Evaluation of Measurement Uncertainty of Some Biochemical Parameters According to ISO/TS 20914 Guidance

Bazı Biyokimyasal Parametrelerin Ölçüm Belirsizliğinin ISO/TS 20914 kılavuzuna Göre Değerlendirilmesi

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Abstract

Aim: Laboratory tests are an important part of clinical diagnostic decision. Therefore, measurement uncertainty stands out in the context of the accuracy of the laboratory results. In the study, different quality objectives and results were evaluated by investigating the measurement uncertainty of 29 routine biochemical analytes.

Material and Methods: The measurement uncertainty calculation model of 29 analytes were analyzed with the Mindray BS-800 autoanalyzer between October 2020 and April 2021, and evaluated according to ISO/TS 20914 Guideline. The external uncertainty and measurement uncertainties consist of definition of the measured values, determination of the factors affecting the measurement, method measurement uncertainty, calibration uncertainty, and quality control data were determined.

Results: The measured analytes as triglyceride, iron, phosphorus, GGT, creatine kinase, uric acid, lipase and CRP were compatible with both levels according to the total allowable error (TEa%) of EFLM and Ricos. Also, it was observed that the other parameters were not compatible with both levels. Further and comprehensive studies are needed.

Conclusion: Measurement uncertainty shows the distribution of measurement results which displays an important role in the evaluation of measurement of laboratory tests. In our study, it was determined that triglyceride, iron, phosphorus, GGT, creatine kinase, uric acid, lipase and CRP analytes were compatible with both levels, according to the total allowable error (TEa%) of EFLM and Ricos. Also, it was observed that the other parameters were not compatible with both levels. Further and comprehensive studies are needed.

Key words: Measurement uncertainty, analysis, quality control, external uncertainty

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INTRODUCTION

Biochemical quantitative analyses are based on the results of many clinical decisions. The measurement uncertainty is a quantitative indicator of the quality of the measured results. From another respect, it may show the expanding to which the result represents the exact value. If the measurement uncertainty presented with the results, correct information about the quality of the measurements will be arrive to the users (1). Measurement uncertainty may provide information on the level of confidence on the measurements. Although the concept of precision expresses uncertainty, more comprehensive uncertainty calculations should be made by evaluating more component effects in biochemical measurements (2). The information which presented with the International Vocabulary of Metrology (VIM2), Guide to the expression of uncertainty in measurement (GUM1), ISO/IEC and VIM3 documents are provide metrological techniques for the calculations. However, there is no consensus on how the calculation should be made, yet (3).

There are many imprecise reasons that may cause measurement uncertainty in laboratories such as sampling method, sample matrix, environment conditions, uncertainty of instruments, errors in calibration, methods and procedures (4). While calculating the measurement uncertainty, the standard uncertainty values from each uncertainty source can also be calculated separately. From the studies, the expanded uncertainty value is calculated by increasing the confidence interval of the uncertainty by multiplying the total uncertainty by the coverage factor ($k$) (5). In addition, the total errors are equal to the sum of the systematic and random error in the measurement procedures (6). Total allowable error (TEa) is an analytical quality requirement that adjust the uncertainty (random error) and bias (systematic error) of a single test result or measurement within tolerable limits. Clinical Laboratory Implementation Amendments 1988 (CLIA’88) criteria specify the legally permissible maximum error limits of the substances which being measured in the laboratories. There are different recommendations for the TEa calculation on the basis of biological variability coefficients in European countries. If the total error for an analyte is within the limits of TEa, it’s assumed that the diagnostic efficiency of the system seems as appropriate (7). The ISO/TS 20914 guide became available in 2019. This ISO document is presented as a guide for the practical application of measurement uncertainty estimation for the clinical laboratory. The use of the coefficient of variation and the potential for misuse requires certain concerns about clarification between changes in measurement conditions and the potentially misleading, pooled variance and uncertainty of measurement, calculation of the international normalized ratio (INR), rules for evaluating unified uncertainty in functional relationships, and some of the clarified measurement uncertainties are also presented in the guidance (8, 9).

