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Can Reference Materials Prepared Following CLSI C37-A Be Utilized Without Commutability Assessment? Perspectives Based on Lipid Measurements

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Background: Ensuring reference material (RM) commutability is crucial for evaluating measurement traceability in order to standardize laboratory tests. However, commutability assessment is not routinely performed. We assessed whether RMs prepared following CLSI C37-A guidelines could be used without assessing commutability by evaluating their commutability for four lipid measurements using the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and CLSI EP14 protocols.

Methods: We analyzed total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in frozen sera from 20 individuals and 11 RMs, prepared by the Korea Disease Control and Prevention Agency-Laboratory Standardization Project (per CLSI C37-A), using six routine measurement procedures (MPs). Regression equations and 95% prediction intervals derived from single-donor sera were analyzed following CLSI EP14. The IFCC protocol was used to assess differences in inter-MP biases between RM and clinical samples. The effect of the TG concentration on commutability was evaluated by analyzing biases between MP results and reference procedure-assigned values.

Results: RMs were commutable for most MP pairs for TC and TG. Commutability for HDL-C and LDL-C varied across RMs, with RM10 and RM11 showing higher TG levels (2.38 and 2.95 mmol/L, respectively) and lower commutability. Increased bias percentages from assigned values were observed for RMs with higher TG levels.

Conclusions: RMs prepared per CLSI C37-A were commutable with most MP pairs for TC and TG. Elevated TG levels affected HDL-C and LDL-C commutability, highlighting the need to consider TG concentrations during RM preparation and assess commutability to standardize laboratory tests.

Key Words: Cholesterol, Commutability, External quality assessment material, Lipids, Reference material, Triglycerides

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INTRODUCTION

Dyslipidemia and cardiovascular disease are major global health issues [1, 2]. Diagnosis and treatment depend on accurate and comparable lipid profile testing [2]. However, clinical guidelines often overlook inter-method differences. Hence, accuracy-based quality assessments for clinical laboratories and reagent manufacturers are crucial. Since 2011, the Korean Society of Laboratory Medicine has collaborated with the Korea Disease Control and Prevention Agency (KDCA) to implement the Laboratory Standardization Project (LSP), aimed at standardizing major tests, including those for creatinine, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), and HbA1c [3]. Reference materials (RMs) prepared from frozen pooled sera were used to evaluate *in vitro* diagnostics and clinical laboratories participating in the KDCA-LSP.

Commutability, a key property of RMs, refers to the equivalence of mathematical relationships between results obtained using different measurement procedures (MPs) for both RMs and clinical samples (CSs) [4]. Commutability may also be defined as the extent to which the relationship between results for an RM using two MPs mirrors that observed between CSs [5], indicating the degree to which processed RMs reflect CS behavior in MP comparisons. Briefly, "commutable" RMs can be regarded as representative of actual CSs when comparing two MPs.

The CLSI C37-A guidelines were developed to support the preparation of commutable RMs for cholesterol [6], later extended to other analytes, and are now considered a standard for preparing commutable RMs [7]. Many standardization projects and external quality assessment (EQA) programs have relied on RMs produced using these guidelines. However, creating large batches of RMs from pooled samples often involves matrix modifications, including spiking with exogenous substances, freezing, or lyophilization [8]. These modifications can affect the commutability of RMs, compromising their suitability [8, 9]. To ensure standardization when evaluating differences between MPs, the RM must be commutable [8–12].

Although efforts to analyze the standardization or harmonization status using EQA data are ongoing [13, 14], the ability to assess RM commutability has been insufficient. Unlike creatinine, gradual performance improvements and the pass rate of laboratories participating in the KDCA-LSP for lipid measurements were unclear [3], likely owing to large biases in certain RMs that affected the mean bias, total error, and EQA scores. A similar trend was noted for accuracy-based lipid proficiency testing (ABL-PT), managed by the Korean Association of External Quality Assessment Service (KEQAS) [15], which used RMs produced using the same process as the KDCA-LSP, with some RMs being shared from the same batch. Previous reports demonstrated bias or non-commutability in cholesterol measurements due to interference factors, such as high TG levels [16–18]. Assessing the commutability of RMs and exploring the possible causes affecting lipid commutability is necessary.

Two major guidelines for assessing commutability were available during this study. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) working group has recommended the difference-in-bias approach [19–21], whereas the CLSI suggests guidelines based on regression [22]. As no consensus was available for determining which guidelines to use for specific purposes, we assessed the commutability of KDCA-LSP lipid RMs (prepared per CLSI C37-A) across multiple MPs employing IFCC and CLSI approaches. We also explored potential factors contributing to bias and suggest an initiative for enhancing the utility of the RMs.

