Harmonization of laboratory results by data adjustment in multicenter clinical trials

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Background/Aims: In multicenter clinical trials, laboratory tests are performed in the laboratory of each center, mostly using different measuring methodologies. The purpose of this study was to evaluate coefficients of variation (CVs) of laboratory results produced by various measuring methods and to determine whether mathematical data adjustment could achieve harmonization between the methods.

Methods: We chose 10 clinical laboratories, including Green Cross Laboratories (GC Labs), the central laboratory, for the measurement of total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), serum triglycerides, creatinine, and glucose. The serum panels made with patient samples referred to GC Labs were sent to the other laboratories. Twenty serum samples for each analyte were prepared, sent frozen, and analyzed by each participating laboratory.

Results: All methods used by participating laboratories for the six analytes had traceability by reference materials and methods. When the results from the nine laboratories were compared with those from GC Labs, the mean CVs for total cholesterol, HDL-C, LDL-C, and glucose analyzed using the same method were 1.7%, 3.7%, 4.3%, and 1.7%, respectively; and those for triglycerides and creatinine analyzed using two different methods were 4.5% and 4.48%, respectively. After adjusting data using Deming regression, the mean CV were 0.7%, 1.4%, 1.8%, 1.4%, 1.6%, and 0.8% for total cholesterol, HDL-C, LDL-C, triglyceride, creatinine, and glucose, respectively.

Conclusions: We found that more comparable results can be produced by laboratory data harmonization using commutable samples. Therefore, harmonization efforts should be undertaken in multicenter trials for accurate data analysis (CRIS number; KCT0001235).

Keywords: Multicenter clinical trial; Harmonization; Comparable data; Traceability

INTRODUCTION

Lasting and continuous competence is imperative as the field of medicine is constantly evolving. The need to investigate multiple populations in medical research has led to multicenter and even multinational trials. In multicenter trials, many challenges arise that are not present in single-institution studies, particularly with respect to clinical laboratory results. To overcome challenges associated with compiling and comparing results...
from different laboratories, standardization or harmonization of laboratory test results is needed. However, standardization and harmonization consume much time and resources, creating obstacles in multicenter trials [1].

In multicenter trials, clinical laboratory testing can be performed in the laboratory of each participating site or in a central laboratory. In general, routine tests, such as complete blood cell counts, general chemistries, and urinalysis, are usually performed at the respective laboratory of each participating site; and special tests, such as drug concentration and genetic testing, are performed in a central laboratory. Because researchers may assume that various methods for measuring or evaluating an analyte produce the same results, they may not be aware of variability in results between methods, which could result from a lack of traceability. As all laboratories do not use the same analytic methods, measurement principles, calibrators, and reagents, test results may vary based on the laboratory, which makes comparison of test results from different laboratories difficult. In multicenter trials carried out to establish diagnostic or therapeutic guidelines or to aid in drug development, variance of clinical laboratory test results caused by the use of different measurement methods should be considered in evaluating results for optimal guidelines and efficient drug development. If these variances are not considered, the accuracy of the analysis may suffer and result in negative clinical, technical, financial, and regulatory consequences [2].

In its 2015 survey, the College of American Pathologists (CAP), one of the largest external quality assessment organizations in the world, showed that the inter-assay coefficient of variation (CV) of total cholesterol was 3.3% across all methods, 3.9% for creatinine, and 3.2% for hemoglobin A1c [3]. Those CVs were much lower than those of other analytes.

As international guidelines were established in hyperlipidemia and diabetes, standardization in measuring glucose and lipids were developed by using reference materials and methods [1]. Standardization and harmonization are processes used to equalize results derived using different methods. Standardization can be accomplished by relating the result to a reference through a documented, unbroken chain of calibration. When such a reference is not available, harmonization is used to equivalize results utilizing a consensus approach, such as application of an agreed-upon method mean [2]. However, accurate standardization rely on securing traceability from reagent manufacturers. Each laboratory in a trial must check the traceability of reagents in clinical laboratory tests before the trial begins. Although traceability may be confirmed, commutability of reference materials should also be considered. Without such standardization, harmonization, and traceability, accurate interpretation of trial results may be difficult. This study was conducted to evaluate the CV of laboratory results produced by various measuring methods and to determine whether mathematical data adjustment could achieve harmonization between the methods.

METHODS

Materials and methods
This study is part of the Cooperative Network Construction of a Nationwide Clinical Trial study [4] to evaluate the characteristics of and treatment strategies in patients with hypertension in 37 Korean centers. Of these 37 centers, the laboratories of nine centers were investigated in this study. This study was approved by the Institutional Review Board of Cheil General Hospital & Women’s Healthcare Center (approval number: CGH-IRB-2013-33) and its associated centers, and written informed consent was obtained from all patients.

