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Measurement Uncertainty of Cardiac Markers: High-Sensitivity Troponin T and Myoglobin

Kardiyak Belirteçlerin Ölçüm Belirsizliği: Yüksek Hassasiyetli Troponin T ve Miyoglobin

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ABSTRACT

Aim: The measurement uncertainty (MU) of high-sensitivity troponin T (hs-TnT) and myoglobin assays was calculated in accordance with ISO/TS 20914:2019. The calculated MU values were subsequently compared with the relevant analytical performance specifications (APS).

Materials and Methods: Internal quality control (IQC) and external quality control (EQC) data collected between January and June 2024 were analyzed. Level 1 and Level 2 control materials for hs-TnT and myoglobin were measured daily. Expanded combined relative measurement uncertainty (Ucrel) were calculated with calibrator uncertainties included, and level-specific estimates were combined to derive the total device-level expanded measurement uncertainty, t(Ucrel).

Results: For the hs-TnT assay, Ucrel values at Level 1 exceeded the allowable APS on both the Cobas 8000 and Cobas Pro analyzers, whereas Level 2 results remained within acceptable limits. t(Ucrel) values on both analyzers (19.63% and 14.24%) exceeded the minimum APS of 13%. For myoglobin, Ucrel values were below the APS limit of 13% at both control levels on the Cobas 8000 analyzer, whereas they slightly exceeded the APS at both levels on the Cobas Pro analyzer. Accordingly, the t(Ucrel) value exceeded the APS on the Cobas Pro analyzer (13.45%) but remained acceptable on the Cobas 8000 analyzer (11.49%).

Conclusion: Routine monitoring and reporting of MU can facilitate more reliable interpretation of laboratory results, particularly those close to clinical decision thresholds. Improved communication of MU between laboratories and clinicians may enhance the clinical utility of cardiac biomarkers and support more informed diagnostic decision-making.

Keywords: Troponin T, Myoglobin, Uncertainty, Quality Control

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ÖZET

Amaç: Yüksek duyarlılıklı troponin T (hs-TnT) ve miyoglobin testlerinin ölçüm belirsizliği (MU), ISO/TS 20914:2019 standardına uygun olarak hesaplandı. Hesaplanan MU değerleri daha sonra ilgili analitik performans spesifikasyonları (APS) ile karşılaştırıldı.

Gereç ve Yöntemler: Ocak–Haziran 2024 döneminde toplanan iç kalite kontrol (IQC) ve dış kalite kontrol (EQC) verileri analiz edildi. hs-TnT ve miyoglobin için Seviye 1 ve Seviye 2 kontrol materyalleri günlük olarak ölçüldü. Kalibratör belirsizlikleri dâhil edilerek seviye-özel genişletilmiş birleşik bağıl ölçüm belirsizlikleri (Ucrel) hesaplandı ve bu seviye-özel tahminler birleştirilerek cihaz düzeyindeki toplam genişletilmiş ölçüm belirsizliği, t(Ucrel), elde edildi.

Bulgular: hs-TnT testi için Seviye 1'deki Ucrel değerleri hem Cobas 8000 hem de Cobas Pro analizörlerinde izin verilen APS sınırlarını aşarken, Seviye 2 sonuçları kabul edilebilir sınırlar içinde kaldı. Her iki analizörde elde edilen t(Ucrel) değerleri (%19,63 ve %14,24), %13 olan minimum APS değerini aştı. Miyoglobin için ise Cobas 8000 analizöründe her iki seviyedeki Ucrel değerleri %13'lük APS sınırının altında kalırken, Cobas Pro analizöründe her iki seviyede de APS'yi hafifçe aştı; buna bağlı olarak Cobas Pro analizöründe t(Ucrel) değeri (%13,45) APS'yi aşarken, Cobas 8000 analizöründe (%11,49) kabul edilebilir düzeyde kaldı.

