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Research Article

Evaluation of measurement uncertainty of coagulation parameters according to two different current guidelines

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Abstract

Objectives: This study aims to calculate the measurement uncertainty values of prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, and fibrinogen tests according to ISO/TS 20914 and Nordtest 2017 guidelines and to compare these values with the total allowable error (TEa%) and maximum expanded allowable measurement uncertainty (MAU) values established by international organizations.

Methods: Normal and pathological level internal quality control data for PT, APTT, D-dimer, and fibrinogen tests performed on the Sysmex CS2100 device between January and May 2024 were obtained from the Laboratory Information System. External quality control data for October 2023 and September 2024 were sourced from the external quality control system. Measurement uncertainty was calculated following ISO/TS 20914 and Nordtest 2017 guidelines.

Results: According to the ISO/TS 20914 guideline, the measurement uncertainty values for PT, APTT, D-dimer, and fibrinogen tests were 10.42%, 3.49%, 4.81%, and 19.10%, respectively. According to the Nordtest guideline, the measurement uncertainty values were 10.44%, 12.64%, 17.94%, and 21.69%, respectively.

Conclusion: Based on the ISO/TS 20914 guideline, it was observed that the measurement uncertainty values for all coagulation tests met the TEa% analytical targets. According to the Nordtest guideline, all tests except fibrinogen met these targets. When evaluated against the MAU criterion, it was determined that D-dimer met the targeted quality specification according to both guidelines; however, the measurement uncertainty values for PT, APTT, and fibrinogen exceeded the allowed targets. Standardization of the measurement uncertainty calculation model and the determination of analytical targets based on laboratory priorities can ensure reliable monitoring of analytical performance.

Keywords: Coagulation, Nordtest, measurement uncertainty, total allowable error

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Since laboratory results are crucial in the diagnosis, treatment, follow-up, and risk assessment of diseases, accurate and reliable measurements are essential. Measurement uncertainty (MU) is a concept that characterizes the distribution of values that can be attributed to a measurement to evaluate the reliability and accuracy of the analysis result [1]. MU is not a doubt about the validity of the measurement but rather a defined confidence limit. MU can also provide laboratory users with confirmation that patient results meet performance specifications [2]. MU components must be identified throughout the entire traceability chain, starting with reference material providers, through *in vitro* diagnostic manufacturers, processes for assigning calibrator values, and finally, the result. In addition to uncertainties in the steps of the metrological chain, test results are also affected by uncertainties arising from random effects in laboratory measurements [3].

Apart from uncertainties due to matrix effects, interferences, environmental factors, reference materials, and calibrators, differences in the methods and procedures used in calculating MU also contribute to MU. The ISO/TS 20914:2019 guideline recommends calculating MU after each of the three main MU sources has been estimated. According to this guideline, the MU value should primarily be calculated based on long-term uncertainty (uRW) and calibrator uncertainty (uCAL),

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and bias (uBias) should be included in the calculation only when a significant medical difference is observed [4].

There is limited literature investigating MU in coagulation parameters. Therefore, the aim of our study was to calculate the MU values of prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, and fibrinogen tests according to ISO/TS 20914 [4] and Nordtest 2017 [5] guidelines and to compare them with the total allowable error (TEa%) and maximum expanded allowable measurement uncertainty (MAU) values determined by international organizations.

Materials and Methods

Approval for our retrospective study was received from the Ethics Committee of Bakircay University Cigli Training and Research Hospital, with the decision dated 03 July 2024 and numbered 1648. Normal and pathological level internal quality control (IQC) data for PT, APTT, D-dimer, and fibrinogen tests (Control Plasma N (Lot: 507924), Control Plasma P (Lot: 556741), Dade Ci-Trol 2 (Lot: 548527), INNOVANCE D-Dimer Controls (Lot: 575611-575506, Siemens Healthcare Diagnostics, Marburg, Germany)) run on the Sysmex CS2100 (Sysmex Corporation, Kobe, Japan) device between January and May 2024 were obtained from the Laboratory Information System. The reagents used for PT, APTT, D-dimer, and fibrinogen tests were Thromborel S, Dade Actin FS, INNOVANCE D-Dimer, and Multifibren U, respectively.

External quality control (EQC) data (External Quality Assurance Services (EQAS) Coagulation Program, Lot: 281000–281100, Bio-Rad Laboratories Inc., Irvine, CA, USA) for October 2023 to September 2024 were obtained from the EQC system.

