EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
IRMM
Institute for Reference Materials and Measurements

IRMM **GE/R/IM/17/03**
Isotope Measurements **March 2004**

CCQM-K14 key comparison
Calcium amount content in serum

Final version

L. Van Nevel, Y. Aregbe, P.D.P. Taylor

Measurement contributions from:
R. Arvizu from CENAM;
A. Barzev from CSIR-NML;
E. Zeiller, R. Schorn, A. Toervenyi and K. Burns from IAEA;
M. Berglund, C. Hennessy and S. Duta from IRMM;
E. Hwang from KRISS;
R. Hearn and L. Simpson from LGC;
S. Long, K. Murphy and G. Turk from NIST;
M. Van Son from NMi-VSL;
D. Schiel and O. Rienitz from PTB

European Commission – Joint Research Centre
Institute for Reference Materials and Measurements
B-2440 GEEL (Belgium)

Abstract

The CCQM-K14 key comparison “Ca in Serum”is a follow-up for the pilot study P14. The aim of the study is to demonstrate and document the capability of National Metrology Institutes to measure the Ca amount content in a serum sample.
The comparison was an activity of the Inorganic Analysis Working Group of CCQM and was piloted by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium).

The following laboratories participated in this key comparison (in alphabetical order):

CENAM (Mexico)
CSIR-NML (South Africa)
IAEA (International Organisation)
IRMM (European Union)
KRISS (South Korea)
LGC (United Kingdom)
NIST (United States of America)
NMi -VSL (The Netherlands)
PTB (Germany)

The majority of participants applied isotope dilution mass spectrometry (IDMS) using thermal ionisation MS (TIMS), sector field or quadrupole inductively coupled plasma MS (ICP-MS) as analytical technique. IAEA reported a combined result of AAS and ICP-OES for the Ca amount content in the serum. The Key Comparison Reference Value (KCRV) was agreed upon during the IAWG meeting in October 2003 at EMPA/St-Gallen as the weighted mean of the reported participants' results. Accordingly the equivalence statements were calculated.

Table of content

1. Introduction

The CCQM-K14 key comparison "Ca in Serum" has been proposed and discussed at the April 2002 CCQM Inorganic Analysis Working Group meeting in Paris as a follow-up for the pilot study P14. The aim of the study is to demonstrate and document the capability of interested National Metrology Institutes to measure the Ca amount content in a serum sample.

1.1. Rationale for the key comparison

1.1.1. Calcium and medical diagnosis

Calcium is the most abundant mineral element in the human body with about 99 percent in the bones. In addition to skeletal functions, calcium is involved in blood coagulation, enzyme activation, preservation of cell membrane integrity and permeability and many other processes.[1]

Determination of the free ($S\text{-}Ca_{free}$) and the total concentration of calcium in serum ($S\text{-}Ca_{tot}$) are common applications in the medical laboratory. The results are used for screening of for example D- and A-vitamin disorders, kidney insufficiency, various bone diseases and leukaemia.

The rationale for the key-comparison is the need for better reference methodology to support routine clinical work. Single tests with current methods are according to clinical experts not fully satisfactory for use in medical diagnosis. The expected total concentration of calcium in serum of healthy adults is in the range 2.25-2.65 mmol/L.* The observed intra- and inter-individual biological variation is about 1.9% and 2.8% respectively. [2]

Analytical procedures as developed for Ca, are also relevant for other elements in serum. Sample pre-treatment, including digestion and separation, and isotope ratio measurement are similar. The measurements in CCQM-K14 are, therefore, likely to be representative for elements such as Mg, Cu, Zn, Li and Fe.

* In laboratory medicine the range is referred to as a "reference interval" or "normal values". It includes biological variation and the uncertainty of the analytical work.

1.1.2. Ca in human serum – results from the IMEP-17 participants

Figure 1 gives the state-of-the practice in Ca measurements in the clinical measuement community world-wide. In Figure 1 results for the total amount content of calcium in the IMEP-17 serum material, obtained from laboratories originating from 35 countries world-wide, are presented[3]. The material used for IMEP-17 is the same material as was used in the CCQM Pilot study P14 for Ca in serum. The reference value for Ca in IMEP-17, displayed as the grey band in Figure 1, was based on measurements done by 5 reference laboratories (results previously reported for CCQM-P14). [4]