Basic data gathering
Nine laboratories (labeled A through I) as well as Green Cross Laboratories (GC Labs), the reference laboratory, were included in this study (Fig. 1). Instruments, analytic methods, reagents, lot information of reagents, and traceability of calibrators were surveyed for the six test items, serum total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, creatinine, and glucose, in all 10 laboratories. The traceability chain is shown in Fig. 2.

Manufacturing serum panel
A serum panel with 20 concentrations for each analyte was created for method comparison. Remaining patient samples at GC Labs were used to make the serum pan-
In this study, the pooled serum samples were centrifuged at 3,000 rpm at 2°C to 8°C for 10 minutes, the supernatant was separated; and the supernatant was mixed for 10 hours under refrigerated conditions. Following filtration with a 0.22 μM filter (MF membrane, Sigma-Aldrich Co. LLC, St. Louis, MO, USA), 300 μL per tube were dispensed and kept in a deep freezer at −70°C before transportation to each participating laboratory under freezing conditions.

Measurement method

Each laboratory was asked to store samples in a deep freezer the day before measurement. All samples transported to each laboratory were analyzed in 14 days after being frozen. The analysis was done at the same time in both GC Labs and each laboratory to exclude bias due to pre-analytical conditions. Samples were thawed in a refrigerator for 1 hour and then were mixed for 30 minutes on a roller mixer. Each sample was analyzed in duplicate. The second measurement was done in reverse order compared to the first one. All measurements were done within 2 hours. Test results were reported in integers for serum total cholesterol, HDL-C, LDL-C, triglycerides, and glucose. Creatinine was reported to two decimal places.

Analytic method and traceability

Instruments used to measure the analytes in this study were as follows: modular analytics (Roche Diagnostics, Manheim, Germany) for GC Labs; Cobas 8000 c702 (Roche Diagnostics) for laboratories A, E, and G; TBA-2000FR (Toshiba Medical System Corporation, Tochigi, Japan) for laboratories B, D, H, and I; ADVIA1800 (Siemens Healthcare Diagnostics, Marburg, Germany) for laboratory C; and TBA 200FR NEO (Toshiba Medical System Corporation) for laboratory F (Supplementary Table 1). Reagent and calibrator information for each analyte are described in Supplementary Table 2. Ranges of CVs for the six analytes at all concentrations are shown in Supplementary Table 3. The number of laboratories using the same calibrator and assigned values as the reference laboratory, GC Labs, was two for total cholesterol, one for LDL-C, two for triglycerides, two for glucose, and none for HDL-C and creatinine.

As seen in Table 1, the analytic method for total cho-
Cholesterol in all 10 laboratories was uniformly enzymatic. All methods were traceable with isotope dilution mass spectrometry or the Abell-Kendall method. For HDL-C and LDL-C measurements, direct methods were used (Table 1). Direct methods do not include pre-analytical processes of ultracentrifugation, precipitation, and calculation steps, which make direct methods suitable for auto-analyzers. Every laboratory used the direct method using cationic detergent, and all laboratories used reagents traceable to the Centers for Disease Control and Prevention reference method (Table 1) [5-7]. All laboratories used enzymatic methods for triglycerides. Five of the 10 laboratories used glycerol blank methods. For serum creatinine measurements, nine laboratories used the Jaffe method, one used an enzymatic method, and two did not use adjustment of pseudo-creatinine chromogen [8,9]. All used the hexokinase method to measure glucose. Traceability was ensured with reference materials for glucose.

**Statistics**

For statistical analysis, EP evaluator release 11 (Data Innovations LLC, South Burlington, VT, USA) and Excel 2000 (Microsoft Corp., Redmond, WA, USA) were used. CV was calculated for central and each laboratory variation from each sample. The interassay CVs for each analyte were calculated by averaging the observed CVs over all 20 samples. We aimed to find the line of best-fit using Deming regression equation, which was defined y-axis as a reference standard and x-axis as each laboratory result (y = βo + β1 × X). To evaluate the effect of harmonization using Deming regression analysis, in-

| Laboratory | Assay principle | Traceability       |
|------------|----------------|--------------------|
| **Total cholesterol** | | |
| GC Labs    | Enzymatic      | IDMS/AK            |
| A          | Enzymatic      | IDMS/AK            |
| B          | Enzymatic      | NIST SRM911        |
| C          | Enzymatic      | NCEP/CDC           |
| D          | Enzymatic      | ReCCS JCCRM211     |
| E          | Enzymatic      | IDMS/AK            |
| F          | Enzymatic      | ReCCS JCCRM211     |
| G          | Enzymatic      | IDMS/AK            |
| H          | Enzymatic      | NIST SRM911        |
| I          | Enzymatic      |                    |
| **HDL-C**  | | |
| GC Labs    | Direct method  | CDC reference method |
| A          | Direct method  | CDC reference method |
| B          | Direct method  | ReCCS JCCRM224     |
| C          | Direct method  | NCEP designated comparison method |
| D          | Direct method  | ReCCS JCCRM224     |
| E          | Direct method  | CDC reference method |
| F          | Direct method  | ReCCS JCCRM224     |
| G          | Direct method  | CDC reference method |
| H          | Direct method  | ReCCS JCCRM224     |
| I          | Direct method  | ReCCS JCCRM224     |