MATERIALS AND METHODS

A schematic of the study workflow is presented in Fig. 1. The

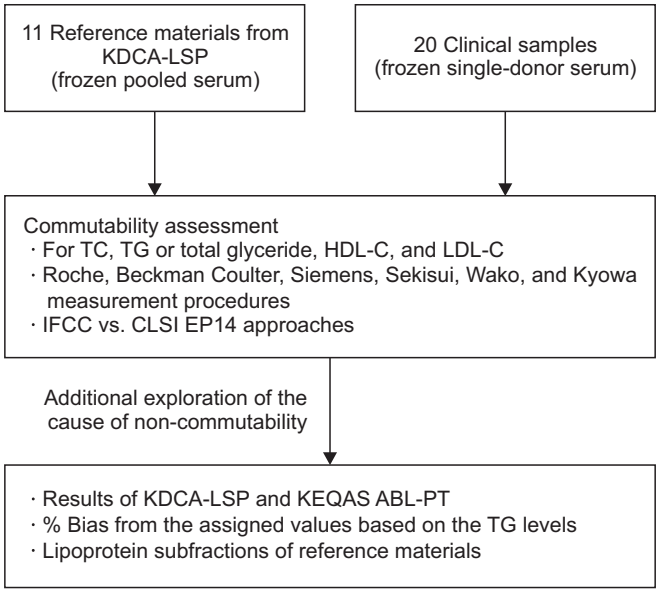

Fig. 1. Study design.
Abbreviations: ABL-PT, accuracy-based lipid proficiency testing; HDL-C, high-density lipoprotein cholesterol; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; KDCA-LSP, Korea Disease Control and Prevention Agency-Laboratory Standardization Project; KEQAS, Korean Association of External Quality Assessment Service; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

CSs were collected after obtaining approval from the Institutional Review Board (IRB) of Chungnam National University Hospital (CNUH), Daejeon, Korea, following the principles of the Declaration of Helsinki (CNUH 2020-04-098-002). Assessment was performed after approval by the Konkuk University Medical Center (KUMC), Seoul, Korea IRB (KUMC 2022-03-037). The study samples were delivered on dry ice in June 2022 to five institutions participating in commutability assessments, namely Seoul National University Bundang Hospital (SNUBH), Gachon University Gil Hospital (GUGH), Seegene Medical Foundation Laboratory (SGLab), Chungnam National University Sejong Hospital (CNUSH), and KUMC. Measurements were recorded in a single run on the day of delivery, immediately after thawing the samples with a roller mixer for approximately 60 min at room temperature (approximately 20–22°C).

RM selection

Eleven frozen pooled serum RMs (each with different lipid concentrations and used for the KDCA-LSP in the second half of 2021) were selected for commutability assessment. The serum RMs were prepared by Severance Hospital, CNUH, and KUMC from 2016 to 2020, according to the procedures outlined in the CLSI C37-A guidelines [6]. Blood bags without anticoagulants were immersed in an ice slurry of collected blood until centrifugation to prevent clotting. Plasma was transferred to 250 mL glass bottles, allowed to clot at room temperature (approximately 20–22°C) for 3 hrs, and transferred to 250 mL plastic centrifuge bottles for centrifugation. Each separated serum sample was transferred to another glass bottle and stored at 4°C, after which the samples were pooled and mixed overnight for 18 hrs to prepare an RM. The resulting mixture was filtered using a 0.2 μm pore hydrophilic membrane filter, aliquoted into 1 mL vials, and frozen at −80°C. Each RM level included sera from 6–8 individuals. The assigned values were determined at accredited reference laboratories using a reference measurement procedure (RMP). Cholesterol values were measured at the Canadian External Quality Assessment Laboratory (CEQAL; Vancouver, Canada) using the Abell–Kendall method for TC (developed by the Centers for Disease Control [CDC]), whereas HDL-C and LDL-C values were determined with the CDC's ultracentrifugation and beta-quantification methods, respectively [23]. Total glyceride values were determined at the National Medical Reference Laboratory (NMRL; Osong, Korea) using isotope dilution mass spectrometry. TG levels were calculated by subtracting the free glycerol levels (measured at the Reference Material Institute for Clinical Chemistry Standards [ReCCS; Yokohama, Japan] using the same method) from the total glyceride levels.

CS collection

Twenty frozen single-donor serum samples were collected as CSs from healthy volunteers who provided informed consent at CNUH in July 2021, following the same collection process used for the RMs, except for the pooling step. Convenience sampling was performed. Inclusion criteria required participants to have an LDL-C concentration of ≤3.36 mmol/L (130 mg/dL) and no prior diagnosis of dyslipidemia. The lipid levels of each serum sample were measured using a TBA-FX8 analyzer (Toshiba, Tokyo, Japan) with dedicated reagents, used in routine MPs at CNUH. The measured levels ranged from 3.52–5.46 mmol/L (136–211 mg/dL) for TC, 1.07–2.42 mmol/L (41.2–93.4 mg/dL) for HDL-C, 1.37–3.35 mmol/L (52.8–129.5 mg/dL) for LDL-C, and 0.39–1.45 mmol/L (34.3–128.3 mg/dL) for TG.

Analytes and MPs for commutability assessments

We assessed the commutability of each lipid profile, including TC, HDL-C, LDL-C, and total glyceride or TG. Six major MPs for lipid measurements in Korea were included [15]: Cobas c702 (Roche Diagnostics GmbH, Mannheim, Germany [Roche]), AU5800 (Beckman Coulter, Brea, CA, USA [Beckman]), Atellica CH 930 (Siemens Healthineers, Forchheim, Germany [Siemens]) with their dedicated reagents, Hitachi Labospect 008AS analyzer with the Sekisui reagent (Sekisui Medical, Tokyo, Japan [Sekisui]), Toshiba TBA-2000FR analyzer with the Wako reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan [Wako]), and Toshiba TBA-FX8 analyzer with the Kyowa reagent (Kyowa Medex Co., Ltd., Tokyo, Japan [Kyowa]) (Supplemental Data Table S1). Five institutions participated, namely SNUBH (Roche and Beckman), GUGH (Siemens), SGLab (Sekisui), CNUSH (Wako), and KUMC (Kyowa), using these MPs for routine lipid profile tests.

