CCQM-K11.2 Determination of Glucose in Human Serum
and
CCQM-K12.2 Determination of Creatinine in Human Serum

Final Report
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SUMMARY

Glucose and creatinine are two of the most frequently measured substances in human blood/serum for assessing the health status of individuals. Because of their clinical significance, CCQM-K11 Glucose in Human Serum and CCQM-K12 Creatinine in Human Serum were the fourth and fifth Key Comparisons (KCs) performed by the Organic Analysis Working Group (OAWG). These KCs were conducted in parallel and were completed in 2001. The initial Subsequent KCs for glucose, CCQM-K11.1, and creatinine, CCQM-K12.1, were completed in 2005. Measurements for the next KCs for these two measurands, CCQM-K11.2 and CCQM-K12.2, were completed in 2013. While designed as Subsequent KCs, systematic discordances between the participants' and the anchor institution's results in both comparisons lead the OAWG to request reference results from two experienced laboratories that had participated in the 2001 comparisons. Based on the totality of the available information, the OAWG converted both CCQM-K11.2 and CCQM-K12.2 to "Track C" KCs where the Key Comparison Reference Value is estimated by consensus.

These comparisons highlighted that carrying out comparisons for complex chemical measurements and expecting to be able to treat them under the approaches used for formal subsequent comparisons is not an appropriate strategy. The approach used here is a compromise to gain the best value from the comparison; it is not an approach that will be used in the future. Instead, the OAWG will focus on Track A and Track C comparisons that are treated as stand-alone entities.

Participation in CCQM-K11.2 demonstrates a laboratory's capabilities to measure a polar ($pK_{ow} > 2$), low molecular mass (100 g/mol to 500 g/mol) metabolite in human serum at relatively high concentrations (0.1 mg/g to 10 mg/g). Participation in CCQM-K12.2 demonstrates capabilities to measure similar classes of metabolites at relatively low concentrations (1 µg/g to 30 µg/g). The capabilities required for the analysis of complex biological matrices include sample preparation (protein precipitation, extraction, derivatization), gas chromatographic (GC) or liquid chromatographic (LC) separation, and quantification using an isotope dilution mass spectrometry (IDMS) approach.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CENAM	Centro Nacional de Metrologia, México
CMC	calibration and measurement capabilities
CRM	certified reference material
DI	designated institute
GC	gas chromatography
GC-MS	gas chromatography with mass spectrometry detection
HSA	Health Sciences Authority, Singapore
ID	isotope dilution
INMETRO	Instituto Nacional de Metrologia, Qualidade e Tecnologia, Brazil
JCTLM	Joint Committee for Traceability in Laboratory Medicine
KC	Key Comparison
KCRV	Key Comparison Reference Value
KRISS	Korea Research Institute of Standards and Science, Republic of Korea
LC	liquid chromatography
LC-MS	liquid chromatography with mass spectrometry detection
LC-MS/MS	liquid chromatography with tandem mass spectrometry detection
LNE	Laboratoire National de Métrologie et d'Essais, France
MADe	median absolute deviation from the median (MAD)-based estimate of s: MADe = 1.4826·MAD, where MAD = median($\|x_i$-median(x_i)$\|$)
NIM	National Institute of Metrology, China
NIMT	National Institute of Metrology (Thailand), Thailand
NIST	National Institute of Standards and Technology, USA
NMI	national metrology institute
NMI/DI	national metrology institute or designated institute
NMIJ	National Metrology Institute of Japan, Japan
OAWG	Organic Analysis Working Group
pK_{ow}	logarithm of the octanol-water partition coefficient
PTB	Physikalisch-Technische Bundesanstalt, Germany
SRM	Standard Reference Material, a NIST CRM
UME	National Metrology Institute of Turkey, Turkey
VNIIM	D.I. Mendeleyev Institute for Metrology, Russian Federation

INTRODUCTION

Cholesterol, glucose, and creatinine are three of the most frequently measured substances in human blood/serum to assist in assessing the health status of individuals. Because of their clinical significance, measurements of cholesterol, glucose, and creatinine were three of the first five key comparisons (KCs) conducted by the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM) Organic Analysis Working Group (OAWG). These KCs were completed in 2000 and 2001 with the National Institute of Standards and Technology (NIST) as the coordinating laboratory [1,2,3]. Subsequent KCs were conducted for the three measurands in 2005 with the Korea Research Institute of Standards and Science (KRISS) or NIST as the coordinating laboratories. Table 1 lists these comparisons.

Table 1: Previous Comparisons of Cholesterol, Glucose, and Creatinine

Comparison	Name of Comparison	Date	Coordinating Laboratory	Number of Participants
CCQM-P6	Cholesterol in Human Serum	1999	NIST	7
CCQM-K6	Cholesterol in Human Serum	2000	NIST	7
CCQM-K6.1	Cholesterol in Human Serum	2005	NIST	2
CCQM-P8	Glucose in Human Serum	2000	NIST	4
CCQM-K11	Glucose in Human Serum	2001	NIST	3
CCQM-K11.1	Glucose in Human Serum	2005	KRISS	3
CCQM-P9	Creatinine in Human Serum	2000	NIST	4
CCQM-K12	Creatinine in Human Serum	2001	NIST	5
CCQM-K12.1	Creatinine in Human Serum	2005	KRISS	3

Since these earlier comparisons were conducted, additional National Metrology Institutes (NMIs) or their designated institutes (DI) are now providing measurement services for one or more of these clinical measurands. At the April 2012 OAWG meeting a proposal was accepted to conduct Subsequent KCs for the three measurands with NIST as the coordinating laboratory. These three comparisons were to be conducted in parallel. They were designed as Subsequent KCs, i.e., one laboratory would serve as the anchor laboratory to which the results of the participants would be compared.

Due to discordant results between the anchor and participant results in CCQM-K11.2 and CCQM-K12.2, KRISS and the Physikalisch-Technische Bundesanstalt (PTB) were requested by the OAWG to provide measurements for both glucose and creatinine. These institutions had participated in the original comparisons. While the results provided by KRISS and PTB for creatinine agreed well with NIST's result, their results for glucose were themselves discordant. Further investigations by NIST, PTB, and the Health Sciences Authority (HSA) failed to identify the cause of the differences. After considerable debate, at its April 2015 meeting the OAWG decided to estimate KCRVs from the consensus of participant and reference laboratory results. The decision to treat CCQM-K11.2 and CCQM-K12.2 as Track C KCs was considered a more practical choice than the alternative of abandoning both comparisons.

This report describes only CCQM K11.2 and K12.2. CCQM-K6.2 met the requirements of a Subsequent KC and the results are described in a separate report.

TIMELINE

The timeline for CCQM-K11.2 and CCQM-K12.2 is summarized in Table 2.

Table 2: CCQM-K11.2 and CCQM K12.2 Timeline

Date	Action
Apr. 2012	OAWG authorized CCQM-K6.2, CCQM-K11.2, and CCQM-K12.2 Subsequent KCs and approved protocols
Nov. 2012	Call for Participation to OAWG members
Dec. 2012	Samples shipped to participants
Apr. 2013	Preliminary results presented to OAWG at Paris meeting. KRISS and PTB asked to provide reference measurements for CCQM-K11.2 and CCQM-K12.2
Nov. 2013	Reference results from PTB and KRISS received and discussed at CCQM meeting in South Africa
Apr. 2014	Further discussion of how to assign KCRV for CCQM-K11.2 and CCQM-K12.2; decision to assign values from participant and reference laboratory results
Apr. 2015	Draft A Report discussed; decision to prepare two Draft A Reports, one for CCQM-K6.2 and a second report for CCQM-K11.2 and CCQM-K12.2, which are to be treated as Track C KCs rather than Subsequent KCs
Oct. 2015	Draft A Report distributed to OAWG
Nov. 2015	Draft B report distributed to OAWG
Sep. 2016	Feedback from CCQM WG chairs
Apr. 2018	Final report delivered to OAWG Chair

MEASURANDS

The measurands for the three clinical comparisons were the mass fractions of cholesterol, glucose, and creatinine in human serum as previously defined in CCQM-K6, CCQM-K11, and CCQM-K12. These three clinical health status markers were selected to be representative of measurement challenges associated with well-defined and low molar mass organic substances in blood.

For CCQM-K11.2 the measurand was the mass fraction of glucose. Glucose (molar mass 180 g/mol) is a polar ($pK_{ow} = 2.8$), highly water-soluble (909 g/L) analyte that is present in human serum at relatively high concentrations (0.5 mg/g to 1.5 mg/g). Glucose is partially associated with serum proteins.

For CCQM-K12.2 the measurand was the mass fraction of creatinine. Creatinine (molar mass 113 g/mol) is a polar analyte ($pK_{ow} = 1.8$) that is present in human serum at relatively low concentrations (1 µg/g to 30 µg/g). Without proper handling, creatine (the open-ring analog) and creatinine can interconvert, leading to biased results.

COMPARISON MATERIALS

The study materials for CCQM-K11.2 and CCQM-K12.2 were subsamples from two serum pools that were either an existing NIST CRM or a candidate CRM intended for the determination of cholesterol. These materials contain naturally occurring concentrations of glucose and creatinine.

The study material for CCQM-K11.2 was SRM 1951b Cholesterol in Frozen Human Serum (Level 2), which has a certified value for cholesterol, but no values assigned for glucose [4]. The inventory of this CRM was depleted in 2012 shortly after the CCQM-K11.2 study results were submitted. For CCQM-K12.2, the study material was candidate SRM 1951c Lipids in Frozen Human Serum (Level 2), which was issued in June 2013 with certified values for cholesterol but no values for creatinine [5].

Participants were provided with three vials of serum for determination of each measurand. Each vial contained 1 mL of human serum. Samples were shipped on dry ice. Participants were instructed that a -20 ºC freezer was adequate for storage up to one week; however, if longer storage time was anticipated, the material should be stored at temperatures of -60 ºC or below.

Homogeneity and Stability Assessment

Based on nearly two decades of experience with frozen serum samples prepared as CRMs for these measurands, the materials used in these comparisons were expected to be adequately homogeneous and stable.

The material used in CCQM-K11.2 had been assessed for cholesterol homogeneity in 2003; the analysis of 18 vials yielded a CV of 0.12 %. Glucose measurements were made in 2012 on three vials, yielding a combined within- and between-vial CV of 1.2 %.