GC Labs, Green Cross Laboratories; IDMS, isotope dilution mass spectrometry; AK, Abell-Kendall; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material; NCEP, National Cholesterol Education Program; CDC, Centers for Disease Control and Prevention; ReCCS, Reference Material Institute for Clinical Chemistry Standards; JCCRM, Japanese Serum Primary Reference Materials; HDL-C, high density lipoprotein cholesterol.
Table 2. Comparison data of the six analytes

| Participant | Total cholesterol | HDL-C | LDL-C | Triglycerides | Creatinine | Glucose |
|-------------|------------------|-------|-------|---------------|------------|---------|
|             | Correlation       | Bias, % | CV, % | Adjusted      | Bias, % | CV, % | Adjusted | Correlation       | Bias, % | CV, % | Adjusted | Correlation       | Bias, % | CV, % | Adjusted |
| A           | 0.9979            | 0.3    | 0.8   | 0.8           | 0.9977    | 2.7   | 0.9970  | 7.7   | 5.2   | 1.9       | 0.9977    | 2.4   | 1.7   | 0.8       |
| B           | 0.9976            | -0.4   | 2.5   | 0.8           | 0.9996    | -9.7  | 0.9980  | -5.6  | 4.1   | 1.5       | 0.9995    | -10.2 | 7.7   | 1.2       |
| C           | 0.9973            | -3.8   | 2.8   | 0.9           | 0.9911    | 0.2   | 0.9864  | 10.4  | 6.7   | 5.3       | 0.9994    | 4.6   | 3.2   | 0.8       |
| D           | 0.9992            | -0.7   | 0.8   | 0.6           | 0.9988    | 3.8   | 0.9996  | 3.9   | 2.7   | 0.9       | 0.9977    | -12.1 | 9.3   | 1.9       |
| E           | 0.9990            | 0.7    | 0.7   | 0.6           | 0.9955    | 9.4   | 0.9982  | 6.6   | 4.4   | 1.9       | 0.9996    | 1.2   | 1.0   | 0.9       |
| F           | 0.9966            | 2.5    | 2.1   | 1.2           | 0.9966    | 4.1   | 0.9955  | 5.5   | 3.8   | 0.9       | 0.9975    | -3.9  | 3.9   | 0.9       |
| G           | 0.9995            | 0.5    | 0.5   | 0.4           | 0.9992    | 3.1   | 0.9978  | 9.4   | 6.3   | 2.0       | 0.9995    | 0.4   | 1.0   | 1.1       |
| H           | 0.9996            | 5.0    | 3.5   | 0.4           | 0.9983    | 0.7   | 0.9991  | 2.4   | 1.8   | 1.1       | 0.9978    | -6.6  | 5.4   | 1.7       |
| I           | 0.9994            | 2.9    | 2.0   | 2.0           | 0.9984    | 2.6   | 0.9991  | 4.8   | 3.3   | 1.0       | 0.9975    | -9.3  | 7.1   | 2.0       |
| Mean        | -                 | -1.7   | 0.7   | -             | -         | -4.3  | 1.8   | -4.5  | 1.4   | -         | -         | -4.5  | 1.6   | -         |

HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; CV, coefficient of variation.

**RESULTS**

Different methods demonstrated differences in results, especially for triglycerides. Deming regression was performed after adjustment, where the correlation coefficients for triglycerides were 0.9977, 0.9980, and 0.9995, respectively. After adjustment, the results from each laboratory were compared with those of GC Labs. The correlation coefficient was over 0.9 for all six analytes tested (Table 2).

**Correlation according to analytic methods**

The mean CVs for total cholesterol, HDL-C, LDL-C, and glucose analyzed by the same method were compared with those of GC Labs. The correlation coefficient was over 0.9 for all six analytes tested (Table 2).