MU calculation according to the ISO/TS 20914 guide [4]

The standard deviation (SD) of the IQC results was calculated. The SD was accepted as long-term precision (uRW). The uRW was calculated using equation:

$uRW = \sqrt{(SD\ (Level\ 1)^2 + SD\ (Level\ 2)^2)} / 2$

Calibrator uncertainty (uCAL) data was obtained from the manufacturer (Table 1). The mean bias (%) was calculated according to equation:

Mean bias (%): Σ bias(%)(EQC) / Number of EQCs

Since the mean bias (%) values calculated from the EQC data were lower than the desirable bias (%) values, ubias was not included in the uncertainty calculation. The combined uncertainty was calculated according to the following equation:

$Combined\ uncertainty = \sqrt{(uRW^2 + uCAL^2)}$

The expanded uncertainty was calculated using equation:

Expanded uncertainty = k x u (At 95% confidence interval, k=2 was taken).

The expanded uncertainty values were compared with TEa% values of international organizations [Clinical Laboratory Improvement Amendments (CLIA), Wisconsin State Laboratory of Hygiene (WLSH), Wadsworth Center of the New York State Department of Health (NYS), and American Association of Bioanalysts (AAB)] and MAU values in the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation database [6,7]. 50% of the total allowable error was taken as desirable bias.

MU calculation according to Nordtest guide [5]

The intermediate precision standard deviation ($S_{RW(Absolute)}$) was calculated from IQC data. The relative intermediate precision standard deviation ($S_{RW(Relative)}$) was calculated according to equation:

$S_{RW(Relative)} = S_{RW(Absolute)} \times 100) / mean$

The uRW is equal to the SRW(Relative).The uRW was calculated using equation:

$uRW = \sqrt{(S_{RW(Relative)}\ (Level\ 1)^2 + S_{RW(Relative)}\ (Level\ 2)^2)} / 2$

RMSbias and uncertainty of nominal values (uCref) were calculated from EQC data according to the following equations:

$RMSbias = \sqrt{\Sigma\ bias\ (EQC)^2} / N$

$uCref = \Sigma(CV(EQC) / \sqrt{N_{Lab}})^2 / N$

(Σbias(EQC): Sum of squares of EQC bias values, CV(EQC): CV% of each EQC, N_{Lab}: Number of participating laboratories in each EQC using the same method and same instrument, N: Number of EQCs).

The standard uncertainty, combined uncertainty, and expanded uncertainty were calculated according to the following equations:

$Standard\ uncertainty = \sqrt{RMSbias^2 + uCref^2}$

$Combined\ uncertainty = \sqrt{uRW^2 + ubias^2}$

Expanded uncertainty = k x u (At 95% confidence interval, k=2 was taken).

The expanded uncertainty values were compared with TEa% values of international organizations (CLIA, WLSH, NYS and AAB) and MAU values in the EFLM Biological Variation database [6, 7].

Results

The MU values of the parameters are shown in Table 2 and Table 3 according to ISO/TS 20914 and Nordtest guidelines, respectively. In our study, the MU values of PT, APTT, D-dimer, and fibrinogen tests according to ISO/TS 20914 guidelines were 10.42%, 3.49%, 4.81%, and 19.10%, respectively (Table 2). The MU values of PT, APTT, D-dimer, and fibrinogen tests calculated according to the Nordtest guideline were 10.44%, 12.64%, 17.94%, and 21.69%, respectively (Table 3).

According to the ISO/TS 20914 guideline, it was observed that all coagulation tests met the TEa% analytical targets. According to the Nordtest guideline, all tests except fibrinogen met the TEa% analytical targets. When evaluated according to the MAU criterion, it was determined that only the D-dimer MU value met the targeted quality specification according to both guidelines.

Discussion

Laboratory results should never be considered absolute values because they are affected by various sources of un-

Table 1. Calibrator uncertainty (uCAL) data was obtained from the manufacturer

Parameter	Product	Reagent	Reference material	Expanded uncertainty of assigned value(s) [%]	uCAL
PT	PT-multi calibrator	Thromborel S	WHO rTF /16, fresh normal plasma samples	L1:2.8 L2:2.9 L3:3.6 L4:6.2 L5:8.1	$\sqrt{(2.8^2+2.9^2+3.6^2+6.2^2+8.1^2)/5} = 5.16$
D-dimer	INNOVANCE D-dimer calibrator	INNOVANCE D-dimer	Master standard	2.4	2.40
Fibrinogen	Fibrinogen calibrator kit	Multifibren U	Internal protein standard	L1:11.1 L2:5.2 L3:10.6 L4:10.6 L5:9.8 L6:8.7	$\sqrt{(11.1^2+5.2^2+10.6^2+10.6^2+9.8^2+8.7^2)/6} = 9.55$

Since the APTT test does not have a calibrator, it is not included in the table. PT: Prothrombin time; APTT: Activated partial thromboplastin time.