Sonuç: Ölçüm belirsizliğinin rutin olarak izlenmesi ve raporlanması, özellikle klinik karar eşiklerine yakın sonuçların daha güvenilir şekilde yorumlanmasını kolaylaştırabilir. Laboratuvarlar ile klinisyenler arasında ölçüm belirsizliğinin daha iyi iletişimi, kardiyak biyobelirteçlerin klinik faydasını artırabilir ve daha bilinçli tanısal kararların verilmesini destekleyebilir.

Anahtar kelimeler: Troponin T, Miyoglobin, Belirsizlik, Kalite kontrol

INTRODUCTION

Clinical laboratories play a crucial role in supporting a wide range of clinical decisions, including early and accurate diagnosis, guiding treatment selection, preventing delays in therapy, and reducing the need for palliative care (1,2). A survey conducted among clinicians in Germany and the United States reported that laboratory test results influence approximately 60–70% of clinical decisions (3). Therefore, clinical laboratories focus on improving and maintaining quality through the use of quality assurance tools. To enhance the clinical value of laboratory tests, results must be reported in a manner that supports effective clinical decision-making. In addition, clinical laboratories should provide consultative services that understand clinicians' needs and collaborate with them regarding test selection and interpretation. To this end, it is essential for laboratory professionals to share clinically relevant information with clinicians through face-to-face meetings, telephone consultations, and written reports in order to enhance the clinical value of laboratory tests (4). Laboratory testing involves numerous potential sources of 'uncertainty' that may significantly influence results, including preanalytical errors in sample collection or transport, biological variation, medication use, and recording or reporting errors (5). The evaluation of measurement uncertainty (MU) is essential for interpreting laboratory results, diagnosing diseases, and monitoring treatment. MU is defined as a parameter that describes the dispersion of values reasonably attributable to a measurand, and reporting MU alongside test results can influence clinical decision-making (6). Determining MU in medical laboratories provides several important benefits. These include offering objective information about the quality of individual laboratory performance, supporting appropriate clinical decisions, identifying tests that require analytical improvement before clinical application, encouraging IVD manufacturers to enhance the quality of their analytical performance, and enabling the discontinuation of analytical methods that demonstrate inadequate quality (7,8). According to the ISO 15189 accreditation standard, if requested, the MU of laboratory tests can be calculated and reported alongside the test results. However, despite these recommendations and guidelines, most calibrator insert documents do not include MU values.

There are two fundamental approaches to calculating MU: the bottom-up and top-down approaches. In the bottom-up approach, all individual factors contributing to uncertainty and their influence ratios are included separately in the calculation. In contrast, the top-down approach relies on existing analytical performance data obtained from quality control materials (9,10). For the calculation of MU, the International Vocabulary of Metrology (VIM2), the Guide to the Expression of Uncertainty in Measurement (GUM1), and the International Organization for Standardization / International Electrotechnical Commission (ISO/IEC) guidelines provide metrological methodologies (11). The ISO/TS 20914:2019 guideline specifically recommends the calculation of MU (9). The Nordtest guide, which is widely used as a standard reference for estimating MU in environmental laboratories across Europe, employs a top-down approach and aims to provide a clear and practical framework for MU calculation (12).

Myocardial infarction (MI), the most severe form of coronary artery disease, is a life-threatening condition and a major cause of global mortality (13). Measurement of cardiac troponins is the cornerstone of MI diagnosis (14). High-sensitivity troponin assays (hs-troponin) enable the rapid exclusion of MI and help prevent unnecessary hospitalizations (15).

Suboptimal analytical performance in troponin testing—including device-to-device variability and differences in reagents and calibrator lots—can significantly affect measurements at low troponin concentrations and lead to patient misclassification within MI diagnostic algorithms used in emergency departments (16). Therefore, medical laboratories should calculate the MU of troponin assays and report it alongside test results to assist clinicians. Clinicians should interpret hs-troponin results near clinical decision cutoffs by taking MU into account (17).

In this study, we aimed to determine the MU of hs-TnT and myoglobin assays performed in our laboratory in accordance with ISO/TS 20914:2019, and to compare the calculated MU values with the analytical performance specifications (APS) for MU.