**Comparison among methods**

To evaluate the correlation among methods at each location, the results from each of the nine laboratories were compared with those of GC Labs. The correlation coefficient was over 0.9 for all six analytes tested (Table 2).
| Participant | Adjustment | No. & CV, % | Cholesterol | HDL-C | LDL-C | Triglycerides | Creatinine | Glucose |
|-------------|------------|------------|--------------|-------|-------|---------------|------------|---------|
|             |            |            | total, ≤ 3.0% | ≤ 4.0% | ≤ 4.0% | ≤ 5.0% | ≤ 3.2% | ≤ 2.8% |
| A           | Before     | Number³    | 20           | 20    | 5     | 20            | 14         | 19      |
|             |            | Mean CV, % | 0.8          | 1.9   | 5.2   | 1.7           | 3.5        | 1.2     |
|             | After      | Number³    | 20           | 20    | 18    | 20            | 18         | 20      |
|             |            | Mean CV, % | 0.8          | 0.6   | 1.9   | 0.8           | 1.9        | 0.9     |
| B           | Before     | Number³    | 13           | 2     | 10    | 6             | 13         | 20      |
|             |            | Mean CV, % | 2.5          | 7.5   | 4.1   | 7.7           | 3.0        | 1.1     |
|             | After      | Number³    | 20           | 20    | 19    | 20            | 19         | 20      |
|             |            | Mean CV, % | 0.8          | 1.0   | 1.5   | 1.2           | 1.1        | 0.6     |
| C           | Before     | Number³    | 10           | 9     | 12    | 19            | 13         | 6       |
|             |            | Mean CV, % | 2.8          | 4.8   | 6.7   | 3.2           | 3.2        | 3.4     |
|             | After      | Numbera    | 19           | 14    | 8     | 20            | 18         | 20      |
|             |            | Mean CV, % | 0.9          | 3.1   | 5.3   | 0.8           | 1.4        | 1.1     |
| D           | Before     | Numbera    | 20           | 17    | 17    | 2             | 0          | 20      |
|             |            | Mean CV, % | 0.8          | 2.9   | 2.7   | 9.3           | 8.2        | 0.7     |
|             | After      | Numbera    | 20           | 19    | 20    | 18            | 20         | 20      |
|             |            | Mean CV, % | 0.6          | 1.4   | 0.7   | 1.9           | 1.0        | 0.6     |
| E           | Before     | Numbera    | 20           | 0     | 11    | 20            | 18         | 19      |
|             |            | Mean CV, % | 0.7          | 6.3   | 4.4   | 1             | 1.4        | 1.4     |
|             | After      | Numbera    | 20           | 20    | 18    | 20            | 18         | 20      |
|             |            | Mean CV, % | 0.6          | 0.8   | 1.9   | 0.9           | 1.4        | 0.9     |
| F           | Before     | Number³    | 16           | 16    | 13    | 15            | 6          | 9       |
|             |            | Mean CV, % | 2.1          | 2.9   | 3.8   | 3.9           | 4.9        | 3.4     |
|             | After      | Number³    | 18           | 19    | 20    | 17            | 18         | 19      |
|             |            | Mean CV, % | 1.2          | 1.5   | 0.9   | 1.9           | 1.3        | 1.2     |
| G           | Before     | Numbera    | 20           | 18    | 4     | 20            | 14         | 20      |
|             |            | Mean CV, % | 0.5          | 2.8   | 6.3   | 1             | 1.8        | 1.0     |
|             | After      | Number³    | 20           | 20    | 16    | 20            | 14         | 20      |
|             |            | Mean CV, % | 0.4          | 1.0   | 2.0   | 1.1           | 1.9        | 0.7     |
| H           | Before     | Number³    | 4            | 18    | 18    | 11            | 6          | 16      |
|             |            | Mean CV, % | 3.5          | 1.9   | 1.8   | 5.4           | 9.9        | 1.8     |
|             | After      | Number³    | 20           | 19    | 19    | 19            | 18         | 20      |
|             |            | Mean CV, % | 0.4          | 1.7   | 1.1   | 1.7           | 1.6        | 0.8     |
| I           | Before     | Number³    | 18           | 18    | 15    | 8             | 10         | 18      |
|             |            | Mean CV, % | 2.0          | 2.2   | 3.3   | 7.1           | 4.3        | 1.3     |
|             | After      | Number³    | 20           | 19    | 20    | 18            | 13         | 20      |
|             |            | Mean CV, % | 0.5          | 1.3   | 1.0   | 2.0           | 2.7        | 0.8     |
| Total (A–I) | Before  | Number³ | 141 (78) | 118 (66) | 105 (58) | 121 (67) | 94 (52) | 147 (82) |
|             | After      | No. (%)d   | 177 (98)    | 170 (94) | 158 (88) | 172 (96) | 156 (87) | 179 (99) |

CV, coefficient of variation; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

³Number of samples within criteria among the 20 samples of each analyte.

²National Cholesterol Education Program test performance guideline [10].

³Desirable specifications for total error, imprecision, and bias, derived from intra- and inter-individual biologic variation [20], recently updated in the database located on the Westgard homepage (https://www.westgard.com/biodatabase1.htm).

⁴Number of samples within criteria among total 180 samples.
Comparison between enzymatic methods and the Jaffe method yielded a large percent difference, 9.60%.

