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Scand J Clin Lab Invest 2004; 64: 309–320

The Nordic Trueness Project 2002: use of reference measurement procedure values in a general clinical chemistry survey

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Pedersen MM, Örnemark U, Rustad P, Steensland H, Loikkanen M, Ólafsdóttir E, Henriksen GM, Jørgensen N, Uldall A, Nordin G, Nordberg U-R. The Nordic Trueness Project 2002: use of reference measurement procedure values in a general clinical chemistry survey. Scand J Clin Lab Invest 2004; 64: 309–320.

Up to 136 laboratories participated in a joint effort to assess the trueness of routine measurements for 14 serum components. An unmodified, fresh-frozen human serum (“IMEP-17 Material 1”), produced for an international inter-laboratory comparison, served as the “master material”. The serum had assigned values of the highest available metrological quality, and is assumed to involve no or negligible commutability problems. The material was used in the assignment of traceable values to two other reference sera, “CAL” and “X”, through parallel measurements on the three materials according to a common protocol. In this transfer process, uncertainty estimates were provided for all values. The material CAL had been supplied with reference measurement procedure values in 1997, and the two sets of assigned values agreed well. A lyophilized control serum “HK02” was also included in the routine analysis series. It, too, had assigned values based on reference measurement procedures. Significant matrix effects were found. The project has provided:

- Assigned traceable values for 14 components in a fresh-frozen serum, available to Nordic laboratories for the coming years as “NFKK reference serum X”.
- Confirmation of earlier assigned reference measurement procedure values for a number of components in CAL, the main calibrator in the Nordic Reference Interval Project (NORIP). The transferred values will now serve as the primary reference.
- Evidence of long-term stability ($\geq$5 years) of the fresh-frozen serum CAL when stored at -80°C.
- Evidence of substantial matrix effects in the processed serum HK02. The findings should be used to discuss to what extent reference measurement procedure values are useful and cost-efficient for this type of material.

DOI 10.1080/00365510410002805

Key words: Commutability; external quality assurance; frozen serum; lyophilized serum; matrix effects; NORIP; reference measurements; value transfer

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INTRODUCTION

Background and objectives

The use of reference measurement procedure values as targets in the External Quality Assurance/Assessment (EQA) has been recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) as the first of six choices [1]. A general guide stresses the importance of such values [2] and the Nordic countries have repeatedly discussed their use on fresh-frozen human sera on a more regular basis. This has, however, only been possible on a few occasions and for a few components. A limiting factor is the small lots of fresh-frozen human sera produced, which has rendered the price per vial rather high. Multi-component lyophilized materials with assigned reference measurement procedure values—available at a more reasonable cost—have been used a few times but with little success.

In December 2001, the EU Institute for Reference Materials and Measurements (IRMM) invited selected EQA scheme organizers to co-ordinate national participation in Round 17 of the International Measurement Evaluation Programme (IMEP-17). That project included two fresh-frozen sera with focus on 20 common components to which traceable values and uncertainties of the highest available metrological quality were assigned [3]. IMEP-17 was intended for clinical biochemistry laboratories and scheduled for spring 2002 [4]. This was a response to the need expressed by the clinical community for more frequent use of reference measurement procedure values to support routine quality assurance (QA) work, e.g. EQA schemes and reference materials (RM) production. This is also in support of the EC directive 98/79/EC [5].

The liaison organization for external quality assurance in the Nordic countries (EQAnord) agreed on a regional expansion of IMEP-17 [6]. The main objective would be to transfer the trueness of the IMEP-17 certified values to three other sera used in the Nordic community [7]. These were "CAL" and "X" (unmodified fresh-frozen materials), used in connection with the Nordic Reference Interval Project (NORIP) [8], and the modified (spiked and lyophilized) "HK02" material, used as a long-term control serum. The transfer would be achieved by measuring, in parallel, the IMEP-17 samples and the other three materials. For HK02, the aim was mainly to test the validity of the assigned reference measurement procedure values when the material is used in routine internal quality control (IQC). If matrix biases were observed, a subsequent objective would be to investigate their size in frequently used routine measuring systems.

