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Estimating the Biases of the Korean National Cholesterol Proficiency Test

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Abstract. It is recommended that clinical laboratories keep the bias of serum total cholesterol analysis at ≤3.0% compared to a reference method. In Korea, national cholesterol proficiency testing has long been available, but there has been little information about the magnitude of analytical bias. The authors calculated the bias of the peer group mean for Korea's national cholesterol proficiency test through an indirect approach that overcomes the potential matrix effect of proficiency test materials. One laboratory was selected among the proficiency test participants to represent Korean laboratories. Total cholesterol levels of six fresh serums spanning a wide range of concentrations were measured by the representative laboratory and three reference laboratories. The relationship between the proficiency test mean and the reference method mean was established by linear regression analysis. The peer group mean of the proficiency test was calculated to have a bias of +2.4 to +2.5% at the medical decision levels. When grouped by instrument and reagent, 29 to 66% of the laboratories showed biases <3.0%. Thus it was determined that the peer group mean of the Korean cholesterol proficiency test has an acceptable level of positive bias. The indirect approach used in this study provides a practical model for estimating cholesterol analytical bias for proficiency testing.

Keywords: cholesterol analysis, proficiency testing, analytical bias

Introduction

In clinical laboratories, the accuracy of serum total cholesterol measurements has been regarded as especially important, because a laboratory result itself is directly used for determining the cardiovascular risk of an individual [1-4]. Therefore, every laboratory should have accurate, reproducible cholesterol measurements, enabling sound medical practice. To achieve that goal, authorized guidelines have been issued for clinical laboratories; prominent among these are recommendations of the Laboratory Standardization Panel (LSP) of the National Cholesterol Education Program (NCEP) [5]. This panel recommends that clinical laboratories achieve a bias of ≤3.0% compared to the Centers for Disease Control and Prevention (CDC) reference method and overall precision consistent with a CV of ≤3.0% [5]. Although these recommendations originated in the United States, they have been accepted worldwide, because the CDC is connected to the World Health Organization Collaborating Center for Reference and Research in Blood Lipids.

In Korea, the national proficiency test program that is provided by the Korean Association of Quality Assurance for Clinical Laboratories (KAQACL) plays a critical role for inter-institutional harmonization of cholesterol measurements. In 2007, more than 700 laboratories participated in the KAQACL serum cholesterol proficiency test

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[6]. According to the report of the KAQACL, about 99% of the participating laboratories used an enzymatic method for measuring serum cholesterol concentration and were grouped together as one peer [6-8]. These laboratories were encouraged to adjust their cholesterol results to agree with the peer group mean obtained from the proficiency materials. However, bias of the peer group mean was not assessed properly; hence, improvement in the accuracy of cholesterol measurement was limited. Although it is desirable for practicing evidence-based medicine, the bias of the peer group mean has not been measured reliably prior to this study. As the degree of inter-laboratory agreement has become progressively higher, the demand for estimating the bias of the peer group mean has become more and more apparent.

The CDC uses the Abel-Kendall version of the Liebermann-Buchard procedure as the reference method for total cholesterol measurement [9]. In addition, the CDC has approved several laboratories worldwide to perform the Abel-Kendall method for certifying the commercial cholesterol assay kits produced by manufacturers. This group of laboratories is known as the Cholesterol Reference Method Laboratory Network (CRMLN) [9,10]. There were nine CRMLN laboratories spanning the United States, Europe, Asia, Canada, and South America at the time of this study [11]. It is deemed inappropriate to apply the Abel-Kendall method directly to the KAQACL proficiency test materials, because commercial proficiency materials are treated with stability-enhancing processes and they may not produce accurate results with the reference method due to matrix bias [12]. According to the laboratory certification protocol issued by CRMLN, any serum preservation method apart from freezing is unacceptable for the Abel-Kendall method [10]. Therefore, estimating the bias of the KAQACL proficiency test program required a scheme different from direct measurement.

Given that background, we adopted an indirect approach by selecting a representative Korean laboratory to serve as an intermediary to correlate the reference method and the peer group means of the proficiency test. Through this indirect approach, we estimated the bias and degree of compliance of Korean laboratories to the LSP recommendations.