In the study, we aimed to evaluate of 29 routine biochemistry analytes according to ISO/TS 20914 measurement uncertainty guidance which studied in Karapınar State Hospital laboratory.

MATERIAL AND METHODS

The study was carried out with 29 biochemical analytes between October 2020 and April 2021. In the study, analytes were measured with Mindray BS-800 autoanalyzer (glucose, total protein, albumin, BUN, uric acid, creatinine, creatinine kinase, cholesterol, HDL-cholesterol, triglyceride, sodium, potassium, chloride, magnesium, calcium, phosphorus, amylase, lipase, lactate dehydrogenase, total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase, GGT, CRP, iron and iron binding capacity). The measurement uncertainty of these analytes was calculated and performed according to the ISO/TS 20914 MU guidance. The bias values were calculated from external quality control results according to the ISO 20914 guideline. In the study, desirable bias values were obtained from the Westgard’s biodatabase (www.westgard.com/biodatabase1.htm). According to the ISO 20914 guideline, if the bias estimated from EQC testing is within allowable error limits, bias can be ignored. The bias (%) values were lower than the desirable bias (%) values in our study (Table 1). The reagent, calibrator and control lot number were followed, and there was no change during this period. This retrospective study was approved by the Necmettin Erbakan University Meram Faculty of Medicine, Non-Invasive Clinical Research Ethics Committee (Decision Number: 2021/3228).

Statistical Analysis

The combined standard uncertainty calculation $U(y)$ was performed using the $\sqrt{(uRw^2 + ucal^2)}$ ($U_{cw}$: long term precision, $U_{cal}$: calibrator uncertainty) formula. Within the scope of ISO/TS 20914 guideline, standard deviation (SD) of internal quality control data was accepted as $uRw$. Ucal data was obtained from Mindray Company. Internal quality control data (between 01.10.2020 and 01.04.2021) were used for
RESULTS

The expanded uncertainty, standard combined uncertainty and measurement uncertainty from sources were calculated for 29 different analytes and analyzed with Mindray BS-800 autoanalyzer (Table 1). The TEa% values of 29 different parameters were calculated in the study and shown in the Table 2. Triglyceride, iron, phosphorus, GGT, creatine kinase, uric acid, lipase and CRP were compatible with EFLM and Ricos total allowable error (TEa%) values at both levels. ALT was compatible with level 2 and level 1 and Ricos total allowable error (TEa%) values at both levels. In addition, LDH and BUN were compatible with level 2 according to EFLM. In the other parameters were not compatible with Westgard and Ricos specification limits at both levels.

DISCUSSION

The measurement uncertainty, characterized by the distribution of values accordance with the measured quantity, is associated with the measurement result. Moreover, each measurement result has a measurement uncertainty which consists of different stages such as analysis, incomplete information and sampling (10). So, the measurement uncertainties together with the measured results can be widely used in the future (11). There is an understanding of the essential role of reference measurement systems in clinical biochemistry, but general agreement has not yet been reached on user-performing secondary tuning via new patient samples to minimize or eliminate the bias. To eliminate bias is important mission for laboratory organizations serving patients and healthcare professionals (12). A study proposed that specifying the measurement uncertainty along with the laboratory results of the patient may affect the interpretation of the test results, as clinically (13). Although the determination of measurement uncertainty is not obligatory, clinical laboratories are required to have measurement uncertainty information related to the tests being studied (14). According to EFLM and Ricos, TEa% values