Links to other projects

In NORIP, the goal is to recommend mutual serum/plasma reference intervals for Denmark, Finland, Iceland, Norway and Sweden, for 25 of the most commonly analysed components in clinical chemistry [8]. The laboratories in NORIP measured CAL and X along with serum samples from several thousand reference individuals. CAL was used to consolidate the trueness of the individual participating laboratories. X will be used when laboratories implement the new reference intervals, to assess trueness of the components included in NORIP and hence the validity for use of the reference intervals.

Lyophilized human control sera are used in many Nordic routine laboratories to monitor long-term performance of measurement procedures for more than 60 components. This involves frequent (e.g. daily) determinations and monthly results reporting. Statistics are reported back to the laboratories by the EQA scheme organizers. In Finland, this activity is known as the "DayTrol program", and in Denmark and Norway as the "HK program". HK02 is expected to be available for five years,

so a link to this Nordic project would enable the laboratories to monitor their level of trueness for a long time.

Matrix problems in EQA

In 1994, Ross *et al.* [9] investigated 11 components in a fresh-frozen pooled serum and several processed sera (with additives and/or lyophilized). All materials were supplied with reference measurement procedure values. The observed bias of the processed samples was separated into method bias and matrix bias. Owing to matrix biases, the reference measurement procedure values appeared to be the correct target value only in 32% of the cases.

Thienpont *et al.* [10] reported large differences in biases for glucose and cholesterol when comparing 14 fresh-frozen single-donation sera with lyophilized sera. All materials had assigned reference measurement procedure values. These researchers found a substantial positive bias for the most commonly used photometric glucose and cholesterol methods in the fresh-frozen sera, whereas the apparent method bias was only small in most of the processed sera tested. These findings suggest a negative matrix effect due to non-commutability of processed materials but that the effect was "hidden" by a positive method bias.

Both studies above illustrate the danger of misinterpretation of trueness assessment when processed materials supplied with reference measurement procedure values are used without correction for matrix bias, i.e. a non-commutable material is used as though it was commutable. The inclusion of HK02 in the Nordic Trueness Project 2002 was done to determine whether matrix bias for frequently used routine methods could be validated and possibly estimated for a selected number of components that were expected not to be too prone to matrix effects. Also the reference measurement procedure values could allow distinction between matrix-dependent and method- (calibration-) dependent biases.

MATERIALS AND METHODS

Preparation and characterization of serum materials

The manufacturing process of the two IMEP-17 materials, X and CAL, are described in detail elsewhere [11]. These materials are of human origin, prepared from pools of serum and subject to no or only a minimum of modification (spiking). No stabilizers are added and the time in thawed state is kept to a minimum. The materials are sterile-filtered prior to their transfer into polypropylene vials for storage at $-80^{\circ}C$. CAL and the IMEP-17 materials are, in principle, out of stock but X is available as a reference material [12].

HK02 is of human origin, spiked with various components, and lyophilized [6]. HK02 was produced by Randox Laboratories Ltd., in 2001. The starting material was plasma from plasmapherese of healthy British blood donors.

The homogeneity of the materials was evaluated according to different protocols [6, 13]. With the exception of carbamide (urea) in HK02, no significant vial-to-vial variation was found for the tested components. The long-term stability (10 years) of X at -20, -80 and $-150^{\circ}C$ is being investigated [14]. The outcome is assumed to be valid for other materials manufactured in a similar way. One aspect of the Nordic Trueness Project 2002 has been to collect evidence of the stability of CAL, results of which are reported below. According to the manufacturer, the HK02 material expires 4 years after production if stored at $+4^{\circ}C$. The Danish Institute for External Quality Assurance for Laboratories in Health Care (DEKS), which has experienced that the stability increases if this type of material is stored at $-20^{\circ}C$, monitors this for HK02 and various other lyophilized materials.

Value assignment

The IMEP-17 materials, CAL and HK02 carry property values assigned through measurements by clinical reference laboratories of the German Society of Clinical Chemistry (DGKC) and/or national metrology institutes [3, 6]. The values are based on procedures, which provide traceability to reference points of higher metrological order [15]. The property values of X were assigned in the transfer process described below.

The uncertainty of the assigned values was evaluated according to principles in an international guide [16]. The capability of the reference laboratories to operate with a lower uncertainty than routine laboratories [17] was, in most

cases, clearly demonstrated and documented [3, 4].