Materials and Methods

Selection of a representative laboratory. Through searching the database of the KAQACL proficiency test in 2007 and personal interviews, we selected a laboratory whose proficiency test result was close to the peer group mean. Compared to the peer group means, the laboratory showed a correlation coefficient of 0.999 and an average difference of -0.1% (Table 1). Due to such a high degree of agreement with the peer group mean, the representative laboratory did not adjust its calibration, thus deviating from the routine practice up to the time of this study. The representative laboratory used Liquid Calibrator (Denka-Seiken Co., Tokyo, Japan), Determiner-C-TC reagent (Kyowa-Medex Co., Tokyo, Japan), and a TBA-200FR analyzer (Toshiba Medical Systems Co., Tochigi-Ken, Japan) to measure total cholesterol concentrations.

Specimen collection and transportation. Cholesterol measurements followed the Certification Protocol for Clinical Laboratories of CRMLN as far as possible [10]. All serum specimens were stored at 2 to 8°C within 8 hr from the time of venipuncture [10]. Six serum cholesterol levels were prepared. Two were between 100 and 200 mg/dl, two were between 200 and 240 mg/dl, and the rest were >240 mg/dl. The volume of each serum specimen exceeded 9 ml. The difference of the cholesterol concentrations between neighboring levels was >20 mg/dl and that between the highest and the lowest was >100 mg/dl [10]. Because sufficient serum could not be obtained from single individuals at all the levels, serums from

Table 1. Comparison between the peer group mean and the result of the representative laboratory of the KAQACL cholesterol proficiency test in 2007.

	Peer group* mean (n = 746)	Representative laboratory
Sample 1 (mg/dl)	246	242
Sample 2 (mg/dl)	236	231
Sample 3 (mg/dl)	125	125
Sample 4 (mg/dl)	236	233
Sample 5 (mg/dl)	100	100
Sample 6 (mg/dl)	100	100
Sample 7 (mg/dl)	261	261
Sample 8 (mg/dl)	104	108
Sample 9 (mg/dl)	105	106
Sample 10 (mg/dl)	260	260
Sample 11 (mg/dl)	265	257
Sample 12 (mg/dl)	106	107
Mean (mg/dl)	178.7	177.5
Corr. coefficient (r^2) with peer group mean:		0.999
Mean difference from peer group mean (%):		-0.1
Mean of absolute difference from peer group mean (%);		1.2

* The peer group consisted of 99% of all laboratories using enzymatic methods.

Table 2. Results of cholesterol concentrations measured by the representative laboratory and the three reference laboratories.

	Total cholesterol concentration (mg/dl)															
	The selected representative laboratory						Osaka Medical Center				University of Washington				Laboratorio Analisi Cliniche	
	Day 1		Day 2		Day 3											
	1st run	2nd run	1st run	2nd run	1st run	2nd run	1st run	2nd run	3rd run	4th run	1st run	2nd run	1st run	2nd run	1st run	2nd run
Serum 1	108	109	106	106	107	106	106	106	106	106	105	105	106	107		
Serum 2	152	151	153	152	152	154	150	150	150	150	149	149	149	151		
Serum 3	200	200	201	201	202	203	197	197	197	197	194	194	196	196		
Serum 4	234	233	230	231	235	233	229	229	228	229	227	227	230	230		
Serum 5	270	269	266	264	269	268	271	271	271	271	271	271	273	273		
Serum 6	288	290	287	290	290	288	282	282	282	282	285	285	285	288		

two persons were pooled for the highest two concentrations as allowed by CRMLN [10]. Three aliquots were made to contain 1 ml of each serum specimen. These aliquots were kept refrigerated until the time of cholesterol measurement by the representative laboratory. Three additional aliquots containing 2 ml of each serum specimen were prepared and each aliquot was sent to the three CRMLN laboratories, which were (i) the Northwest Lipid Metabolism and Diabetes Research Laboratories of University of Washington (Seattle, WA, USA), (ii) Diagnostica e Ricerca S. Raffaele (Milan, Italy), and (iii) the Lipid Reference Laboratory of Osaka Medical Center for Health Science and Promotion (Osaka, Japan). During transport to the CRMLN laboratories, the specimens were kept frozen at < -20°C.