The material used in CCQM-K12.2 was assessed for cholesterol homogeneity as part of the certification measurements; the analysis of 15 vials yielded a CV of 0.47 %. In April 2013, creatinine homogeneity was assessed using two aliquots each from three vials, yielding a CV of 1.65 %. Analyses of ten vials of the CRM in February 2014 confirmed this assessment, yielding a CV of 2.00 %. The mean values of the 2013 and 2014 assessments agreed well within the measurement uncertainty, confirming the CRM's creatinine stability.

PARTICIPANTS AND INSTRUCTIONS

Participants were requested to analyze two vials of material for each measurand; the number of subsamples from each vial was left up to the laboratories. Participants were encouraged to use an appropriate serum-matrix CRM as a control material. Participants were to report the mass of glucose (CCQM-K11.2) and creatinine (CCQM-K12.2) per mass of serum (mg/g) in the reporting form provided. The reporting form also requested descriptions of methods used, number and order of measurements, reference compounds used as calibrants with purity corrections, control materials used, method of calculating results, and a description of their uncertainty calculations.

Nine NMIs or DIs (NMI/DIs) participated in CCQM-K11.2 and eight NMI/DIs in CCQM-K12.2. NIST was designated as the anchor laboratory for these two comparisons. However, due to discordance of the results for glucose and creatinine between NIST and most participants, KRISS and PTB were asked to analyze the comparison materials and act as additional reference laboratories. The participants and reference laboratories for both comparisons are listed in Table 3.

Table 3: Participants and Reference Laboratories in CCQM-K11.2 and CCQM-K12.2

NMI/DI	CCQM-K11.2 Glucose	CCQM-K12.2 Creatinine
CENAM	Participant	Participant
HSA	Participant	Participant
INMETRO	Participant	Participant
LNE	Participant	Participant
NIM	Participant	*a*
NIMT	Participant	Participant
NMIJ	Participant	Participant
UME	Participant	Participant
VNIIM	Participant	Participant
NIST	Reference	Reference
KRISS	Reference	Reference
PTB	Reference	Reference

a Did not participate in CCQM-K12.2

CCQM-K11.2 GLUCOSE IN HUMAN SERUM

Methods Used

Results were received from nine participants for CCQM-K11.2 Glucose in Human Serum. The participants used either ID GC-MS (five labs) or isotope dilution liquid chromatography tandem mass spectrometry (ID LC-MS/MS) (six labs). HSA used both ID GC-MS and ID LC-MS/MS; they reported their result as a combination of the two methods. The analytical methods including sample preparation, analytical technique, and quantification approach are summarized for all participant and reference laboratories in Appendix A.

Participant Results

The results for CCQM-K11.2 as received from the participants for measurements on each of two vials are summarized in Table 4. The evaluation of the results including the combination of the measurements on each vial and the corresponding uncertainties are summarized in Table 5. Figure 1 displays the participants' evaluated results and robust consensus location and dispersion estimates. All results appear to be members of an approximately Gaussian (normal) distribution.

Table 4: Results for CCQM-K11.2 Glucose in Human Serum as Received

		Mass Fraction, mg/g			Coverage Factor (k)
NMI/DI	**Vial**	x	$u(x)$	$U_{95}(x)$	
CENAM	1	1.146	0.006	0.019	3.182
	2	1.142	0.006	0.019	3.186
HSA	1	1.155	0.0060	0.012	2
	2	1.156	0.0060	0.012	2
INMETRO	1	1.14	0.0080	0.02	2.57
	2	1.14	0.0056	0.01	2.16
LNE	1	1.161	0.005	0.011	2
	2	1.157	0.006	0.012	2
NIM	1	1.159	0.0062	0.013	2
	2	1.158	0.0051	0.011	2
NIMT	1	1.171	0.017	0.034	2.06
	2	1.184	0.020	0.040	2.03
NMIJ	1	1.172	0.0081	0.017	2
	2	1.171	0.0081	0.017	2
UME	1	1.159	0.0070	0.014	2
	2	1.149	0.0061	0.012	2
VNIIM	1	1.168	0.013	0.026	2
	2	1.170	0.013	0.026	2

Table 5: Participant Results for CCQM-K11.2 Glucose in Human Serum as Combined

	Mass Fraction, mg/g				
NMI/DI	$\bar{x}$	s	$\bar{u}(x)$	$u(\bar{x})$	$U_{k=2}(\bar{x})$
CENAM	1.144	0.003	0.010	0.007	0.014
HSA	1.156	0.001	0.006	0.004	0.009
INMETRO	1.14	0.000	0.008	0.006	0.011
LNE	1.159	0.003	0.006	0.005	0.009
NIM	1.159	0.001	0.006	0.004	0.009
NIMT	1.178	0.009	0.019	0.015	0.029
NMIJ	1.172	0.001	0.009	0.006	0.012
UME	1.154	0.007	0.007	0.007	0.014
VNIIM	1.169	0.001	0.013	0.009	0.018

$\bar{x}$ Mean of the two reported results, x_1 and x_2, where the index indicates the vial

s Standard deviation of the two reported results

$\bar{u}(x)$ Pooled standard uncertainties: $\sqrt{\left(\frac{U_{k=2}^2(x_1)+U_{k=2}^2(x_2)}{2}\right)/2}$

$u(\bar{x})$ Standard uncertainty of the mean: $\left(\sqrt{s^2+\bar{u}^2(x)}\right)/\sqrt{2}$

$U_{k=2}(\bar{x})$ $k = 2$ expanded uncertainty of the mean: $2 \cdot u(\bar{x})$

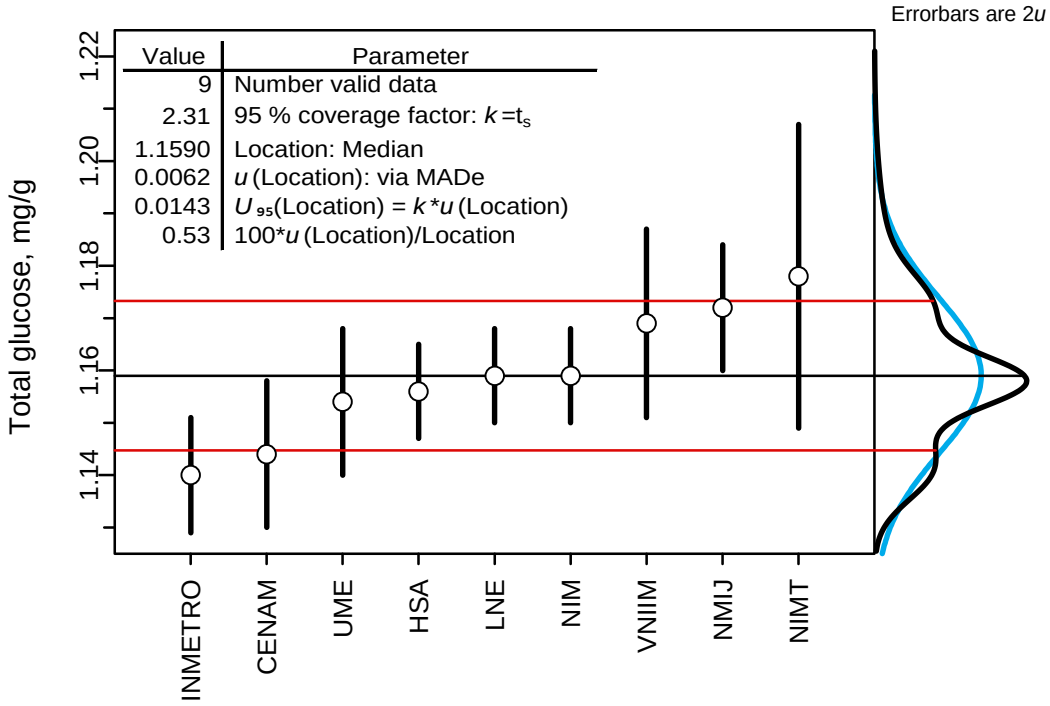

Figure 1: Participant results for CCQM-K11.2

Dots represent the combined values; the vertical bars on the dots span the $k = 2$ expanded uncertainties. The black horizontal line represents the median. The red horizontal lines bracket a robust estimate of the 95 % coverage interval about the median, U_{95}. This interval is estimated as the product of the: standard uncertainty, u, estimated as the median absolute deviation from the median scaled to have the same coverage of a normal distribution as provided by the standard deviation (MADe) [6]; a factor of 1.25 reflecting the efficiency of the median as an estimator of the location for normally distributed data; and the 2.31 expansion factor of the Student's t distribution for 8 degrees of freedom. The black curve to the right edge is the empirical probability density for the reported results; the blue curve to the right edge is the Gaussian probability density function with the robust location and dispersion estimates.

Results from NIST, the Intended Anchor Laboratory

The NIST glucose measurements were made using a modified definitive isotope dilution GC-MS procedure [7,8]. The method is summarized in Tables A1 and A2 in Appendix A.

Measurements for glucose in the CCQM-K11.2 material were completed in January 2012. The NIST value for glucose, (121.46 ± 1.74) mg/dL, was based on measurements of duplicate GC-MS injections for duplicate subsamples from one vial and single subsamples for two additional vials. The density of the material, (102.26 ± 0.02) g/dL, was determined by the Lang-Levy pipet method at ambient balance room temperature (21.5 °C). Combining the two values, the NIST value for CCQM-K11.2 is (1.188 ± 0.017) mg/g.

First Report and Evaluation of Results

The difference between the consensus and NIST results, |1.159-1.188| = 0.029 mg/g (approximately 2.5 %) is greater than the expanded uncertainty of the difference, $\sqrt{0.015^2 + 0.017^2}$ = 0.023 mg/g. The NIST value is also higher than all participant results. The situation was discussed at the April 2013 meeting of the OAWG. The expectation was that there

should be better agreement and potential explanations for this disagreement were explored. It was suggested that there could be some equilibration issues with extraction of serum samples containing high lipid content. The CCQM-K11.2 material has a relatively high lipid content with cholesterol at (266.58 ± 0.84) mg/dL and total glycerides at (264.6 ± 3.2) mg/dL. Therefore, information on equilibration times was requested from all participants for evaluation and discussion at the November 2013 OAWG meeting. The updated information on equilibration time, calibrants, internal standards used, CRMs used, and analytical technique are summarized in Table A3 in Appendix A.

Because this was intended as a Subsequent KC rather than a Track A core competency KC, the participants in CCQM-K11, including KRISS and PTB, did not participate in CCQM-K11.2. However, to assist in determining the appropriate mass fraction of glucose in the K11.2 material, PTB and KRISS were requested to analyze the CCQM-K11.2 material as reference laboratories. Samples were sent to KRISS and PTB in July 2013 and the results of their analyses were received in October 2013. The methods used by KRISS and PTB are summarized in Table A1 and A2 in Appendix A.