| Test | Level 1 SD | Level 1 Mean | Level 2 SD | Level 2 Mean | CAL C | CAL U | Level 1 combined expanded uncertainty | Level 1 expanded measurement uncertainty | Level 2 combined expanded uncertainty | Level 2 expanded measurement uncertainty | Bias (%) | Desirable Bias (%) |
|------|------------|--------------|------------|--------------|-------|-------|-------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------|---------------------|
| Albumin (g/dL) | 0.10 | 31.3 | 0.14 | 48.0 | 33.7 | 1.31 | 1.31 | 2.63 | 1.32 | 2.63 | 1.32 | 2.63 | 1.32 | 2.63 |
| Magnesium (mg/dL) | 0.08 | 2.05 | 0.14 | 3.37 | 1.22 | 0.08 | 0.11 | 0.23 | 0.16 | 0.31 | -0.9 | 3.2 |
| T. Protein (g/dL) | 0.26 | 46.6 | 0.76 | 78.7 | 50.8 | 1.22 | 1.25 | 2.50 | 1.44 | 2.87 | -2.1 | 1.36 |
| ALP (U/L) | 6.33 | 97.5 | 11.3 | 225 | 248 | 8.58 | 10.7 | 21.3 | 14.2 | 28.4 | -2.7 | 6.72 |
| ALT (U/L) | 2.57 | 57.6 | 4.86 | 123 | 104 | 4.78 | 5.43 | 10.9 | 6.89 | 13.8 | -2.2 | 11.48 |
| Amylase (U/L) | 2.84 | 85.7 | 4.10 | 205 | 194 | 7.74 | 8.25 | 16.5 | 8.76 | 17.5 | 0.9 | 26.2 |
| Creatinine (mg/dL) | 0.09 | 1.03 | 0.19 | 3.73 | 3.99 | 0.15 | 0.17 | 0.35 | 0.24 | 0.48 | -1.5 | 3.96 |
| AST (U/L) | 1.54 | 49.5 | 3.89 | 145 | 109 | 5.97 | 6.16 | 12.3 | 10.3 | 20.6 | -3.0 | 6.54 |
| T. chol (mg/dL) | 2.21 | 84.7 | 4.84 | 188 | 158 | 5.14 | 5.60 | 11.2 | 7.13 | 14.3 | 1.4 | 4.1 |
| Calcium (mg/dL) | 0.29 | 8.59 | 0.83 | 12.8 | 10.9 | 0.23 | 0.37 | 0.74 | 0.86 | 1.71 | 0.6 | 0.82 |
| HDL-chol (mg/dL) | 1.09 | 28.3 | 2.86 | 78.6 | 69.2 | 2.63 | 2.85 | 5.69 | 3.88 | 7.77 | -1.3 | 5.61 |
| Potassium (mg/dL) | 1.18 | 3.55 | 0.17 | 6.66 | 3.48 | 0.02 | 0.18 | 0.36 | 0.17 | 0.35 | -1.6 | 4.12 |
| Triglyceride (mg/dL) | 3.87 | 114 | 5.42 | 200 | 131 | 4.78 | 4.78 | 10.9 | 6.89 | 14.5 | 1.0 | 9.57 |
| T. Bilirubin (mg/dL) | 0.08 | 1.01 | 0.26 | 3.60 | 5.08 | 0.20 | 0.22 | 0.43 | 0.33 | 0.86 | 1.1 | 8.95 |
| D. Bilirubin (mg/dL) | 0.05 | 0.81 | 0.15 | 2.17 | 2.68 | 0.13 | 0.14 | 0.29 | 0.20 | 0.40 | 0.6 | 5.6 | 14.2 |
| Iron (µg/dL) | 11.5 | 104 | 21.1 | 216 | 37.8 | 0.93 | 11.5 | 23.0 | 21.2 | 42.3 | -4.7 | 8.8 |
| LDH (U/L) | 8.04 | 170 | 3.89 | 307 | 266 | 6.76 | 10.5 | 21.0 | 10.8 | 21.5 | -1.5 | 2.2 |
| Iron-binding (µg/dL) | 12.6 | 155 | 15.7 | 213 | 105 | 2.68 | 12.9 | 25.8 | 16.9 | 31.9 | 0.4 | 6.4 |
| Sodium (mEq/L) | 2.20 | 113 | 1.49 | 135 | 120 | 0.39 | 2.23 | 4.46 | 1.54 | 3.08 | -2.2 | 3.1 |
| BUN (mg/dL) | 3.16 | 40.8 | 2.89 | 113 | 100 | 3.83 | 4.97 | 9.93 | 4.80 | 9.59 | 0.2 | 5.57 |
| Potassium (mg/dL) | 0.18 | 4.56 | 0.24 | 9.62 | 1.68 | 0.09 | 0.20 | 0.40 | 0.26 | 0.51 | 1.2 | 4.6 |
| GGT (U/L) | 3.16 | 102 | 7.14 | 232 | 195 | 9.98 | 10.5 | 20.9 | 12.3 | 24.5 | 1.4 | 1.8 |
| Creatine kinase (U/L) | 4.70 | 145 | 10.1 | 266 | 350 | 11.6 | 12.5 | 25.0 | 15.4 | 30.8 | -1.9 | 11.5 |
| Uric acid (mg/dL) | 0.30 | 5.07 | 0.26 | 9.54 | 5.09 | 0.29 | 0.42 | 0.83 | 0.39 | 0.79 | 2.8 | 4.87 |
| Glucose (mg/dL) | 3.16 | 102 | 7.14 | 232 | 195 | 9.98 | 10.5 | 20.9 | 12.3 | 24.5 | 1.4 | 1.8 |
| Lipase (U/L) | 2.00 | 44.4 | 2.96 | 103 | 97.4 | 4.40 | 4.83 | 9.86 | 5.30 | 10.6 | 0.1 | 11.31 |
| CRP (mg/dL) | 0.89 | 9.63 | 2.52 | 53.2 | 9.10 | 0.75 | 1.02 | 2.04 | 2.63 | 5.26 | 12.2 | 21.8 |
| Chloride (mEq/L) | 3.29 | 87.20 | 2.31 | 108 | 85.10 | 0.48 | 3.33 | 6.66 | 2.36 | 4.71 | -0.9 | 0.5 |