Commutability assessment protocol

Commutability was assessed following IFCC working group protocols [20] and the CLSI EP14 guidelines [22]. Across six routine MPs, 15 MP pairs (six combinations of two MPs) were assessed for three analytes each, except for glycerides. For glycerides, MPs were grouped based on the test principle into three free glycerol blanking methods (Sekisui, Wako, and Kyowa) and three non-blanking methods (Roche, Siemens, and Beckman). Commutability was assessed only within each group (three MP pairs, i.e., three combinations of two MPs), resulting in six MP pairs (2×3).

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IFCC commutability working group protocol

Triplicate measurements were performed on 20 levels of CSs with varying lipid concentrations, each analyzed in a single position, and 11 RM levels, each placed in five positions within the analyzer. The commutability criterion was established as the range obtained by adding the allowable difference—defined by the National Cholesterol Education Program's accuracy criteria for lipids (±3% for TC, ±5% for HDL-C, ±4% for LDL-C, and ±5% for total glycerides [TG]) [24–27]; applied in the KDCA-LSP)—to the mean % bias between two MPs for CSs. An RM was considered commutable when the bias between MPs, including its uncertainty, fell within these predefined limits. Conversely, RMs with biases that exceeded these limits were classified as non-commutable. RMs with bias values overlapping the decision limit were categorized as having inconclusive commutability at the specified significance level.

CLSI EP14 protocol

Sample measurements were shared in accordance with the IFCC protocol. We calculated the mean of triplicate measurements for each CS and the mean of 15 repeated measurements for each RM (triplicate×five positions). Using the CLSI design, we determined the 95% prediction interval (PI) of the Deming regression between MPs based on CS values as the criterion for assessing commutability. An RM was considered commutable for an MP pair when it fell within this PI, indicating inclusion in the same population as the CSs; RMs falling outside the PI were considered non-commutable.

Additional exploration of non-commutability

The accuracy decisions of each MP confirmed in the KDCA-LSP during the second half of 2021 were reviewed to verify whether the non-commutable RMs yielded 'less acceptable' results in the KDCA-LSP.

Three RMs (RM1, RM4, and RM11) were used in the third ABL-PT program operated by KEQAS in the first half of 2017, which involved over 160 clinical laboratories [15]. The pass rates of the laboratories in the ABL-PT were reviewed to determine whether the commutability of RMs affected their EQA scores.

The % bias of each RM for the four lipid tests in each MP was reviewed based on the TG level. The % bias was calculated as follows:

$$\%\ \text{bias} = ([\text{measured value} - \text{assigned value}]/\text{assigned value}) \times 100\ (\%).$$

All lipoprotein fractions of RMs, previously confirmed using the Lipoprint LDL Subfractions Test (Quantimetrix, Redondo Beach, CA, USA), were reviewed to identify subfractions that could influence measurement bias and commutability.

Statistical analysis

Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA), a supplemental file provided by the IFCC working group [20], and Analyse-it (Analyse-it Software, Ltd., Leeds, UK) programs were used for statistical analysis.

RESULTS

RM characteristics

Table 1 shows the characteristics of RMs 1–11 (numbered in ascending order of TG levels). The assigned values ranged from 3.79–6.35 mmol/L (146.5–245.6 mg/dL) for TC, 1.05–1.83 mmol/L (40.5–70.7 mg/dL) for HDL-C, 2.07–4.17 mmol/L (79.9–161.3 mg/dL) for LDL-C, 0.86–3.13 mmol/L (76.6–277.1 mg/dL) for total glycerides, and 0.76–2.94 mmol/L (67.3-260.7 mg/dL) for TG.

Commutability assessment

Supplemental Data Fig. S1 shows the commutability assessment of RMs for each analyte in each MP pair, and Fig. 2 shows the decisions for TC and glycerides. When RM commutabilities for TC measurements were assessed using the IFCC approach, RM1, RM2, RM3, RM5, RM7, RM9, and RM11 were commutable in 10 of 15 MP pairs. However, five MP pairs involving a Siemens procedure for pairwise analysis (Roche–Siemens, Beckman–Siemens, Siemens–Sekisui, Siemens–Wako, and Siemens–Kyowa) resulted in non-commutable or inconclusive decisions. RM6, RM8, and RM10 were commutable with nine MP pairs, and RM4 was commutable with eight MP pairs. With the CLSI approach, the RMs showed commutable decisions from a minimum of seven MP pairs (RM4) to a maximum of 15 MP pairs (RM2, RM3, RM5, and RM9).

Commutability for total glycerides or TGs was assessed for six MP pairs and was determined to be commutable for four to six MP pairs, depending on the IFCC and CLSI approaches.