In parallel with the additional analyses by KRISS and PTB, HSA undertook an extensive study addressing the possibility of equilibration time as a cause of discrepancies between NIST results and the participant results. HSA studied two serum pool samples from their quality assurance program with high and low lipid content as well as additional units of the CCQM-K12.2 material. HSA evaluated the mass fraction of glucose and creatinine determined using equilibration times of 2 h, 6 h, 21 h, and 30 h. Their conclusion was that equilibration time had no effect on measurement results for either low or high lipid content samples. In addition, after compiling the equilibration time and calibrant information (see Table A3), it was shown that participants used equilibration times of between 0 h to 20 h with no indication of a significant effect.

Results from KRISS and PTB, Reference Laboratories

Results from KRISS and PTB were received in October 2013 just prior to the November 2013 CCQM meeting in South Africa. KRISS analyzed three aliquots from each of two vials using LC-MS/MS, reporting values and 95 % expanded uncertainties of (1.186 ± 0.015) mg/g and (1.185 ± 0.014) mg/g. Combining the values reported for the two vials, the KRISS result for CCQM-K11.2 is (1.186 ± 0.011) mg/g. KRISS also analyzed KRISS CRM 111-01-008 as a control material; the measured value and 95 % expanded uncertainty of (1.199 ± 0.018) mg/g was in excellent agreement with the certified value, (1.195 ± 0.025) mg/g. PTB analyzed single aliquots from six vials using ID GC-MS, reporting a combined value of (1.141 ± 0.013) mg/g. PTB also performed an equilibrium time study for glucose in the study material with equilibrium times of 0 h, 2 h, 4 h, 6 h, 8 h, and 12 h. In agreement with the HSA study, PTB concluded that equilibration time has no significant effect on measurement results.

The results from KRISS, NIST, and PTB are summarized in Table 6 and are displayed relative to the participant results in Figure 2.

Table 6: Reference Results for CCQM-K11.2 Glucose in Human Serum

	Mass Fraction, mg/g		
Reference Laboratory	**Value**	***u*(Value)**	**$U_{k=2}$(Value)**
KRISS	1.186	0.006	0.011
NIST	1.188	0.008	0.017
PTB	1.141	0.007	0.013

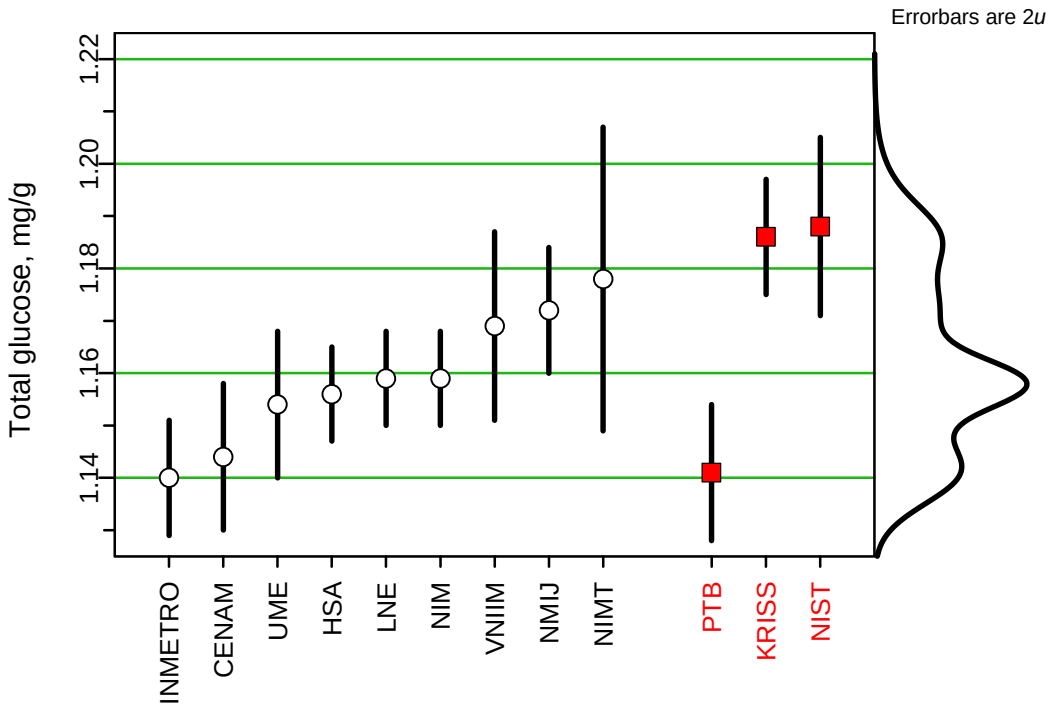

Figure 2: Comparison of KRISS, NIST, PTB, and CCQM-K11.2 Participant Results.

Format as in Figure 1, but with results for the three reference laboratories displayed as red squares. The green horizontal lines are for visual guidance.

Key Comparison Reference Value (KCRV)

Because the range of NIST, PTB, and KRISS results encompassed the participant results, there was considerable discussion within the OAWG at the April 2014 meeting on how to assign a KCRV. Despite assessment by the participants of their methodologies, no clear reason was established for the relatively large range of values. The decision was made to assign the KCRV from the mean, standard deviation, and pooled standard uncertainty of the results reported by the three reference laboratories and the nine participants. It was agreed that although all results would be used to calculate the KCRV, degrees of equivalence would not be estimated for the three reference laboratories.

Table 7 lists the results and statistics used to assign the KCRV. The participant results, both as reported and as combined, and the reference laboratory results are displayed in Figure 3 relative to the KCRV. Figure 4 displays only the participants combined results relative to the KCRV.

Table 7: Assignment of KCRV for K11.2 Glucose in Human Serum

Mass Fraction, mg/g

NMI/DI	Result	u(Result)	$U_{k=2}$(Result)
INMETRO	1.140	0.006	0.011
PTB	1.141	0.007	0.013
CENAM	1.144	0.007	0.014
UME	1.154	0.007	0.014
HSA	1.156	0.005	0.009
LNE	1.159	0.005	0.009
NIM	1.159	0.005	0.009
VNIIM	1.169	0.009	0.018
NMIJ	1.172	0.006	0.012
NIMT	1.178	0.015	0.029
KRISS	1.186	0.006	0.011
NIST	1.188	0.008	0.017

Parameter [a]	Value	Units
n	12	
$\bar{x}$	1.1642	mg/g
s	0.0158	mg/g
$\bar{u}(x)$	0.0074	mg/g
$u(x)$	0.0174	mg/g
$u(\bar{x})$	0.0050	mg/g
$U_{k=2}(\bar{x})$	0.0101	mg/g
KCRV	1.164	mg/g
$U_{k=2}$(KCRV)	0.010	mg/g

a Statistics:

n	Number of results included in calculations
$\bar{x}$	Mean of results
s	Standard deviation of results
$\bar{u}(x)$	Pooled standard uncertainties of the reported values: $\sqrt{\left(\sum_i \frac{U_{k=2}^2(x_i)}{2}\right)/n}$
$u(x)$	Combined standard uncertainty of the reported values: $\sqrt{s^2 + \bar{u}^2(x)}$
$u(\bar{x})$	Standard uncertainty of the mean: $u(x)/\sqrt{n}$
$U_{k=2}(\bar{x})$	$k = 2$ expanded uncertainty of the mean: $2 \cdot u(\bar{x})$

Degrees of Equivalence

The absolute degrees of equivalence for the participants in CCQM-K11.2 are estimated as the signed difference between the combined value and the KCRV: $d_i = x_i$ – KCRV. The expanded uncertainty on the d_i, $U_{k=2}(d_i)$, can be estimated as the square root of the sum of the squares of the expanded uncertainties of the two components: $U_{k=2}(d_i) = \sqrt{U_{k=2}^2(x_i) + U_{k=2}^2(\text{KCRV})}$.

To enable comparison with the degrees of equivalence estimates from CCQM-K11 and CCQM-K11.1, it is convenient to express the d_i and $U_{95}(d_i)$ as percentages relative to the KCRV: $\%d_i = 100 \cdot d_i/\text{KCRV}$ and $U_{k=2}(\%d_i) = 100 \cdot U_{k=2}(d_i)/\text{KCRV}$. Table 8 lists the numeric values of d_i, $U_{k=2}(d_i)$, $\%d_i$, and $U_{k=2}(\%d_i)$ for all participants in CCQM-K11.2. The absolute $d_i \pm U_{k=2}(d_i)$ for CCQM-K11.2 is displayed in Figure 5. The relative $\%d_i \pm U_{k=2}(\%d_i)$ for CCQM-K11, CCQM-K11.1, and CCQM-K11.2 is displayed in Figure 6.

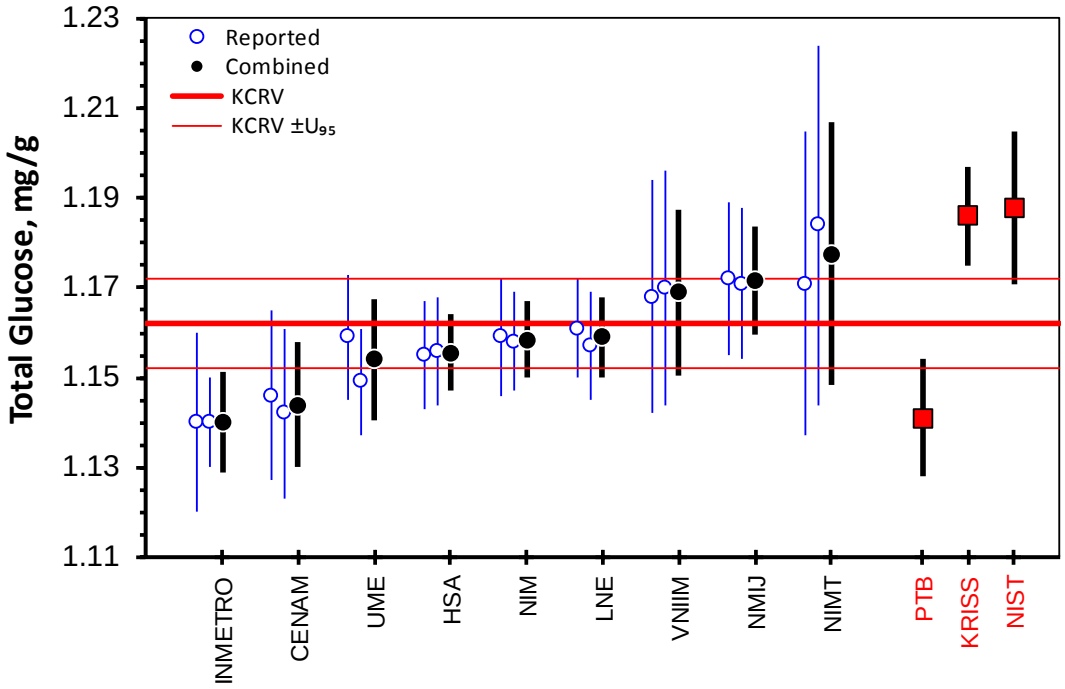

Figure 3: Participant results for CCQM-K11.2 relative to the KCRV.

