the possibility of false-positive urine protein detection by dry chemical method, a confirmatory test of sulfosalicylic acid should be performed on the specimens in conjunction with clinical diagnosis.
16	10	0.037	C	3	0	7	0	Seven false positive cases were caused by epithelial cells, mucus filaments, and crystalline interference.
17	14	0.052	R	5	8	1	0	No urinary cast was detected in any of the 14 specimens.
18	1	0.004						The patient was advised to re-retain the sample.
19	11	0.041	YLC	8	0	3	0	Three false positive cases were caused by red blood cell interference.

Note: The intelligent audit rules were triggered 412 times in total. All specimens were examined by manual microscopy, and the original results of the instrument were judged by the gold standard of manual microscopy. True positive (TP): the original results of the instrument and manual microscopy were both positive; False positive (FP): the original results of the instrument were positive while the manual microscopy was negative; True negative (TN): the original results of the instrument and manual microscopy were both negative; False negative (FN): the original results of the instrument were negative while the manual microscopy was positive. Abbreviation: Red blood cells, R; white blood cells, W; epithelial cells, SEC; yeast-like spores, YLC; urine casts, C.

3.4. Analysis of false negative samples

One hundred and sixty-three specimens did not trigger the microscopic review rule (rules 1–8) but had positive results by manual microscopy, of which 110 specimens did not trigger any audit rule (i.e., positive specimens do not need retest) and the remaining 53 were false-negative specimens. According to the microscopic examination of the content of leukocytes, erythrocytes, and tubular type, while considering factors like yeast, the analysis results are shown in Table 2. Of the 53 false negative samples, 52 triggered the manual

review rule, and the other sample was intercepted due to triggering the instrument alarm review. Among the 53 false negative specimens, 13 samples triggered manual verification rule number 9 (indicating abnormal erythrocyte morphology), respectively. Since there is no mandatory requirement for the classification of anomalous erythrocytes in our laboratory, these 13 samples should be classified as true positive samples, and the false negative rate was reduced to 3.51% (40/1139). By using the manual microscopy results as a criterion, 4 specimens with negative instrument raw results without triggering any intelligent audit rules had positive manual microscopy results. Three cases were tubular misses, 2 from the rheumatology department, and 1 from a patient with allergic purpura in the pediatric hematology department. The last case was a RBC miss specimen. The actual leakage rate was 4/1139 = 0.35%.

Table 2. False negative analysis

Factor	Total	TP	TN	FP	FN	Description
W	7	7	0	0	0	Six of the seven true positive cases triggered manual verification rule 14, and one triggered an instrument alarm.
R	34	29	4	1	0	Among them, 19, 5, 4, and 6 cases triggered the manual verification rule 9, 10, 11, and 12 respectively.
C	10	4	2	4	0	Six cases triggered the manual verification rule 16, the other 4 cases triggered rule 17.
Y	7	1	0	6	0	Seven cases all triggered manual verification rule 19.

3.5. Analysis of false positive specimens

Seventy-four false positive specimens triggered the microscopic review rules (1–8) but had negative manual microscopy results, which were analyzed according to the microscopic review rules as shown in Table 3. Among the 91 false-positive specimens, there were 36 cases of erythrocyte-related factors, 12 of which triggered rule number 2 of the review rules, but the microscopic results were negative under the original results. Among the remaining 24 cases, 22 cases triggered retest rule number 1, and 2 cases triggered retest rule number 3, of which 19 cases were crystalline interference causing false positive erythrocytes. There were 17 cases of leukocyte-related factors, of which 9 cases triggered retest rules number 4 or 5, and had negative microscopic results in agreement with the original instrument results. Seven of the other 8 specimens in which retest rule number 6 was triggered, were false positives for leukocytes caused by epithelial cells or crystalline interference. The relevant factor in 21 cases was cast, which triggered retest rules number 7 and 8, of which 2 cases were false positives for cast due to mucus filament interference.