Comparison of disease prevalence before and after lab harmonization

According to our proposed lab harmonization method, prevalence of diseases in 593 lab data from nine centers was compared before and after adjustment. The prevalence of dyslipidemia increased to 46.2% from 39.63% ($p = 0.0012$) and the prevalence of chronic kidney disease (defined as estimated glomerular filtration rate $< 60 \text{ mL/min/1.73 m}^2$) dropped to 8.26% from 20.57%.

**DISCUSSION**

This study showed that result variation caused by different analytical methods can be reduced by harmonization. Harmonization may become a prerequisite in multicenter trials. The compatibility of data generated by multiple laboratories is not guaranteed due to different methods, reagents, calibrators, etc., used; and management of data from multiple sites is difficult and requires more effort for statistical analysis than what is needed in single-center studies [10].

When clinical laboratory tests may be performed by the laboratory of each participating site, more discussion and consideration in the planning stage of research should be given to whether a single, central clinical laboratory should be used instead of the laboratories at each center. If multiple laboratories will be analyzing samples, researchers should explore how to adjust or compare data prior to initiating the study.

This study was carried out to establish a method of postanalytical harmonization using data from various laboratories. The clinically acceptable total error, including precision and bias, is reported in international guidelines, the literature, and reports from external quality assessment organizations [11-13]. For example, in the CAP survey (external quality assessment), inter-assay CV among all methods for total cholesterol is 3.0% to 3.4%, 3.9% to 15.5% for creatinine, and 3.2% to 9.2% for glucose, which are rather good [3]. Accuracy of the analytic method used in clinical laboratories is established by a standardization process using the results from patient samples, the hierarchy structure, and traceability of the analytical measurement system (Fig. 3).

**Table 4. CV before and after adjustment by deming regression**

| Test item       | Calibrator and assigned value | Same group | Different group |
|-----------------|-------------------------------|------------|----------------|
|                 |                               | Mean CV, % | Mean CV after deming regression, % | Mean CV, % | Mean CV after deming regression, % |
| Total cholesterol| 0.8                           | 0.7        | 2.0            | 0.7        |
| LDL-C           | 5.2                           | 1.9        | 4.1            | 1.8        |
| Triglycerides   | 1.3                           | 0.9        | 5.4            | 1.5        |
| Glucose         | 1.3                           | 0.9        | 1.8            | 0.8        |

CV, coefficient of variation; LDL-C, low density lipoprotein cholesterol.

**Figure 3.** The comparison of inter-assay coefficient of variation (CV) before and after adjustment (CVadj). With the data adjusted based on Deming regression analyses between the central laboratory and each laboratory, mean CVs of all analytes decreased. HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.
1) [14]. This association ensure traceability of analytical methods. Traceability applies a primary reference material with proper accuracy and precision, by cascades of reference material, to the calibrator by the manufacturer, and finally to the patient samples (Fig. 2). The interassay CV of test results from different laboratories is small for an analytical method with traceability that uses the same calibrator adjusted with the same primary reference material. This is standardization. All reagents used in this study have traceability. Thus, when compared with external quality assessment scheme results, all analytes had better or similar results except triglycerides (Supplementary Table 3).

Our study results are also concerning. Although interassay CVs were within acceptable limits according to external quality assessment standards and comparison studies revealed good correlation coefficients, the results of some of the participating laboratories should be evaluated for adjustment against international guidelines like those from the National Cholesterol Education Program. International guidelines propose strict criteria, and not all our results could meet those requirements. In studies where the analysis of the crude data could influence the study’s conclusion, failure to adjust the results could lead to challenges to the integrity of the study. Traceability is particularly important in multicenter trials where the different laboratories utilize various testing methods. However, even where the methods are traceable, differences between the various methods should not be ignored because commutability of reference material, application of the calibrated assigned value, and method compensation, such as the creatinine determination method, could be problematic [2]. Thus, traceability of methods coupled with studies comparing the results from different laboratories during the planning stages of multicenter trials would guide adjustments of results so that analysis of results from multiple sites would be accurate. As inter-assay CVs of the laboratories using the same calibrator and assigned value showed lower variance than the CVs of those that did not, traceability should be checked first when selecting analytical methods in multicenter trials.

Different methods impact CV differently. As the analytic method for triglycerides is affected by the use of a glycerol blank, the method use by each laboratory should be surveyed and considered in the interpretation of test results. Measuring triglyceride concentration without a glycerol blank has a high positive correlation with free glycerol concentration, which was also shown by regression analysis [15], so that methods with or without glycerol blank could be compared by regression analysis. In the current study, when results from the nine participating laboratories were adjusted based on the method used in the central laboratory, interassay CVs were then greatly improved. Compared with the 2015 CAP survey, interassay CVs of the participating laboratories were high. The reason may be that, in our group, 50% used a method with a glycerol blank, whereas, in the CAP group, only 10% used a method with a glycerol blank [5]. Data adjustment would greatly improve accuracy when analytic methods of participating laboratories demonstrate systemic bias.