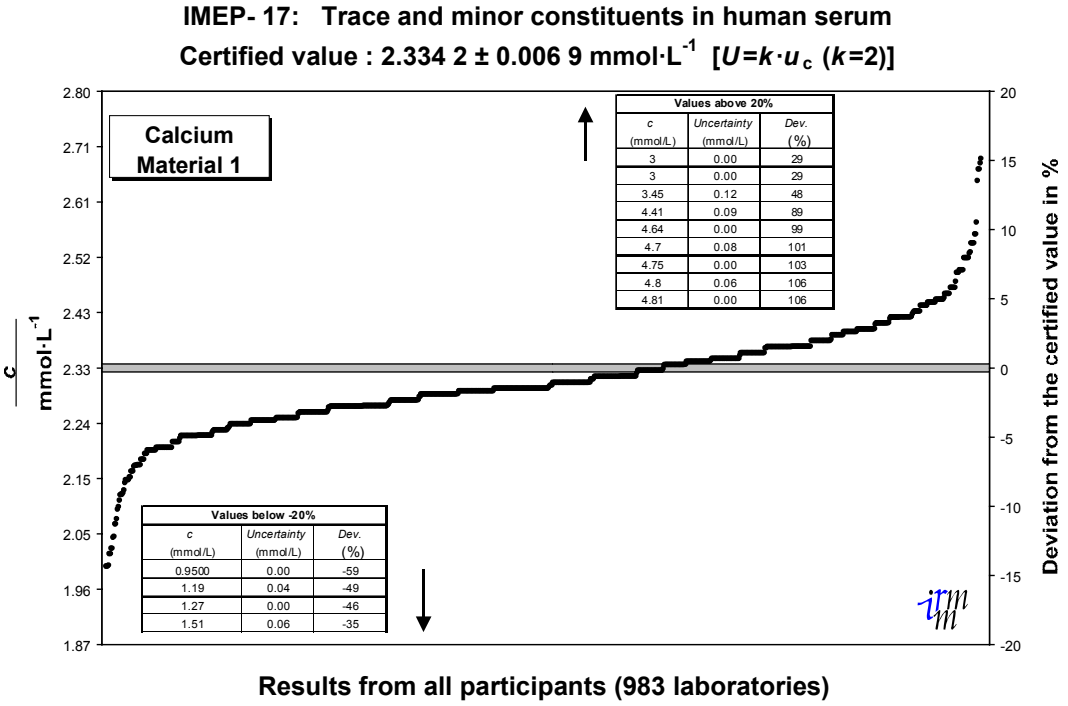

Figure 1 Reported results for the total amount content of Ca, measured by 983 field laboratories from 35 countries worldwide

2. CCQM-K14

2.1. Objective

The objective of CCQM-K14 was to determine the total amount content ($mol \cdot kg^{-1}$) of calcium in a human serum material. The participants were free to choose the analytical procedure, provided it was fit for purpose.

2.2. Participation

In September 2002 the CCQM inorganic working group members and other interested parties were informed by e-mail about the organisation of the key-comparison. Five weeks later, a reminder was sent to all, except those who already registered for participation. The latter were contacted on an individual basis.

Finally nine institutes participated in CCQM-K14 (Table 1). VNIIFTRI, Russia expressed their regrets not to be able to participate in this key comparison for technical reasons.

Table 1. Participants in CCQM-K14

Institution/organisation	Country/region organisation	Contact person
CENAM Centro Nacional de Metrología	Mexico	R. Arvizu
CSIR-NML National Metrology Institute	South Africa	A. Barzev
IAEA International Atomic Energy Agency	International Organisation	K.I. Burns
IRMM Institute for Reference Materials and Measurements	European Commission	M. Berglund
KRISS Korean Research Institute of Standards and Science	South Korea	E. Hwang
LGC Laboratory of the Government Chemist	United Kingdom	R. Hearn
NIST National Institute of Standards and Technology	USA	G. Turk
NMi VSL Nederlands Meetinstituut Van Swinden Laboratorium B.V	The Netherlands	M. van Son
PTB Physikalisch-Technische Bundesanstalt	Germany	D. Schiel

3. The test material

3.1. Production

The CCQM-K14 sample is an unmodified human liquid serum. The material was produced by DEKS[†] (Danmark) from voluntary Danish donors, who were under medical supervision according to WHO[‡] recommendations. The donors were tested individually for absence of HIV antibodies, Hepatitis B antigen and Hepatitis C antibodies. The pool was sterile-filtered (0.2 μm filter) and the procedure has been outlined to keep the material sterile and to avoid contamination.