The blue symbols and vertical bars represent the results as reported; the black symbols and bars represent the combined results. The bars are approximate 95 % expanded uncertainties. The horizontal lines represent the KCRV and the KCRV ± U_{95}(KCRV) interval. The red squares denote the reference laboratory results.

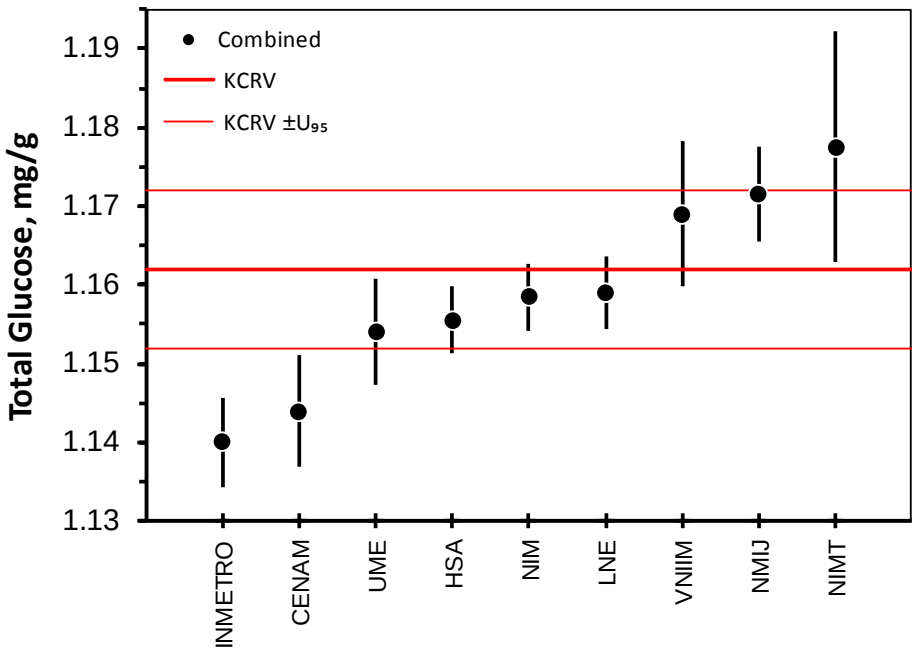

Figure 4: Participant results for CCQM-K11.2 relative to the KCRV.

As above, but showing only the combined results for the participants and with the bars representing standard uncertainties.

Table 8: Degrees of Equivalence for CCQM-K11.2 Glucose in Human Serum

	mg/g		%	
NMI/DI	d_i	$U_{k=2}(d_i)$	$\%d_i$	$U_{k=2}(\%d_i)$
CENAM	-0.018	0.017	-1.5	1.5
HSA	-0.007	0.013	-0.6	1.1
INMETRO	-0.022	0.015	-1.9	1.3
LNE	-0.003	0.014	-0.3	1.2
NIM	-0.004	0.013	-0.3	1.1
NIMT	0.015	0.031	1.3	2.7
NMIJ	0.009	0.016	0.8	1.3
UME	-0.008	0.017	-0.7	1.5
VNIIM	0.007	0.021	0.6	1.8

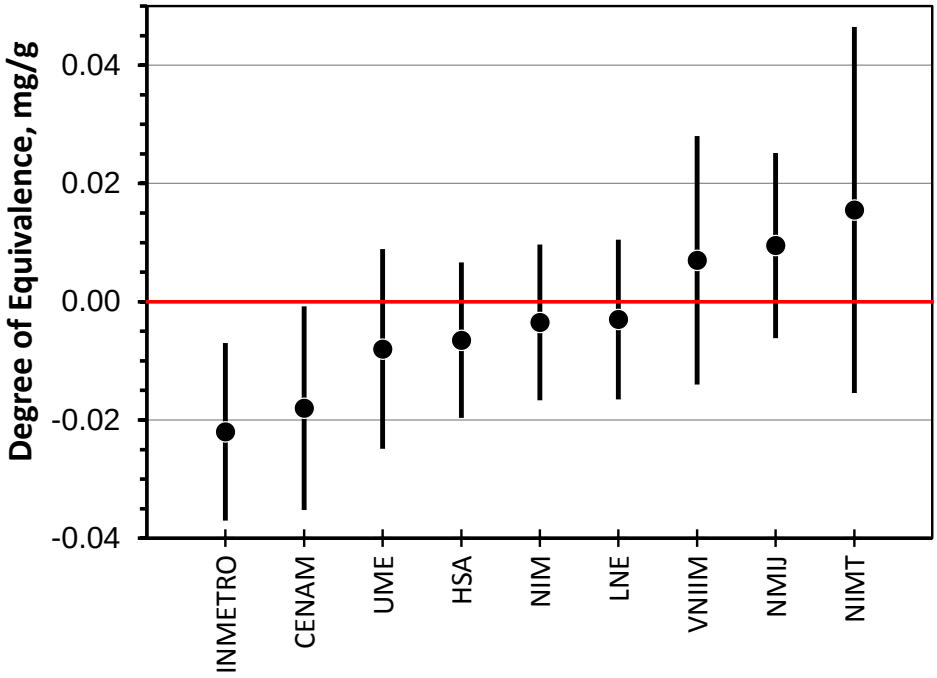

Figure 5: Absolute degrees of equivalence for CCQM-K11.2.

The black symbols and vertical bars represent the $d_i \pm U_{95}(d_i)$. The horizontal line marks the ideal zero deviation from the KCRV.

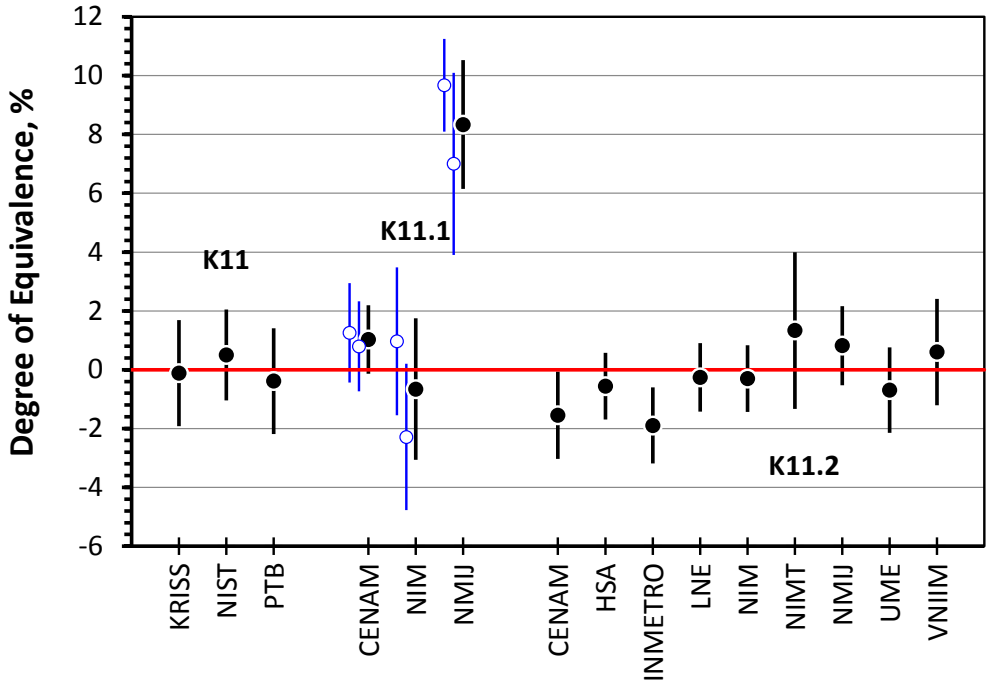

Figure 6: Relative degrees of equivalence for the CCQM-K11 comparisons.

The blue symbols and bars represent $\%d_i \pm U_{95}(\%d_i)$ for individual materials distributed in CCQM-K11.1; the black symbols and vertical bars represent the combined $\%d_i \pm U_{95}(\%d_i)$. The red horizontal line marks the ideal zero deviation from the KCRV; the light grey lines are for visual guidance.

Use of CCQM-K11.2 to Support Calibration and Measurement Capability (CMC) Claims

CCQM-K11.2 Glucose in Human Serum was intended as a Subsequent KC for NMIs and DIs that had not participated in earlier comparisons for determination of glucose. However, due to unexplained systematic discordance between the participants' and anchor institution's results, the comparison did not meet the OAWG's expectations for a Subsequent KC and in consequence is now treated as a Track C KC. The KCRV is estimated from the combined results of the nine participants and the three reference institutions. The $d_i \pm U_{95}(d_i)$ for seven of the nine participants includes zero. The $|\%d_i|$ for all participants is better than 2 %.

The KC demonstrates a laboratory's capabilities to measure a polar ($pK_{ow} > 2$), low molecular mass (100 g/mol to 500 g/mol) metabolite in human serum at relatively high concentrations (0.1 mg/g to 10 mg/g). The concentration of glucose found in normal human populations is typically 0.5 mg/g to 1.5 mg/g. At the time this KC was conducted, the OAWG had not formalized the reporting of "core competencies". However, participation in CCQM-K11.2 demonstrates capabilities in analysis of complex biological matrices including sample preparation (protein precipitation, extraction, derivatization), LC or GC separation, and quantification using an isotope dilution mass spectrometry (IDMS) approach.

K12.2 CREATININE IN HUMAN SERUM

Methods Used

Results were received from eight participants for K12.2 Creatinine in Human Serum. All participants used LC-based methods with either ID LC-MS (two labs) or ID LC-MS/MS (six labs). Two of the laboratories use UPLC rather than conventional LC. The KRISS, NIST, and PTB reference laboratories used ID LC-MS/MS, an ID LC-MS procedure [9] based on the method of Stokes and O'Connor [10], and ID GC-MS, respectively. The analytical methods used by the participants and the reference laboratories are summarized in Table C1 and C2 in Appendix C.

