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ORIGINAL ARTICLE / ARAŞTIRMA YAZISI

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Calculation of Measurement Uncertainty of 20 Clinical Chemistry Analytes According to the Practical ISO Approach

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ABSTRACT

Purpose: Measurement Uncertainty (MU) is a valuable tool for evaluating analytical performance and interpreting results in clinical laboratories. The International Organization for Standardization (ISO) has proposed a practical approach for MU calculation in its ISO/TS 20914:2019 guide. This study aimed to calculate the MU values of 20 clinical chemistry analyses per the ISO guideline and compare them with the Maximum expanded allowable measurement uncertainty (MAU) values.

Methods: The study was performed using long-term imprecision (uRw) obtained from 6-month internal quality control (IQC) values, and calibrator uncertainty (ucal) in line with the recommendations of the ISO/TS 20914:2019 guideline. The pooled MU value was calculated for 20 clinical chemistry tests on two identical devices, Roche Cobas 6000 c501 (Roche Diagnostics, Mannheim, Germany) analyzers. The calculated MU values for the tests were compared with the current MAU values in the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation database (the current Clinical Laboratory Improvement Amendments/CLIA recommendation for Ethanol has been selected).

Results: MU values for Alanine aminotransferase, C-reactive Protein, Iron, Ethanol, Total Bilirubin, Triglyceride, and Blood urea nitrogen remained within the MAU limits. The MU values of the other 13 tests (excluding Aspartate aminotransferase, Glucose, and Potassium Level 2 IQC) exceeded the MAU values.

Conclusion: It was observed that the uRw value affected the MU value the most. Close monitoring and evaluation of uRw and thus IQC and implementation of corrective and preventive actions may reduce MU.

Keywords: Measurement uncertainty, internal quality control, quality management, laboratory medicine, clinical chemistry

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20 Klinik Biyokimya Analitinin pratik ISO yaklaşımına göre ölçüm belirsizliği hesaplaması

ÖZET

Amaç: Ölçüm belirsizliği (MU), klinik laboratuvarlarda analitik performansın değerlendirilmesi ve sonuçların yorumlanması için değerli bir araçtır. The International Organization for Standardization (ISO), ISO/TS 20914:2019 kılavuzunda MU hesaplaması için pratik bir yaklaşım önermiştir. Bu çalışmada ISO kılavuzu doğrultusunda 20 klinik kimya analizinin MU değerlerinin hesaplanması ve İzin verilebilir genişletilmiş ölçüm belirsizliği değerleri (MAU) değerleriyle karşılaştırılması amaçlanmıştır.

Yöntemler: Çalışma ISO/TS 20914:2019 kılavuzu önerileri doğrultusunda, 6 aylık iç kalite kontrol (İKK) değerlerinden elde edilen uzun vadeli belirsizlik bileşeni (uRw) ve kalibratör belirsizliği (ucal) kullanılarak gerçekleştirilmiştir. İki özdeş cihaz olan Roche Cobas 6000 c501 (Roche Diagnostics, Mannheim, Almanya) analizörleri üzerinden 20 klinik kimya testi için ortak MU değeri hesaplanmıştır. Testler için hesaplanan MU değerleri The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biyolojik Varyasyon veri tabanındaki güncel MAU değerleriyle (Etanol için güncel Clinical Laboratory Improvement Amendments/CLIA önerisi seçilmiştir) kıyaslanmıştır.

Sonuçlar: Alanin aminotransferaz, C-reaktif Protein, Demir, Etanol, Total Bilirubin, Trigliserid ve Kan üre nitrojeni için MU değerleri MAU sınırları içerisinde kalmıştır. Diğer 13 testin MU değerleri (Aspartat aminotransferaz, Glukoz ve Potasyum Level 2 IQC hariç) MAU değerlerini aşmıştır.

Sonuç: MU değerini en fazla uRw değerinin etkilediği görülmüştür. uRw'nin dolayısıyla İKK'nın yakın takibi, değerlendirilmesi ve düzeltici önleyici faaliyetlerin uygulanması MU'nun azaltılabilmesini sağlayabilir.