MATERIALS AND METHODS

Study Setting

Six months of internal quality control (IQC) data collected between January and June 2024 were used to determine the MU of hs-TnT and myoglobin assays. MU values were calculated separately for the Roche cobas® pro and Roche cobas® 8000 analyzers (Roche Diagnostics, Mannheim, Germany) used in our laboratory.

The IQC materials consisted of Roche PreciControl Troponin (Lot No. 79059301) and Roche PreciControl Cardiac II (Lot No. 79446901). Level 1 and Level 2 IQC samples for the hs-TnT and myoglobin assays were analyzed in duplicate on a daily basis. For hs-TnT, a total of 904 Level 1 and 928 Level 2 IQC results were evaluated. For the myoglobin assay, 450 IQC results were obtained for each control level.

External quality control (EQC) was performed monthly using Cardiac RQ9186 and Cardiac Plus RQ9190 samples (RIQAS, Randox Laboratories Ltd., Crumlin, UK). Although EQC results were reviewed to assess long-term analytical stability, they were not included in the MU calculation, as ISO/TS 20914:2019 recommends the use of internally generated control data for estimating standard MU (18).

Calculating Measurement Uncertainty

For each control level, the relative standard MU due to imprecision (uRw%) was calculated using IQC data. The relative calibrator uncertainty (ucal%) was obtained from the manufacturer-provided uncertainty information given in Table 1. Each calibrator had an assigned concentration value and an expanded uncertainty (U) with a coverage

factor of k = 2. The corresponding standard uncertainty was calculated by dividing U by k, and ucal% was expressed as a percentage of the assigned calibrator concentration.

Table 1. Calibrator uncertaintiy values
Tablo 1. Kalibratör belirsizlik değerleri

Parameter	ucal% (k=1)
Troponin T Calibrator (C1)	0.64
Troponin T Calibrator (C2)	0.97
Myoglobin Calibrator (C1)	0.74
Myoglobin Calibrator (C2)	2.17

For each control level, the combined relative standard measurement uncertainty (ucrel) was calculated by combining uRw% and ucal%. These level-specific combined uncertainties were then expanded using a coverage factor of k = 2 and reported as the expanded combined relative measurement uncertainty (Ucrel) (Table 2).

To estimate the total device-level measurement uncertainty, the ucrel values obtained from Level 1 and Level 2 were combined using the pooled variance approach. The resulting total combined relative standard uncertainty t(ucrel) was then multiplied by a coverage factor of k = 2 to obtain the total device-level expanded combined relative measurement uncertainty, denoted as t(Ucrel) (Table 3).

The ucrel was calculated separately for each control level using the following formula:

$$u_{crel} = \sqrt{u_{Rw}^2 + u_{cal}^2}$$

The expanded ucrel for each control level was then calculated as:

$$U_{crel} = u_{crel} \times k$$

The t(ucrel) was calculated at the device level by combining the ucrel values from both control levels and multiplying the resulting value by the coverage factor (k = 2) to obtain t(Ucrel).

$$u_{crel,\,combined}(\%) = \sqrt{\frac{(n_{level1} - 1)\,u_{crel,level1}^2 + (n_{level2} - 1)\,u_{crel,level2}^2}{n_{level1} + n_{level2} - 2}}$$

Table 2. Measurement uncertainty values for hs-troponin T and myoglobin
Tablo 2. hs-troponin T ve miyoglobin için ölçüm belirsizliği değerleri

Test	Analyzer	Level	uRw (%)	ucal (%)	u_{crel} (%)	U_{crel} (k=2, %)
hs-troponin T	Cobas 8000	Level 1	13.37	0.64	13.39	26.78
hs-troponin T	Cobas 8000	Level 2	3.82	0.97	3.94	7.89
hs-troponin T	Cobas Pro	Level 1	8.23	0.64	8.26	16.52
hs-troponin T	Cobas Pro	Level 2	5.72	0.97	5.80	11.61
Myoglobin	Cobas 8000	Level 1	5.53	0.74	5.58	11.16
Myoglobin	Cobas 8000	Level 2	5.48	2.17	5.90	11.81
Myoglobin	Cobas Pro	Level 1	6.65	0.74	6.69	13.39
Myoglobin	Cobas Pro	Level 2	6.40	2.17	6.76	13.52