The material will be available for some years in an External Quality Assurance Scheme. (DEKS identification FHK 0108). It is provided in a polypropylene vial with a teflon-coated stopper and an outer metal seal. Each vial contains about 5 ml serum.

3.2. Initial characterisation of the test material

3.2.1. Homogeneity

The homogeneity of the serum was assessed by determining the concentration of calcium in twenty vials (two duplicates). Data was treated using ANOVA one-way analysis. The homogeneity study was performed with routine clinical methods and resulted in a value of 1.04 %[5]. These methods do not have the same high precision as those used by the participants in CCQM-K14. However, results from analyses of calcium by isotope dilution mass spectrometry (IDMS) indicated that the homogeneity was sufficient for the intended intercomparison.

3.2.2. Stability

Several previous studies have indicated that liquid serum material of this type is stable for months to years if stored frozen (<20 °C to –80 °C). Long term stability studies for Calcium are done at DEKS using similar types of materials. [6]

3.3. Sample distribution and deadline for reporting results

The serum material arrived at IRMM beginning of July 2002. It was stored at –80 ^{o}C until dispatch to the participants.

The samples were distributed from IRMM on dry ice in November 2002 to all participants except one. Due to customs regulations, the parcel to Mexico was only delivered to CENAM by the end of December 2002. The parcels were delivered by express courier (DHL or FedEx) or using schedule flights with pick-up in the airport. All participants reported the receipt of the samples in frozen condition. The use of temperature monitors for Korea, South Africa and Mexico (schedule flights) proved the unbroken cool chain.

[†] Danish Institute for External Quality Assurance for Laboratories in Health Care
[‡] WHO : World Health Organisation

Each participant received nine vials and was recommended to store them at –20 ^{o}C until analysis. In January 2003, NIST received on request 5 additional vials.

The deadline for reporting results was set to 15 March 2003 in order to enable IRMM to present the first overview of results at the CCQM meeting in Paris in April 2003. All participants reported their results in time for this purpose.

3.4. Instructions to the participants

The information package was sent by e-mail and regular mail in November 2003 and included:

- accompanying letter and scope of the study (1+1 page)
- general instructions and guidelines (2 pages)
- results report form (1 page)
- questionnaire (1 page)

An additional e-mail informed the participants about the density of the serum material.

3.4.1. Accompanying letter and scope

The two pages gave the background and rationale for the comparison. Information such as registered institutes, information about the sample (origin, clinical test, characteristics of the material) and timing of the key comparison were given.

3.4.2. General Instructions and guidelines

The instructions contained recommendations concerning the sample storage, a request for feedback on the condition of the samples on arrival, guidelines for sampling and the approximate total amount of calcium in the serum material. The participants were free to choose the measurement procedure. No target value for uncertainty (U, k=2) was recommended as was the case in the pilot study. In addition some specific points of IDMS were highlighted and recommendations for reporting were included.

3.4.3. Results report form and instructions for uncertainty evaluation

Participants were asked to report results and uncertainty in mol·kg^{-1} (amount content). The instructions were prepared in order to obtain consistency in the evaluation of the uncertainty and stressed that the uncertainty was to be evaluated according to the guidelines from ISO-GUM[7] and from Eurachem/CITAC.[8] The reported uncertainty should be an expanded uncertainty with coverage factor 2 (U, k=2).

4. The participants' measurement procedures

All participants have submitted the result report form and the questionnaire. They all stated a functional relationship and described the various uncertainty contributions of the uncertainty budget. Some of them also submitted a complete and detailed analysis report. In the following paragraphs, information as derived from the questionnaire is summarised.

4.1. Sample preparation

All participants prepared the blends or subsamples gravimetrically. The sample weight used and the individual sample preparation procedures are summarised in Table 2.

The matrix of the test sample is complex. Serum contains approximately 1% salts and 7% proteins. Except for LGC where a dilution step was performed before analysis, all sample preparation procedures involved an acid digestion with HNO_3 in combination with H_2O_2 or $HClO_4$. This was done using either high-pressure bombs with microwave oven, digestion on hotplate or the high pressure asher. As extra step, some participants also included cation exchange chromatography in order to separate the analyte from other interfering ions,e.g. Na.