Anahtar kelimeler: Ölçüm belirsizliği, iç kalite kontrol, kalite yönetimi, laboratuvar tıbbı, klinik biyokimya

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Analysis results performed in clinical laboratories play a critical role in the patient's diagnosis, treatment, monitoring, and risk assessment. Therefore, there is a need for precise and accurate routine measurements that can ensure the reliability of the measurement result and the appropriate treatment for the patient (1). Total error (TE) is the first approach to evaluate measurement reliability and accuracy. TE consists of a combination of random and systematic errors and indicates the deviation of the measured value from the exact value (2). Based on these error components, Westgard et al. formulated the TE value as the absolute value of the measured bias plus 2 standard deviations (3). The TE concept requires knowing the exact value of the measurement results; otherwise, the TE cannot be calculated. Another approach to the assessment of measurement accuracy, the concept of measurement uncertainty (MU), is expressed as a non-negative parameter associated with the result of the measurement, which characterizes the distribution of values that can reasonably be attributed to the measurement (2). MU assumes that the exact value of the test results cannot be known, and the lack of a fully accurate value of the results is highlighted (1). MU indicates the range in which the measurable value is determined and that the measurement of values in this range can occur with the same probability for that analyte (4). As with the TE concept, the MU concept requires comparison with allowable analytical performance specifications (APS) to determine whether a result deviates significantly from accuracy (5). International accreditation bodies such as The International Organization for Standardization (ISO), Joint Committee for Guides in Metrology (JCGM), and The International Laboratory Accreditation Cooperation (ILAC) state that the MU values of test results should be evaluated appropriately in routine laboratory practices (6–8).

Many factors can contribute to the MU value, including matrix effects, interferences, environmental factors, uncertainties from reference materials, the uncertainty of commercial system calibrators, and measurement uncertainty methods and procedures (9). Literature data points to two models in the approaches that can be used to estimate measurement uncertainty. The first is the bottom-up model proposed by JCGM (6). In this model, all potential sources of uncertainty that significantly affect the outcome for a given measurement procedure (e.g., calibration, weighing, pipetting, temperature, and instrument fluctuations) are identified, and the uncertainty of each is considered. However, this model is unsuitable for use in routine laboratory medicine, as it is necessary to identify a large number of sources and use complex mathematical models (1). The other model is the top-down approach, where measurement uncertainty is calculated from internal and external quality control data or method verification data (10). A practical approach to MU calculation is proposed in conjunction with the ISO/TS 20914:2019 guide. According to this guideline, it is recommended to calculate the MU value mainly based on long-term imprecision (uR_w) and calibrator uncertainty (u_{cal}) and add the bias (u_{bias}) to the MU calculation only in cases where it creates a significant medical difference (9).

This study aimed to compare the MU values of the biochemistry parameters studied in two identical devices of the same brand and model in our laboratory, based on the ISO/TS 20914:2019 guideline, with the allowable analytical performance specifications and is to evaluate the impact of results on possible clinical decisions over clinical decision thresholds.

MATERIAL AND METHOD

This retrospective and single-center study was approved by the Gaziosmanpaşa Training and Research Hospital Clinical Research Ethics Committee (Decree Date and No: 22 December 2021/393) and was conducted per the Declaration of Helsinki principles.

We calculated the MU values in line with the "Combined standard uncertainties and expanded uncertainties ISO/TS 20914:2019" guideline (9). We determined the definitions of the quantities for 20 clinical chemistry analytes from which MU values were to be calculated (Table 1). The analysis was carried out using two identical (A and B measurement systems) Roche Cobas 6000 c501 (Roche Diagnostics, Mannheim, Germany) biochemistry auto analyzer and the manufacturer's original reagents in the Medical Biochemistry Laboratory of Gaziosmanpaşa Training and Research Hospital.

Table 1. Measurands Definitions

Test (Abbreviations)	Method	Sample type
Albumin (Alb)	Bromocresol green colorimetric method	Serum
Alanine aminotransferase (ALT)	IFCC method without pyridoxal phosphate activation	Serum
Amylase (Amy)	IFCC method, enzymatic colorimetric	Serum
Aspartate aminotransferase (AST)	IFCC method without pyridoxal phosphate activation	Serum
C-reactive protein (CRP)	Immunoturbidimetric method with expanded particle surface	Serum
Iron (Fe)	Ferrozine colorimetric method	Serum
Ethanol (EtOH)	Enzymatic method with alcohol dehydrogenase	Serum
Glucose (Glu)	Enzymatic hexokinase, colorimetric method	Serum
HDL - Cholesterol (HDL-C)	Homogeneous enzymatic colorimetric method	Serum
Calcium (Ca)	Colorimetric method, o-cresolphthalein complex	Serum
Chloride (Cl)	Indirect method using ion-selective electrodes	Serum
Creatinine (Crea)	Jaffe kinetic colorimetric method	Serum
Potassium (K)	Indirect method using ion-selective electrodes	Serum
Sodium (Na)	Indirect method using ion-selective electrodes	Serum
Total Bilirubin (T.Bil)	Diazo method	Serum
Total Cholesterol (Cholesterol)	Enzymatic colorimetric method	Serum
Total Protein (TP)	Colorimetric	Serum
Triglyceride (TG)	Enzymatic colorimetric	Serum
Blood urea nitrogen (BUN)	Kinetic test with urease and glutamate dehydrogenase	Serum
LDL- Cholesterol (LDL-C)	Homogeneous enzymatic colorimetric method	Serum