Table 3. Total Expanded Combined Relative Measurement Uncertainty t(Ucrel)
Tablo 3. Toplam Genişletilmiş Birleşik Bağıl Ölçüm Belirsizliği t(Ucrel)

Test	Analyzer	Level	t (U_{crel}) (k=2, %)	Target (%)	Status
hs-Troponin T	Cobas 8000	Total	19.63	Min 13 / Desirable 9.4	Not acceptable
hs-Troponin T	Cobas Pro	Total	14.24	Min 13 / Desirable 9.4	Not acceptable
Myoglobin	Cobas 8000	Total	11.49	13	Acceptable
Myoglobin	Cobas Pro	Total	13.45	13	Not acceptable

RESULTS

Six-month EQC data for both tests were within acceptable limits for all results, confirming the absence of clinically significant bias. The calibrator uncertainty values (ucal%) for hs-TnT and myoglobin are listed in Table 1. The MU values for hs-TnT and myoglobin at both control levels, including uRw, ucal, ucrel, and Ucrel, are summarized in Table 2. For hs-TnT, the APS values for standard MU were defined as 13% at the minimum level and 9.4% at the desirable level. Because the APS for the standard MU of myoglobin is not clearly defined in the literature, the maximum allowable standard MU (MAu) was estimated using a biological variation–based approach (20). Within-subject biological variation (CVI) values were obtained from the referenced study, and MAu was defined according to the criterion MAu < 2 × 0.5 × CVI (i.e., MAu < CVI). Accordingly, myoglobin results were evaluated using an APS threshold of 13%. For hs-TnT, Level 2 results remained within acceptable limits on both analyzers, whereas Level 1 and t(Ucrel) values exceeded the threshold of 13% on both analyzers. For myoglobin, Ucrel values on the Cobas 8000 analyzer were below 13% at both control levels. In contrast, Ucrel values on the Cobas Pro analyzer slightly exceeded the MAu at both Level 1 (13.39%) and Level 2 (13.52%). The t(Ucrel) value for myoglobin was acceptable on the Cobas 8000 analyzer but exceeded the MAu limit of 13% on the Cobas Pro analyzer (Table 3).

DISCUSSION

In this study, MU of hs-TnT and myoglobin was evaluated across two analyzer platforms and two control levels using an extensive IQC dataset. For myoglobin, the t(Ucrel) value exceeded the current APS limit of 13% only on the Cobas Pro analyzer. The t(Ucrel) value for hs-TnT exceeded the minimum allowable APS threshold on both analyzers, indicating unacceptable MU according to the predefined criteria. Notably, the Cobas 8000 analyzer exhibited a higher t(Ucrel) value (19.63%) than the Cobas Pro analyzer (14.24%), suggesting greater overall analytical variability for hs-TnT on this platform. In addition, Level 1 results for hs-TnT on the Cobas 8000 exceeded the acceptable APS threshold, which may partly reflect the increasingly stringent clinical performance requirements applied to cardiac troponin assays at low concentration levels over the past two decades (21).

Previous studies have reported that MU values for cardiac biomarkers vary depending on the analyte and analytical methodology. For instance, studies on troponin I assays have shown that high sensitivity methods generally remain within acceptable MU limits across clinically relevant concentration ranges (17). In contrast, myoglobin has frequently been reported to exhibit higher analytical variability, which has been attributed to its wide physiological distribution and limited cardiac specificity (22). These findings indicate that MU performance characteristics can vary markedly across biomarkers and analytical platforms, especially near clinical decision thresholds, consistent with the observations of the present study. Laboratory information plays an increasingly central role in diagnosis and treatment; however, as with all clinical data, the inherent limitations of diagnostic tests may influence clinical interpretation. Effective communication of MU can strengthen collaboration between clinicians, patients, and medical laboratories (21). Previous studies have emphasized the critical role of MU reporting in clinical decision-making, and MU has also been incorporated into quality assessment criteria and international standards. Nevertheless, unless MU calculations are readily applicable in routine laboratory practice, their widespread implementation remains limited. Therefore, practical MU models that can be calculated using existing data without requiring additional resources or budget allocation are both important and valuable (22). The CCLM guidelines also emphasize the importance of MU assessment in supporting compliance

