Commutability of a whole-blood external quality assessment material for point-of-care C-reactive protein, glucose, and hemoglobin testing

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Abbreviations: POC, point-of-care; EQA, external quality assessment; EQAS, external quality assessment scheme; CLSI, Clinical and Laboratory Standards Institute; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; Noklus, Norwegian Quality Improvement of Laboratory Examinations; CRP, C-reactive protein; Hb, hemoglobin; PI, prediction interval; ID/GC/MS, isotope dilution/gas chromatography/mass spectrometry; CRM, certified reference material; IVD, in vitro diagnostic.

Abstract

BACKGROUND: The optimal situation in external quality assessment (EQA) is to use commutable materials. No previous study has examined the commutability of a whole-blood material for point-of-care (POC) testing. The aim of this study was to determine the commutability of the Norwegian Quality Improvement of Laboratory Examinations (Noklus) organization's "in-house" whole-blood EQA material for C-reactive protein (CRP), glucose, and hemoglobin for frequently used POC instruments in Norway and to determine the possibility of using a common target value for each analyte.

METHODS: The study was performed according to the Clinical and Laboratory Standards Institute guidelines. The EQA material was pooled stabilized EDTA venous whole-blood containing different concentrations of the analytes. The EQA material and native routine patient samples were analyzed using 17 POC and 3 hospital instruments. The commutability was assessed using Deming regression analysis with 95% prediction intervals for each instrument comparison.

RESULTS: The EQA material was commutable for all CRP and hemoglobin POC instruments, whereas for glucose the material was commutable for all POC instruments at the lowest concentration analyzed [126.0 mg/dL (7.0 mmol/L)] and for 3 POC instruments at all of the concentrations analyzed.

CONCLUSIONS: Noklus EQA participants using CRP and hemoglobin POC instruments now receive results that are compared with a reference target value, whereas the results for participants using glucose POC instruments are still compared with method-specific target values. Systematic deviations from a reference target value for the commutable glucose POC instruments can be calculated, and this additional information can now be offered to these participants and to the manufacturers.

Introduction

The quality of point-of-care (POC) testing can be monitored regularly by participating in an external quality assessment (EQA) scheme (EQAS). The extent to which an EQA provider is able to assess the performance of participants and instruments depends on several factors, including the quality of the EQA material used. The optimal situation is to use EQA material that is commutable, meaning that the EQA samples have the same numeric relationship between 2 measurement methods as a panel of representative patient samples (1). When a commutable EQA material is circulated to the participants, the probability of detecting true analytical errors increases, as well as the possibility of assessing the between-measurement methods variation. If a reference method or reference material is used to assign the target value, the participant results can be compared with a true value (2). The increasing interest in the harmonization and standardization of instruments has also led to an increased interest in using EQASs to assess the agreement of results between different measurement methods (1).

Using a commutable EQA material is important for identifying analytes that need to be standardized and harmonized, and for monitoring the success of the standardization and harmonization efforts (3, 4). To assess the commutability of the whole-blood EQA material used in the present study, we used the method delineated in the Clinical and Laboratory Standards Institute (CLSI) Guideline EP14-A3 (1).

The Norwegian Quality Improvement of Laboratory Examinations (Noklus) organization provides EQA programs for most analytes used in primary health care (5). The approximately 3100 participants who are voluntarily enrolled in the programs come mainly from general-practitioners clinics and nursing homes (6).

C-reactive protein (CRP), glucose, and hemoglobin (Hb) are among the most commonly performed laboratory tests in primary health care in Norway. All Noklus participants analyzing CRP, glucose, and Hb receive EQA material manufactured "in-house" by Noklus that consists of fresh stabilized venous whole-blood. The EQAS is performed biannually, and in each survey the participants receive 2 samples with different concentrations of the 3 analytes.

To our knowledge, the commutability of whole-blood EQA material for POC instruments has not been demonstrated previously. The aim of the present study was to determine the commutability of the Noklus whole-blood EQA material for CRP, glucose, and Hb for the most frequently used POC instruments in primary healthcare in Norway, and to investigate the possibility of using a common EQA target value for each analyte.