Participant Results

The results for K12.2 as received from the participants for measurements on each of two vials are summarized in Table 9. CENAM reported their result, for only one vial, after the April 2013 cutoff date. The evaluation of the results including the combination of the measurements on each vial and the corresponding uncertainties are summarized in Table 10. The participants' evaluated results and robust consensus location and dispersion estimates are displayed in Figure 7. Results from seven of the eight participants appear to be members of an approximately Gaussian (normal) distribution; the remaining result is a potential technical outlier.

Table 9: Results for CCQM-K12.2 Creatinine in Human Serum as Received

		Mass Fraction, µg/g			Coverage Factor (k)
NMI/DI	**Vial**	x	$u(x)$	$U_{95}(x)$	
CENAM [a]	1	7.245	0.091	0.190	2.10
HSA	1	7.34	0.055	0.11	2
	2	7.36	0.055	0.11	2
INMETRO	1	7.95	0.17	0.40	2.306
	2	7.70	0.11	0.23	2.080
LNE	1	7.368	0.076	0.152	2
	2	7.432	0.057	0.114	2
NIMT	1	7.45	0.13	0.26	2.02
	2	7.43	0.13	0.27	2.02
NMIJ	1	7.43	0.07	0.15	2
	2	7.43	0.07	0.13	2
UME	1	7.472	0.037	0.075	2
	2	7.481	0.039	0.077	2
VNIIM	1	7.477	0.084	0.168	2
	2	7.473	0.084	0.168	2

a Information only, result submitted after cutoff date.

Table 10: Participant Results for CCQM-K12.2 Creatinine in Human Serum as Combined

	Mass Fraction, µg/g				
NMI/DI	$\bar{x}$	s	$\bar{u}(x)$	$u(\bar{x})$	$U_{k=2}(\bar{x})$
CENAM [a]	7.245		0.091		0.190
HSA	7.35	0.014	0.055	0.040	0.08
INMETRO	7.83	0.177	0.163	0.170	0.34
LNE	7.400	0.045	0.067	0.057	0.115
NIMT	7.44	0.011	0.131	0.093	0.19
NMIJ	7.43	0.000	0.070	0.050	0.10
UME	7.477	0.006	0.038	0.027	0.054
VNIIM	7.475	0.003	0.084	0.059	0.119

a Information only, result submitted after cutoff date.

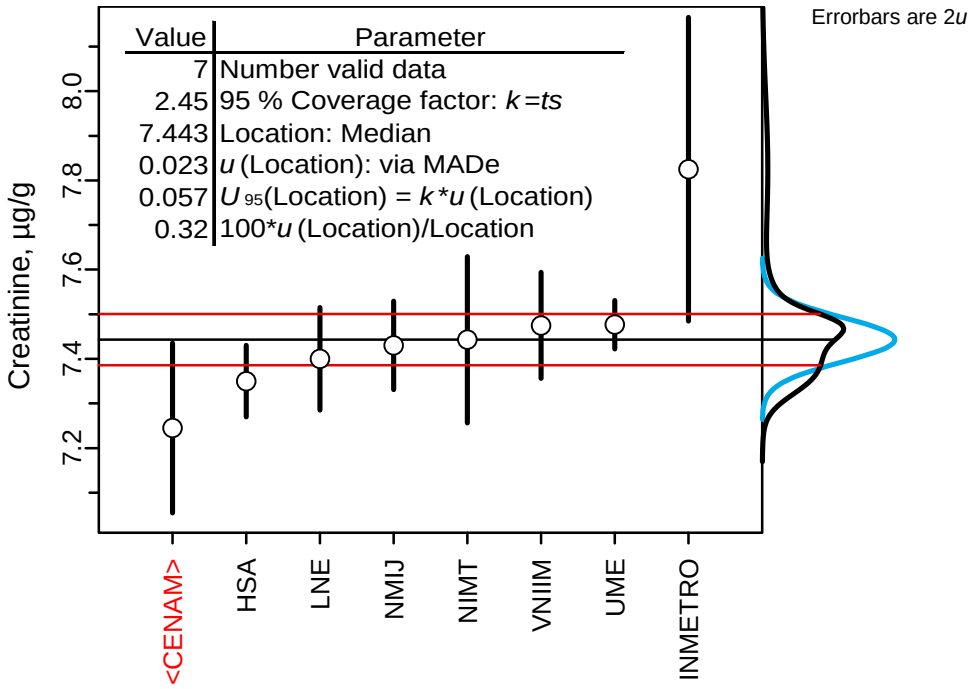

Figure 7: Combined participant results for CCQM-K12.2.

Dots represent the combined values; the vertical bars on the dots span the k = 2 expanded uncertainties. The black horizontal line represents the median. The red horizontal lines bracket a robust estimate of the 95 % coverage interval about the median, U_{95}. This interval is estimated as the product of the: standard uncertainty, u, estimated as the median absolute deviation from the median scaled to have the same coverage of a normal distribution as provided by the standard deviation (MADe) [6]; a factor of 1.25 reflecting the efficiency of the median as an estimator of the location for normally distributed data; and the 2.31 expansion factor of the Student's t distribution for 8 degrees of freedom. The black curve to the right edge is the empirical probability density for the reported results; the blue curve to the right edge is the Gaussian probability density function with the robust location and dispersion estimates. The CENAM result was received after the comparison's cutoff date and is not included in either the robust estimates or the empirical PDF.

Results from NIST, the Intended Anchor Laboratory

The material used for K12.2 was a candidate CRM that was to be certified for cholesterol content [5]. Creatinine measurements for this material were performed in April 2013. The NIST assigned value for creatinine was based on measurements using ID LC-MS with duplicate subsamples from three vials. The NIST value for creatinine in was (7.67 ± 0.15) μg/g. NIST analyzed single vials of SRM 909c and SRM 967a in duplicate as control materials; the measured values and standard deviations, (8.07 ± 0.18) μg/g and (8.41 ± 0.12) μg/g, were in good agreement with the certified values, (8.05 ± 0.18) μg/g and (8.28 ± 0.18) μg/g. Prior to the April 2014 OAWG meeting, NIST confirmed their original value with the analysis of 10 additional vials of the CRM in duplicate obtaining a value of (7.632 ± 0.072) μg/g.

First Report and Evaluation of Results

The difference between the consensus and NIST results, |7.443-7.67| = 0.23 μg/g (approximately 3.0 %) is greater than the expanded uncertainty of the difference, $\sqrt{0.057^2 + 0.15^2}$ = 0.16 μg/g. The NIST value is also larger than seven of the eight participant results. As with CCQM-K11.2, the situation was discussed at the April 2013 meeting of the OAWG. The expectation was again that there should be better agreement, potential explanations for this disagreement were explored, and it was pointed out that there could be some equilibration issues with extraction of serum samples containing high lipid content. The CCQM-K12.2 material had a relatively high lipid content (241.4 ± 2.8) mg/dL and total glycerides (145.4 ± 3.2) mg/dL. Information on equilibration times was requested from all participants for evaluation and discussion at the November 2013. The updated information on equilibration time, calibrants, internal standards used, CRMs used, and analytical technique are summarized in Table C3 in Appendix C.

Because CCQM-K12.2 was designed as a Subsequent rather than a Track A core competency KC, the laboratories from the initial CCQM-K12 KC, including KRISS and PTB, did not participate. After the discordance between the participant's and the NIST result was recognized, KRISS and PTB were asked to analyze the CCQM-K12.2 material to assist in determining an appropriate KCRV.

Results from KRISS and PTB, Reference Laboratories

Samples were sent to KRISS and PTB in July 2013 and the results of their analyses were received by NIST in October 2013 just prior to the November 2013 CCQM meeting in South Africa. The analytical methods used by KRISS and PTB are summarized in Tables C1 and C2 of Appendix C.

KRISS analyzed four aliquots from each of two vials using ID LC-MS/MS, reporting values and 95 % expanded uncertainties of (7.531 ± 0.098) μg/g and (7.525 ± 0.104) μg/g. Combining the values reported for the two vials, the KRISS result for CCQM-K12.2 is (7.529 ± 0.092) μg/g. KRISS also analyzed CRM 111-01-001 as a control material; the measured value and 95 % expanded uncertainty, (6.09 ± 0.06) μg/g, was in excellent agreement with the certified value, (6.08 ± 0.08) μg/g. PTB analyzed single aliquots from six vials using ID GC-MS, reporting a combined value of (7.64 ± 0.12) μg/g. The results from KRISS, NIST, and PTB are summarized in Table 11 and are displayed relative to the participant results in Figure 8.

Table 11: Reference Results for CCQM-K12.2 Creatinine in Human Serum

	Mass Fraction, µg/g		
Reference Laboratory	**Value**	***u*(Value)**	**$U_{k=2}$(Value)**
KRISS	7.529	0.046	0.092
NIST	7.67	0.07	0.15
PTB	7.64	0.06	0.12

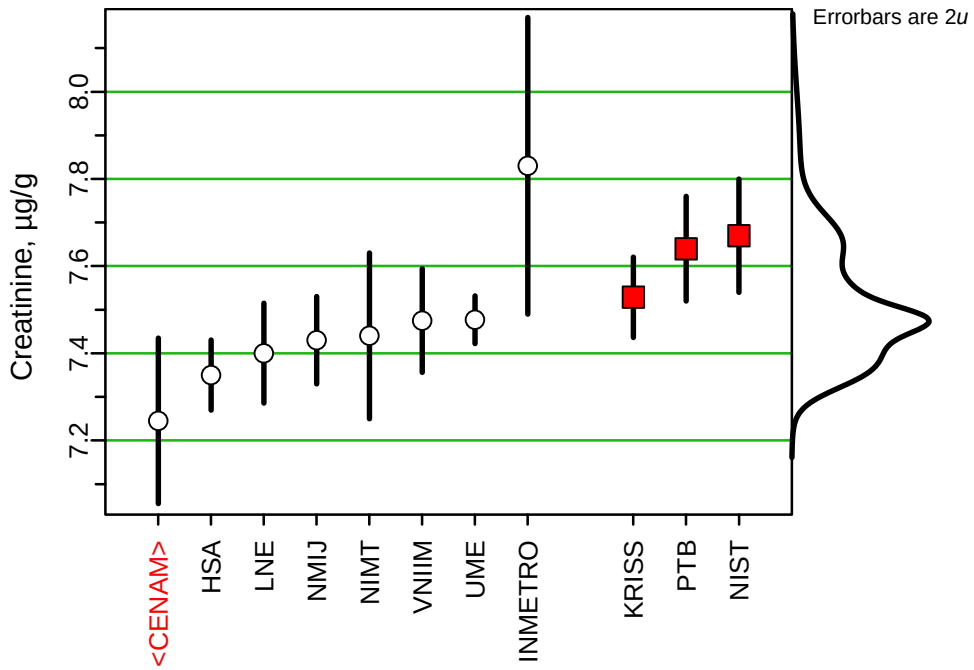

Figure 8: Comparison of KRISS, NIST, PTB, and CCQM-K12.2 Participant Results.