Table 2. Overview of digestion and separation procedures in CCQM-K14

Participant	Main steps in sample preparation	Amount of sample
CENAM	1. 2 mL conc. HNO_3 + 2mL conc. $HClO_4$ 2. Cation exchange chromatography (Eluent HCL 6N) 3. Evaporation of HCL, redissolving in HNO_3 (2%) to reach final weight	1.0 g
CSIR-NML	1. Mixture high purity HNO_3/H_2O_2 (6:2) 2. Sample dilution of approximately 1000 before measurement	1.0 g
IAEA	(For ICP-OES) 1. High pressure bombs in microwave oven 2. $HClO_4$ and HNO_3	
	(For AAS) 1. 2 ml HNO_3 (14.6 M suprapur) – cold digestion stage – overnight 2. Addition of 1 ml HNO_3 + 0.5 ml H_2O_2 (30%) 3. Three-stage microwave digestion 4. Diluted to 100 ml (de-ionised H_2O)	2.5 ml
IRMM	1. 5 mL conc. HNO_3 (reflux) – predigestion in open vessels 2. 5 mL conc. HNO_3, high pressure asher 3. evaporation, dissolving in H_20	2.0 g

	4. Precipitation as calcium oxalate, re-dissolve in HCl 5. Cation exchange chromatography	
KRISS	1. 2 ml HNO_3 + 1ml H_2O_2, 2. microwave digestion, 3. dilution to about 200 with de-ionised H_2O	4.5- 5 g
LGC	1. Dilution to total mass of 10 g by addition of HNO_3 (1%)	0.5 g
NIST	1. 4 g conc. HNO_3 (reflux) – hot plate 2. 2 g conc. $HClO_4$ and 1 g conc. HNO_3 (reflux) 3. Heating to dryness after redissolving twice in 1 ml HCl and with high purity H_2O 4. Cation exchange chromatography 5. Evaporation, re-dissolving in 1 ml high purity H_2O 6. Cation exchange chromatography 7. Residue redissolved in diluted HNO_3	0.5 to 2.0 g
	1. 6 g conc. HNO_3 2. Microwave digestion (600W-10 minutes, 100 W-20 minutes) 3. Evaporation of HNO_3 on a hotplate to dryness 4. Redissolved twice in 2ml HCl 5. Redissolved in 1 ml high-purity H_2O 6. Cation exchange chromatography 7. Residue redissolved in diluted HNO_3	0.5 to 2.0 g
NMi VSL	1. 1,5 mL ultrapure HNO_3 + 3 mL H_2O (MilliQ) 2. Microwave digestion, 300 W-25 min 3. Dilution to 10 ml	1.0 g
PTB	1. 10 mL conc. HNO_3 (65 %)+ 6 mL H_2O_2 (31 %) 2. Microwave digestion 3. Evaporation, re-dissolving in water 4. Cation exchange chromatography 5. Evaporation, re-dissolving in 0.005 % HCl (2*200µl)	2.0 g

4.2. Methods and instrumentation

Eight participants used isotope dilution mass spectrometry (IDMS). Low- and high-resolution (HR) mass spectrometers with quadrupole or magnetic sector, and with thermal ionisation (TI) and inductive coupled plasma (ICP) were employed. LGC applied exact matching double IDMS; CSIR-NML, KRISS and NMI-VSL applied double IDMS. NIST used reverse IDMS for the spike solution. One participant reported the value based on optical emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS) (flame-AAS using conventional pneumatic nebulisation and nitrous oxide-acetylene flame). Table 3 gives for the reporting laboratories, an overview of the analytical method and the instrumentation used.

Table 3. Methods and instrumentation used by CCQM-K14 participants.

Participant	Method	Instrumentation
CENAM	IDMS	HR-ICP-MS, Finnigan MAT Element 1
CSIR-NML	IDMS	ICP-Magnetic sector-MS
IAEA	ICP-OES AAS	Jobin Yvon 38S ICP-OES upgraded with a simultaneous spectrometer AAnlyst 800 Atomic absorption Spectrometer (Perkin-Elmer)
IRMM	IDMS	Thermal ionisation magnetic sector mass spectrometry
KRISS	IDMS	HR-ICP/Magnetic sector MS (Finnigan Element 1)
LGC	IDMS	ICP-MS with octapole collision cell (Agilent 7500 c)
NIST	IDMS	ICP-MS ("cold plasma")-VG Plasmaquad 3
NMi-VSL	IDMS	Sector-field ICP-MS applying resolution R=approx.3000
PTB	IDMS	TIMS, multicollector, magnetic sector field-Triton TI, Thermo Finnigan MAT