Material and Methods

INSTRUMENTS

This study included 5 CRP, 7 glucose, and 5 Hb POC instruments (Table 1) that are the most commonly used POC instruments for these analytes in Norway. One accredited hospital instrument for CRP and glucose (Cobas 6000, Roche Diagnostics) and another accredited for Hb (Advia 2120, Siemens Healthcare)— both of which are available at the laboratory at Haraldsplass Deaconess Hospital (Bergen, Norway)— were included as the comparison instruments in the present study (1). The hospital instruments have been assessed through EQASs and have documented good analytical quality. The ERM/DA474/IFCC certified reference material (CRP in processed human serum) and the standard reference material SRM 965b (glucose in frozen human serum) were used to validate the trueness of the Cobas 6000 instrument in the present study (7, 8).

WHOLE-BLOOD EQA MATERIAL

The commutability studies were performed in association with the Noklus EQASs in 2014 (CRP and glucose) and 2015 (Hb). The EQA material was manufactured by Noklus to cover the clinically relevant concentration ranges: 2 concentrations of Hb and 3 concentrations of CRP and glucose.

To produce the EQA material, AB0-compatible EDTA venous whole-blood from healthy blood donors was collected from the blood bank at Haukeland University Hospital (Bergen, Norway). At Noklus (Haraldsplass Deaconess Hospital) the whole-blood was pooled, and plasma or erythrocytes were added to achieve Hb concentrations of 9.6 and 13.2 g/dL; human CRP (in.vent Diagnostica) was added to achieve CRP concentrations of 23, 58, and 73 mg/L; and D(+)-glucose monohydrate dissolved in sodium chloride (VWR) was added to achieve glucose concentrations of 126.0, 234.0, and 306.0 mg/dL (7.0, 13.0, and 17.0 mmol/L). Finally, iodoacetic acid sodium salt (VWR) and chloramphenicol succinate (VWR) were added to stabilize the glucose concentration and prevent bacterial growth. The whole-blood material was stored overnight in a refrigerator before distributed into 2-mL cryovials (Sarstedt) and stored at room temperature until the next day. The stability and homogeneity were tested according to ISO 13528 (9).

An overview of the procedures of the commutability studies is shown in Fig. 1.

PATIENT SAMPLES

At the laboratory of Haraldsplass Deaconess Hospital, 22 CRP and 25 Hb routine patient samples were collected during a single day. All CRP and Hb patient samples were “leftover” samples form the laboratory where CRP and Hb had been requested. The patient samples

were selected to cover the concentration range of the EQA material, with a range of 10 to 119 mg/L for CRP and 7.9 to 16.1 g/dl for Hb.

For glucose, fresh capillary whole-blood samples were collected from 23 healthy volunteers (with and without diabetes mellitus) over a 6-day period. All participating persons gave informed consent to donate blood for the glucose measurements. A biomedical laboratory scientist collected capillary blood from a finger prick using ACCU-Chek Safe-T-Pro Plus (Roche Diagnostics), after wiping off the first blood drop. One finger prick was used when possible, but in some cases 2 and sometimes 3 finger pricks were needed to collect the required amount of capillary blood. For all the 23 patients, the sequence to collect capillary blood was as follows: First, 1 sample was collected for later analysis on the hospital instrument; the next samples were collected for analysis on the 7 POC instruments; and finally, a last sample was collected for later analysis on the hospital instrument. The time spent on each sequence varied between 5 and 14 min. The samples for use in the hospital instrument were collected in Microvette Lithium Heparin tubes (300 μL) (Sarstedt) and centrifuged immediately for 3 min at 10 000*g* (Minispin AG 5452 Model) to separate the plasma and to prevent glycolysis. The plasma was then frozen within the next 20 min at –80°C and stored until analysis on the hospital instrument. The stability of the glucose concentration during sampling was examined by calculating the mean values for the duplicate capillary samples obtained at the start and end of each measurement sequence. The concentration was considered stable if the difference was <10% (10). All the patient samples had a difference <5% except 3 samples which had a difference of 5% to 8%. No significant difference was found between the first and the last glucose measurements (Student *t*-test, $P>0.05$). The glucose concentration in the whole-blood patient samples ranged from 72 to 432 mg/dL (4–24 mmol/L).

MEASUREMENTS

The CRP and Hb patient samples and the EQA material were analyzed in triplicate on the same day using the POC and hospital instruments. For CRP, serum samples were analyzed on the hospital instrument and EDTA whole-blood was analyzed on the POC instruments. For Hb, EDTA whole-blood samples were analyzed both on the POC instruments and on the hospital instrument. The measurements were performed at Noklus (POC) and at the laboratory of Haraldsplass Deaconess Hospital (hospital instruments). The patient samples were analyzed immediately after collection and the EQA material 2 days after production, stored 1 night in the refrigerator and additionally 1 night at room temperature to mimic the delay associated with mail delivery of the EQA material (Fig. 1). The EQA material and the patient samples for CRP were centrifuged before being analyzed using the Cobas 6000 instrument.