Table 2. Measurement uncertainty values calculated according to ISO/TS 20914 guideline

	IQC material	n	Mean	SD	uRW	uCAL	Combined uncertainty	Expanded uncertainty	Mean bias (%)	Desirable bias (%)	TEa%	MAU
PT (sec)	Level 1	355	12.1	0.5	0.73	5.16	5.21	10.42	-0.3	±7.5	±15 (CLIA, WSLH, NYS, AAB)	2.6
	Level 2	505	20.1	0.9								
APTT (sec)	Level 1	212	26.0	0.9	1.75	*	1.75	3.49	3.7	±7.5	±15 (CLIA, WSLH, NYS, AAB)	2.8
	Level 2	564	47.5	2.3								
D-dimer (mg/L)	Level 1	404	0.36	0.03	0.14	2.40	2.40	4.81	2.4	±15	±30 (AAB)	25.2
	Level 2	231	2.73	0.19								
Fibrinogen (g/L)	Level 1	100	2.72	0.19	0.15	9.55	9.55	19.10	-0.3	±10	±20 (CLIA, WSLH, NYS, AAB)	10.2
	Level 2	100	0.84	0.10								

*: Since the APTT test does not have a calibrator, the uCAL value was not added. ISO/TS: International organization for standardization / technical specification; IQC: Internal quality control; n: Average number of laboratories using the same method and the same device in the EQC program; SD: Standard deviation; uCAL: Calibrator uncertainty; TEa: Total allowable error; MAU: Measurement uncertainty; PT: Prothrombin time; APTT: Activated partial thromboplastin time; CLIA: Clinical Laboratory Improvement Amendments; WLSH: Wisconsin State Laboratory of Hygiene; NYS: Wadsworth Center of the New York State Department of Health; AAB: American Association of Bioanalysts.

Table 3. Measurement uncertainty values calculated according to Nordtest guideline

Test	IQC level	N	Mean	SD	$S_{RW\ (Relative)}$	uRW	RMS bias	uCref	Standard uncertainty	Combined uncertainty	Expanded uncertainty	TEa%	MAU
PT (sec)	Level 1	355	12.1	0.5	3.8	4.27	3.00	0.18	3.00	5.22	10.44	±15 (CLIA, WSLH, NYS, AAB)	2.6
	Level 2	505	20.1	0.9	4.7								
APTT (sec)	Level 1	212	26.0	0.9	3.6	4.30	4.63	0.11	4.63	6.32	12.64	±15 (CLIA, WSLH, NYS, AAB)	2.8
	Level 2	564	47.5	2.3	4.9								
D-dimer (mg/L)	Level 1	404	0.36	0.03	8.6	7.78	4.36	0.93	4.46	8.97	17.94	±30 (AAB)	25.2
	Level 2	231	2.73	0.19	6.87								
Fibrinogen (g/L)	Level 1	100	2.72	0.19	7.0	9.95	4.30	0.46	4.32	10.85	21.69	±20 (CLIA, WSLH, NYS, AAB)	10.2
	Level 2	100	0.84	0.10	12.2								

IQC: Internal quality control; N: Numbers of internal quality control data; SD: Standard deviation; uRW: Standard uncertainty component for the within-laboratory reproducibility; RMS: Root mean square; uCref: Uncertainty of nominal values; TEa: Total allowable error, MAU: Maximum expanded allowable measurement uncertainty; PT: Prothrombin time; APTT: Activated partial thromboplastin time; CLIA: Clinical Laboratory Improvement Amendments; WLSH: Wisconsin State Laboratory of Hygiene; NYS: Wadsworth Center of the New York State Department of Health; AAB: American Association of Bioanalysts.

certainty in preanalytical, analytical, and postanalytical processes. MU is crucial for physicians to compare test results accurately and reliably with reference ranges, decision limits, or the patient's previous results. Today, many accreditation programs and international organizations state that MU should be calculated and reported [2, 8–10].