Calculations

Standard deviation (SD), which measures the distribution of values obtained from precision studies under long-term precision conditions, is called standard uncertainty (u) in measurement uncertainty calculations (SD = u). To estimate the overall (combined) uncertainty of the result, it is necessary to combine values from different uncertainty sources. According to the ISO/TS 20914:2019 guideline, under long-term precision conditions, which contribute to uncertainty in the calculation of u(y) of the Y analyte measured in the laboratory, the uncertainty of the measurement procedure (uR_w), the uncertainty of the value assigned to the calibrator (u_{cal}) and the uncertainty (u_{bias}) of the bias from the specified value are combined (Formula 1). We calculated the uRw component of uncertainty based on the last six months' internal quality control (IQC) results of normal and pathological control materials studied in the auto analyzer (PreciControl ClinChem Multi 1 Lot no: 47572405, 46149001 and 49417305; PreciControl ClinChem Multi 2 Lot no: 46159701, 46160304 and 46160305, Roche Diagnostics, Mannheim, Germany). We included the ucal values in the calculations per the manufacturer's declaration. We did not add the ubias component to the uncertainty calculation as no medically significant bias was observed. Because the mean values of the different IQC lots differ from each other, we calculated the pooled average uRw over three different lots (Formula 2).

$$u(y)=\sqrt{(uR_w^2 + u_{cal}^2 + u_{bias}^2)} \text{ (Formula 1).}$$

$$\text{Pooled average } uR_w(\text{lot1, lot2, lot3}) = \frac{\sqrt{(u^2\text{-}1+u^2 2+u^2 3)}}{3} \text{ (Formula 2).}$$

Since it is not known in advance which measurement system the samples will be studied in laboratories with more than one identical device, it is recommended to calculate a single pooled average standard uncertainty [u(pooled)] that can be applied to two devices. We calculated the means $\dot{x}$(A), $\dot{x}$(B), $\dot{x}$(A, B) and variances uR_w^2(A), uR_w^2(B), uR_w^2(A, B) for each measurement system from the IQC data used on two identical instruments A and B in our laboratory. We then calculated the variance u^2(A, B) of the two mean values between the two measurement systems (Formula 3). For u(pooled) calculation, we combined u^2(A, B) and uR_w^2(A, B) (Formula 4). For a single u(y) value, the u(pooled) value could now be combined with u_{cal} and u_{bias} (in the case of a medically significant bias) (Formula 1).

$$\text{Variance } SD^2(A, B) = u^2(A, B) = [\sum \dot{x}\text{- } \dot{x}\ (A, B)]/(n\text{-}1) \text{ (Formula 3).}$$

$$u(\text{pooled}) = \sqrt{(u^2(A, B) + uR_w^2(A, B))} \text{ (Formula 4).}$$

We calculated the expanded uncertainty (U) by multiplying the calculated u(y) value for each analyte with k (coverage factor) and the percentage relative expanded uncertainty value ($\%U_{rel}$) according to the mean value (Formulas 5 and 6). We set the k value as 2 to represent the 95% confidence interval.

$U(y) = 2 \times u(y)$ (Formula 5).

$$\%U(y)\ \text{rel} = \frac{(U(y))}{\text{mean}} \times 100 \text{ (Formula 6).}$$

We obtained maximum expanded allowable measurement uncertainty (MAU) targets by selecting desirable targets from The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation database for tests other than ethanol (11). We determined the MAU value for ethanol as 20%, which is the current acceptance limit of Clinical Laboratory Improvement Amendments (CLIA) (12). Microsoft Office 365 (Microsoft Excel Software, Microsoft Corporation, US) was used to perform the calculations and create the tables.