Total cholesterol measurement. The representative laboratory measured the cholesterol concentrations of all six specimens twice a day consecutively for 3 days, from the day following venipuncture. For every level, one aliquot was used on each day of measurement. During the study, routine maintenance of the analyzer, calibration, and internal quality control was done. The model of the analyzer and the reagent brand were the same as those in the 2007 KAQACL proficiency test.

Each CRLMN laboratory measured the total cholesterol levels of the six specimens repeatedly by the Abel-Kendall method. Once 0.5 ml of a serum specimen was saponified with 5.0 ml of 0.36 mol/L ethanolic KOH at 50°C for 60 min, 10 ml of hexane was added for extraction. After removing water from the extract, 3.2 ml of Liebermann-Buchard reagent was added. Absorbance was measured at 620 nm after 30 min of incubation [13]. To minimize the effect of a possible random error, one CRMLN laboratory ran the specimens in quadruplicate, while the other laboratories ran the specimens in duplicate as recommended for the usual certification of assays for a clinical laboratory [10].

Statistical analysis. Traceability was determined by the CDC criteria [14]. Linear regression analysis was used for comparisons between the reference method mean, the representative laboratory mean, and the peer group mean of the KAQACL proficiency test. First, the representative laboratory mean was correlated with the reference method mean and the peer group mean, respectively. Then, the peer group mean was correlated with the reference method mean, using the representative laboratory mean as an intermediary. Based on the regression equation between the reference method mean and the peer group mean, a reference method concentration of each proficiency material was calculated. Then, the calculated reference method concentration was compared against the proficiency test result of an individual laboratory or a group of laboratories. The difference between the two was regarded as the predicted bias.

Results

Measurement of total cholesterol. Total cholesterol concentrations of the six fresh serum specimens measured by the representative laboratory and the 3 CRMLN reference laboratories are listed in Table 2. The cholesterol concentrations measured by each reference laboratory differed by <1.0% from the mean concentrations of all three reference laboratories. That was judged to be acceptable, considering the limit of bias applied to CRMLN laboratories [14]. Also, the representative laboratory was found to fulfill the traceability criteria of the CDC having a mean bias of +1.3%.

Table 3. Calculated bias of the cholesterol proficiency test result of a group of laboratories using the same model of analyzer.

Instrument model	No. of labs.	Linear regression of the proficiency test result between the group and the representative laboratory*		Linear regression of the group's proficiency test result and the calculated reference interval concentration†	Bias at 200 mg/dl (%)	Bias at 240 mg/dl (%)	Proportion of proficiency test results with a bias ≤3.0%
		Regression formula	r				
Hitachi 7180	43	y = 1.012x - 2.328	1.000	y = 0.983x + 5.726	1.1	0.7	275/468 (58.8%)
Olympus AU400	38	y = 1.077x - 10.548	0.999	y = 0.924x + 13.016	-1.1	-2.2	188/459 (41.0%)
Hitachi 7060	37	y = 1.014x + 0.772	1.000	y = 0.982x + 2.659	-0.5	-0.8	188/444 (42.3%)
Olympus AU640	35	y = 1.053x - 6.981	0.999	y = 0.945x + 9.923	-0.6	-1.4	186/417 (44.6%)
Hitachi 7080	34	y = 1.014x +0.073	1.000	y = 0.981x + 3.347	-0.2	-0.5	173/396 (43.7%)
Toshiba TBA-200FR	29	y = 1.000x - 0.945	1.000	y = 0.995x + 4.414	1.7	1.4	187/330 (56.7%)
Roche Modular D/P	23	y = 1.032x - 7.433	1.000	y = 0.964x + 10.565	1.7	0.8	176/273 (64.5%)
Siemens Advia 1650	15	y = 1.016x - 7.438	1.000	y = 0.979x +10.736	3.3	2.4	111/176 (63.1%)

*The variable 'x' is the proficiency test result of the representative laboratory and the variable 'y' is the proficiency test mean of the group of laboratories using the same model of analyzer.
†The variable 'x' is the calculated reference concentration, and the variable 'y' is the proficiency test mean of the group of laboratories using the same model of analyzer.

Table 4. Calculated bias of the cholesterol proficiency test result of a group of laboratories using the same brand of reagent.