with ISO 15189 and in interpreting results close to clinical decision thresholds (23–24). Unlike bias, MU cannot be reduced to zero; therefore, the objective is to keep uncertainty within predefined targets to prevent excessive uncertainty that could compromise the clinical utility of test results. (25). Taken together, these findings underscore that MU is not merely a statistical parameter but a clinically relevant factor that may affect result interpretation, particularly in borderline cases. Differences in t(Ucrel) values between analyzers underscore the need to account for uncertainty when evaluating cardiac biomarkers, as such differences may influence clinical judgment in distinguishing acute myocardial injury from non-cardiac causes of biomarker elevation in emergency settings.

This study has several limitations. First, only two analyzers and two levels of IQC materials were evaluated, and variability related to patient samples was not assessed. Second, long-term trends, lot-to lot variability, and the potential impact of reagent or calibrator changes were not investigated. Despite these limitations, the study has notable strengths. It is based on a large IQC dataset collected over a six-month period, applies the most recent ISO/TS 20914:2019 framework, and provides a detailed comparison of MU across two widely used analyzers. Collectively, these features enhance the practical relevance of the findings and support their applicability in routine laboratory practice.

CONCLUSION

Routine monitoring and reporting of MU can facilitate more reliable interpretation of laboratory results, particularly those close to clinical decision thresholds. Furthermore, improved communication of MU between laboratories and clinicians may enhance the clinical utility of cardiac biomarkers and support more informed diagnostic decision making.

Conflict of Interests

The authors declare no conflicts of interest in this study.

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REFERENCES

1. Price CP. Application of the principles of evidence-based medicine to laboratory medicine. Clin Chim Acta. 2003;333(2):147-154. doi:10.1016/S0009-8981(03)00179-7.
2. The Lewin Group. The value of laboratory screening and diagnostic tests for prevention and health care improvement. September 2009. Available from: https://www.lewin.com/content/dam/Lewin/Resources/Site_Sections/Publications/Lewin_Value_LabTesting_Sept_2009.pdf. Accessed February 26, 2023.
3. Rohr UP, Binder C, Dieterle T, Giusti F, Messina CG, Toerien E, et al. The value of in vitro diagnostic testing in medical practice: a status report. PLoS One. 2016;11(3):e0149856. doi:10.1371/journal.pone.0149856.
4. Sikaris KA. Enhancing the clinical value of medical laboratory testing. Clin Biochem Rev. 2017; 38(3):107-114.
5. White GH, Farrance I; AACB Uncertainty of Measurement Working Group. Uncertainty of measurement in quantitative medical testing: a laboratory implementation guide. Clin Biochem Rev. 2004;25(4):1-24.
6. Yavuz H, Yaman H, Karahan S, Katkat M, Aliyazıcıoğlu Y, Örem A. Evaluation of measurement uncertainty of first trimester prenatal screening. Turk Klin Biyokim Derg. 2021;19(2):122-129.
7. Braga F, Panteghini M. The utility of measurement uncertainty in medical laboratories. Clin Chem Lab Med. 2020;58(9):1407-1413. doi:10.1515/cclm-2019-1336.
8. Milinković N, Ignjatović S, Šumarac Z, Majkić-Singh N. Uncertainty of measurement in laboratory medicine. J Med Biochem. 2018;37(3):279-288. doi:10.2478/jomb-2018-0002.
9. College of American Pathologists. Measurement uncertainty guide ISO 15189 accreditation program. Available from: https://documents.cap.org/ documents/

cap15189-accreditation-program-measurement-uncertainty-guide.pdf. Accessed November 28, 2025.