The glucose capillary whole-blood patient samples were analyzed in triplicate immediately after collection using the 7 POC instruments over a 6-day period. The same order of POC instruments was used in each measurement sequence. The EQA material was analyzed 2 days after production, including an overnight storage at room temperature to mimic mail delivery. All patient plasma samples were analyzed in one run on the hospital instrument on the same day.

Internal quality control was performed at the beginning and end of each measurement sequence, which indicated that all of the POC instruments were stable throughout the analysis period for all analytes. In accordance with the EP14-A3 guideline (1), the fresh patient samples and the EQA material were analyzed in triplicate using all of the instruments.

STATISTICS

The statistical analyses were performed according to the EP14-A3 guideline (1). A short overview is shown in Fig. 1. The data were first checked for outliers within the triplicate measurements. One outlier detected for each of glucose and Hb was excluded from further analysis, and no outliers were detected for CRP.

When assessing the homogeneity of variance, the mean and SD of the triplicate measurements were presented in a scatter plot for each instrument, and if the plot revealed that SD increased with the analyte concentration, the results were $\log_{10}$-transformed. The results from 4 CRP instruments underwent $\log_{10}$ transformation to obtain homogeneity of the variance. All of the glucose and Hb results showed homogeneity of the variance.

The linearity between the paired instruments was assessed visually using ordinary linear regression, which revealed that all of the instrument combinations showed linearity.

Difference plots were displayed for every instrument combination, and $\log_{10}$ transformation was applied if the scattering increased with the concentration: This was done for 14 of 15 CRP, 10 of 28 glucose, and 4 of 15 Hb instrument comparisons. The differences appeared constant in a second difference plot using the $\log_{10}$-transformed values, and so the $\log_{10}$-transformed values were applied.

Outliers between the methods were detected visually in the difference plots and excluded from the subsequent analysis. One outlier was excluded from 6 of the 15 CRP instrument combinations; 1 outlier was excluded from 3 of the 15 Hb instrument combinations; and 1 outlier was excluded from 3 of the 28 instrument comparisons for glucose (i.e., 12 of 1349

data points were excluded) (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://wwwclinchem.org/content/vol65/issue6).

Deming regression analyses using the mean of triplicate patient sample measurements (log_{10}-transformed or untransformed values) were performed for each analyte and each instrument combination. A 95% prediction interval (PI) was calculated and then plotted graphically along with the mean values of the triplicate measurements of the EQA samples (Fig. 2 here and also Fig. 2 in the online Data Supplement). The EQA material was assessed as being commutable if the means of each EQA sample were within or touching the PI limits; otherwise, it was considered noncommutable.

The calculations were performed using the following packages of R software (version 3.1.2, R Development Core Team, 2007): MCR (method comparison regression, version 1.2.1), MethComp (functions for analyzing the agreement in method comparison studies, version 1.22.2), and Boot (bootstrap functions, version 1.3.13).

Results

When the CRP and Hb POC instruments were compared with the hospital instruments, the EQA material was commutable for all instrument combinations except QuikRead GO at the highest CRP concentration (Fig. 2 and Table 1).

For glucose, the EQA material was commutable between the hospital instrument and all of the POC instruments at the lowest concentration, but for the 2 higher concentrations, commutability was demonstrated for only the Contour and HemoCue instruments (Table 1).

Pairwise comparisons of the POC instruments revealed that the EQA material was commutable for nearly all instrument combinations for CRP and Hb, whereas 37 of 63 combinations showed commutability with the EQA material for glucose, also mainly at the lowest concentration (see Table 1 in the online Data Supplement).

Discussion

This study examined the commutability of the Noklus whole-blood EQA material for CRP, glucose, and Hb POC instruments. The results showed that the EQA material can be considered commutable for CRP and Hb, as well for about half of the glucose POC instruments.

Noklus aims to provide participants with EQA material that is as close to native patient samples as possible. However, for practical purposes, some constituents and stabilizers have to be added, which can jeopardize the commutability of the material (11). In the present EQA material, iodoacetic acid sodium salt in combination with EDTA and chloramphenicol succinate was used to stabilize the concentration of glucose, as iodoacetic acid has been shown to interfere less with whole-blood materials than does fluoride (12). However, it seems unlikely that any of these constituents contributed to the observed noncommutability seen between some of the instruments at some of the concentrations analyzed because the amounts of the components added were similar at all concentrations of the analytes in the EQA material. Thus, the only varying quantity was the concentration of the analytes themselves. However, because the analytes added were highly purified, it is difficult to understand that this could be the cause of the noncommutability (2).