RESULTS

The $\%U_{rel}$ (pooled) values of two identical devices for ALT, CRP, Fe, EtOH, T. Bil, TG, and BUN remained within the MAU values. The $\%U_{rel}$ (pooled) values of the two identical devices for AST, Glu, and K remained within the MAU values only for Level 2 IQC. The $\%U_{rel}$ (pooled) values of two identical devices for the other ten tests exceeded the MAU values for both levels (Table 2). u^2 (A, B), u^2R_w (A, B), u^2cal, u(y) (A, B), $\%U_{rel}$ (pooled), and MAU values calculated for two identical devices are presented in Table 2. The IQC control number (n), %CV, u^2R_w, u^2_{cal}, u(y), and $\%U_{rel}$ values of all tests on both devices can be found in Supplemental Tables 1 and 2.

DISCUSSION

The present study revealed that the MU values calculated for only 7 of the 20 clinical biochemistry analytes (ALT, CRP, Fe, EtOH, T. Bil, TG, and BUN) remained within the MAU values. When we reviewed the MU components, we determined that the uRw values obtained from the internal quality control studies were the biggest contributors to the combined standard uncertainty. Similar to our study, uRw appears as a basic component in current MU approaches (9,13). Hence, it is of great importance to evaluate the IQC, follow up on inappropriate results, and take corrective and preventive actions.

Keeping MU values within as a narrow range as possible means producing quality and reliable test results suitable for patient care. Although it is not obligatory to present MU values in laboratory result reports, laboratories must have MU information about the tests to inform clinicians upon their request. For example, if the clinician has a request for MU for a patient with a glucose value of 120 mg/dl (U_{rel}=10%), the possible options for reporting MU would be 120±12 mg/dl, 120 mg/dl ±10% or 120 mg/dl (108 – 132 mg/dl) (k=2, 95% CI) (9,14).

The MU calculation we made for glucose in our study revealed the $\%U_{rel}$ (pooled) values, including identical A and B measurement systems in our laboratory, as 10.4% and 4.2% for IQC-1 and 2, respectively. We determined that the MU we calculated for the IQC-2 met the targeted quality specification (5%), but the MU value for the IQC-1 material exceeded the allowable targets. Measurement of blood glucose plays a central role in the diagnosis and follow-up of diabetes and the assessment of organ damage risks due to glucose metabolism disorders (15). Therefore, clinicians expect accurate and reliable glucose results to be provided with the highest level of consistency with the clinical condition. In this context, evaluating MU together with test results can help clinicians interpret patient results to understand whether a glucose test result measurably exceeds the medical decision limit.

The present study found the $\%U_{rel}$ (pooled) values for creatinine to be 11.9% and 7.6% for IQC-1 and 2, respectively. We determined that the MU values did not meet the allowable quality specification (4.5%). It was demonstrated that very small changes in serum creatinine concentrations, widely used for diagnosing and treating kidney diseases, are directly related to the severity of acute kidney injury (16,17). Therefore, knowing with what uncertainty the creatinine test result is measured will make valuable contributions to the proper management of acute kidney injury. Also, we saw that $\%U_{rel}$ (pooled) values for Na, K, and Cl remained outside the MAU (0.5%, 4.1%, and 1.1% for Na, K, and Cl, respectively). These ions are tightly controlled by metabolic and renal mechanisms (5). In particular, the K value may change due to the drugs used, and for this reason, close follow-up is recommended in terms of both its effect on the cardiac system and the evaluation of renal functions (15). Keeping the MU values of these tests within the allowable range may benefit clinicians in managing diseases.

Table 2. Within-laboratory precision, calibration uncertainty and percent relative expanded uncertainty values of two identical devices calculated according to ISO guideline