Format as in Figure 7 but with results for the three reference laboratories displayed as red squares. The green horizontal lines are for visual guidance.

Key Comparison Reference Value (KCRV)

While the results of the three reference laboratories (KRISS, NIST, and PTB) were in good agreement, they are larger than six of the seven participant results. After considerable discussion within the OAWG at the April 2014 meeting, the decision was made to assign the KCRV from the mean, standard deviation, and pooled standard uncertainty of the results reported by the three reference laboratories and the seven participants who reported results before the cutoff date. The results and statistics used to assign the KCRV are summarized in Table 12. The participant results, both as reported and as combined, and the reference laboratory results are displayed in Figure 9 relative to the KCRV. Figure 10 displays the combined participant results relative to the KCRV.

Table 12: Assignment of KCRV for K12.2 Creatinine in Human Serum

Mass Fraction, µg/g

NMI/DI	Result	u(Result)	$U_{k=2}$(Result)
CENAM [a]	7.245	0.095	0.190
HSA	7.35	0.040	0.08
LNE	7.400	0.057	0.115
NMIJ	7.43	0.050	0.10
NIMT	7.44	0.094	0.19
VNIIM	7.475	0.059	0.119
UME	7.477	0.027	0.054
KRISS	7.529	0.046	0.092
PTB	7.64	0.060	0.12
NIST	7.67	0.073	0.15
INMETRO	7.83	0.170	0.34

Parameter [b]	Value	Units
n	10	
$\bar{x}$	7.524	µg/g
s	0.147	µg/g
$\bar{u}(x)$	0.078	µg/g
$u(x)$	0.166	µg/g
$u(\bar{x})$	0.053	µg/g
$U_{k=2}(\bar{x})$	0.105	µg/g
KCRV	7.52	µg/g
$U_{k=2}$(KCRV)	0.10	µg/g

a Information only, result submitted after cutoff date.

b Statistics:

n	Number of results included in calculations
$\bar{x}$	Mean of results
s	Standard deviation of results
$\bar{u}(x)$	pooled standard uncertainties of the reported values: $\sqrt{\left(\sum_i \frac{U_{k=2}^2(x_i)}{2}\right)/n}$
$u(x)$	Combined standard uncertainty of the reported values: $\sqrt{s^2 + \bar{u}^2(x)}$
$u(\bar{x})$	Standard uncertainty of the mean: $u(x)/\sqrt{n}$
$U_{k=2}(\bar{x})$	k = 2 expanded uncertainty of the mean: $2 \cdot u(\bar{x})$

Degrees of Equivalence

The absolute degrees of equivalence for the participants in CCQM-K12.2 are estimated as the signed difference between the combined value and the KCRV: $d_i = x_i$ – KCRV. The expanded uncertainty on the d_i, $U_{k=2}(d_i)$, can be estimated as the square root of the sum of the squares of the expanded uncertainties of the two components: $U_{k=2}(d_i) = \sqrt{U_{k=2}^2(x_i) + U_{k=2}^2(\text{KCRV})}$.

To enable comparison with the degrees of equivalence estimates from CCQM-K12 and CCQM-K12.1, it is convenient to express the d_i and $U_{95}(d_i)$ as percentages relative to the KCRV: $\%d_i = 100 \cdot d_i/\text{KCRV}$ and $U_{k=2}(\%d_i) = 100 \cdot U_{k=2}(d_i)/\text{KCRV}$. Table 13 lists the numeric values of d_i, $U_{k=2}(d_i)$, $\%d_i$, and $U_{k=2}(\%d_i)$ for all participants in CCQM-K12.2. Figure 11 displays the absolute $d_i \pm U_{k=2}(d_i)$ for CCQM-K12.2; Figure 12 displays the relative $\%d_i \pm U_{k=2}(\%d_i)$ for CCQM-K12, CCQM-K12.1, and CCQM-K12.2.

Since the CENAM result for creatinine was received after the CCQM-K12.2 cutoff date, the values listed in Table 13 and displayed in Figures 11 and 12 are for information only.

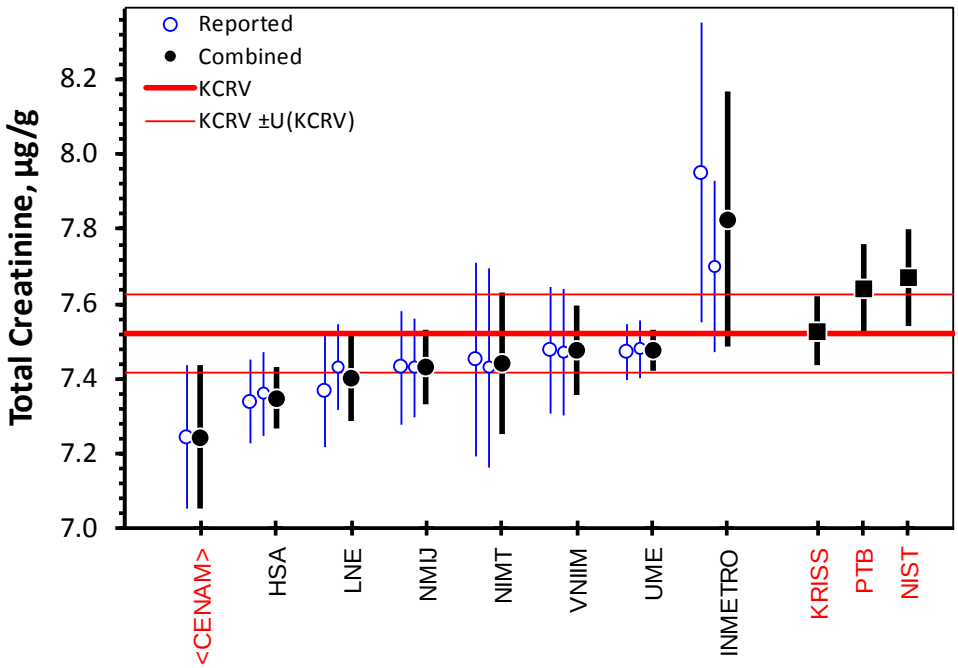

Figure 9: Participant results for CCQM-K11.2 relative to the KCRV.

The blue symbols and vertical bars represent the results as reported; the black symbols and bars represent the results as combined by NIST. The bars are approximate 95% expanded uncertainties. The horizontal lines represent the KCRV and the KCRV ± U_{95}(KCRV) interval. The red squares represent the reference laboratory results. The CENAM result is for information only.

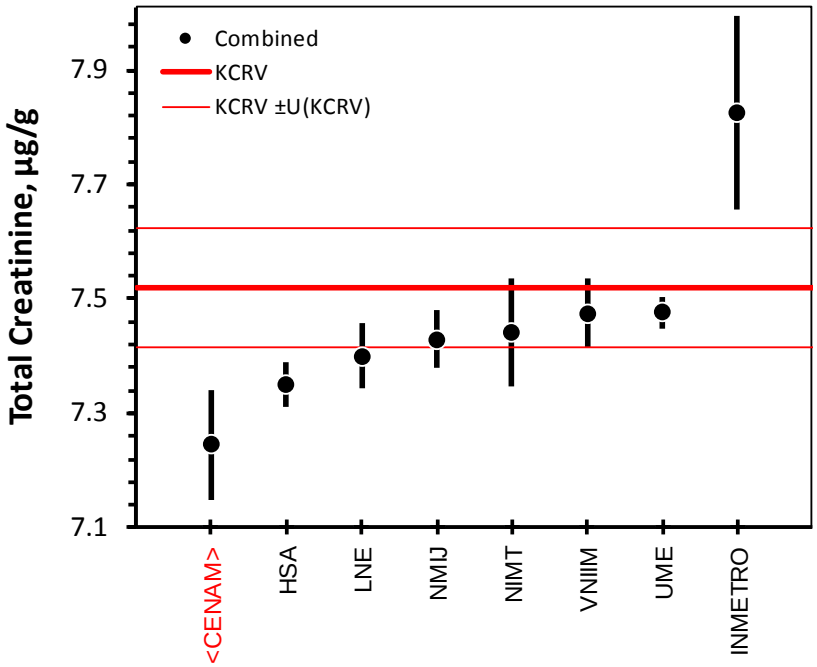

Figure 10: Participant results for CCQM-K11.2 relative to the KCRV.

The format is otherwise the same as in Figure 9. The CENAM result is for information only.

Table 13: Degrees of Equivalence for CCQM-K12.2 Creatinine in Human Serum

	µg/g		%	
NMI/DI	d_i	$U_{k=2}(d_i)$	$\%d_i$	$U_{k=2}(\%d_i)$
CENAM [a]	-0.27	0.22	-3.7	2.9
HSA	-0.17	0.13	-2.3	1.7
INMETRO	0.31	0.36	4.1	4.7
LNE	-0.12	0.15	-1.6	2.1
NIMT	-0.08	0.22	-1.1	2.9
NMIJ	-0.09	0.14	-1.2	1.9
UME	-0.04	0.12	-0.6	1.6
VNIIM	-0.04	0.16	-0.6	2.1

a Information only, result submitted after cutoff date.