In creatinine analysis, the Jaffe method and enzymatic method can be used. The differences between the analytic methods depend on avoiding interference of pseudo-creatinine chromogen, such as proteins, antibiotics, and ketones. Different measurements using the Jaffe method may yield significant analytic errors depending on whether compensation was made for those chromogens [8] because creatinine is a very low-concentration analyte. For such reasons, interassay CVs before and after adjustment were higher for creatinine than for other analytes. After adjustment, the interassay CV for creatinine decreased dramatically, and the correlation coefficient was good.

To measure glucose, all laboratories used the hexokinase method. As traceability was secured with reference methods and materials, the mean CV for glucose was excellent at 1.7% (0.7% to 3.4%).

Using a single, assigned analytic method in a central laboratory would be best in multicenter trials. However, because of the logistical challenges to sample transportation and the need for timely analysis, analyzing samples in the laboratories of the participating center where the sample was taken is how many multicenter trials currently manage the analysis. In this situation, data harmonization is an option. Laboratory data harmonization is especially needed when reference methods or materials are absent or when reference material is non-commutable. As described above, calibrator values and method characteristics can influence results and, thus, analysis of data. For detecting these possible ob-
stacles to accurate analysis and deciding on harmonization, pre-study surveys of the participating laboratories should be considered. In multicenter trials, direct comparison without data adjustment is best practice provided no variance in results existed; but, in older studies, traceability was seldom addressed, complicating comparisons between data sets. This study confirms the need to compare assays and methods used in obtaining older data and to adjust data if needed. In the future, if a central laboratory could compare its results with those of a reference laboratory, such as Cholesterol Reference Method Laboratory Network, and calculate bias, the central and participating laboratories could coordinate using a hierarchical structure permitting the central laboratory to communicate the reference material obtained to the participating laboratories and creating consistency in measurement of analytes across the participating laboratories.

The major limitation of our study is no external validation of this model in clinic. Although the prevalence of diseases such as dyslipidemia and chronic kidney disease changed significantly after application of our proposed lab harmonization method, it is still strongly required to test whether this regression reduces the interlaboratory gap. Further studies are needed.

**Perspective**
This study will encourage researchers to focus on traceability and improve the quality of study results through harmonization. For analytes with available reference materials and methods, standardization would be the best approach; and harmonization should be applied to the analytes for which reference materials and reference methods are not available or cannot be developed. Additional studies on harmonization of reference ranges may broaden the scope of the interpretation of clinical laboratory results [16].

**KEY MESSAGE**
1. By comparing the mean coefficients of variation of laboratory results between participating laboratories and central laboratory, more comparable results were produced.
2. Laboratory data harmonization can be a worthy alternative to compare all participating laboratories and so strongly recommended for accurate data analysis in multicenter clinical trials.

**Conflict of interest**
No potential conflict of interest relevant to this article was reported.

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**REFERENCES**
1. Miller WG, Tate JR, Barth JH, Jones GR. Harmonization: the sample, the measurement, and the report. Ann Lab Med 2014;34:187-197.
2. Greg Miller W, Myers GL, Lou Gantzer M, et al. Roadmap for harmonization of clinical laboratory measurement procedures. Clin Chem 2011;57:1108-1117.
3. Horowitz GL, Alter DN, Baskin LB, et al. Hemoglobin A1c (5 Challenge) Participant Summary. Northfield (IL): College of American Pathologists, 2015.
4. Kim SA, Kim JY, Park JB. Significant interarm blood pressure difference predicts cardiovascular risk in hypertensive patients: CoCoNet study. Medicine (Baltimore) 2016;95:e3888.
5. Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz ID Jr. Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clin Chem 1992;38:150-160.
6. Sugiuichi H, Uji Y, Okabe H, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem 1995;41:717-723.
7. Matsuzaki Y, Kawaguchi E, Morita Y, et al. Evaluation of two kinds of reagents for direct determination of HDL-cholesterol. Seibutsu Shiryo Bunseki 1996;19:419-427.
8. Chung HJ, Chun S, Min WK. Creatinine determination with minimized interference. J Lab Med Qual Assur 2008;30:229-231.
eire N, Engel W. Arithmetic compensation for pseudo-creatinine Jaffé method and its effect on creatinine clearance results. Clin Chem 2001;47:A148-A149.

10. Chahal AP. A knowledge-based process for offshoring clinical trials. J Clin Res Best Pract 2006;2. http://www.firstclinical.com/journal/2006/0601_Offshoring.pdf.