Test	Material	u^2(A,B)	u^2R_w(A,B)	u^2cal	U(y) (A,B)	U_{rel}% (pooled) (k=2)	MAU(%)
Alb	IQC-1 (A,B)	0,02	1,8939	0,0882	1,42	**8,65**	2,5
	IQC-2 (A,B)	0	3,0553	0,0882	1,77	**7,01**	
ALT	IQC-1 (A,B)	0,005	3,7445	0,1018	1,96	8,29	10,1
	IQC-2 (A,B)	0,08	15,2767	0,1018	3,93	6,36	
Amy	IQC-1 (A,B)	0,845	40,639	0,49	6,48	**16,15**	6,6
	IQC-2 (A,B)	0,5	84,013	0,49	9,22	**9,78**	
AST	IQC-1 (A,B)	0,02	4,9199	0,1845	2,26	**9,74**	9,6
	IQC-2 (A,B)	0,08	25,1047	0,1845	5,04	7,03	
CRP	IQC-1 (A,B)	0,005	0,1229	1,051	1,09	22,98	34,1
	IQC-2 (A,B)	0,002	5,5586	1,051	2,57	10,04	
Fe	IQC-1 (A,B)	64,98	3007	164,4	56,89	10,33	20,7
	IQC-2 (A,B)	320	8008,86	164,4	92,16	7,71	
EtOH	IQC-1 (A,B)	0,13	6,6218	1,452	2,86	11,3	20*
	IQC-2 (A,B)	0,18	23,231	1,452	4,99	6,7	
Glu	IQC-1 (A,B)	0,08	27,5749	0,637	5,32	**10,4**	5
	IQC-2 (A,B)	1,445	21,7093	0,637	4,88	4,2	
HDL-C	IQC-1 (A,B)	0,32	1,625	0,3399	1,51	**9,5**	5,8
	IQC-2 (A,B)	1,125	17,1055	0,3399	4,31	**14,2**	
Ca	IQC-1 (A,B)	0,02	0,0901	0,00188	0,34	**7,52**	1,8
	IQC-2 (A,B)	0,045	0,1093	0,00188	0,4	**5,83**	
Cl	IQC-1 (A,B)	0,245	6,9892	0,16	2,72	**6,41**	1,1
	IQC-2 (A,B)	0,125	5,1305	0,0625	2,31	**4,52**	
Crea	IQC-1 (A,B)	0,00005	0,0031	0,00083	0,06	**11,9**	4,5
	IQC-2 (A,B)	0,00005	0,0226	0,00083	0,15	**7,6**	
K	IQC-1 (A,B)	0	0,0111	0,000625	0,11	**6,02**	4,1
	IQC-2 (A,B)	0	0,0157	0,0001	0,13	3,7	
Na	IQC-1 (A,B)	0,02	7,5145	0,16	2,77	**4,92**	0,5
	IQC-2 (A,B)	0,005	5,7189	0,0625	2,401	**3,58**	
T.Bil	IQC-1 (A,B)	0,005	0,0049	0,000337	0,1	19,27	20
	IQC-2 (A,B)	0,02	0,0651	0,000337	0,29	15,8	
Cholesterol	IQC-1 (A,B)	4,81	10,6041	0,4563	3,98	**7,9**	5,3
	IQC-2 (A,B)	12	30,3426	0,4563	6,54	**7,8**	
TP	IQC-1 (A,B)	1,125	4,4217	0,0181	2,36	**9,7**	2,6
	IQC-2 (A,B)	2,645	8,1977	0,0181	3,3	**8,5**	
TG	IQC-1 (A,B)	0,72	12,8189	0,64	3,77	6,3	20
	IQC-2 (A,B)	1,28	34,3906	0,64	6,03	5,7	
BUN	IQC-1 (A,B)	0,5	1,825	0,194	1,59	8,06	13,9
	IQC-2 (A,B)	4,205	11,2289	0,194	3,95	6,9	
LDL-C	IQC-1 (A,B)	2,38	4,41	0,596	2,72	**8,9**	8,3
	IQC-2 (A,B)	3	41,37	0,596	6,71	**13,7**	

Alb – Albumin, ALT – Alanine aminotransferase, Amy – Amylase, AST – Aspartate aminotransferase, CRP – C-reactive protein, Fe – Iron, EtOH – Ethanol, Glu – Glucose, HDL-C – HDL Cholesterol, Ca – Calcium, Cl – Chloride, Crea – Creatinine, K – Potassium, Na – Sodium, T.Bil – Total Bilirubin, Cholesterol – Total Cholesterol, TP – Total Protein, TG – Triglyceride, BUN – Blood Urea Nitrogen, LDL-C – LDL Cholesterol.

Mean (A, B) – Mean of two measurement systems mean values, u^2 (A, B) – variance of two mean values between two measurement systems, u^2Rw (A, B) - standard uncertainty component for the long-term precision obtained from six months' internal quality control, u^2cal - uncertainty of calibrator values provided by manufacturer, U(y) - expanded uncertainty, %Urel (pooled) - percent relative expanded uncertainty, MAU - Maximum expanded allowable measurement uncertainty.