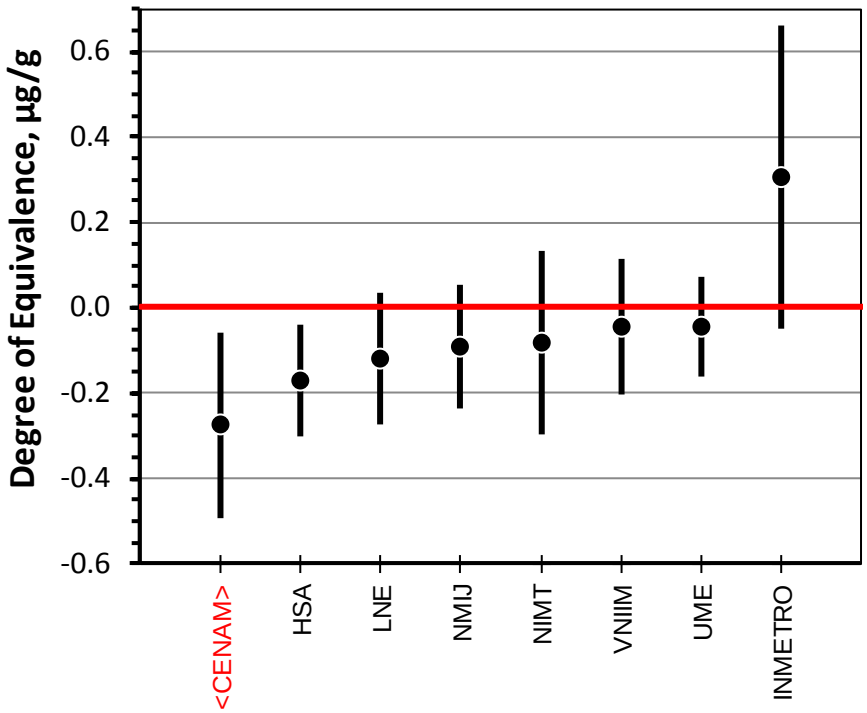

Figure 11: Absolute degrees of equivalence for CCQM-K12.2.

The black symbols and vertical bars represent the $d_i \pm U_{95}(d_i)$. The horizontal line marks the ideal zero deviation from the KCRV. The CENAM result is for information only.

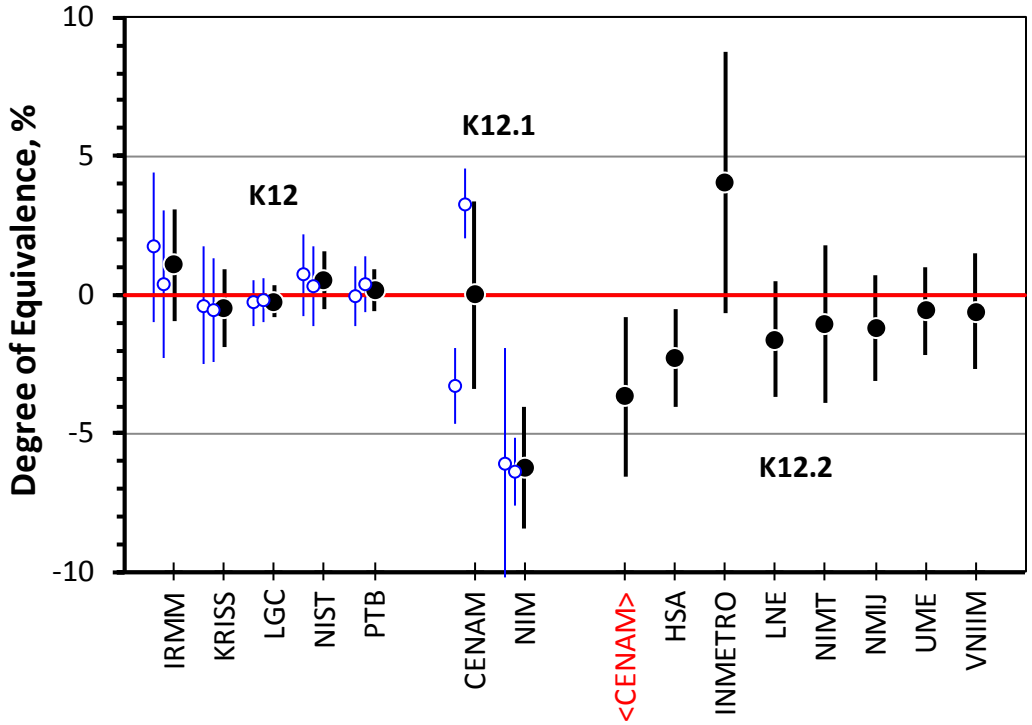

Figure 12: Relative degrees of equivalence for the CCQM-K12 comparisons

The blue symbols and bars represent $\%d_i \pm U_{95}(\%d_i)$ for individual materials distributed in CCQM-K11.1; the black symbols and vertical bars represent the combined $\%d_i \pm U_{95}(\%d_i)$. The red horizontal line marks the ideal zero deviation from the KCRV; the light grey lines are for visual guidance. The CENAM result for CCQM-K12.2 is for information only.

Use of CCQM-K12.2 to Support Calibration and Measurement Capability (CMC) Claims

CCQM-K12.2 Creatinine in Human Serum was intended as a Subsequent KC for NMIs and DIs that had not participated in earlier comparisons for determination of creatinine. However, due to unexplained systematic discordance between the participants' and anchor institution's results, the comparison did not meet the OAWG's expectations for a Subsequent KC. KRISS and PTB were requested by the OAWG to provide reference measurements. Although their results substantially agreed with the NIST result, the discordance among the reference results in CCQM-K11.2 led the OAWG to also convert CCQM-K12.2 as a Track C KC. The KCRV is estimated from the combined results of the seven participants that reported result before the cutoff date and the three reference institutions. The $d_i \pm U_{95}(d_i)$ for six of the eight participants includes zero. The $|\%d_i|$ for all participants is better than 4.1 %.

CCQM-K12.2 demonstrates a laboratory's capabilities to measure a polar (pK_{ow} >2), low molecular mass (100 g/mol to 500 g/mol) metabolite in human serum at relatively low concentrations (1 µg/g to 30 µg/g) found in normal populations. At the time of this study, the OAWG had not formalized the reporting of "core competencies". However, participation in this study demonstrates capabilities in analysis of complex biological matrices including sample preparation (protein precipitation, extraction, derivatization), LC or GC separation, and quantification using an isotope dilution mass spectrometry approach.

THOUGHTS ON SUBSEQUENT KEY COMPARISONS

CCQM-K11.2 and CCQM 12.2 exemplify the intrinsic disadvantages of the Subsequent KC design:

- Under the best of outcomes, such as the excellent agreement between nearly all participants' and anchor laboratory's results in CCQM-K6.2, a Subsequent KC costs the anchor laboratory considerable resources without providing much if any benefit. Because the anchor results are the reference value, it is not appropriate to assign degrees of equivalence to the anchor results or otherwise use those results to defend CMCs.
- If the participants' and anchor's results are not in substantial agreement, the study must either be retrospectively redesigned, redone, or abandoned.

Identifying the cause of systematic discordances requires additional resources from the anchor and at least some of the KC participants. When the cause(s) cannot be quickly identified, additional measurements from expert institutions that were not participants will be needed. Such "honest brokers" may not be available, may not have the needed resources, or may not be willing to expend their resources for little benefit beyond goodwill. Because the participant and anchor results are known before such reference measurements are made, it is not appropriate to assign degrees of equivalence to such results or to otherwise use them to defend CMCs.

In CCQM-K11.2, the reference laboratory results were as or more discordant than were the participant and anchor results. Either there was unexpected excess sample inhomogeneity or the expected extent of agreement was unrealistic. Because all available units of the comparison material were used in the follow-up studies, no root-cause can be assigned. Using a consensus KCRV appears to be the only alternative to abandonment of the KC.

In CCQM-K12.2, the reference laboratory results substantially confirmed the anchor result. However, if the anchor result or any consensus estimate using just the anchor and reference results were used as the KCRV, then most of the participant results would be assigned unacceptable degrees of equivalence. Correcting this would require performing another KC to enable them to make and defend CMCs, effectively rendering the CCQM-K12.2 results moot. Using a consensus KCRV appears to be the only viable alternative to redoing the KC.

Given the cost and risks of systematic differences between anchor and participant results, "Subsequent KCs" should in general be designed from their inception to use consensus KCRVs, with all interested institutions encouraged to participate. That is, they should be designed as Track A or Track C KCs.

ACKNOWLEDGEMENTS

The study coordinators thank all participants for providing additional information during the evaluation of these comparisons. We thank HSA for their in-depth study addressing the question regarding sample preparation equilibration time. We greatly appreciate the willingness and rapid response of KRISS and PTB to provide measurements to assist in establishing the KCRVs.

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APPENDIX A: Analytical Methods Used in K11.2

Table A1. Summary of Extraction and Chromatographic Techniques in CCQM-K11.2

NMI/DI	Sample Size (g)	Extraction Method	Post Extraction Cleanup	Analytical Technique	Chromatographic Column
CENAM	0.5	Protein precipitation with ethanol	After centrifugation, sample was filtered and solvent evaporated; add hydroxylamine hydrochloride in pyridine, heat and add acidic anhydride and heat again; evaporated to dryness and reconstitute in chloroform for injection into GC-MS.	GC-MS	WCOT fused silica column, CP-Sil 13 CB, 25 m × 0.25 mm id, 0.20 µm film stationary phase; 14% phenyl methylpolysiloxane (medium polarity)
HSA	0.2	Protein precipitation: sample allowed to equilibrate for 2 h then proteins precipitated with 3 equivalent volumes of acetonitrile; centrifuge and filter supernatant	For GC-MS: Evaporate to dryness, add hydroxylamine hydrochloride in pyridine, heat 90 C for 40 min; acidic anhydride added and heated to 90 C for 60 min; evaporated to dryness and reconstitute in methylene chloride for injection in GC-MS. For LC-MS: Dilute filtered sample with acetonitrile/water (9:1) and inject in LC-MS/MS	GC-MS; LC-MS	GC-MS: DB5-MS, 15 m × 0.25 mm id, 0.25 µm thickness LC-MS: Imtakt Unison UK-Amino, 100 mm × 2.0 mm, 3 µm particles

NMI/DI	Sample Size (g)	Extraction Method	Post Extraction Cleanup	Analytical Technique	Chromatographic Column
INMETRO	0.05	Protein precipitation: sample vortexed and allowed to equilibrate for 2 h at 4 ºC, then proteins precipitated with equivalent volumes of methanol; centrifuge and evaporate to dryness	Dry extract reconstituted with pyridine and BSTFA at 80 ºC for 40 min	GC-MS	VF1ms, 10 m × 110 mm id, 0.1 µm thickness
KRISS	0.01	Cut off filtration; spike with IS; equilibrate for 6 h; filtration	None	LC-MS/MS	Shodex Asahi pak NH2P-50 2D, 50 mm × 2.1 mm, 3 µm particles
NIM	0.1	Protein precipitation with acetonitrile 3:1(v:v) for 20 min	Mixture vortexed and centrifuged; supernatant filtered and diluted with (90:10) acetonitrile:water for injection in LC-MS/MS	LC-MS/MS	Unison UK Amino Column, 100 mm × 2.0 mm
NIMT	0.1	Protein precipitation with acetonitrile for 5 min; centrifugation	No further cleanup	LC-MS/MS	Clipeus Cyano 100 mm × 3.0 mm, 5 µm particles
NIST	0.16 to 0.36	Sodium azide added and equilibrate overnight (20 h) at room temperature; ethanol to precipitate proteins	Centrifuge; supernatant evaporated to dryness at 40 ºC to 50 ºC under N_2; samples derivatized with butylboronic acid in pyridine with heating at 95 ºC for 50 min to 60 min. Acetic anhydride added and equilibrate for 45 min. Pyridine removed by evaporation 40 ºC to 50 ºC under N_2; reconstituted in iso-octane with 1% acetic anhydride	GC-MS	30 m nonpolar capillary column, DB-5-MS.