4.3. Experimental details

Experimental details such as the number of vials examined, the number of sample blends prepared, the choosen reference isotope in case of IDMS and the (reference) materials were used as assay materials or for calibration purposes, are summarised in Table 5. Every participant received 9 vials of serum material. The number of vials analysed ranged from 3 to 6 vials. The number of sample blends prepared (subsamples for ICP-OES and AAS) ranged from 5 to 9 except for one participant who prepared 20 blends. As reference isotope, 3 IDMS laboratories used ^{42}Ca, 3 the isotope ^{40}Ca and 2 the isotope ^{44}Ca. For the primary assay material for IDMS all laboratories relied on a $CaCO_3$ material. As part of the method development some participants also performed measurements on reference materials certified for the calcium content.

In addition Table 5 presents the experimental reproducibility of c_x for the different sample aliquots. This confirms that the sample homogeneity was adequate for the

intended purpose. All laboratories using IDMS use square root of n for type A uncertainty contributions.

5. Results and discussion

5.1. Results

The participants' results and uncertainties are listed in Table 4 and graphically displayed in Figure 2. All participants, except KRISS, reported their results with an expanded uncertainty with coverage factor k=2.

Table 4. CCQM-K14 participants' measurement results for calcium.

Participant	Amount content 10^{-3} mol·kg^{-1}	U 10^{-3} mol·kg^{-1}	k factor	Relative Uncertainty (%)
CENAM	**2.312**	**0.090**	**2**	**3.9**
CSIR-NML	**2.113 3**	**0.035**	**2**	**1.7**
IAEA	**2.24**	**0.096**	**2**	**4.3**
IRMM	**2.24**	**0.015**	**2**	**0.7**
KRISS	**2.265**	**0.021**	**2.57**	**0.9**
LGC	**2.236**	**0.012**	**2**	**0.5**
NIST	**2.239 5**	**0.011 5**	**2**	**0.5**
NMi VSL	**2.222**	**0.029**	**2**	**1.3**
PTB	**2.248**	**0.012**	**2**	**0.5**

Table 5. Experimental details.

Participant	Number of vials examined	Number of sample blends	Reference isotope [§] (for IDMS)	Materials used for assay and/or for calibration purposes and/or for method validation purposes	Ca isotope ratios (calibrated)	Use of square root of n for type A uncertainty contributions	Experimental reproducibility of c_x from different sample aliquots (in %)
CENAM	3	6	^{42}Ca	• $^{42}CaCO_3$, Oak Ridge National Laboratory • DMR 55b, spectrometrical standard reference solution certified by CENAM (994.6 ± 8.3 mg/kg)	$^{42}Ca/^{44}Ca$	yes	1.38
CSIR-NML	3	6	^{44}Ca	• $^{42}CaCO_3$, ^{42}Ca=87.8%, Cambridge Isotopic laboratories, Inc. • CRM BCR-304 • $CaCO_3$, Puratronic, 99.999%, Alfa Aesar;		yes	0.65 (n=6)
IAEA	2*4 vials	2*8		• ICV2A (Spex) multielement calibration standard (ICP-OES) • Single element Ca stock solution Merck (AAS) • BCR CRM 304	Not applicable	no	ICP-OES= 0.85 AAS= 2.44
IRMM	3	9	^{40}Ca	• IM 6008 based on NIST SRM 915, $CaCO_3$	$^{44}Ca/^{40}Ca$=0.021 530 ± 0.000 034	yes	0.85
KRISS	5	5	^{44}Ca	• Oak Ridge National Laboratory - Batch 139693 ^{42}Ca=94.41%		yes	0.74
LGC	3	9	^{44}Ca	• $CaCO_3$ -^{42}Ca=93.58% Oak Ridge National Laboratory • $CaCO_3$, 99.99% - Alfa Spec pure Spectra. • NIST SRM 3109a (aqueous Ca solution)	$^{44}Ca/^{42}Ca$	yes	0.57
NIST	4	7	^{40}Ca	• NIST SRM 915a, $CaCO_3$;		yes	0.28

[§] The reference isotope is the isotope to which the spike is ratioed.