SD: standard deviation, uRw: long term precision, CAL C: calibrator concentration, CAL U/Ucal: calibrator uncertainty, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate transaminase, LDH: lactate dehydrogenase, BUN: blood urea nitrogen, GGT: gamma glutamyl transferase, CRP: C-reactive protein.
of triglyceride, iron, phosphorus, GGT, creatine kinase, uric acid, lipase and CRP analytes as were observed compatible with both levels. While ALT was compatible with level 2 and level 1 according to Ricos, amylase, AST, total cholesterol, HDL, potassium, total bilirubin were compatible with level 2. LDH and BUN were compatible with level 2 according to EFLM in our study. In a study, Kutukcu et al. reported that albumin, amylase, alanine transaminase, total bilirubin, direct bilirubin, blood urea nitrogen, calcium, creatinine kinase, chlorine, creatinine, glucose, potassium, lipase, magnesium, sodium, total protein, phosphorus, CRP, aspartate transaminase and troponin-I test results were compatible with CLIA'88 limits, but the MU results of albumin, calcium, chlorine, magnesium, sodium and total protein were not compatible with Westgard limits (15). In another study, the uncertainty of HbA2 measurement was found to be as 12.4% in the Tosoh HLC 723 G8 device (16). Dulgeroglu et al. (17) declared that there was ± 0.4 measurement uncertainty at the 6% medical decision level for HbA1c, and they proposed that these result may affect the clinical decision. From the reports, it is assumed that the uncertainty calculations of interlaboratory and intralaboratory performance data may facilitate the calculation of measurement uncertainty (18). Recently, ISO is implementing a new project to evaluate measurement uncertainty in medical laboratories (ISO/NP TS 20914 Medical Laboratories-Practical Guide for the measurement of uncertainty) (19). Ayyildiz et al. (20) were evaluate the measurement uncertainty of dihydroepiandrosterone sulfate analysis (DHEA-S), and they found measurement uncertainty of DHEA-S as 95%, and the confidence interval as +15.5%. They declared that the calculation of measurement uncertainty in tests may provide transparency in the evaluation of the results.