*%Urel (pooled) values exceeding the MAU are indicated in bold. All MAU values obtained from The EFLM Biological Variation Database (11), except EtOH. *The MAU value of EtOH obtained from updated CLIA (Clinical Laboratory Improvement Amendments) Proposed Acceptance Limits (12).*

Supplemental Table 1. MEASUREMENT UNCERTAINTY COMPONENTS OF CLINICAL CHEMISTRY ANALYTES IN ANALYZER A								
Analyte	Material	n	%CV	u^2R_w	u^2cal	u (y)	$\%U_{rel}$ (k=2)	MAU
Alb	IQC-1	177	3,61	1,42	0,0882	1,23	**7,48**	2,5
	IQC-2	175	2,6	1,74	0,0882	1,35	**5,35**	
ALT	IQC-1	175	4,01	3,69	0,1018	1,95	8,23	10,1
	IQC-2	175	3,37	17,81	0,1018	4,23	6,86	
Amy	IQC-1	169	6,83	31,25	0,49	5,63	**13,93**	6,6
	IQC-2	189	4,25	69,22	0,49	8,35	**8,83**	
AST	IQC-1	176	3,73	3,03	0,1845	1,79	7,73	9,6
	IQC-2	175	3,05	19,18	0,1845	4,4	6,15	
CRP	IQC-1	175	3,56	0,11	1,051	1,08	22,91	34,1
	IQC-2	174	4,22	4,67	1,051	2,39	9,32	
Fe	IQC-1	197	4,88	2917,08	164,4	55,5	10,02	20,7
	IQC-2	187	3,7	7896,1	164,4	89,8	7,47	
EtOH	IQC-1	173	5,03	6,55	1,452	2,83	11,1	20*
	IQC-2	175	3,03	2,37	1,452	1,96	2,6	
Glu	IQC-1	176	2,53	6,71	0,637	2,71	**5,3**	5
	IQC-2	175	1,85	18,32	0,637	4,35	3,8	
HDL - C	IQC-1	175	3,17	1	0,3399	1,16	**7,4**	5,8
	IQC-2	173	7,31	17,81	0,3399	4,26	**14,2**	
Ca	IQC-1	180	3,39	0,096	0,00188	0,31	**6,96**	1,8
	IQC-2	177	2,3	0,096	0,00188	0,31	**4,57**	
Cl	IQC-1	414	2,93	6,25	0,16	2,53	**5,94**	1,1
	IQC-2	407	2,2	5,06	0,0625	2,26	**4,42**	
Crea	IQC-1	187	4,52	0,0025	0,00083	0,058	**10,8**	4,5
	IQC-2	183	3,42	0,0196	0,00083	0,143	**7,1**	
K	IQC-1	430	3,18	0,0121	0,000625	0,113	**6,27**	4,1
	IQC-2	416	1,87	0,0169	0,0001	0,13	3,83	
Na	IQC-1	425	2,59	8,53	0,16	2,95	**5,22**	0,5
	IQC-2	411	1,91	6,55	0,0625	2,57	**3,83**	
T.Bil	IQC-1	193	6,46	0,005	0,000337	0,07	13,16	20
	IQC-2	189	6,9	0,068	0,000337	0,26	13,72	
Cholesterol	IQC-1	178	3,06	9,99	0,4563	3,23	**6,3**	5,3
	IQC-2	177	2,95	25,4	0,4563	5,09	**6**	
TP	IQC-1	177	3,09	2,34	0,0181	1,54	**6,22**	2,6
	IQC-2	176	2,63	4,29	0,0181	2,07	**5,27**	
TG	IQC-1	175	3,05	13,18	0,64	3,72	6,3	20
	IQC-2	173	2,56	29,59	0,64	5,5	5,2	
BUN	IQC-1	180	3,52	1,96	0,194	1,47	7,36	13,9
	IQC-2	177	2,96	11,77	0,194	3,46	5,96	
LDL-C	IQC-1	192	4,3	7,07	0,596	2,77	**9**	8,3
	IQC-2	195	5,25	27,23	0,596	5,28	**10,6**	