Participant	Number of vials examined	Number of sample blends	Reference isotope [§] (for IDMS)	Materials used for assay and/or for calibration purposes and/or for method validation purposes	Ca isotope ratios (calibrated)	Use of square root of n for type A uncertainty contributions	Experimental reproducibility of c_x from different sample aliquots (in %)
				• NIST SRM 3109a, Calcium standard solution			
NMi VSL	3	8	^{42}Ca	• 87 % enriched in ^{42}Ca (spike) • 99.999% $CaCO_3$ (reference solutions) • Calibration using an isotopic reference material was not considered to be necessary	$^{43}Ca/^{42}Ca=0.204\ 5 \pm 0.006\ 1$ $^{44}Ca/^{42}Ca=3.162 \pm 0.085$ $^{46}Ca/^{42}Ca=0.006\ 45 \pm 0.000\ 69$ $^{48}Ca/^{42}Ca=0.289\ 3 \pm 0.006\ 9$ k=2 and non-calibrated ratios	yes	0.70
PTB	6	20	^{40}Ca	• No isotopic reference material was used for calibration • Chemotrade ^{44}Ca #115-1 • NIST SRM 915a, $CaCO_3$	not determined	yes	0.40

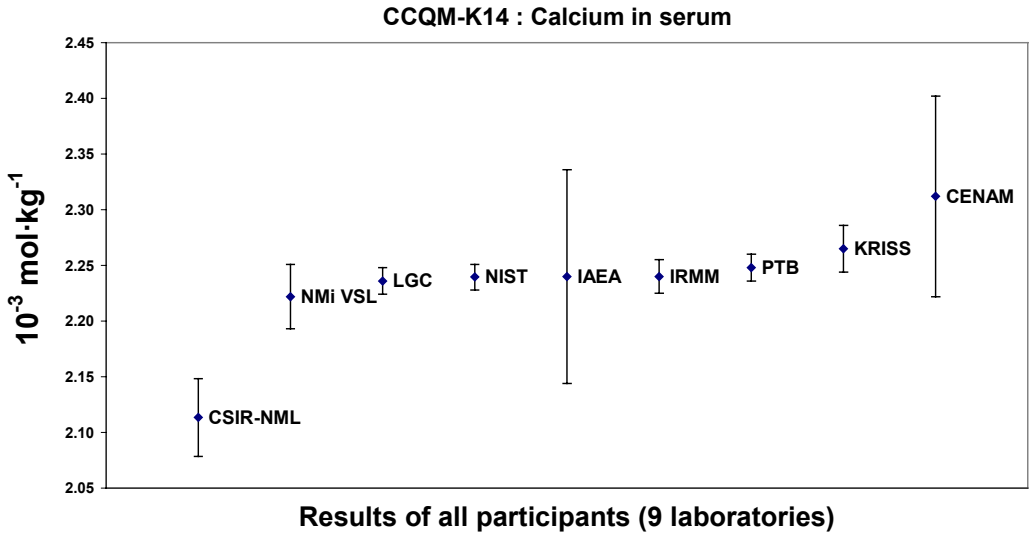

Figure 2. CCQM-K14 participants' measurement results for calcium.

5.2. Calculation of the Key Comparison Reference Value (KCRV)

As known from BIPM and from previous key comparisons there is no rule as to the choice of average in the calculation of the KCRV. This is agreed upon by the CCQM working groups. Table 6 lists the arithmetic mean, the median and the weighted mean for the 9 reported values. The use of the median as KCRV is recommended if there are results reported with significant lower or higher amount content compared to the other results of the participants. The weighted mean is applicable for independent normal distributed results of the same population. During the meeting of the CCQM-IAWG in October 2003 in St. Gallen (Switzerland), the weighted mean was selected as KCRV for the reason that it takes into account the reported uncertainties.

Table 6. Arithmetic mean, median and the weighted mean of the reported K-14 results

	CCQM-K14 KCRV proposal 10^{-3} mol·kg^{-1} Uncertainty (k=2)
Aritmic mean*	2.235 ± 0.035 (1.6%)
Median**	2.240 0 ± 0.007 9 (0.35%)
Weighted Mean***	2.239 ± 0.011 (0.51%)

*uncertainty of the mean estimated as standard deviation of the mean
**uncertainty of the median was estimated applying "robust statistics" [9]
***uncertainty of the weighted mean estimated as standard deviation of the weighted mean