Organization and logistic arrangements

The project was conducted in the frame of national EQA schemes. Samples were shipped on dry ice in Denmark and Finland (to enable also analyses of phosphate and triglycerides), and by normal mail in the other countries. The IMEP-17 samples as well as CAL and X were ready to use and HK02 had to be reconstituted in cold, distilled water. The laboratories were instructed to measure CAL and/or X in parallel with HK02 and the IMEP-17 materials. Single (CAL, X, HK02) or duplicate determinations (IMEP-17 materials) over 5 days were recommended, with a minimum of 3 days. Between measurement series, the samples were to be stored at 4°C.

A report form in Microsoft® Excel was designed to handle the data. The form enabled the participants to describe their measuring systems in detail [4]. The information about available methods, instruments and calibrators came from Labquality [18]. Up to 136 laboratories (Denmark 55, Norway 50, Sweden 18, Finland 12, and Iceland 1) submitted results. Each report form was checked for inconsistencies, after which the information was extracted with a Microsoft® Visual Basic macro [4].

Calculation of transferred values

Transferred values and uncertainties for CAL, X and HK02 were calculated in the same way as outlined here for CAL. For each laboratory a factor F_i was calculated

$$F_i = \frac{C_m}{I_m} \tag{1}$$

where C_m and I_m are the mean values of a laboratory's measurements of CAL and IMEP-17 Material 1, respectively. The mean value, F of these factors for each component was calculated after removal of outliers using Grubb's test [19]. A maximum of two laboratories were excluded for any of the components and materials, but in most cases all data were kept.

$$F = \frac{1}{n}\sum_{i=1}^{n} F_i \tag{2}$$

Transferred values from IMEP-17 Material 1 were calculated as

$$C_{\text{Trans}}^{\text{CAL}} = C_{\text{Ref}}^{\text{IMEP}} \cdot F \tag{3}$$

where $C_{\text{Ref}}^{\text{IMEP}}$is the assigned (certified) value for the IMEP material (Table I). The standard uncertainty for the transferred value was calculated as

$$u(C_{\text{Trans}}^{\text{CAL}}) = C_{\text{Trans}}^{\text{CAL}} \sqrt{\left(\frac{u(C_{\text{Ref}}^{\text{IMEP}})}{C_{\text{Ref}}^{\text{IMEP}}}\right)^2 + \left(\frac{u(F)}{F}\right)^2} \tag{4}$$

where $u(F)$ is the standard deviation of the mean $(s/\sqrt{n})$ of the factors F_i.

Estimation of method (calibration bias)

The total observed bias for a method is in principle the sum of the laboratory bias, the method bias ($B_{\text{Meth.}}$) and a possible matrix bias (B_{Matrix}). In the transferred values described above, the laboratory bias is eliminated. In fresh-frozen unmodified (commutable) sera, the matrix bias is considered negligible and, therefore, $B_{\text{Meth.}}$ may be calculated as the difference between the mean value for a specific routine measurement procedure and the assigned value based on an applied reference measurement procedure.

The following other symbols are used for the assigned values of the two fresh-frozen reference sera, CAL and X:

$C_{\text{Trans}}^{\text{CAL}}$ Assigned (transferred) value for CAL
$C_{\text{Trans}}^{\text{X}}$ Assigned (transferred) value for X
M_{Routn}Mean (average) value on a specific material (identified by an upper index X or CAL) from laboratories using the same routine measurement procedure.

The relative method bias ($B_{\text{Meth.}}$) may be estimated from Eq. 5 for laboratories using the same routine measurement procedure:

$$\begin{aligned} B_{Meth.} &= \frac{M_{\text{Routn.}}^{\text{IMEP}}}{C_{\text{Ref}}^{\text{IMEP}}} - 1 \approx \frac{M_{\text{Routn.}}^{\text{CAL}}}{C_{\text{Trans}}^{\text{CAL}}} - 1 \\ &\approx \frac{M_{\text{Routn.}}^{\text{X}}}{C_{\text{Trans}}^{\text{X}}} - 1 \end{aligned} \tag{5}$$

Estimation of matrix bias (for specific methods)

In a processed serum, the matrix bias (B_{Matrix}) can have some magnitude. The

TABLE I. Values assigned to four sera. The values for IMEP-17 Material 1, CAL and HK02 are based on reference measurement procedures. Transferred values are based on data for the IMEP-17 Material 1 or CAL together with participants' results in the Nordic Trueness Project 2002 or in NORIP. The approximate level of confidence is 95% (expanded uncertainties U with coverage factor $k=2$).