Reagent brand	No. of labs.	Linear regression of the proficiency test result between the group and the representative laboratory*		Linear regression of the group's proficiency test result and the calculated reference interval concentration†	Bias at 200 mg/dl (%)	Bias at 240 mg/dl (%)	Proportion of proficiency test results with a bias ≤3.0%
		Regression formula	r				
Daiichi Preauto S	112	y = 1.014x - 2.291	1.000	y = 0.982x + 5.682	1.0	0.5	701/1317 (53.2%)
Wako CHO	68	y = 1.014x + 1.817	1.000	y = 0.981x + 1.628	-1.1	-1.2	235/804 (29.2%)
Olympus OSR	63	y = 1.017x - 4.652	0.997	y = 0.978x + 7.985	1.8	1.2	340/763 (44.6%)
Asan 701 LQ DIA	59	y = 1.014x - 0.575	1.000	y = 0.981x + 3.987	0.1	-0.2	366/720 (50.8%)
Shinyang SICDIA	53	y = 0.988x + 1.482	1.000	y = 1.001x + 2.010	1.7	1.6	388/658 (59.0%)
DiaSys Cholesterol	48	y = 1.053x - 6.927	0.999	y = 0.945x + 9.870	-0.6	-1.4	204/551 (37.0%)
Roche CHOD-PAP	33	y = 1.039x - 8.781	1.000	y = 0.9575x + 11.790	1.6	0.7	246/372 (66.1%)

*The variable 'x' is the proficiency test result of the representative laboratory and the variable 'y' is the proficiency test mean of the group of laboratories using the same brand of reagent.
†The variable 'x' is the predicted reference concentration and the variable 'y' is the proficiency test mean of the group of laboratories using the same brand of reagent.

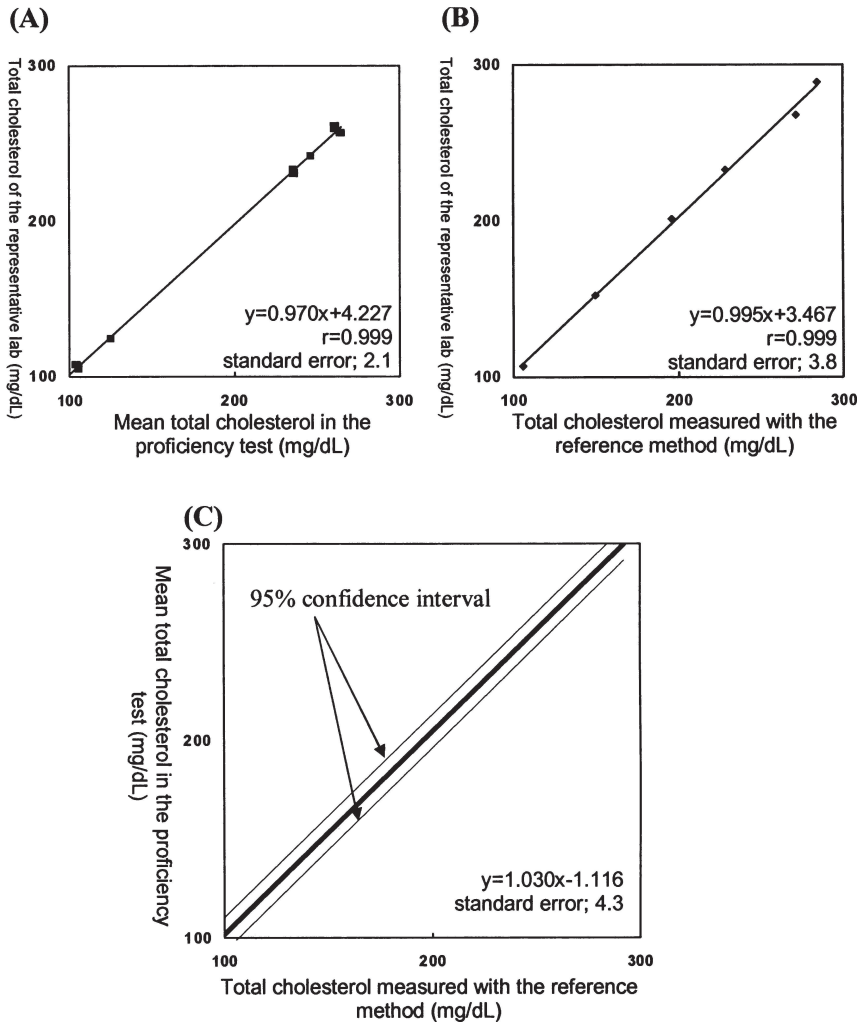

Fig. 1. This chart shows the x-y plots and linear regression lines for cholesterol means analyzed by the representative laboratory, the peer group of the proficiency test, and the reference Abel-Kendall method. Note that plot (C) contains statistically defined 95% confidence limits but no data points, because the linear regression line was drawn by indirect analysis by way of the representative laboratory.