The present study examined CRP and glucose at each of the 3 concentrations and Hb at 2 concentrations. It is interesting that in all cases, the EQA material showed commutability for all analytes at the lowest concentration. This means that if only the lowest concentration had been examined, the EQA material would have been assessed as commutable for all POC instruments. This finding highlights the importance of including several concentrations when assessing the commutability of an EQA material. This aspect is also emphasized in the recent recommendations published by the IFCC working group on commutability (13). Those recommendations allow the inclusion of several concentrations and suggests that when assessing a panel of EQA materials, the EQA material should be individually evaluated at each concentration (13). For practical reasons, it cannot be expected, in an EQAS, that each concentration in each survey is examined for commutability. Additionally, the concentrations in the different surveys will always vary somewhat. Knowledge about when an EQA material is considered commutable or noncommutable (or at least the concentration ranges in which it can be considered commutable) is important for the EQA organizers. Therefore, for practical reasons, a discussion on how to apply the results from a commutability study in every day practice is needed.

There is no agreed reference method for CRP, but the Cobas 6000 uses a method that is traceable to isotope dilution/GC-MS (ID/GC/MS), which is a method that fulfills all of the requirements for use as a higher-order reference measurement procedure. Additionally, the ERM/DA474/IFCC certified reference material (CRM) for CRP was used to validate the trueness of the Cobas 6000 (7). All CRP POC instruments showed commutability with the EQA material, except for QuikRead GO displaying borderline noncommutability at the highest concentration (Fig. 2 here and also Table 1 in the online Data Supplement). Nevertheless, because this CRP concentration is rather high and because the EQA material

shows commutability around the commonly used decision limits, Noklus has decided to calculate a common target value that is traceable to the CRM for all the CRP POC instruments.

The reference ID/GC-MS method is available for glucose, but this method is not suitable for routine measurements due to the time-consuming sample preparation required (14). The Cobas 6000 used in the present study is traceable to the ID/GC-MS method (15), and additionally the trueness of the Cobas 6000 was verified using the SRM 965b (8). The results for the commutability of the glucose EQA material turned out to vary with the concentration. Three of the 7 POC instruments in this study showed commutability for all concentrations, and in theory a true value can be obtained for these instruments. However, it is difficult to explain to the participants that instruments for the same analyte will be evaluated against different types of target values; therefore, as a routine, a peer-group target value is still used in the Noklus EQAS. Nonetheless, Noklus will calculate the systematic deviation from the true target value for the commutable POC instruments, and this information will be communicated to the in vitro diagnostic (IVD) manufacturers and participants when needed.

For Hb, a reference target value for the Noklus EQA evaluation is established by the Odense University Hospital (Odense, Denmark), which uses a cyanmethemoglobin reference method (16). Although based on a cyanide-free colorimetric method, the Advia 2120 used in the present study has been confirmed in EQASs as having good analytical quality. The Noklus EQA material showed commutability for all Hb POC instruments included in the present study; therefore, it can be evaluated using a common target value that is traceable to the reference method.

Miller et al. suggested a system of ranking into categories from 1 to 6 depending on the ability of an EQAS to evaluate the performance of participants and instruments (2). The results from the present study allow a target assignment based on the results for CRM for all of the CRP POC instruments and for 3 of the glucose POC instruments, and a target value assignment traceable to a reference measurement procedure for all Hb POC instruments. Thus, when using the Noklus EQA material, participants in the Noklus EQA program using these POC instruments can be categorized as using a category 1 scheme. Hence, the reproducibility, calibration traceability, and uniformity between participants and instruments can be evaluated (2). This is a considerable improvement, and it enhances the opportunity to guide both participants and the IVD industry, as well as contributing to achieving the goal of standardized and harmonized clinical laboratory results.

The data evaluation was performed in the present study using the Deming regression model as advised by the EP14-A3 guideline (1). When using a linear model, the regression line (and therefore the PI) is determined by all of the data points for the selected population. The statistical approach suggested by the EP14-A3 guideline has been brought into question by the recent IFCC recommendations, which argue that the model might not be optimal when assessing the commutability of an EQA material (13). Whereas the IFCC suggests calculating the difference in bias between the EQA material and the patient samples along with error bars for the uncertainty (17), the CLSI method depends on visual inspection of where the data points for the EQA material are located in relation to the patient samples and the limits of the PI. In the present study, it was challenging to evaluate whether the data points were located on, or partly on, the limits of the PI in the commutability plots (Fig. 2 here and also Fig. 2 in the online Data Supplement), as the EP14-A3 guideline does not provide advice on how to

interpret such data points. Calculating error bars might have helped the evaluations, and slightly different conclusions could have been reached.