		Serum material													
		IMEP-17 Material 1		CAL				X				HK02			
		IRMM 2002		1997 DGKC		IMEP transferred		CAL transferred		IMEP transferred		DGKC 2002		IMEP transferred	
Component	Unit	Value	U, $k=2$	Value	U, $k=2$	Value	U, $k=2$	Value	U, $k=2$	Value	U, $k=2$	Value	U, $k=2$	Value	U, $k=2$
Calcium	mmol/L	2.334 2	0.006 9	2.267		2.266 0	0.008 0			2.325	0.008	2.272	0.050	2.340	0.011
Iron	μmol/L	19.39	0.54			21.16	0.59			20.00	0.56			16.60	0.55
Potassium	mmol/L	3.735	0.021	3.700		3.738	0.022			3.732	0.022	3.789	0.130	3.995	0.031
Magnesium	mmol/L	0.812 3	0.005 6	0.807		0.797 0	0.006 4			0.810 0	0.006 5	0.855	0.050	0.886 0	0.007 8
Sodium	mmol/L	140.36	0.95	137.6		137.36	0.732			140.65	0.751	137.1	1.7	140.13	0.77
Glucose	mmol/L	4.412	0.033	4.49		4.464	0.035			4.405	0.034	7.003	0.046	6.820	0.057
Cholesterol	mmol/L	5.111	0.021	4.94	0.055	4.901	0.023			5.22	0.023	4.570	0.066	4.290	0.039
Creatininium	μmol/L	74.57	0.57	69.6	1.05	70.57	0.58			73.9	0.60	162.6	3.3	141.7	1.5
Carbamide	mmol/L	4.772	0.049			4.80	0.050			4.910	0.026				
Urate	μmol/L	308.9	5.7	285	4.7	290.2	5.4			309.9	5.8	416	13	416.3	7.8
Thyroxine (T4)	nmol/L	97.6	1.3	98.0	1.4			99.4	3.1						
Albumin	g/L	41.5	2.7	40.8		40.52	2.64			41.5	2.7			34.06	2.3
Gamma-glutamyltransferase	U/L	34.70	0.93	35.9		35.83	0.96			35.42	0.95	56.7	2.4	58.71	1.65
Triglyceride	mmol/L			1.31	0.019			1.287	0.038						

NORIP = Nordic Reference Interval Project.

observed difference between the mean value of a routine measurement procedure (M_{Routn}) and the assigned reference measurement procedure value (C_{Ref}) may be termed *the apparent method bias*, which is the sum of $B_{\text{Meth.}}$ and B_{Matrix}.

The matrix phenomena can be illustrated for HK02. If the concentration level for a given component in HK02 does not differ too much from that in the fresh-frozen sera, a corrected mean value $M_{\text{Corr.}}^{\text{HK02}}$ may be calculated. This is corrected for the method bias observed on a non-processed reference serum.

$$M_{\text{Corr.}}^{\text{HK02}} = M_{\text{Routn.}}^{\text{HK02}} \cdot (1 - B_{\text{Meth.}}) \tag{6}$$

The difference between $M_{\text{Corr.}}^{\text{HK02}}$ and $C_{\text{Ref}}^{\text{HK02}}$ is the "matrix bias" caused by the difference in composition of the materials. The bias caused by matrix differences, B_{Matrix}, can be estimated from the following expression:

$$B_{\text{Matrix}} = \frac{\left(M_{\text{Routn.}}^{\text{HK02}} \cdot (1 - B_{\text{Meth.}})\right)}{C_{\text{Ref}}^{\text{HK02}}} - 1 \tag{7}$$

Although the matrix bias here is expressed relative to HK02, one should bear in mind that matrix bias is generally considered not to be proportional to the component concentration, but rather as constant (by nature).

RESULTS AND DISCUSSION

Assigned values

Assigned property values for components in the four sera are summarized in Table I. The transferred values (levels and uncertainties) are similar for CAL and X. This is due to the origin of the two sera (analogous serum pools from more than 100 healthy blood donors each) and the same conditions for transfer of values from IMEP-17. A similar technique was used in the preparation, dispensing, storage and mailing of all the frozen materials in this study. Since up to 136 laboratories contributed to the measurements, the increase in the transferred uncertainties (see Eq. 4) to CAL is very small. The 14 values for X in Table I are certified. There exist also indicative values for 12 other components [12] in this material. Those values were established via consensus mean values in NORIP, or transferred from reference measurement procedure values for material CAL in NORIP.