The HDL-C and LDL-C measurements were commutable for fewer MP pairs, especially for some RMs (Fig. 3). With the IFCC approach, RMs were commutable from 3–13 pairs of all 15 MP pairs for HDL-C measurements and 1–11 MP pairs for LDL-C. With the CLSI approach, RMs were commutable for 5–15 MP pairs for HDL-C and 1–15 for LDL-C. These 'less commutable'

Table 1. The characteristics of 11 frozen pooled serum-based RMs

RM	Institution*	Year†	Assigned value (mg/dL)‡				
			TC	HDL-C	LDL-C	Total glyceride	TG
RM1	KUMC	2016	185.3	70.7	98.8	76.6	67.3
RM2	CNUH	2020	172.3	60.0	94.8	78.3	71.6
RM3	SH	2020	146.5	52.0	79.9	78.5	71.8
RM4	KUMC	2016	164.6	55.3	88.5	86.2	76.9
RM5	SH	2020	245.6	61.8	161.3	102.2	93.4
RM6	KUMC	2016	158.2	44.1	90.0	118.5	110.0
RM7	SH	2017	154.8	51.3	83.8	128.8	119.7
RM8	CNUH	2018	161.3	40.5	90.6	165.3	161.5
RM9	CNUH	2019	176.1	49.0	93.4	200.9	194.7
RM10	CNUH	2020	183.0	44.2	96.1	220.2	210.8
RM11	KUMC	2016	178.6	43.0	87.2	277.1	260.7

*The institution refers to the entity that prepared the frozen pooled serum-based RMs.
†The year indicates when the frozen pooled serum-based RMs were prepared.
‡TC, HDL-C, and LDL-C values were assigned by CEQAL Inc., a participating member of the CRMLN, using the Abell–Kendall, ultracentrifugation, and beta-quantification methods, respectively. The total glyceride results were measured at the NMRL laboratory (a participating member of the CRMLN), using isotope dilution mass spectrometry without free glycerol blanking, and were regarded as assigned values. TG values were obtained by subtracting free glycerol values, measured at the ReCCS, a JCTLM member organization, using the same method, from total glyceride values.
Abbreviations: CNUH, Chungnam National University Hospital; CRMLN, Cholesterol Reference Measurement Laboratory Network; HDL-C, high-density lipoprotein cholesterol; JCTLM, Joint Committee for Traceability in Laboratory Medicine; KUMC, Konkuk University Medical Center; LDL-C, low-density lipoprotein cholesterol; NMRL, National Medical Reference Laboratory; ReCCS, Reference Material Institute for Clinical Chemistry Standards; RM, reference material; SH, Severance Hospital; TC, total cholesterol; TG, triglyceride.

properties were particularly pronounced for RM10 and RM11, which had the highest TG levels.

Additional exploration of non-commutability

Samples from the KDCA-LSP showed differences in LDL-C biases with the same MP across the 11 RMs. Accuracy acceptances for the measured values of each run from six MPs are shown in Supplemental Data Table S2. Biases exceeding the acceptance criteria were common for LDL-C measurements using Siemens MP, revealing many non-commutable decisions.

The KEQAS ABL-PT pass rates for each test were compared between RM1 and RM4 (which showed good commutability between MP pairs) and RM11 (which showed some non-commutable decisions) (Table 2). RM11 exhibited large measurement biases and lower pass rates for LDL-C in laboratories using certain MPs.

Fig. 4 presents the % bias compared with the assigned value for each RM in the lipid tests across six MPs. For LDL-C measurements, a large positive bias was noted for RMs 10 and 11 with TG levels exceeding 2.26 mmol/L (200 mg/dL). The increased bias of the RMs was consistent with that in non-commutable decisions in the commutability assessments. For other analytes (TC, HDL-C, total glyceride, and TG), the % bias did not show a clear tendency for changed TG levels, unlike LDL-C.

Because the measurement bias and non-commutability were not entirely dependent on TG levels, we performed electrophoresis lipoprotein fraction testing to investigate other factors affecting cholesterol measurements (Supplemental Data Table S3 and Fig. S2). RM10 and RM11, which had large biases in LDL-C measurements, showed a small mean LDL-C particle size (< 265 Å) and were classified as subtype B.

DISCUSSION

To assess the commutability of 11 RMs, we used the IFCC working group and CLSI EP14 guidelines, which have distinct features. The IFCC protocol uses a fixed decision criterion adopted from external information for all MP pairs. In contrast, the CLSI protocol employs a statistical value derived from the CSs and varies with each MP pair [20, 22].

Although the PI broadened due to widely scattered biases originating from differences in non-selectivity (DINS) between MPs, which can increase the chance of RMs being judged as commutable [12], integrating results from these MPs is inappro-