The strengths of the present study are that it included the most commonly used POC instruments for CRP, glucose, and Hb in Norway. Because 99% of the Norwegian general practitioner clinics participate in Noklus, all CRP, glucose, and Hb laboratory instruments in primary healthcare were covered in this study. All of the patient samples were freshly collected and were not pooled before the analysis. Furthermore, all of the reagents used were from the same lot number in order to avoid any effects of between-lot variations (18, 19).

A limitation of the present study is the predominance of patient samples covering the lowest concentration of glucose. The low availability of patient samples at higher concentrations made it difficult to obtain a sufficient number of samples in this range, and it may have contributed to the noncommutability observed at the higher glucose concentrations.

In conclusion, the Noklus whole-blood EQA material was commutable for all the CRP and Hb POC instruments and for about half of the glucose POC instruments used in primary healthcare in Norway. As a consequence, the participants in Noklus now get their EQA CRP and Hb results evaluated against a common reference value. For glucose, owing to educational challenges, all participants will still get method-specific target values although true values for the commutable instruments are calculated and assessed by Noklus. The possibility to discover true systematic differences between the POC instruments can be disclosed, which enhances the EQA providers' opportunity to give feedback to the IVD manufacturers and provide better guidance to the participants on which instrument to buy, as

well as improving the efforts to monitor standardization and harmonization of clinical laboratory results.

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Table

Table 1.

Commutability of the Noklus whole-blood EQA material at different concentrations between the 5 CRP, 7 glucose, and 5 Hb POC instruments and the corresponding hospital instruments (Cobas 6000 and Advia 2120).

CRP	**23 mg/L**	**58 mg/L**	**73 mg/L**
Instrument	**Cobas 6000**	**Cobas 6000**	**Cobas 6000**
ABX Micros CRP 200 *(Horiba)*	C	C	C
Afinion AS 100 Analyzer *(Axis-Shield)*	C	C	C
i-Chroma *(BodiTech Med.Inc.)*	C	C	C
NycoCard CRP Single Test *(Axis-Shield)*	C	C	C
QuikRead GO CRP+Hb *(Orion Diagnostica)*	C	C	NC
Glucose	**126.0 mg/dL** *(7.0 mmol/L)*	**234.0 mg/dL** *(13.0 mmo/L)*	**306.0 mg/dL** *(17.0 mmol/L)*
Instrument	**Cobas 6000**	**Cobas 6000**	**Cobas 6000**
Accu-Chek Performa *(Roche Diagnostics)*	C	NC	NC
Ascensia Contour *(Bayer Healtcare)*	C	NC	NC
Contour *(Bayer Healtcare)*	C	C	C
Contour XT *(Bayer Healtcare)*	C	NC	NC
FreeStyle Freedom Lite	C	NC	NC

(Abbott Diabetes Care Inc)			
HemoCue Glucose 201+ *(HemoCue AB)*	C	C	C
HemoCue Glucose 201RT *(HemoCue AB)*	C	C	C
Hb	**9.6 g/dl**	**13.2 g/dL**	
Instrument	**Advia 2120**	**Advia 2120**	
ABX Micros CRP 200 *(Horiba)*	C	C	
HemoControl *(EKF diagnostic GmbH)*	C	C	
HemoCue Hb 201+ *(HemoCue AB)*	C	C	
Sysmex PocH-100i *(Sysmex)*	C	C	
QuikRead GO CRP+Hb *(Simens Healtcare)*	C	C	

C = commutable, NC = noncommutable

Figures legends

Figure 1.

The procedure for determining the commutability of the whole-blood EQA material for CRP, Hb, and glucose. The figure is modified from the CLSI EP14-A3 guideline (1).

Figure 2.

Examples of commutability plots for the whole-blood EQA material for 2 POC glucose (A and B), 1 POC CRP (C), and 1 POC Hb (D) instruments compared with the designated hospital instruments. Patient samples are indicated as grey squares, the EQA material as black dots, Deming regression lines as solid grey lines and the 95% prediction intervals as black dotted lines.