Stability of CAL and consolidation of the accuracy of NORIP data

A comparison of the values assigned to CAL (DGKC, 1997) with those obtained in the value transfer in this project (Table II-A) shows that the differences are generally small. This indicates good stability properties of the material over the 5-year period and similar commutability properties as IMEP-17 Material 1. For the three components where uncertainties were available also in 1997, there is no significant difference between the values ($p = 0.05$). The transferred values and their uncertainties are therefore used in NORIP to obtain traceability to the highest available metrological level.

Estimation of method and matrix bias in fresh-frozen and lyophilized sera

It is generally assumed that reference measurement procedure values assigned to processed sera like HK02 cannot be used as targets in the routine laboratory unless a well-established correction for bias due to method matrix phenomena is done. Therefore, when processed control sera are used in traditional EQA, consensus values obtained by related analytical procedures are the preferred targets, because data for matrix corrections are not usually available. A comparison of the transferred values for HK02 with the values assigned by DGKC is shown in Table II-B. The differences are larger than those for the fresh-frozen material CAL. This confirms that values cannot be transferred to HK02 in the same way as with sera like CAL and X, most likely because of matrix effects.

However, since these data include all methods used, also dry chemistry methods (Ortho Vitros systems) which are known to be especially vulnerable to matrix effects in processed sera, there were good reasons to separate the dry and wet chemistry methods. This is shown in Table III. As expected, the value difference (%) between the transferred wet chemistry values and the DGKC reference values are considerably smaller than between the transferred Ortho Vitros values and the DGKC reference values.

TABLE II. Column A: Comparison of values assigned to the fresh-frozen material CAL by the DGKC (1997) with those transferred in the Nordic Trueness Project 2002. The value difference indicates good stability over a 5-year period. Column B: Comparison of directly assigned values to the lyophilized material HK02 (DGKC 2002) with those transferred in the Nordic Trueness Project 2002. In the transfer process, IMEP-17 Material 1 served as the reference. The value difference demonstrates the magnitude of the matrix effect.

Component	Unit	A Value difference (%)	B Value difference (%)
Calcium	mmol/L	−0.044	3.0
Potassium	mmol/L	1.0	5.4
Magnesium	mmol/L	−1.2	3.6
Sodium	mmol/L	−0.17	2.2
Glucose	mmol/L	−0.58	−2.6
Cholesterol	mmol/L	−0.79	−6.1
Creatininium	μmol/L	1.4	−13
Urate	μmol/L	1.8	0.07
Albumin	g/L	−0.69	–
Gamma-glutamyltransferase	U/L	−0.19	3.5

DGKC = German Society of Clinical Chemistry.

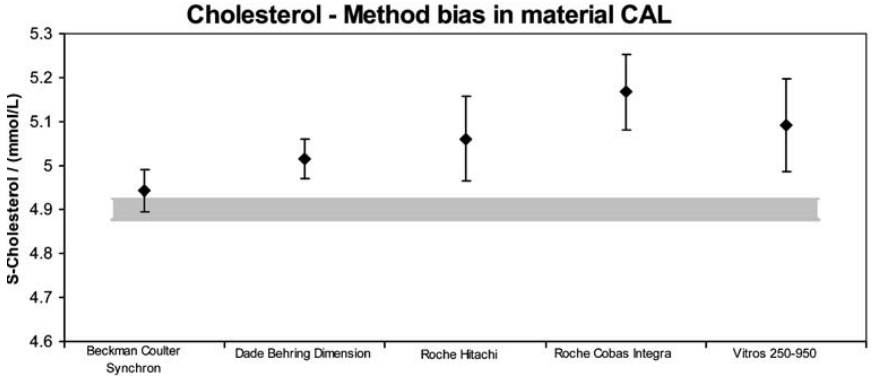

FIG. 1. Cholesterol: results for CAL with five measuring systems given as means ±2 standard deviations of the mean. The shaded area incorporates the assigned value (transferred from IMEP-17 Material 1) and its expanded uncertainty.

