INTRODUCTION

Laboratory medicine plays an important role in clinical diagnosis, treatment, and monitoring. Laboratory test results influence medical decision-making in two-thirds to three-quarters of cases. The goal of all clinical laboratorians is to provide high-quality reported results in order to secure correct diagnosis, prediction, and decision-making during treatment and follow-up. The quality of results usually includes accuracy and reproducibility. The accuracy of laboratory tests is usually monitored through EQA (2-3 times per year), while reproducibility of test results is commonly monitored through IQC. Internal quality control (IQC) represents an essential...
risk management tool within the total testing pathway (TTP) that contributes to the overall objective of assuring the quality of results produced in medical laboratories. IQC is primarily utilized in routine practice to monitor system performance (ie, make comparisons to what is expected under stable conditions), and allow analytical failures that affect performance to be detected. Reliable tests are contingent on passing both IQC and external quality assessment (EQA).

Here, we report an incident in which a batch of creatinine test results was found to be unreliable due to a change in reagent lots, even though IQC results were satisfactory.

2 | MATERIAL AND METHODS

2.1 | Experimental design

Twenty patients’ serum specimens with serum creatinine concentrations that span the reportable range of the test method were obtained. The serum creatinine was tested with two reagent lots (hereinafter referred to as lot A and lot B). Samples were tested within 4 hours after collection and were stored at room temperature before tested. After each lot reagents had been changed, the creatinine was recalibrated and IQC was performed. Lot-to-lot differences in creatinine results were compared.

Consecutive patients’ serum creatinine data from October 16 to November 15, 2017, were extracted from the Laboratory Information System (Neusoft), which were assayed using lot A. The median of the 360 patients’ results that had been measured with the new lot on November 16 was calculated and compared with daily medians using lot A. All these data were from patients, including outpatient clinics and hospital wards.

2.2 | Methods and equipment

Serum creatinine was measured by an enzymatic method on a Beckman Coulter AU5821 biochemical analyzer, using reagents and calibrators (lot 20170222 and lot 20170422) from Kehua Biological Products Co., Ltd, Shanghai. IQC materials were third-party controls from Bio-Rad Laboratories (lot 26400). To assess whether creatinine results are in control, the Westgard multiplication rules (1_3, 2_2, R_4, 4_1) are used.

2.3 | Statistical analysis

Statistical analyses were performed with SPSS 19.0. The S-W test was used to evaluate the normality of distribution. Wilcoxon signed-rank test was used for significance testing between groups of continuous data (data are not normally distributed). P values <.05 were considered statistically significant.

Lot-to-lot comparison was performed with the Deming regression analysis for estimation of the slope and intercept. Difference between paired samples, and mean percentage difference between the results obtained with the two reagent lots were evaluated too. The acceptance criteria were slope between 0.90 and 1.10, intercept <6 μmol/L (<50% of lowest reportable value), R² > .95, and <10% mean difference between reagent lots.

3 | RESULTS

3.1 | Lot-to-lot differences

Serum creatinine levels of patients tested varied between the two reagent lots are shown in Table 1. The creatinine levels measured using two lots showed significantly different (P < .001). Although IQC was consistent and satisfactory, serum creatinine concentrations measured were higher using lot B (median: 153 μmol/L; interquartile range: 122-522 μmol/L) than using lot A (median: 133 μmol/L; interquartile range: 76-508 μmol/L) for 19 out of those 20 patients. According to the Analytical Quality Specification for Routine Analytes in Clinical Chemistry (WS/T 403-2012, China) requirements, accepted total error for serum creatinine was 12%. Out of 20 patients, 10 were unacceptable (relative difference >12%). The difference and percentage difference decreased with increasing creatinine concentration measured (Table 1). The variation was remarkably higher (>10%, 10 of 11) for the specimens with creatinine concentration below 150 μmol/L.

Figure 1 shows a linear regression analysis of the relationships between two lots. The correlation was 0.9444 and was statistically significant (P < .0001). The Deming linear regression showed a best fit of y = 0.9394 × x + 45.66, R² = .8919, and mean % difference was 34% (Table 1), so lot B was considered unacceptable on the basis of the predetermined criteria.