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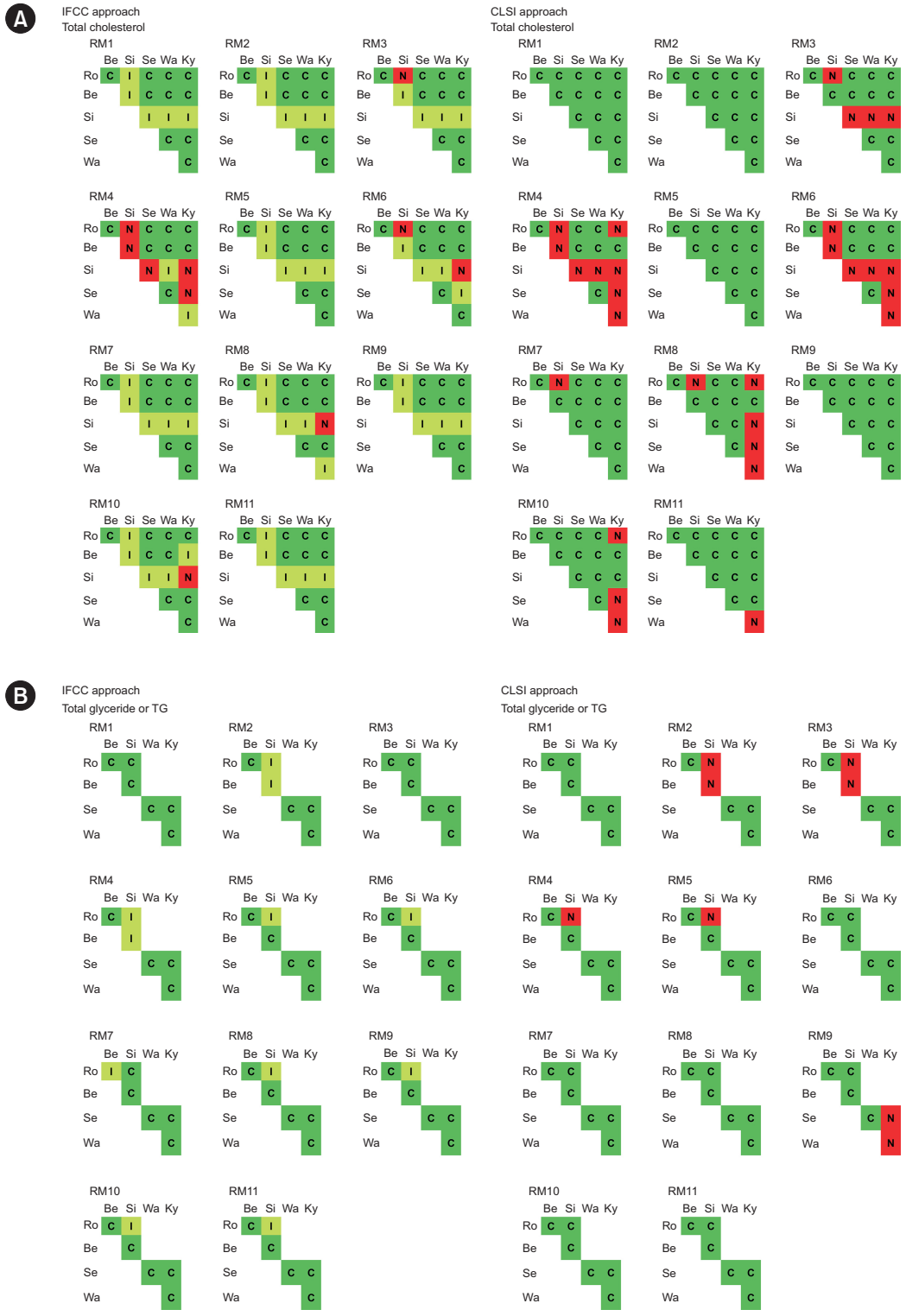

Fig. 2. Pairwise commutability assessment results for each RM across different MP pairs. (A, B) Total cholesterol (A) and total glyceride or TG (B) measurements. The decisions made are indicated with a green "C" for commutable, a yellowish-green "I" for inconclusive, and a red "N" for non-commutable.

Abbreviations: Be, Beckman Coulter measurement procedure; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; Ky, Kyowa measurement procedure; RM, reference material; Ro, Roche measurement procedure; Se, Sekisui measurement procedure; Si, Siemens measurement procedure; TG, triglyceride; Wa, Wako measurement procedure.