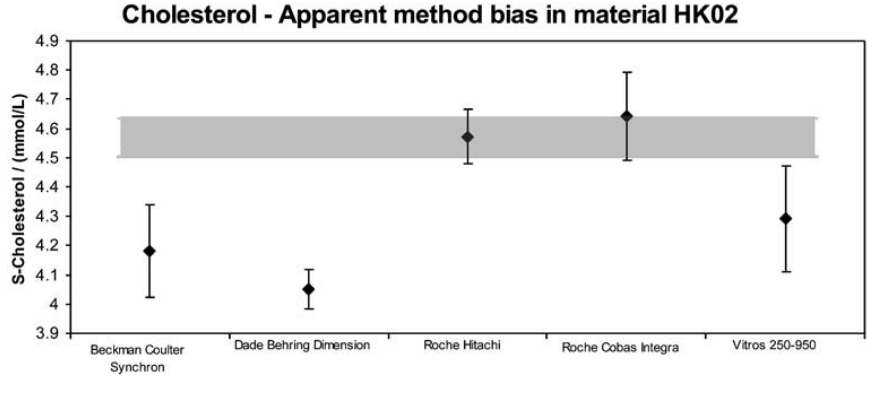

FIG. 2. Cholesterol: results for HK02 with five measuring systems given as means ±2 standard deviations of the mean. The shaded area incorporates the reference measurement procedure value (DGKC) and its expanded uncertainty.

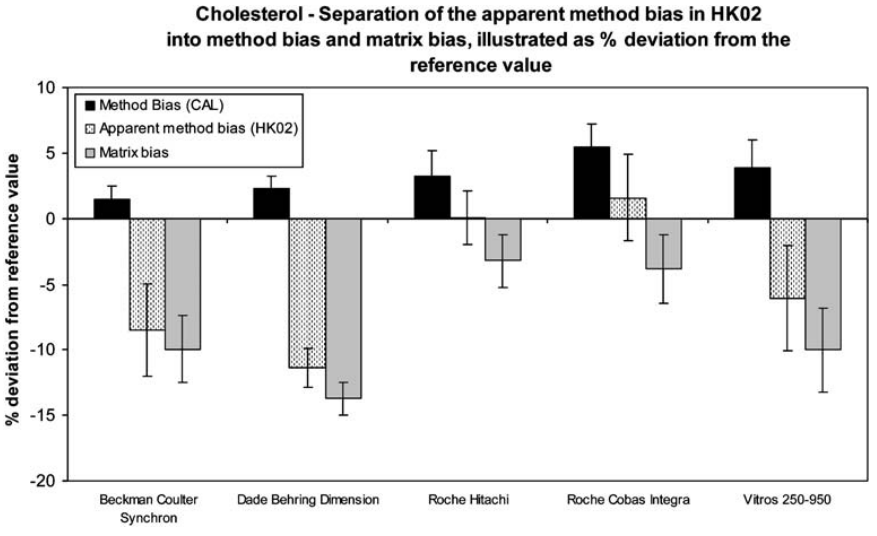

FIG. 3. Cholesterol: estimation of matrix bias in HK02 with five measuring systems. Black columns: the estimated method bias (%) for CAL relative to the value transferred from IMEP-17 Material 1. Speckled columns: the "apparent method bias" (%) for HK02 relative to the German Society of Clinical Chemistry (DGKC) reference measurement procedure value. Grey columns: the matrix bias (%) for HK02, which is the difference between the apparent method bias for HK02 and the method bias for CAL.

However, in most cases, the wet chemistry transferred values include different measuring systems. These "integral" deviations may therefore, cover deviations connected to the individual systems. Further breakdown of the results into individual analysis systems could illustrate this assumption.

A special problem is seen for creatininium. The 17% deviation for this component when measured with photometric Jaffe methods includes an error owing to the contribution from non-creatininium chromogens. The reference value (DGKC) was obtained by a more selective method (isotope dilution mass spectrometry).