Figure 2 shows the difference in serum creatinine results below 150 μmol/L (lot A) measured using different lots of reagents. The results measured using lot B_{1A}A_{2} (Reagent 1 is lot B and Reagent 2 is lot A) and lot B_{1}B_{2} were both obviously higher than those of lot A_{1}A_{2} when creatinine was less than 150 μmol/L (both P values were .003). The difference in creatinine results measured between lot A_{1}B_{2} and lot A_{1}A_{2} was not statistically significant (P = .050). Nevertheless, the quality control results measured using lot B_{1}A_{2}, lot B_{1}B_{2}, and lot A_{1}B_{2} were satisfactory and showed no difference with using lot A_{1}A_{2}.

3.2 | Medians of patients’ data

The daily medians of consecutive patients’ creatinine results (analyzed using lot A) from October 16 to November 15, 2017, are shown in Figure 3. The median number of daily serum creatinine tests on the instrument was 714 (range: 298-954). The median of daily medians was 66 μmol/L (range: 61-70 μmol/L). Figure 3 shows that the median of the 360 patients’ creatinine concentrations analyzed using lot B was 102 μmol/L (the last dot point in Figure 3), which was significantly higher than the daily medians of patients’ results using lot A in the previous month.

4 | DISCUSSION

We noticed on November 16, 2017, that laboratory tests on serum creatinine level of several patients did not match their clinical
symptoms and deviate from their previous test results, although IQC had met the criteria in that run. And there were no changes in the procedure used on November 16 with reagent lot B. After reviewing the laboratory procedure and protocol, we found that the reagent for testing serum creatinine was accidentally changed to new lot (lot B) without verification on that day. The old lot had been used for more than 3 months successfully in our laboratory. Given the lot A also passed the proficiency test organized by National Center for Clinical Laboratories (NCCL, China) on September 2017 (PT score was 100%), it is plausible to speculate that the significant variation in test results may have resulted from the new lot. When replacing Reagent 2 of the lot A with Reagent 2 of the lot B, the deviation of the test results was negligible (Figure 2), demonstrating that Reagent 1 of the lot B skewed the test.

The change in reagent component materials, the instability, or deterioration of reagent composition during transportation or storage may cause new lot failure. The occurrence of noncommut- able results for QC materials was frequent enough that the QC results could not be used to verify the consistency of results for patient samples when changing lots of reagents. The results showed that IQC worked as expected in spite of big variations in specimen test results between two lots of reagent. The verification of new reagent lot performance is not only a routine but also an important laboratory task. The Clinical and Laboratory Standards Institute (CLSI) EP26-A guideline provides a lot-to-lot verification protocol to detect significant changes in test performance. Our laboratory’s protocol for lot-to-lot verification consists of simultaneously testing five samples from both current and new lots. The relative difference for each sample is calculated. The new reagent lot is deemed acceptable only if the relative difference in at least four samples was less than the predefined rejection limit. Even so, verification of the new lot was overlooked by the technician and caused significant variations in test