Correlation between the KAQACL proficiency test and the reference method. The correlation coefficient and the standard error of the estimate between the results of the representative laboratory and the reference method mean were 0.999 and 3.8 mg/dl, respectively (Fig. 1B). Based on this correlation, the relationship between the peer group means of the KAQACL proficiency test and the reference method mean could be plotted, and the standard error of the estimate was 4.3 mg/dl (Fig. 1C). Based on the plot, it was predicted that the KAQACL proficiency test had biases of +2.4 and +2.5% at the medical decision levels of 200 mg/dl and 240 mg/dl, respectively.

Among the 12 trials of the proficiency test using different materials, a trial with the mean concentration close to the medical decision level was chosen to show a typical distribution of the results (Fig. 2). The peer group mean was estimated to have a positive bias of 2.5% referenced to the calculated reference concentration. Among the 686 participating laboratories, 59.2% reported a result <3.0% different from the peer group mean. However, only 49.9% were found to have a predicted bias of ≤3.0%. This finding was partly caused by the high proportion of the results clustered around the peer group mean.

For major groups of laboratories using the same model of analyzer and the same reagent, the proportion of results with predicted bias of ≤3.0% is shown inTable 3 and Table 4. The proportions were remarkably variable, ranging from 29.2% and 66.1%. All but one of the groups had predicted biases ≤3.0% at the medical decision levels.

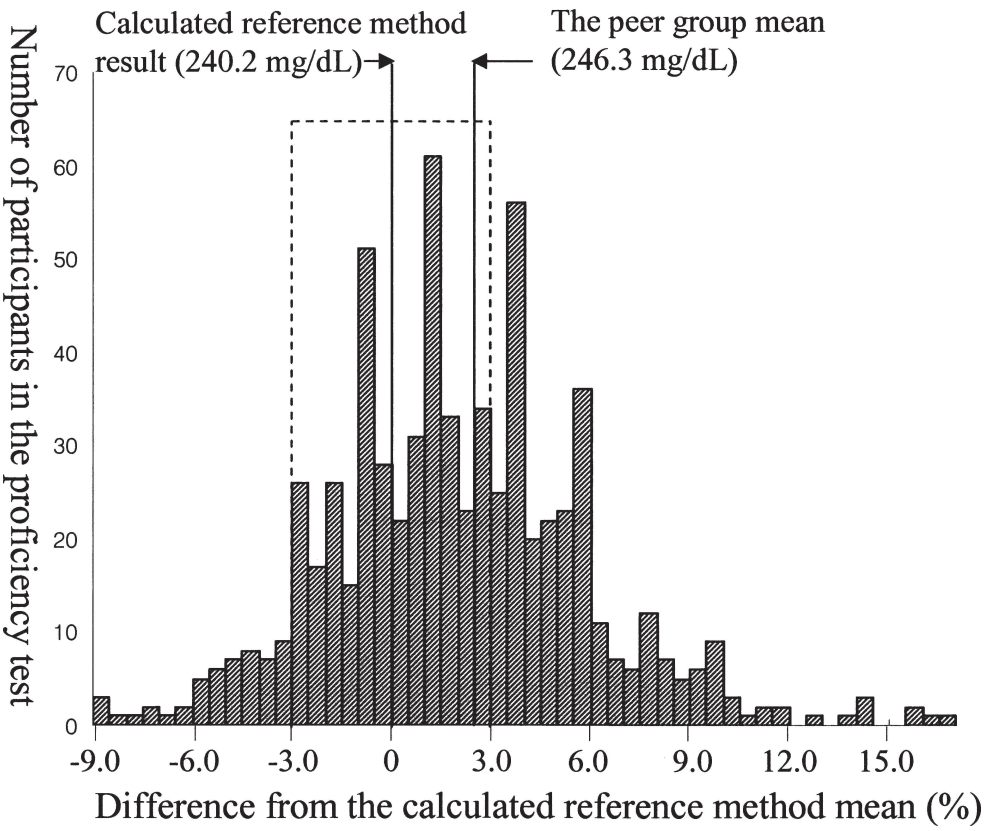

Fig. 2. Distribution of cholesterol concentration of a selected trial of the proficiency test. The peer group mean was estimated to have a positive bias of 2.5%. A dotted square was drawn to delineate the proficiency test results having a bias of 3.0% or less.