The algorithms above were used to estimate the bias for routine methods applied to the fresh-frozen serum CAL, and to estimate the matrix bias of the processed serum HK02. Figures 1–6 illustrate the estimated biases on cholesterol and calcium for the two sera for 4–5 commonly used measuring systems. In Figure 1 it can be seen that all the routine systems show a positive method bias for

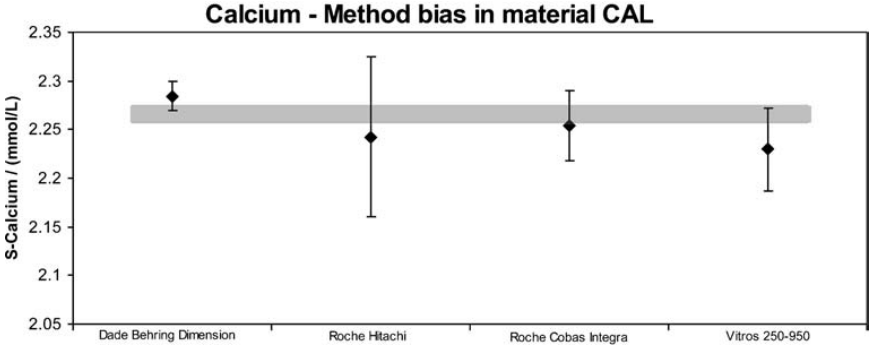

FIG. 4. Calcium: results on CAL with four measuring systems given as means ± 2 standard deviations of the mean. The shaded area incorporates the assigned value (transferred from IMEP-17 Material 1) and its expanded uncertainty.

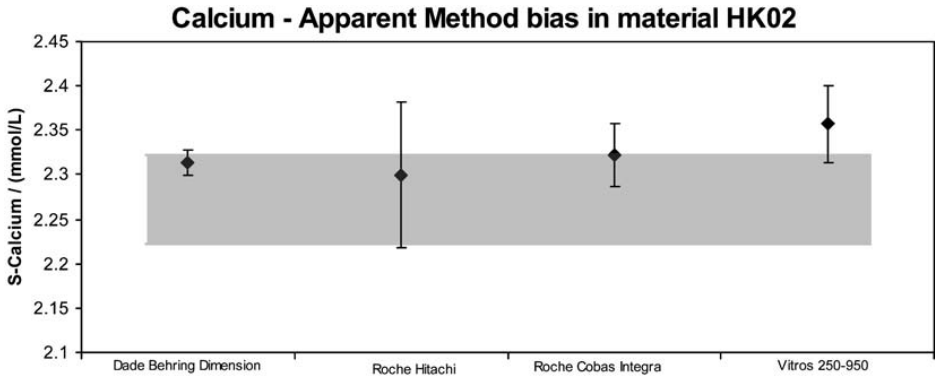

FIG. 5. Calcium: results on HK02 with four measuring systems given as means ± 2 standard deviations of the mean. The shaded area incorporates the reference measurement procedure value (DGKC) and its expanded uncertainty.

cholesterol in CAL. The *apparent* method bias in HK02 shown in Figure 2, however, expose a quite different pattern, which again gives rise to the estimated matrix bias for cholesterol in HK02 shown in Figure 3. If the estimated uncertainties are taken into account, the matrix effects are statistically significant. This is much in line with earlier findings [4, 9].

For calcium (Figs 4–6), the estimated method bias, the *apparent* method bias as well as the matrix bias are considerably smaller for all the wet chemistry systems, and with the uncertainties taken into account, the estimated biases for these systems are hardly statistically significant.

These two examples show how matrix bias may be estimated in a processed serum, but this kind of explorations is demanding, and serves here only to illustrate that reference procedure values might or might not be useful in processed sera and that the usefulness sometimes is related to the analytical systems and their robustness.