6. Graphical display results and KCRV

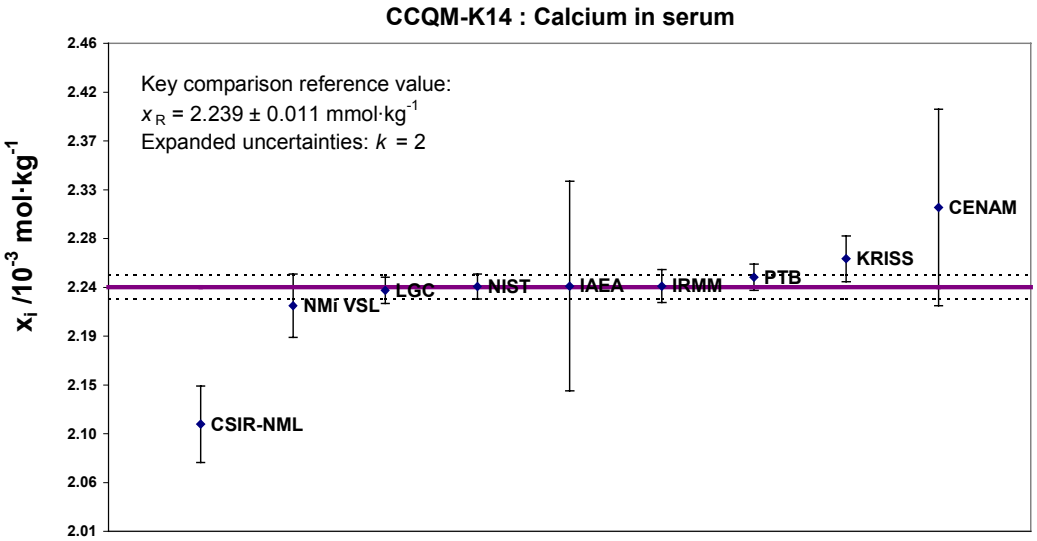

Figure 3 : Graphical display of the reported results together with the KCRV

7. Equivalence statements

The equivalence statements are calculated according to the BIPM guidelines. The degree of equivalence (and its uncertainty) between a NMI result and the KCRV is calculated according to the following equations:

$$D_i = (x_i - x_R) \qquad U_i^2 = 2^2 (u_i^2 + u_R^2)$$

where D_i is the degree of equivalence between the NMI result x_i and the KCRV x_R and U_i is the expanded uncertainty (k=2) of the D_i calculated by the combined uncertainty (k=1) of the NMI result u_i and the uncertainty (k=1) of the KCRV u_R.

The equivalence statements of the CCQM-K14 as graphical display is presented in Figure 4. At the CCQM meeting on 15th April 2002 in Paris it was decided that the matrix representing the degree of equivalence between two NMI results is not a part of a final key-comparison report anymore.

The degree of equivalence (and its uncertainty) between two NMI results is calculated **on request only**, according to the following equations:

$$D_{ij} = (x_i - x_j) \qquad U_{ij}^2 = 2^2 (u_i^2 + u_j^2)$$

where D_{ij} is the degree of equivalence between the NMI results x_i and x_j and U_{ij} is the expanded uncertainty (k=2) of the D_{ij} calculated by the combined uncertainty (k=1) of the NMI results u_i and u_j.