Supplemental Table-2: MEASUREMENT UNCERTAINTY COMPONENTS OF CLINICAL CHEMISTRY ANALYTES IN ANALYZER B								
Analyte	Material	n	%CV	u^2R_w	u^2cal	u (y)	$\%U_{rel}$ (k=2)	MAU
Alb	IQC-1	177	4,73	2,37	0,0882	1,57	**9,62**	2,5
	IQC-2	178	4,17	4,37	0,0882	2,117	**8,34**	
ALT	IQC-1	176	4,13	3,8	0,1018	1,98	8,34	10,1
	IQC-2	177	2,88	12,75	0,1018	3,58	5,79	
Amy	IQC-1	175	8,44	47,06	0,49	6,9	**17,33**	6,6
	IQC-2	182	5,25	98,8	0,49	9,97	**10,6**	
AST	IQC-1	177	5,52	6,81	0,1845	2,65	**11,35**	9,6
	IQC-2	178	3,85	31,03	0,1845	5,59	7,78	
CRP	IQC-1	178	3,81	0,14	1,051	1,09	22,94	34,1
	IQC-2	179	4,91	6,45	1,051	2,74	10,72	
Fe	IQC-1	211	5,08	3097	164,4	57,11	10,42	20,7
	IQC-2	208	3,79	8122	164,4	91,03	7,66	
EtOH	IQC-1	176	5,37	7,29	1,452	2,96	11,7	20*
	IQC-2	167	4,47	44,09	1,452	6,75	9,1	
Glu	IQC-1	177	6,99	48,44	0,637	7,01	**13,7**	5
	IQC-2	187	2,16	25,1	0,637	5,07	4,4	
HDL - C	IQC-1	174	4,63	2,25	0,3399	1,61	**10**	5,8
	IQC-2	176	6,55	16,4	0,3399	4,09	**13,3**	
Ca	IQC-1	178	3,31	0,08	0,00188	0,29	**6,66**	1,8
	IQC-2	178	2,63	0,12	0,00188	0,35	**5,26**	
Cl	IQC-1	392	3,3	7,73	0,16	2,81	**6,65**	1,1
	IQC-2	374	2,24	5,2	0,0625	2,29	**4,5**	
Crea	IQC-1	194	5,76	0,0036	0,00083	0,07	**12,6**	4,5
	IQC-2	190	4,04	0,0256	0,00083	0,16	**8,1**	
K	IQC-1	400	2,81	0,01	0,000625	0,1	**5,73**	4,1
	IQC-2	383	1,7	0,0144	0,0001	0,12	3,54	
Na	IQC-1	393	2,28	6,5	0,16	2,58	**4,58**	0,5
	IQC-2	379	1,64	4,88	0,0625	2,22	**3,31**	
T.Bil	IQC-1	193	7,3	0,0049	0,000337	0,07	14,47	20
	IQC-2	194	6,88	0,0625	0,000337	0,25	13,93	
Cholesterol	IQC-1	182	3,32	11,22	0,4563	3,42	**6,8**	5,3
	IQC-2	184	3,51	35,28	0,4563	5,98	**7,2**	
TP	IQC-1	189	5,28	6,5	0,0181	2,55	**10,66**	2,6
	IQC-2	190	4,52	12,11	0,0181	3,48	**9,12**	
TG	IQC-1	174	2,95	12,46	0,64	3,62	6,1	20*
	IQC-2	176	2,96	39,18	0,64	6,31	6	
BUN	IQC-1	177	3,34	1,69	0,194	1,37	7,06	13,9
	IQC-2	180	2,88	10,69	0,194	3,3	5,84	
LDL-C	IQC-1	194	2,22	1,752	0,596	1,53	5,1	8,3
	IQC-2	195	7,68	55,5	0,596	7,49	**15,4**	

Alb – Albumin, ALT – Alanine aminotransferase, Amy – Amylase, AST – Aspartate aminotransferase, CRP – C-reactive protein, Fe – Iron, EtOH – Ethanol, Glu – Glucose, HDL-C – HDL Cholesterol, Ca – Calcium, Cl – Chloride, Crea – Creatinine, K – Potassium, Na – Sodium, T.Bil – Total Bilirubin, Cholesterol – Total Cholesterol, TP – Total Protein, TG – Triglyceride, BUN – Blood Urea Nitrogen, LDL-C – LDL Cholesterol.

u^2Rw - standard uncertainty component for the long-term precision obtained from six months internal quality control, u^2cal - uncertainty of calibrator values provided by manufacturer, U(y) - expanded uncertainty, %Urel (y) - percent relative expanded uncertainty, MAU - Maximum expanded allowable measurement uncertainty.

*%Urel values exceeding the MAU are indicated in bold. All MAU values obtained from The EFLM Biological Variation Database, except EtOH. *The MAU value of EtOH obtained from updated CLIA (Clinical Laboratory Improvement Amendments) Proposed Acceptance Limits.*

Lipid metabolism disorders are independent risk factors for developing atherosclerotic cardiovascular diseases (18). Therefore, considering MU may change the way of diagnosis and treatment, especially if the patient's lipid profile is at medical decision levels. Our study found $\%U_{rel}$(pooled) values for LDL-C to be 8.9% and 13.7% for IQC-1 and 2, respectively. We determined $\%U_{rel}$ (pooled) values for HDL-C to be 9.5% and 14.2% for IQC-1 and 2, respectively, and $\%U_{rel}$ (pooled) values for triglyceride as 6.3% and 5.7% for IQC-1 and 2, respectively. We found the $\%U_{rel}$ (pooled) values for total cholesterol to be 7.9% and 7.8% for IQC-1 and 2, respectively. The MU values for triglyceride met the quality target (20%). However, none of the MU values for HDL-C, LDL-C, and Total cholesterol met the allowable quality targets (5.8%, 8.3%, and 5.3% for HDL-C, LDL-C, and Total cholesterol, respectively).