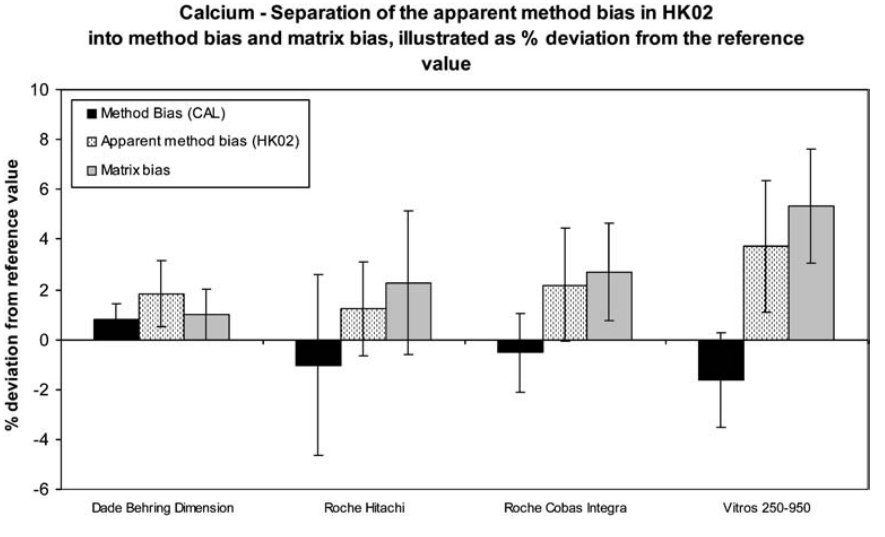

FIG. 6. Calcium: estimation of matrix bias in HK02. Black columns: the estimated method bias (%) for CAL relative to the value transferred from IMEP-17 Material 1. Speckled columns: The "apparent method bias" (%) for HK02 relative to the German Society of Clinical Chemistry (DGKC) reference measurement procedure value. Grey columns: the matrix bias (%) for HK02 alone, which is the difference between the apparent method bias for HK02 and the method bias for CAL.

TABLE III. The participants' results for individual components in the lyophilized material HK02 after recalibration against IMEP-17 Material 1. Results for wet and dry chemistry separated. Apparent method bias: the difference (in %) between the arithmetic mean value and the certified value.

Component	DGKC certified value and expanded uncertainty (U, $k=2$)	Participants' results					
		Method name [18]	Mean	s	CV%	No. of labs	Difference (%)
Calcium	2.272±0.050	Photometry	2.32	0.03	1.2	47	2.1
		Vitros 250–950	2.40	0.03	1.2	23	5.6
Cholesterol	4.570±0.066	Enzymatic: Roche	4.43	0.05	1.1	26	−3.1
		Enzymatic: Roche Cobas Integra	4.32	0.08	1.8	18	−5.5
		Vitros 250–950	4.13	0.09	2.2	20	−9.6
Creatininium	162.6±3.3	Photometry, Enzymatic	158	11	7.0	4	−2.8
		Photometry, Jaffe	135.7	6.2	4.6	55	−17
		Vitros 250–950	152.8	5.8	3.8	26	−6.0
Glucose	7.003±0.046	Photometry	6.91	0.13	1.8	57	−1.3
		Vitros 250–950	6.53	0.10	1.5	21	−6.8
Gamma-glutamyltransferase	56.7±2.4	IFCC comp. methods	56.2	1.7	3.1	39	−0.88
		Vitros 250–950	65.4	1.3	2.0	18	15
Magnesium	0.855±0.050	Photometry	0.87	0.02	2.2	38	1.8
		Vitros 250–950	0.91	0.01	1.1	20	6.4
Potassium	3.789±0.130	ISE direct	4.00	0.11	2.8	13	5.6
		ISE indirect	4.10	0.20	4.8	48	8.2
		Vitros 250–950	3.88	0.04	1.2	26	2.4
Sodium	137.1±1.7	ISE direct	139.5	2.3	1.6	13	1.8
		ISE indirect	140.0	2.3	1.6	48	2.1
		Vitros 250–950	139.0	1.2	0.9	26	1.4
Urate	416±13	Photometry, Enzymatic	420.2	9.2	2.2	55	1.0
		Vitros 250–950	409.0	5.1	1.2	24	−1.7

DGKC = German Society of Clinical Chemistry.

The usefulness of assigning reference measurement procedure values to lyophilized materials is thus questionable. It seems that such assignments should preferably be reserved to fresh materials where commutability is maintained. This is also in line with what is reported by Ross *et al.* [9] and Thienpoint *et al.* [10]. However, since we have not yet investigated the results for individual methods for all components, we can at this stage only recommend that values assigned to HK02 by DGKC should not be used directly as target values for routine methods unless additional supporting data are available. Lyophilized sera, being stable and easy to store, are still of considerable importance in long-term quality control.

ACKNOWLEDGEMENTS

We express our gratitude to the participating laboratories for their comprehensive and careful analytical work. We are also indebted to the DGKC for assigning reference values to CAL in 1997 as a joint DGKC-DEKS project and to Per Hyltoft Pedersen, Odense University Hospital, for his input and discussions.

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Received: 19 December 2003
Accepted: 30 April 2004