10. Lee JH, Choi JH, Youn JS, Cha YJ, Song W, Park AJ. Comparison between bottom-up and top-down approaches in the estimation of measurement uncertainty. Clin Chem Lab Med. 2015;53(7):1025-1032. doi:10.1515/cclm-2014-0801.
11. Aytekin M, Cevlik T, Emerk K. Describing an ideal model for calculating the uncertainty of measurements in a clinical laboratory. Clin Biochem. 2009;42(3):321-322. doi:10.1016/j.clinbiochem.2008.09.039.
12. Magnusson B, Naykki T, Hovind H, Krysell M, Sahlin E. Handbook for calculation of measurement uncertainty in environmental laboratories. NordTest Report TR 537, 4th ed. 2017. Available from: www.nordtest.info.
13. Salari N, Morddarvanjoghi F, Abdolmaleki A, Rasoulpoor S, Khaleghi AA, Hezarkhani LA, et al. The global prevalence of myocardial infarction: a systematic review and meta-analysis. BMC Cardiovasc Disord. 2023;23(1):206. doi:10.1186/s12872-023-03231-w.
14. Kleemeier S, Abildgaard A, Ladefoged SA, Sørensen JT, Stengaard C, Adelborg K. High-sensitivity troponin T and I in patients suspected of acute myocardial infarction. Scand J Clin Lab Invest. 2022;82(2):96-103. doi:10.1080/00365513.2022.2033310.
15. Westwood M, Ramaekers B, Grimm S, Worthy G, Fayter D, Armstrong N, et al. High-sensitivity troponin assays for early rule-out of acute myocardial infarction: a systematic review and economic evaluation. Health Technol Assess. 2021;25(33):1-276. doi:10.3310/hta25330.
16. Krintus M, Panteghini M. Judging the clinical suitability of analytical performance of cardiac troponin assays. Clin Chem Lab Med. 2023;61(5):801-810. doi:10.1515/cclm-2023-0027.
17. Ünal K, Atikeler GF. The importance of evaluating measurement uncertainty for troponin I. J Clin Anal Med. 2019;10(1):5-8. doi:10.4328/jcam.5835.
18. Farrance I, Frenkel R, Badrick T. ISO/TS 20914:2019 – a critical commentary. Clin Chem Lab Med. 2020;58(8):1182-1190. doi:10.1515/cclm-2019-1209.
19. Panteghini M. Analytical performance specifications for combined uncertainty budget in the implementation of metrological traceability. Clin Chem Lab Med. 2024;62(8):1497-1504. doi:10.1515/cclm-2023-1325.
20. Sandberg S, Coskun A, Carobene A, et al. Analytical performance specifications based on biological variation data: considerations, strengths and limitations. Clin Chem Lab Med. 2024;62(8):1483-1489. doi:10.1515/cclm-2024-0108.
21. Plebani M, Padoan A, Sciacovelli L. Measurement uncertainty: light in the shadows. Clin Chem Lab Med. 2020;58(9):1381-1383. doi:10.1515/cclm-2020-0134.
22. Tan R, Yilmaz M, Kurtulmuş Y. A practical approach example to measurement uncertainty: evaluation of 26 immunoassay parameters. Biochem Med (Zagreb). 2022;32(3):030705. doi:10.11613/BM. 2022.030705.
23. Akşit M, Terzioğlu M, Zeytinli Akşit M, Kansu Altan T, İşbilen Başok B, Çolak A. Measurement uncertainty in specific hormone tests. Turk Klin Biyokim Derg. 2023;21(3):119-127. doi:10.56615/tkbd.2023.15.
24. Braga F, Infusino I, Panteghini M. Performance criteria for combined uncertainty budget in the implementation of metrological traceability. Clin Chem Lab Med. 2015;53(6):905-912. doi:10.1515/cclm-2014-1240.
25. Braga F, Panteghini M. Defining permissible limits for the combined uncertainty budget in the implementation of metrological traceability. Clin Biochem. 2018;57:7-11. doi:10.1016/j.clinbiochem.2018.03.007.