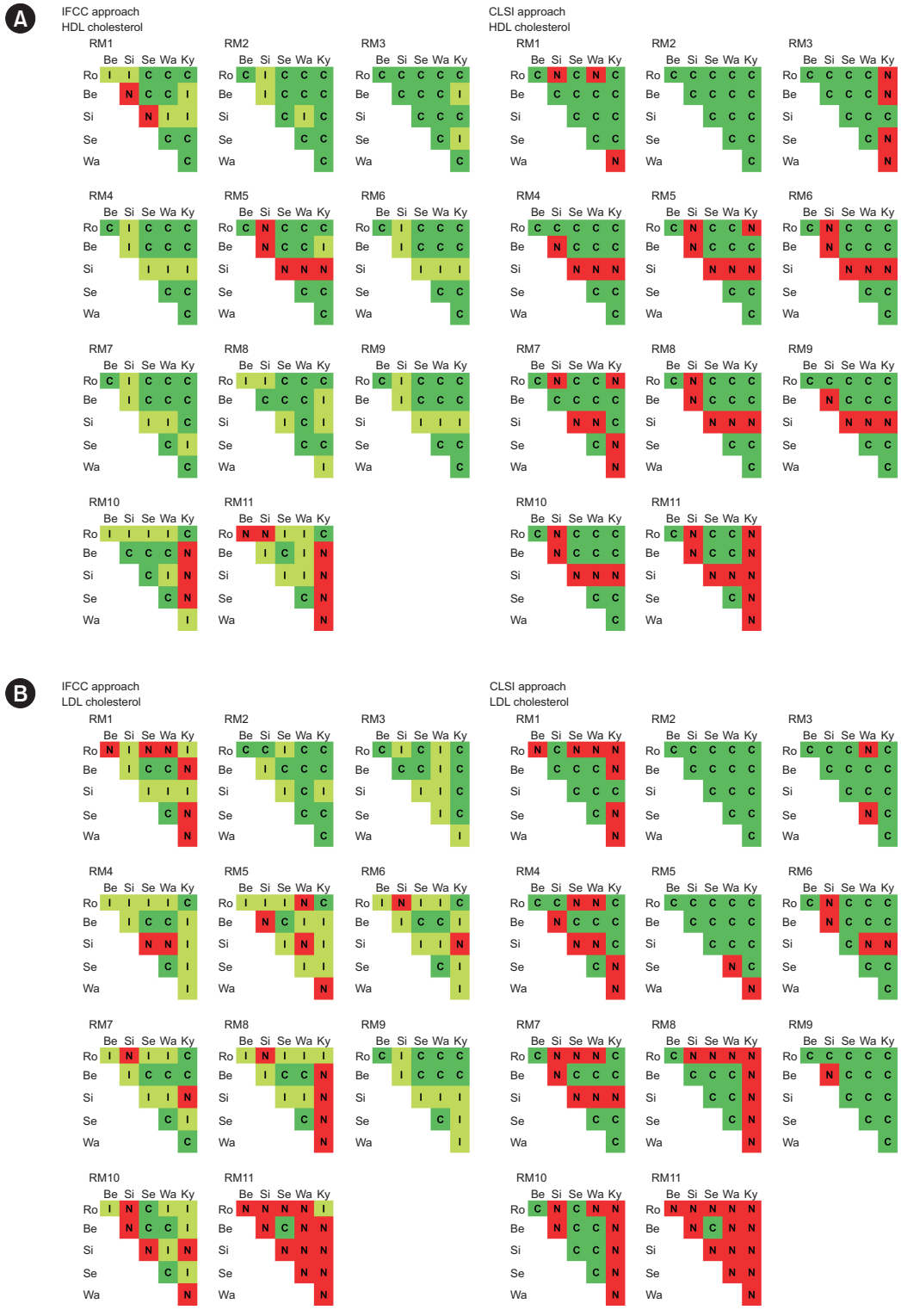

Fig. 3. Pairwise commutability assessment results for each RM across different MP pairs. (A, B) High- (A) and low-density (B) lipoprotein cholesterol measurements. The decisions made are indicated with a green "C" for commutable, a yellowish-green "I" for inconclusive, and a red "N" for non-commutable.

Abbreviations: Be, Beckman Coulter measurement procedure; HDL, high-density lipoprotein; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; Ky, Kyowa measurement procedure; LDL, low-density lipoprotein; MP, measurement procedure; RM, reference material; Ro, Roche measurement procedure; Se, Sekisui measurement procedure; Si, Siemens measurement procedure; Wa, Wako measurement procedure.

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Table 2. The pass rates (%) of clinical laboratories participating in accuracy-based lipid proficiency testing for three RMs used by the KEQAS during the first half of 2017

MP	RM1 (TG: 0.76 mmol/L)				RM4 (TG: 0.87 mmol/L)				RM11 (TG: 2.94 mmol/L)			
	TC*	HDL-C	LDL-C	TG	TC	HDL-C	LDL-C	TG	TC	HDL-C	LDL-C	TG
Roche	100	100	96	100	100	100	96	100	100	98	78	100
Beckman	100	100	74	97	100	100	61	100	100	100	32	100
Siemens	100	100	100	100	100	100	100	100	100	100	0	100
Sekisui	100	100	97	100	100	100	100	95	96	100	13	100
Wako	100	86	80	100	100	100	100	100	100	100	80	100
Kyowa	100	100	100	100	100	100	100	100	100	100	100	100

*The external quality assessment results were assessed based on the total error criteria of the National Cholesterol Education Program: 9% for TC, 13% for HDL-C, 12% for LDL-C, and 15% for TG.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; KEQAS, Korean Association of External Quality Assessment Service; MP, measurement procedure; RM, reference material; TC, total cholesterol; TG, triglyceride.

priate due to low harmonization. Recent IFCC guidelines recommend assessing the DINS between MPs in CSs to evaluate whether regression-based commutability assessment is appropriate [28]. The IFCC recommends their design over the CLSI procedure due to associated limitations [20], including (1) criteria misaligned with 'medically relevant' differences, (2) an inability to quantify the closeness of agreement for RMs with the average relationship for CSs at the level of interest, and (3) challenges in assessing the significance level of commutability decisions by neglecting uncertainty. However, recently updated CLSI EP30 guidelines incorporate the concept of a confidence interval for the RM mean [29].

These different decision criteria can result in conflicting decisions for the same RM, analyte, and MP pair. We found that the LDL-C commutability for RM5 in Roche–Wako, Beckman–Siemens, and Siemens–Wako MP pairs was non-commutable with the IFCC approach but commutable with the CLSI approach. Conversely, TC measurements for RM10 with Roche–Kyowa, Sekisui–Kyowa, and Wako–Kyowa MP pairs were commutable using the IFCC approach but non-commutable with the CLSI design. Because the EQA program evaluates multiple MPs simultaneously, assessing the commutability of panel RMs with the IFCC approach is appropriate, given its consistent criteria across all MPs.