Table 3. False positive analysis

Rules	Times	Factor	TP	TN	FP	FN	Description
1	22	R	0	0	22	0	17 of the 22 false positive cases were caused by crystal interference with red blood cell count
2	12	R	0	12	0	0	No erythrocytes were detected in any of the five specimens
3	2	R	0	0	2	0	2 false positive cases were caused by crystal interference
4	5	W	0	5	0	0	Epithelial cells but not leukocytes were detected in all 5 specimens
5	4	W	0	4	0	0	All four specimens were from elderly women with negative leukocyte counts and positive leukocyte esterase.
6	8	W	0	1	7	0	7 cases of false positive leukocyte count due to epithelial cells or other impurities
7	1	C	0	0	1	0	Only 1 case of false positive due to mucus filament interference
8	20	C	0	19	1	0	Only 1 case of false positive due to mucus filament interference

3.6. TAT time analysis

The adjusted intelligent audit rules are shown in Table 4. After the application of the intelligent audit rules, a total of 32545 specimens were hospitalized from November 2021 to January 2022, with an automated audit pass rate of 41.91%, an intercept rate of 58.09%, and a retest rate of 14.92%. The turnaround time (TAT) time from specimen collection in the laboratory to result review decreased from a median of 29 min to 20 min and from 33 min to 23 min, with a decrease rate of 31.03% and 30.30%, respectively.

Table 4. Adjusted intelligent audit rules

Microscopic review rules	Manual verification rules
1. RBC ≥ grade 1 and BLD ≤ grade 0 (grade difference ≥ 2)	9. RBC ≥ grade 1 and BLD ≥ grade 1 (grade difference ≥ 2)
2. RBC ≤ grade 0 and BLD ≥ grade 1 (grade difference ≥ 2)	10. RBC ≥ grade 1 and BLD ≤ grade 0 (grade difference < 2)
3. RBC = grade 0 and BLD = grade 0 (grade difference ≥ 2) and heterogeneous red blood cells	11. RBC ≤ grade 0 & BLD ≥ grade 1 (grade difference < 2)
4. WBC-&SEC ≥ 1+	12.WBC ≥ grade 1 & LEU - (grade difference < 2)
5. WBC-& LEU ≥ 2+	13.WBC ≥ grade 1 & LEU ≥ grade 1 (grade difference ≥ 2)
6. WBC ≥ grade 1 & LEU - (grade difference ≥ 2)	14. PRO = 1+ and CAST ≤ 1.96 (male); PRO =1+ & CAST≤1.62 (female)
7. PRO ≤1+ and CAST > 1.96 (male); PRO ≤ 1+ and CAST > 1.62 (female)	15. PRO = ± and BLD = grade 0
8. PRO ≥ 2 +	16. SG < 1.005
	17.YLC ≥ 6.0 or ≥ 1+

Abbreviation: Red blood cell, RBC; occult blood, BLD; white blood cell, WBC; squamous epithelial cell, SEC; leukocyte esterase, LEU; protein, PRO; tubular type, CAST; urine density, SG; yeast, YLC.

4. Discussion

At present, with the continuous development and improvement of fully automated analyzers and laboratory information systems (LIS), automatic audit of test results has been applied in many fields such as biochemistry, immunology, and routine blood tests, and has shown good results [9,10]. Although fully automated urinalysis systems (including urine dry chemistry and urine formed-element analysis) have been widely used, the audit of routine urine test results is mostly at the 100% manual audit stage as the results of urine dry chemistry, formed-element analysis, and microscopic examination need to be taken into consideration during the audit [11–13]. The familiarity and application of the audit rules vary widely among laboratory personnel at different skill levels or even among different staff in the same laboratory, and the subjectivity in the audit of urinalysis results can easily result in incorrect or inconsistent reports [14]. Specimens for this study were obtained from Tai'an Central Hospital in Shandong Province, China. The hospital currently has 3,066 open beds. The large volume of ward specimens for urinalysis (about 300 cases per day) and the concentration of ward specimens sent for testing puts a lot of pressure on the timeliness of report review by the staff. Inspectors spent a lot of time on nearly 80% of the specimens that do not need to be retested, while on 20% of the specimens that need to be retested. However, manual retesting is not meticulous, which may lead to missed inspections. This phenomenon is also common in many large hospitals, hence the implementation of an automated audit of urinalysis is urgently needed.