In our study, it was determined that all coagulation tests met TEa% analytical targets according to the ISO/TS 20914 guideline, and PT, APTT, and D-dimer tests met TEa% analytical targets according to the Nordtest guideline. The MU value of the fibrinogen test was found to be borderline higher than the TEa% criterion. When evaluated according to the MAU criterion, the D-dimer MU met the targeted quality specification according to both guidelines, but PT, APTT, and fibrinogen MU values exceeded the allowed targets. It was determined that there was a significant difference between the MU and MAU values of PT and fibrinogen tests in particular. The calibrator uncertainty values of PT and fibrinogen being higher than the analytical target make it challenging to achieve the desired analytical performance. For evaluation according to MAU criteria, *in vitro* diagnostic (IVD) system manufacturers must first optimize their calibrators and improve MU performance.

In a study by Lapić et al. [11], the MU values obtained using the D-dimer HS 500 calibrator on Sysmex CS-5100 and Atellica COAG 360 devices were 12.6% and 15.6%, respectively, and 12.0% and 10.0% when the INNOVANCE D-dimer calibrator was used. Therefore, it was reported that it met the targeted TEa criterion (28.04%). The MU calculated using the INNOVANCE D-dimer calibrator on the ACL TOP 550 device was 28.1% and was found to be borderline higher than the targeted TEa criterion. The results show that MU is significantly affected by changes in instruments and calibrators.

In the study by Qin et al. [10], where MU was evaluated using only external quality assessment program data (Beijing Center for Clinical Laboratory proficiency testing/external quality assessment, Beijing, China), MU values of PT, APTT, and fibrinogen tests on the Sysmex device were found to be 13.6, 15.0, and 11.7, respectively. The calculated MU values of all three parameters were below the CLIA TEa criteria. When evaluated according to Ricos TEa criteria, although the MU value of fibrinogen met the targeted criterion, the MU of PT and APTT tests was higher than the target.

In the study by Lim et al. [12], the MU value of fibrinogen on the ACL TOP 750 CTS device was reported to be 9.9% when certified reference materials with a target value of 270 mg/L were used. It was suggested that when the fibrinogen test was calculated using EQC material at target values of 306.6 mg/L, 120.1 mg/L, and 83.8 mg/L, MU values were determined as 12.2%, 17.0%, and 21.3%, respectively. For the APTT test, MU values calculated using EQC material at target values of 27.9 sec, 58.0 sec, and 79.5 sec were found to be 13.5%, 15.1%, and 9.0%, respectively.

In the study by Matar et al. [13], where MU was investigated only with external quality assessment program data (ProBioQual, France, member of EQALM), MU values of fibrinogen and APTT tests calculated according to the group average of all participat-

ing laboratories were 13.4% and 18.1%, respectively. According to the peer group average (laboratories using the same test method, the same reagents, and the same device), MU values were 11.1% and 6.4%, respectively. When MU values calculated for both groups were evaluated according to Ricos TEa criteria, fibrinogen met the analytical target, while APTT did not.

The literature investigating MU in coagulation parameters is limited, and no study has evaluated MU for all four parameters in our study simultaneously. The strengths of our study are that it is the first to investigate MU in coagulation parameters in our country, calculate MU according to ISO/TS 20914 and Nordtest guidelines, and evaluate MU according to MAU analytical targets in addition to TEa%.

One limitation of our study is that preanalytical and postanalytical sources of uncertainty were not evaluated. Another is that calculations were based only on five months of data due to IQC lot changes.

Since different results can be obtained by using different MU calculation models for the same analyte in clinical laboratories, and there are multiple allowable analytical performance specification options for comparing MU values, it is essential to standardize MU calculation methods and performance targets in laboratories. The calculation needs to be revisited in cases of operator, equipment, calibrator, and reagent changes, as these may affect MU. Furthermore, to achieve more reliable MU values, we believe that in addition to analytical uncertainty, studies should include all measurable sources of uncertainty attributable to preanalytical and postanalytical steps that may affect test results.

In conclusion, our study demonstrates the importance of standardizing the MU calculation model in laboratories and carefully managing MU sources such as calibrator uncertainty to improve the analytical performance of coagulation tests. Reducing assay uncertainty through improved calibrators and methodological standardization should be a priority for both laboratories and IVD manufacturers. Standardizing the calculation of MU and aligning it with laboratory-specific performance criteria is critical to ensure the reliability of coagulation tests.

Ethics Committee Approval: The study was approved by The Bakircay University Cigli Training and Research Hospital Non-interventional Clinical Research Ethics Committee (No: 1648, Date: 03/07/2024).

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