Discussion

Each clinical laboratory should maintain a bias of cholesterol measurement within the globally recommended limit of 3.0% [5]. A long-standing national proficiency testing program has helped to reduce inter-laboratory differences of cholesterol assays in Korean laboratories. However, to enhance the significance of inter-laboratory harmonization, it was necessary to determine the bias of the peer group mean. Because the KAQACL materials were lyophilized commercial products, the authors had to develop an indirect approach to estimate the bias of the proficiency test samples. For a simpler procedure, fresh frozen sera could have been used for the proficiency test. But in that case, pooling multiple sera would have been necessary for distribution to a large number of laboratories, and that would have complicated comparison with the reference method [9,15]. Even if it were possible to obtain sufficient serum from a single individual to cover both the reference method and proficiency test, that would be feasible only for a limited range of concentrations [16]. Therefore direct comparison between the KAQACL proficiency test and the reference method was considered to be an unrealistic option for estimating the bias.

Ross et al. [12] showed the existence of the matrix effect, which results in a different behavior of a proficiency test material compared to that of fresh serum. Based on that finding, we used an indirect method to compare the reference method mean and the peer group mean of the proficiency test. Through this indirect approach, it was possible to assess the degree of compliance of Korean laboratories to the LSP recommendation for bias.

Through the correlation between the peer group mean and the reference method mean, it was found that the bias of the peer group mean at the medical decision level was <3.0%, which was regarded as acceptable in respect to the LSP recommendation. However, considering that the proficiency test results tended to cluster around the biased peer group mean, further efforts were deemed necessary to reduce the bias. In addition, the distribution of the proficiency test results was skewed, having predominantly positive biases. That finding suggests that the prevalence of hypercholesterolemia in Koreans could be over-estimated. Although further investigation seemed necessary before reaching a definite conclusion, it was envisioned that reducing the bias of cholesterol measurements in Korean clinical laboratories might change the current epidemiological data.

Grouping laboratories according to the analyzer and the brand of reagent revealed that variable proportions of each group showed a bias of ≤3.0% at the medical decision levels. Remarkably, the group of users of Advia 1650 (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA) showed a mean bias >3.0% at 200 mg/dl, although the analyzer itself had been certified by CRMLN [17]. On the other hand, users of Wako CHO (Wako Pure Chemical Industries, Osaka, Japan) showed the lowest proportion of results with bias <3.0%, although the reagent itself was certified by CRMLN [17]. Users of reagents without CRMLN certification, Asan 701 LQ DIA, (Asan Pharmaceutical, Seoul, Korea) and Shinyang SICDIA (Shinyang Chemical, Seoul, Korea) had relatively higher proportions of bias <3.0%. Based on these findings, the bias of an individual clinical laboratory was affected by the quality of laboratory practice.

Considering that most peer group means of the proficiency test were estimated to be <3.0%, it was assumed that the proficiency test had improved accuracy as well as laboratory harmonization. However, the analysis also revealed that almost half of the proficiency test results demonstrated less than acceptable bias. Such an unsatisfactory level of compliance was thought to warrant heightened awareness of inaccuracy and further efforts to be exerted by the relevant academic societies.

For an individual laboratory, an acceptable bias should be combined with a satisfactory level of precision to achieve accuracy of cholesterol measurement. In future investigations, information about bias, estimated by the method described in this study, can be supplemented by the laboratory's own internal quality control data. That will allow a better estimate of the impact of the bias on the diagnosis and treatment of hypercholesterolemia and on the disease prevalence among Koreans.

In conclusion, we estimated the bias of the peer group mean of the Korean cholesterol proficiency test using an indirect approach. The strategy employed in this study can serve as a model for determining the bias of proficiency tests for other analytes as well. The peer group mean of the KAQACL cholesterol proficiency test was shown to have mostly positive biases <3.0%.

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