Ethanol analysis, which is one of the tests performed in the forensic toxicology laboratory, significantly affects the status of individuals in terms of clinical and forensic decisions (19). We determined the $\%U_{rel}$ (pooled) values for ethanol as 11.3% and 6.7% for IQC-1 and 2, respectively, and met the quality specification (20%). To ensure the accuracy and reliability of a result measured in a laboratory that measures ethanol, especially within the limits of medical and forensic decisions, giving the test result together with MU can change the shape of the decisions to be taken.

Different results can be obtained in clinical laboratories using different MU models for the same analyte. Using two separate MU calculation models for glucose, Chen et al. found 7.38% and 13.58% values (20). Therefore, laboratories should standardize their MU calculation methods. Recently, Coskun et al. reported that only *u* (SD) value could be used for MU calculation, which would be sufficient for routine clinical laboratory operations (13). This new model, named MU for practical use (MUPU), is a very facilitating tool for laboratories to calculate and evaluate MU. Besides, the authors think that the main component of MU is u value, as seen in our study. However, in this approach, ignoring the ucal value and using a single level (especially normal level) IQC material can be stated as aspects of the MUPU approach that need to be developed (21). Also, there are multiple APS options with which MU values can be compared (22). Recently, MAU values on a BV basis have been published by EFLM (11). Since the most recent recommended MAU values are in the EFLM BV database, we used these values in our study, except for the EtOH test. However, APS options may be different for each laboratory and analyte (23). Therefore, it is thought that laboratories can make APS selections by determining their priorities and considering Milan models (24).

In laboratories using more than one device, the MU values calculated for the same analyte in each device must not exceed the allowable APS values separately to keep the analytical difference between the devices within acceptable limits. However, it is known that the result obtained from the given laboratory can be obtained from different devices. Hence, it is considered that the *u* (pooled) calculation suggested by the ISO/TS 20914:2019 guide will be more useful in terms of evaluating the effect of MU on the reported results. However, the *u* (pooled) value will be higher or lower than the individual *u* values of the devices. This is one of the problems with reporting MU with results because the MU value calculated over u (pooled) will not fully reflect the analytical performance of the instruments. Since there are two identical devices in our laboratory in our study, we evaluated over *u* (pooled) per the recommendation of the ISO guideline, but it should not be overlooked that we calculated the *u* (pooled) value when evaluating the results of this study. For example, in our study, the MU value of the LDL-C test in the 1st device was calculated as 5.1% and in the 2nd device as 9%. The MU value of the two devices was calculated as 8.9%, and it was observed to exceed the MAU value of 8% (Supplemental Table 1-2). For all these reasons, we think that the MU and MAU evaluation can be used mainly to evaluate the analytical performance of the devices and that we are just at the beginning of the way in adding the MU values calculated from identical devices to the result reports.

CONCLUSION

The present study demonstrated that the component with the most significant effect on the MU value was the uR_w value. To solve this problem, it may be suggested to follow the IQC values of the relevant method more closely and to change the calibration frequency. With the help of MU, laboratories can reliably monitor their analytical performance. By knowing the MU concept, clinicians can accurately perceive the measurement result and provide reliable patient care. Therefore, we hypothesize that understanding the MU concept and adapting it to routine laboratories may increase the reliability of the results.

DECLARATIONS

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Ethics Approval: All protocols for this study were approved by the Gaziosmanpaşa Training and Research Hospital Clinical Research Ethics Committee (Decree Date and No: 22 December 2021/393)

Availability of Data and Material: All data is available.

Authors' Contributions: Establishing the main idea and hypothesis of the study: A.Ç.; Developing the hypothesis and designing the materials and methods section: A.Ç. and K.T.U.; Evaluation of data: A.Ç. and K.T.U.; Writing the draft of the article: A.Ç. and K.T.U..; Assessing the final version of the article and making necessary corrections: A.Ç. and K.T.U.

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