Frozen pooled serum RMs produced based on CLSI C37-A guidelines were commutable (with most MP pairs using the IFCC design), especially for TC and TG measurements. However, some high-TG materials were non-commutable with certain MP pairs, particularly for LDL-C measurements. High TG levels or small dense LDLs (sdLDLs) in samples interfered with LDL-C measurements, leading to varying results among MPs. This observation aligns with previous findings [16, 17, 30].

RMPs (CDC ultracentrifugation for HDL-C and beta-quantification for LDL-C) are time-consuming and labor-intensive. Automated assays in clinical laboratories have replaced RMPs by inhibiting reactions of non-target substrates or by depleting other substrates first with target-analyte reaction inhibition and subsequently detecting the target-analyte signal. Several factors, including high TG or sdLDL levels, can interfere with this pre-analytical process. With MPs that are largely affected by such interference, the analytical performance might be influenced by whether such samples are included in the EQA program panel. The accuracy acceptance for KDCA-LSP using 11 RMs as panel samples and the ABL-PT results obtained by KEQAS in the first half of 2017 (for RM1, RM4, and RM11) [15] showed large pass-rate differences, depending on the RM, analyte, and MP.

The impact of atypical samples on measurement bias differed by the MP used. The Siemens MPs showed fewer commutable decisions for cholesterol measurements (particularly LDL-C) and exhibited relatively low pass rates for LDL-C for some samples (Supplemental Data Table S2 and Table 2), with a large positive bias. Laboratories using Siemens MPs demonstrated lower rates of acceptable performance than those using other MPs due to an increased mean % bias observed in EQAs, including the ABL-PT performed by KEQAS [15] and the KDCA-LSP. Our ABL-PT results, obtained using target values determined using RMPs, suggest that factors influencing differences in inter-MP bias between RMs and CSs affect commutability decisions and EQA scores. Evaluating the performance of such MP is challenging because EQA scores can be largely influenced by the composition of the panel RM, and the presence or proportion of such samples.