11. Sacks DB, Arnold M, Bakris GL, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34:e61-e99.

12. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-3421.

13. Ricos C, Alvarez V, Cava F, et al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491-500.

14. Myers GL, Kimberly MM, Waymack PP, Smith SJ, Cooper GR, Sampson EJ. A reference method laboratory network for cholesterol: a model for standardization and improvement of clinical laboratory measurements. Clin Chem 2000;46:1762-1772.

15. Choi JS, Shon BR, Kim YK. A clinical study on the need for glycerol-blanking in triglycerides measurement. Korean J Clin Pathol 1995;15:204-211.

16. Ichihara K. Statistical considerations for harmonization of the global multicenter study on reference values. Clin Chim Acta 2014;432:108-118.
## Supplementary Table 1. Instruments in each participating laboratory

| Laboratory | Instrument       | Manufacturer                                           |
|------------|------------------|--------------------------------------------------------|
| GC Labs    | Modular analytics | Roche Diagnostics, Manheim, Germany                    |
| A          | Cobas 8000, c702  | Roche Diagnostics, Manheim, Germany                    |
| B          | TBA-200FR        | Toshiba Medical Systems Corporation, Tochigi, Japan    |
| C          | ADVIA1800        | Siemens Healthcare Diagnostics, Marburg, Germany       |
| D          | TBA-2000FR       | Toshiba Medical Systems Corporation, Tochigi, Japan    |
| E          | Cobas 8000, c702  | Roche Diagnostics, Manheim, Germany                    |
| F          | TBA 200FR NEO    | Toshiba Medical Systems Corporation, Tochigi, Japan    |
| G          | Cobas 8000, c702  | Roche Diagnostics, Manheim, Germany                    |
| H          | TBA-2000FR       | Toshiba Medical Systems Corporation, Tochigi, Japan    |
| I          | TBA-2000FR       | Toshiba Medical Systems Corporation, Tochigi, Japan    |

GC Labs, Green Cross Laboratories.
## Supplementary Table 2. Reagents and calibrator information for the 10 laboratories

| Participant laboratory | Reagent | Manufacturer | Calibrator | Calibratorlot no. | Calibrator assigned value, mg/dL |
|------------------------|---------|--------------|------------|-------------------|----------------------------------|
| **Total cholesterol**  |         |              |            |                   |                                  |
| GC Labs                | CHOL    | Roche⁴       | C.f.a.s    | 175190            | 158                              |
| A                      | CHOL₂   | Roche        | C.f.a.s    | 175190            | 158                              |
| B                      | Determiner-C-TC | Kyowa⁵   | HDL.LDL-C  | 235ADG            | 202                              |
| C                      | Total cholesterol | Siemens⁶ | Chemistry CAL | 459400 | 183                             |
| D                      | Determiner-C-TC | Kyowa    | Liquid cal. | 153081            | 221                              |
| E                      | CHOL₂   | Roche        | C.f.a.s    | 175190            | 158                              |
| F                      | Determiner-C-TC | Kyowa    | Liquid cal. | 164651            | 221                              |
| G                      | CHOL₂   | Roche        | C.f.a.s    | 176123            | 160                              |
| H                      | Cholesterol N | Denka Seiken⁷ | C.f.a.s   | 177953            | 175                              |
| I                      | Determiner-C-TC | Kyowa    | HDL.LDL-C  | 235ADG            | 202                              |
| **HDL-C**              |         |              |            |                   |                                  |
| GC Labs                | HDLC3   | Roche        | C.f.a.s Lipids | 172156 | 76.5                             |
| A                      | HDLC3   | Roche        | C.f.a.s Lipids | 174327 | 75                               |
| B                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 74.9                             |
| **LDL-C**              |         |              |            |                   |                                  |
| GC Labs                | LDL-C   | Roche        | C.f.a.s Lipids | 172156 | 146                             |
| A                      | LDL-C   | Roche        | C.f.a.s Lipids | 174327 | 146                             |
| B                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 110                             |
| C                      | LDL-C   | Siemens      | HDL/LDL    | 308123            | 128                             |
| D                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 110                             |
| E                      | LDL-C   | Roche        | C.f.a.s Lipids | 178923 | 166                             |
| F                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 110                             |
| G                      | LDL-C   | Roche        | C.f.a.s Lipids | 175839 | 159                             |
| H                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 110                             |
| I                      | Determiner-L | Kyowa     | HDL.LDL-C  | 235ADG            | 110                             |
### Supplementary Table 2. Continued