Ricos et al. (21) defined bias and total error to adjust analytical quality within acceptable limits in terms of desirable properties for uncertainty. In a measurement uncertainty study which conducted by Cubukcu et al. (22) they evaluated 14 parameters and found that the measurement uncertainties of TSH, estradiol, LH, progesterone, prolactin and vitamin B12 were within the allowable limits, U-unilateral

| Test              | Level 1, Urel% | Level 2, Urel% | Ricos (TEa%) | EFLM (TEa%) |
|-------------------|----------------|----------------|--------------|-------------|
| Albumin           | 8.39           | 5.48           | 4.07         | 3.40        |
| Magnesium         | 11.08          | 9.29           | 4.80         | 4.00        |
| T.Protein         | 5.36           | 3.65           | 3.63         | 3.50        |
| ALP               | 21.88          | 12.62          | 12.04        | 10.50       |
| ALT               | 18.84          | 11.16          | 27.48        | 16.10       |
| Amylase           | 19.24          | 8.55           | 14.60        | 13.20       |
| Creatinine        | 33.72          | 12.94          | 8.87         | 7.40        |
| AST               | 24.92          | 14.17          | 16.69        | 13.60       |
| T.cholesterol     | 13.22          | 7.56           | 9.01         | 8.70        |
| Calcium           | 8.65           | 13.41          | 2.55         | 2.30        |
| HDL-cholesterol   | 20.09          | 9.88           | 11.63        | 11.10       |
| Potassium         | 10.20          | 5.24           | 5.61         | 4.80        |
| Triglyceride      | 10.75          | 7.20           | 25.99        | 27.00       |
| T. Bilirubin      | 42.69          | 18.23          | 26.94        | -           |
| D. Bilirubin      | 35.38          | 18.39          | 44.50        | -           |
| Iron              | 22.17          | 19.63          | 30.70        | -           |
| LDH               | 12.34          | 7.02           | 11.40        | 7.70        |
| Iron-binding      | 16.66          | 14.95          | -            | -           |
| Sodium            | 3.96           | 2.27           | 0.73         | 0.70        |
| BUN               | 24.37          | 8.46           | 15.55        | 17.80       |
| Phosphorus        | 8.87           | 5.30           | 10.11        | 9.70        |
| GGT               | 17.10          | 13.43          | 22.11        | 18.90       |
| Creatine kinase   | 17.23          | 11.57          | 30.30        | 22.60       |
| Uric acid         | 16.46          | 8.25           | 11.97        | -           |
| Glucose           | 20.47          | 10.57          | 6.96         | 6.50        |
| Lipase            | 21.75          | 10.25          | 37.88        | 14.20       |
| CRP               | 21.22          | 9.88           | 56.60        | 50.70       |
| Chloride          | 7.63           | 4.37           | 1.50         | 1.30        |

TEa: Total Allowable Error, Urel %: expanded uncertainty of measurement
FT3 and U-unilateral ferritin exceeded the defined TEa%, and also U-FT3 and U-ferritin (pUEQAS%) were at low values. Moreover, they concluded that the measurement uncertainties of FT4, cortisol, DHEAS, FSH, testosterone and folate were not within the specification limits. The adequate application of modern medicine is unlikely without the results of tests carried out in clinical laboratories. The measurement of these tests is performed by a series of complex precision instruments and various automated electronic equipment using test procedures. However, no test result is completely certain. These uncertainties and errors in test results may also vary depending on the operator skill, measurement system, environmental situations, and other factors. So, the concept of uncertainty of measurements was needed to precise the uncertainty (23, 24).

The principal assumption in calculating measurement uncertainty is related with information for the identification and correction of all systematic errors. The quality of a measurement is associated with the uncertainty about random and systematic error is taken into account on the correct basis (25). The U values calculated from all the tests which analyzed by Mindray BS-800 device was within the Ricos and EFLM total error limits, and we proposed that these values could be used in our laboratory. Also, it is suggested that improvements should be made to reduce error sources for these tests.

CONCLUSION

In our study, we aimed to compare the TEa % values of EFLM and Ricos according to the current ISO/TS 20914 guideline by calculating the measurement uncertainty of clinical biochemistry parameters that are frequently studied in our hospital. All conditions in the laboratory may affect the uncertainty. For this reason, uncertainty should be constantly monitored. Errors related medical decisions are becoming more important than before. For this reason, it is important to report the measurement uncertainty to the clinician with the results. To the best of our knowledge, there are very limited studies on this topic. So, comprehensive and multicenter studies are needed.

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