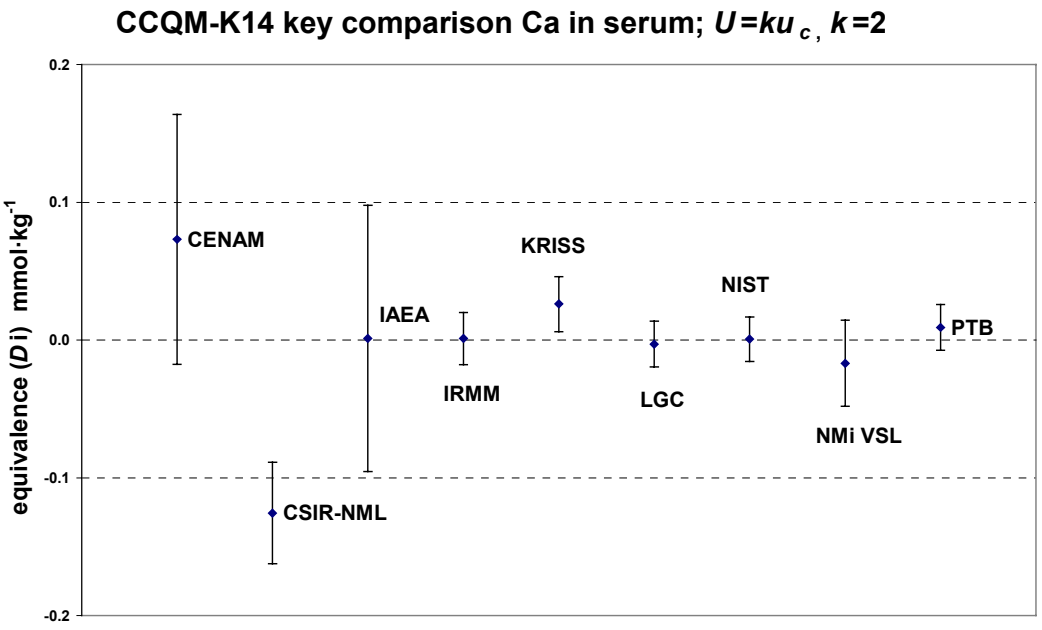

Figure 4: Graphical display of the equivalence statements of the participatin measurement institutes in alphabetical order

8. Discussion

In the pilot study CCQM-P14, a target value for uncertainty (U, k=2) of 2 % had been set. It was decided not to set a target value for uncertainty (U, k=2) for this key comparison. Nevertheless it is observed that all except 2 laboratories reported results that reached the target value of uncertainty as set for the pilot study. Moreover 5 of the 9 laboratories reported a result with an expanded uncertainty lower than 1 %.

The outcome of CCQM-K14 shows hence that several participants have sufficient measurement capability to support the clinical community with SI-traceable reference values for S-Ca_{tot} at the required uncertainty level.

Results of CCQM-K14 are going to be used for documentation of measurement capability in view of the CIPM MRA. The Key Comparison Reference Value (KCRV), and based on this the calculated equivalence statements will be placed in the Appendix B of the MRA after approval by CCQM.

The material of this key comparison will be available for some years in an External Quality Assurance Scheme. (DEKS identification FHK 0108). Therefore field laboratories will be able to compare their results with the KCRV hence establishing traceability of measurement results up to the highest level of the international measurement infrastructure.

9. Acknowledgement

The work described here contains the contributions of many scientists and contact persons at the various participating institutes: R. Arvizu from CENAM; A. Barzev from CSIR-NML; E. Zeiller, R. Schorn, A. Toervenyi and K. Burns from IAEA; M. Berglund, C. Hennessy and S. Duta from IRMM; E. Hwang from KRISS; R. Hearn and L. Simpson from LGC; S. Long, K. Murphy and G. Turk from NIST; M. Van Son from NMi-VSL; D. Schiel and O. Rienitz from PTB. Special thanks is ought to all colleagues at IRMM who gave assistance throughout this project and to U. Örnemark for his valuable suggestions. The pilot laboratory acknowledges in particular the efforts of A. Uldall and G. Henriksen from DEKS for supplying the samples and of M. Sargent (LGC) for his assistance as chairman of the Inorganic Analysis WG of CCQM.

References

1 D. Fraser, G. Jones, S.W. Kooh, I.C. Radde, Calcium and phosphate metabolism, in: Tietz NW. ed., Fundamentals of Clin. Chem, 3rd ed., Philadelphia, WB Saunders; 1987, pp. 705-728

2 U. Örnemark,Y. Aregbe, L. Van Nevel, I. Papadakis, P.D.P. Taylor, A. Uldall, CCQM-P14 report, Internal IRMM report number GE/R/IM/27/01

3 L. Van Nevel, et al., IMEP-17 Trace and minor constituents in human serum. Report to participants. Part 1 International comparability, EUR 20657 EN - Internal IRMM report GE/R/IM/42/02 (available on www.imep.ws)

4. U. Örnemark, A. Uldall, Y. Aregbe, L. Van Nevel, P.D.P. Taylor, IMEP-17, Trace and minor constituents in human serum - Certification report, internal report GE/R/IM/36/01, IRMM, Geel, Belgium (available on www.imep.ws)

5 Gitte M. Nielsen (DEKS, Denmark): personal communication (e-mail 04/06/2002)

6 Gitte M. Henriksen (DEKS, Denmark): personal communication (e-mail 19/06/2003)

7 Guide to the Expression of Uncertainty in Measurement, ISBN 92-67-10188-9 ISO, Geneva (corrected and reprinted 1995)

8 Eurachem/CITAC, Quantifying uncertainty in analytical measurement, 2nd ed., www.eurachem.bam.de

9 Royal Society of Chemistry, Robust Statistics, *Analyst* (1989) 114