| Participant laboratory | Reagent | Manufacturer | Calibrator | Calibrator lot no. | Calibrator assigned value, mg/dL |
|------------------------|---------|--------------|------------|-------------------|----------------------------------|
| **Triglycerides**      |         |              |            |                   |                                  |
| GC Labs                | TG      | Roche        | C.f.a.s    | 175190            | 127                              |
| A                      | TRIGL   | Roche        | C.f.a.s    | 175190            | 127                              |
| B                      | Determiner-C-TG | Kyowa | Liquid Calibrator | 235ADG    | 77                                |
| C                      | TG2     | Siemens      | Chemistry CAL | 459400   | 186                              |
| D                      | Determiner-C-TG | Kyowa | Liquid Calibrator | 133081    | 151                              |
| E                      | TRIGL   | Roche        | C.f.a.s    | 175190            | 127                              |
| F                      | Determiner-C-TG | Kyowa | Liquid Calibrator | 164051    | 153                              |
| G                      | TRIGL   | Roche        | C.f.a.s    | 176123            | 134                              |
| H                      | Determiner-C-TG | Kyowa | Liquid Calibrator | 17795301 | 146                              |
| I                      | Determiner-C-TG | Kyowa | Liquid Calibrator | 235ADG    | 77                                |
| **Creatinine**         |         |              |            |                   |                                  |
| GC Labs                | CREA    | Roche        | C.f.a.s    | 175190            | 4.29                             |
| A                      | CREJ2   | Roche        | C.f.a.s    | 175190            | 4.2                              |
| B                      | CREA    | Roche        | Liquid Calibrator | 133081   | 4.3                              |
| C                      | Creatinine | Siemens | Chemistry CAL | 459400   | 9.12                             |
| D                      | Creatinine FS | DiaSys | Trucal U     | 19846    | 3.65                             |
| E                      | CREJ2   | Roche        | C.f.a.s    | 175190            | 4.21                             |
| F                      | CREA    | Roche        | Liquid calibrator | 164051   | 4.15                             |
| G                      | CREJ2   | Roche        | C.f.a.s    | 176123            | 4.06                             |
| H                      | Creatinine FS | DiaSys | C.f.a.s | 177953    | 3.9                              |
| I                      | L-CRE-L | Shinyang     | C.f.a.s    | 175190            | 4.04                             |
| **Glucose**            |         |              |            |                   |                                  |
| GC Labs                | GLU     | Roche        | C.f.a.s    | 175190            | 200                              |
| A                      | GLUC3   | Roche        | C.f.a.s    | 175190            | 200                              |
| B                      | Glucose | Denka Seiken | Liquid Calibrator | 133081   | 203                              |
| C                      | Glucose | Siemens    | Chemistry CAL | 459400  | 244                              |
| D                      | Glucose | Denka Seiken | Liquid Calibrator | 133081  | 203                              |
| E                      | GLUC3   | Roche        | C.f.a.s    | 175190            | 200                              |
| F                      | Glucose | Denka Seiken | Liquid Calibrator | 164051  | 188                              |
| G                      | GLUC3   | Roche        | C.f.a.s    | 176123            | 195                              |
| H                      | L-type glucose 2 | Wako | C.f.a.s | 17795301 | 205                              |
| I                      | Glucose Hexokinase FS | DiaSys | C.f.a.s | 175 190-01 | 196                              |

GC Labs, Green Cross Laboratories; C.f.a.s, calibrator for automated systems; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; CAL, calibrator; TG, triglyceride; TRIGL, triglyceride; CREA, creatinine; CREJ2, creatinine Jaffe generation 2; FS, fluid stable; GLU, glucose; GLUC3, glucose hexokinase generation 3.

aRoche Diagnostics, Manheim, Germany.
bKyowa, Tokyo, Japan.
cSiemens Healthcare Diagnostics, Marburg, Germany.
dDenka Seiken, Tokyo, Japan.
eDiaSys, Waterbury, CT, USA.
fShinyang, Seoul, Korea.
gWako, Richmond, VA, USA.
### Supplementary Table 3. Ranges of coefficients of variance for the six analytes at all concentrations

| Test item     | CAP 2015 results | Current investigation |
|---------------|------------------|-----------------------|
|               | CV, %            | No. of participants   | CV, %                  | No. of participants |
| Total cholesterol | 3.0–3.4          | 4,524–4,536           | 0.9–3.7                | 10                 |
| HDL-C         | 11.4–29.2        | 4,430–4,445           | 1.5–8.1                | 10                 |
| LDL-C         | 15.1–29.9        | 2,525–2,542           | 1.4–15.2               | 10                 |
| Triglycerides | 4.4–5.1          | 4,510–4,545           | 1.7–16.5               | 10                 |
| Creatinine    | 3.9–15.5         | 5,427–5,449           | 1.3–14.5               | 10                 |
| Glucose       | 3.2–9.2          | 5,425–5,448           | 0.4–4.2                | 10                 |

Values are presented as range.
CAP, College of American Pathologists; CV, coefficient of variation; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.