Reports of automatic urine audit rules are very rare. We chose the intelligent audit rules of the UF5000+UC3500 fully automated urine analysis line from Sysmex, Japan, which is the same model as our laboratory urine analyzer, as reported by Cui *et al*. This rule was developed based on the latest requirements in

the industry standard - WS/T 616 -2018 “Automatic Audit of Quantitative Test Results in Clinical Laboratories” issued by the National Health and Health Commission in 2018, which united 8 large hospitals in different regions of China [15]. Divided into retest rules and manual audit rules, it covers outpatients and inpatients and the vast majority of departments and diseases, thus ensuring the reliability and standardization of the intelligent audit of urine test results.

To avoid missed urine analysis of outpatients, our laboratory used an automated urine analyzer for image analysis to test outpatient urine specimens and has achieved the purpose of microscopic examination of urine samples from all outpatients. The 1139 urine specimens used for the intelligent audit rule validation were all collected from the wards. Since urinalysis testing is a routine screening test for patients admitted to the hospital, the vast majority of specimens came from patients with non-urinary tract infections and kidney-related diseases, so the manual audit rate (36.17%) was lower and the automatic audit rate (63.82%) was higher in our laboratory compared to the manual audit rate (50.89%) and automatic audit rate (49.11%) reported by Cui *et al*. For rule 9 (RBC ≥ grade 1 & BLD ≥ grade 1 (grade difference < 2) & dysmorphic RBC) of the intelligent rules reported by Cui *et al*., it was directly deleted because the classification of abnormal erythrocytes was not routinely examined in our laboratory. In this study, 7 specimens violating this rule number 14 (WBC ≥ grade 1 & LEU ≥ 1+ (grade difference ≥ 2)) were true negatives, but Cui *et al*. reported one false positive in 14 specimens violating this rule, so this rule was still retained. Seven of the 9 specimens that violated rule number 16 (PRO ≤ ± & CAST > 1.96 (male), PRO ≤ ± & CAST >1.62 (female)) were tubular false positives, and the high rate of false positives was due to the high number of mucus filaments and epithelial cells and other formed fractions in the first-morning urine collected from inpatients. Therefore, we combined rule 16 with rule 7 (PRO = 1 + & CAST > 1.96 (male), PRO = 1 + & CAST > 1.62 (female)), and the changed rule 7 for retesting is PRO ≤ 1 + & CAST > 1.96 (male), PRO ≤ 1 + & CAST > 1.62 (female). After the adjustment of the intelligent audit rules, the true positive rate, false positive rate, true negative rate, and false negative rate of retest rules were 27.65% (315/1139), 7.9% (91/1139), 60.84% (693/1139), 3.51% (40/1139), and the retest rate was 25.63% (292/1139), respectively. The approval consistency rate of manual verification rules was 87.38% (748/856), the approval inconsistency rate was 0% (0/856), the interception consistency rate was 12.62% (108/856), and the interception inconsistency rate was 0%.

5. Conclusion

The intelligent rules for urinalysis reported by Cui *et al*. have been adjusted in our laboratory to solve the problems of the heavy load of testing personnel and irregular audit reports caused by the large volume of specimens. This ensured the quality of tests while shortening the reporting time, which has good applicability in our laboratory. Our lab will continue to validate this intelligent audit rule and train staff regularly to meet clinical needs. All in all, the standardization and automation of urinalysis review were realized and the turnaround time of laboratory testing was shortened.

Disclosure statement

The authors declare no conflicts of interest.

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