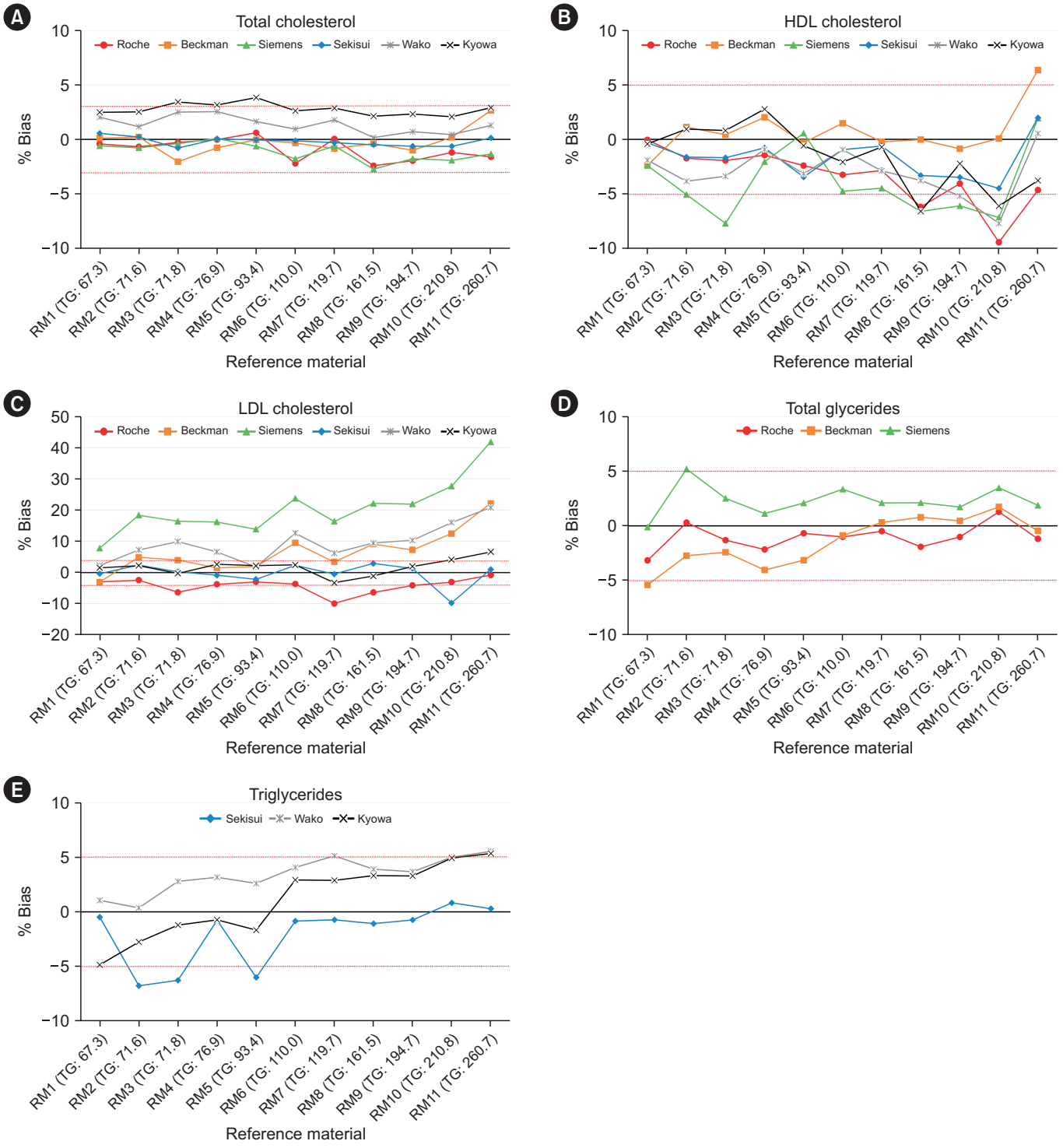

Fig. 4. Percent bias of the assigned values for the second round of the 2021 KDCA-LSP is exhibited for each RM and arranged in ascending order of TG levels, presented within parentheses as units of mg/dL in the lipid tests. (A–E) The total cholesterol (A), HDL cholesterol (B), LDL cholesterol (C), total glyceride (D), and TG (E) levels measured across six different measurement procedures. Data points from the Roche, Beckman, Siemens, Sekisui, Wako, and Kyowa measurement procedures are indicated with red dots, orange squares, green triangles, blue diamonds, gray asterisks, and black crosses, respectively. The broken red lines indicate the National Cholesterol Education Program's accuracy criteria for the tests.

Abbreviations: HDL, high-density lipoprotein; KDCA-LSP, Korea Disease Control and Prevention Agency-Lipids Standardization Program; LDL, low-density lipoprotein; RM, reference material; TG, triglyceride.

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Because preparation following the CLSI C37 guidelines may not fully ensure RM commutability, we considered potential causes of non-commutability and differences from CSs to enhance EQA efficacy. First, matrix effects introduced during processing, owing to the influence of surrounding components—proteins, electrolytes, and lipids—on physicochemical properties, can alter measurement results [31], as demonstrated by previous findings on the commutability of processed RMs [9, 32–35]. However, current EQA systems require large RM volumes for distribution to numerous laboratories, limiting the feasibility of producing RMs from single-donor serum [8] and complicating system-wide modifications. Second, increasing positive bias in LDL-C measurements for high-TG samples with some MPs can introduce systemic error and affect analytical performance evaluations. In this study, some RMs had higher TG levels than the CSs, and commutability decisions for RMs outside the CS coverage range were based on extrapolated criteria from CSs. This approach may lead to inaccurate conclusions, especially when an RM falls near the decision threshold. Although the composition of CSs may impact assessment outcomes [18, 30], adjusting the CS selection alone does not offer a fundamental solution for improving EQA efficacy. Third, unpredictable bias between RMs and CSs, driven by sample-specific effects from DINS among MPs, complicates analytical performance evaluations [29]. Enhancing MP specificity is essential and requires dedicated efforts from manufacturers. However, when short-term improvements are not feasible, selecting RMs that align with EQA objectives provides a practical alternative. When the EQA goal is to identify MP non-selectivity using real-world samples, the inclusion of such samples may be effective. Previous researchers using the same RMP recommended including samples from patients with hypertriglyceridemia and/or using fresh serum to accurately assess measurement trueness in real-world settings [36, 37]. However, obtaining sufficient volumes and ensuring the stability of fresh RMs present substantial challenges for both EQA bodies and manufacturers. When the objective is to identify poorly calibrated MPs within peer groups, excluding RMs with TG levels >2.26 mmol/L (200 mg/dL) (where large biases and distorted mean % bias are expected) from HDL-C and LDL-C evaluation panels may enable more accurate analytical performance assessment and improve EQA program effectiveness.

This study had some limitations. First, the IFCC working group recommended using more than 30 CSs for commutability assessment; however, our study included 20 CSs due to limited donor recruitment, which potentially reduced the statistical power. Second, although analyte levels in CSs ideally cover those in RMs, difficulties in obtaining "abnormal" samples from healthy donors may lead to inaccurate decisions for RMs outside the CS coverage. Third, although recent CLSI EP30 guidelines recommend incorporating confidence intervals and the IFCC proposed novel methods to address non-selectivity in regression approaches, we followed the CLSI EP14 guidelines available at the time of analysis. Future studies should address these limitations and adopt these advancements to improve commutability assessments, particularly for evaluating EQA materials. However, this study was the first to assess the commutability of RMs used in Korean EQA programs, providing a basis for enhancing their clinical utility.

In conclusion, RMs prepared per CLSI C37-A guidelines were more likely to be commutable for TC and TG across MP pairs. However, commutability was less consistent for HDL-C and LDL-C, especially for RMs with high TG levels and a higher proportion of sdLDLs, showing large LDL-C biases relative to assigned values. A review of previous PT programs using these RMs revealed that these RMs showed larger biases from assigned values and lower pass rates. When preparing RMs, the TG concentration and lipid-particle size should be carefully considered, and a commutability assessment should be conducted before their use.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2025.0010.

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AUTHOR CONTRIBUTIONS

Seo JD and Yun YM contributed to conception and design of the study; Yun YM and Kim S were involved in investigation and supervision; Seo JD collected, analyzed, and visualized the data and wrote the original draft; Yun YM acquired funding and administered the project; Seo JD, Yun YM, and Kim S interpreted data and reviewed and edited the manuscript draft; Kwon GC,

Kim JH, and Cho CI supported method development; Lee SG collected and analyzed data; and Song J, Park PW, An D, and Choi Q collected data. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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