| Patients | A (A_R1A_R2) | B (B_R1B_R2) | B_R1A_R2 | A_R1B_R2 | Difference (B from A) | % Difference (B from A, %) | Result |
|----------|--------------|--------------|----------|----------|-----------------------|---------------------------|--------|
| 1        | 43.5         | 94.8         | 98.4     | 46.2     | 51.3                  | 117.9                     | Fail   |
| 2        | 51.7         | 121.1        | 125      | 55.2     | 69.4                  | 134.2                     | Fail   |
| 3        | 57.1         | 90           | 91.6     | 60.1     | 32.9                  | 57.6                      | Fail   |
| 4        | 66.6         | 145.1        | 146.3    | 69.4     | 78.5                  | 117.9                     | Fail   |
| 5        | 76           | 106.9        | 108.1    | 77.2     | 30.9                  | 40.7                      | Fail   |
| 6        | 76.6         | 99.9         | 103.8    | 80.2     | 23.3                  | 30.4                      | Fail   |
| 7        | 105          | 131.7        | 134.3    | 106.8    | 26.7                  | 25.4                      | Fail   |
| 8        | 108.2        | 126.1        | 129.1    | 110.8    | 17.9                  | 16.5                      | Fail   |
| 9        | 122.7        | 135.6        | 137.5    | 128.8    | 12.9                  | 10.5                      | Pass   |
| 10       | 132.1        | 237.3        | 246.8    | 122.1    | 105.2                | 79.6                      | Fail   |
| 11       | 134.3        | 142          | 146.9    | 159.4    | 7.7                   | 5.7                       | Pass   |
| 12       | 151.3        | 160.1        | 166.3    | 166.9    | 8.8                   | 5.8                       | Pass   |
| 13       | 267.6        | 334.1        | 337.8    | 264.4    | 66.5                  | 24.9                      | Fail   |
| 14       | 350.1        | 386.3        | 386.9    | 359.4    | 36.2                  | 10.3                      | Fail   |
| 15       | 428.3        | 439.3        | 444.8    | 441.4    | 11.0                  | 2.6                       | Pass   |
| 16       | 534.7        | 550          | 559.9    | 550.8    | 15.3                  | 2.9                       | Pass   |
| 17       | 645.7        | 605.8        | 644.2    | 656      | −39.9                 | −6.2                      | Pass   |
| 18       | 654.9        | 663.6        | 664.3    | 680.4    | 8.7                   | 1.3                       | Pass   |
| 19       | 778.7        | 781.6        | 795.9    | 798.4    | 2.9                   | 0.4                       | Pass   |
| 20       | 887.9        | 892.1        | 904.3    | 918.6    | 4.2                   | 0.5                       | Pass   |
| QC       | QC1          | 135          | 134.6    | 142      | 137.6                | −0.4                      | −0.3   |
|          | QC2          | 449.5        | 445      | 455      | 451.5                | −4.5                      | −1.0   |
| Statistics of 20 patients’ data | Median | 133.2 | 157.6 | 134.1 |
| Interquartile range | 133.2 | 157.6 | 134.1 |
| P valuea | 0.001 | 0.000 | 0.002 |

Lot BR1A_R2: Reagent 1 is lot B and Reagent 2 is lot A.
Compared with lot A (A_R1A_R2), Wilcoxon signed-rank test.

| Statistics of 20 patients’ data |
|--------------------------------|
| Median | 133.2 | 152.6 | 156.6 |
| Interquartile range | 76.15-508.10 | 122.35-522.33 | 126.03-525.13 |
| P valueb | 0.001 | 0.000 | 0.002 |

TABLE 1 Lot-to-lot reagent differences for serum creatinine (unit: μmol/ L, rejection limit ± 12%)
results. Fortunately, the issue was found before the patients’ test reports were issued, and serum creatinine concentrations of these 360 patients were reanalyzed using the old reagent.

There are several reports on the use of patient results as a tool in monitoring analytical quality on the daily internal control. We keep all laboratory data in our laboratory information system. Median value or average of normal (AON) can be easily calculated using statistical programs such as Excel, SPSS, or SAS. Any significant deviation from the median value or AON of previous test results can serve as an indicator for potential analytical error. IQC materials with a different matrix from clinical specimens may be insensitive to reagent changes, as observed here. Clinical specimens contain no artificial components and therefore are a useful component in assuring analytical performance. Moreover, all patients’ results can be extracted from the laboratory information system without involving extra cost and labor as compared to the preparation of IQC materials. So, median value or AON can be used as another aspect of QA. In this study, with the reagent problems, the daily median of patient outcomes had changed significantly while IQC results were satisfactory, which suggested that the daily median could be a good complement to IQC.

**CONCLUSIONS**

In summary, our findings indicate that satisfactory IQC does not necessarily mean reliable analytical results in clinical laboratories. However, in practice, many laboratory staff heavily rely on IQC to assess the reliability of their test results. As reported here, some laboratory testing errors could not be revealed only through IQC and EQA, which could negatively impact on clinical diagnosis and treatment. To ensure the reliability of test results, reagent verification and analysis of patient previous test records should be implemented besides IQC and EQA.

**AUTHOR CONTRIBUTIONS**

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission.
ETHICAL APPROVAL AND PATIENT CONSENT

Ethical clearance for this study was obtained from the Ethics Committee at the First Affiliated Hospital of Nanjing Medical University. Because all the samples used in this study were collected from clinical residual specimen, written informed consent from each patient was waived.

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