NMI/DI	Sample Size (g)	Extraction Method	Post Extraction Cleanup	Analytical Technique	Chromatographic Column
NMIJ	0.1		Derivatize with butylboronic acid and acetic anhydride	GC-MS	DB5ms, 30 m × 25 mm id with 0.25 µm stationary phase thickness
PTB	0.46	Spike with IS; equilibrate for 20 h; deproteinize with ethanol	Clean-up with C18 SPE cartridge, lyophilize sample and derivatize to form α-D-glucofuranose cyclic 1,2:3,5-bis-butylboronate-6-acetate	GC-MS	
UME	0.3	Add IS, vortex for 1 min and equilibrate 2 h; protein precipitation with acetonitrile, vortex 1 min and centrifuged; supernatant filtered	No further cleanup	LC-MS/MS	Luna 5 µm NH2 100 A, 250 mm × 2.0 mm
VNIIM	0.1	Protein precipitation with acetonitrile for 15 min; centrifugation	No further cleanup	LC-MS/MS	YMC-Pack NH2 150 mm × 4.6 mm, 5 µm particles

Table A2. Summary of Detection and Quantification Techniques in CCQM-K11.2

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
CENAM	80 ºC for 1 min, 20 ºC/min to 300 ºC and hold 3 min. Split injection at 270 ºC; helium carrier gas at 1.0 mL/min constant flow from column. Mass Selective Detector: quadrupole at 150 ºC, source at 300 ºC. Ions monitored: *m/z* 242 and *m/z* 246 (IS)	IDMS	Bracketing	$^{13}C_6$-glucose (Sigma Aldrich) of 99% ^{13}C purity added before sample preparation	Purity assessed at CENAM using HPLC, DSC, and Karl Fischer (moisture); CENAM DMR-263a used as control
HSA	GC-MS: Inlet at 270 ºC, 100 ºC for 1 min, then 30 ºC/min to 230 ºC hold 5 min. Flow at 1.0 mL/min; transfer line at 270 ºC. Ions monitored: *m/z* 314 and *m/z* 319 (IS) (quantifying ions) and *m/z* 242 and *m/z* 246 (IS) (confirmatory ions). LC-MS: Mobile phase (A) 5 nmol/L ammonium formate with 0.05% formic acid and (B) acetonitrile, 60%A/40% B at 0.3 mL/min. Ions Monitored: *m/z* 225/89 and *m/z* 231/92 (IS) (quantifying ions) and 225/59 and 231/61 (IS) (confirmatory ions)	IDMS	6-point calibration	$^{13}C_6$-glucose (Cambridge Isotopes) of 99.4% ^{13}C purity added during gravimetric preparation of the samples	

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
INMETRO	140 ºC initial then 20 ºC/min to 320 ºC and hold 2 min. Split mode injection (1:50); helium carrier gas at 0.5 mL/min Mass Selective Detector: Ions monitored: *m/z* 191, 204, 217, and 435 (quantifying ions) and *m/z* 192, 206, 220, and 441 (IS quantifying ions)	IDMS	Bracketing	$^{13}C_6$-glucose added before sample preparation	NIST SRM 917c
KRISS	Mobile phase: isocratic 20 mmol/L ammonium acetate in water/ACN (50/50) at 0.3 mL. Ions monitored: *m/z* 225/89 glucose and *m/z* 231/92 (IS)	ID LC-MS/MS	Bracketing	glucose-$^{13}C_6$ (Cambridge Isotopes)	NIST SRM 917c KRISS CRM 111-01-008
NIM	90:10 acetonitrile:water (5 mmol/L ammonium formate with 0.05% formic acid. Ions monitored: *m/z* 225/89 and *m/z* 231/92 (IS)	IDMS	4-point linear regression calibration curve	$^{13}C_6$-glucose added after weighing	GBW10062 (NIM)
NIMT	Mobile phase: (A) 1% formic acid in water, (B) 0.1% formic acid in acetonitrile; 15% A and 85% B held at 1 min with gradient to 95% B in 0.05 min held for 1.55 min, then decreased to 85% B in 0.05 min at flow rate of 0.5 mL/min.	IDMS	Exact matching double IDMS with 1-point calibration for bracketing	Glucose-d_2 added prior to protein precipitation	NIST SRM 956b used for matrix matched calibration blends

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
NIST	Split injection (20:1) at 200 ºC, MS quadrupole at 150 ºC, MS source at 230 ºC. Temperature program: 150 ºC 1 min hold; 40 ºC/min to 200 ºC, 10 min hold. Ions monitored: *m/z* 297 for glucose and *m/z* 303 for labeled glucose	ID GC-MS	Bracketing	$^{13}C_6$ labeled glucose (Isotec, Miamisburg, OH)	NIST SRM 917c
NMIJ	150 ºC for 1 min then 5 ºC/min to 230 ºC and 20 ºC/min to 320 ºC hold 3 min. Mass Selective Detector: Ions monitored: Ions monitored: *m/z* 297 and *m/z* 303 (IS)	IDMS	2- point calibration curve	$^{13}C_6$-glucose	NIST SRM 917c
PTB	Ions monitored: *m/z* 297 glucose and *m/z* 303 (IS)	ID GC-MS		glucose-$^{13}C_6$	NIST SRM 917c
UME	Mobile phase: (A) 10 mmol ammonium formate in water (B) acetonitrile; 50% A and 50% B isocratic. Ions monitored: *m/z* 225/89 and *m/z* 231/92 (IS)	IDMS	3-point calibration curve	$^{13}C_6$-glucose added at beginning of extraction	NIST SRM 965b
VNIIM	Mobile phase: isocratic 50% water with 10mM ammonium acetate and 50% acetonitrile Ions monitored: *m/z* 225/89 and *m/z* 231/92	IDMS	1-point	$^{13}C_6$-glucose (Cambridge Isotopes) added at beginning of extraction	NIST SRM 917a

Table A3. Comparison of Methods Potentially Critical Parameters in CCQM-K11.2

NMI/DI	Equilibration Time	Internal Standard	Calibrant	CRMs Used as Control	Analytical Technique
CENAM	NR	Glucose-$^{13}C_6$	CENAM		GC-MS
HSA	2 h	Glucose-$^{13}C_6$	SRM 917c		LC-MS/MS, GC-MS
INMETRO	2 h	Glucose-$^{13}C_6$	SRM 917c		GC-MS
KRISS	6 h	Glucose-$^{13}C_6$	SRM 917c	KRISS CRM 111-01-008	LC-MS/MS
LNE	1 h	Glucose-$^{13}C_6$	SRM 917c		GC-MS
NIM	NR	Glucose-$^{13}C_6$	GBW10062	SRM 965a	LC-MS/MS
NIMT	1 h	Glucose-d_2	SRM 965b*		LC-MS/MS
NIST	20 h	Glucose-$^{13}C_6$	SRM 917c	SRM 956b	GC-MS
NMIJ	NR	Glucose-$^{13}C_6$	SRM 917c		GC-MS
PTB	20 h	Glucose-$^{13}C_6$	SRM 917c		GC-MS
UME	2 h	Glucose-$^{13}C_6$	SRM 965b*		LC-MS/MS
VNIIM	NR	Glucose-$^{13}C_6$	SRM 917c		LC-MS/MS

NR Not Reported
* Matrix CRM used as calibrant

APPENDIX B: Summary of Uncertainty Estimation Methods in CCQM-K11.2

The following are pictures of the uncertainty-related information provided by the participants in the "Analytical Information" worksheet of the "Reporting Form" Excel workbook. Information is grouped by participant and presented in alphabetized acronym order.

CCQM-K11.2 Uncertainty Information from CENAM

$$w_x = \left(\frac{\left(\frac{m_2}{m_{I2}}\right) \cdot w_2 \cdot (\overline{R_x} - \overline{R_1}) - \left(\frac{m_1}{m_{I1}}\right) \cdot w_1 \cdot (\overline{R_x} - \overline{R_2})}{\overline{R_2} - \overline{R_1}} \right) \cdot \frac{m_{Ix}}{m_x}$$

Symbol	Description
w_1	Mass fraction of the solution calibration standard (low level) (mg/g)
w_2	Mass fraction of the solution calibration standard (high level) (mg/g)
R_1	Response relationship of low level solution
R_2	Response relationship of high level solution
m_{I1}	Mass of the isotope solution added to the low level solution calibration standard (g)
m_1	Mass of the analyte standard solution of low level calibration (g)
m_{I2}	Mass of the isotope solution added to the high level solution calibration standard (g)
m_2	Mass of the analyte standard solution of high level calibration (g)
m_x	Mass of sample to be measured (g)
m_{Ix}	Mass isotope of the solution added to the sample (g)
R_x	Instrument response relationship (GC or LC) between the analyte in the sample and its isotope added (dimensionless)

Symbol	Description	Value	Units	Uncertainty source	Type of distribution	Standard uncertainty	Units	Relative uncertainty ui(y)
w_1	Mass fraction of the solution calibration standard (low level)	**1.0000**	mg/g	Experimental	normal type A	0.0033	mg/g	0.3287%
w_2	Mass fraction of the solution calibration standard (high level)	**1.2010**	mg/g	Experimental	normal type A	0.0036	mg/g	0.3021%
R_1	Response relationship of low level solution	**0.9536**		Experimental	normal type A	0.0009		0.0942%
R_2	Response relationship of high level solution	**1.1389**		Experimental	normal type A	0.0013		0.1180%
m_{I1}	Mass of the isotope solution added to the low level solution calibration standard (g).	**0.5096**	g	Experimental	normal type B	0.00003	g	0.0057%
m_1	Mass of the analyte standard solution of low level calibration (g)	**0.5107**	g	Experimental	normal type B	0.00002	g	0.0045%
m_{I2}	Mass of the isotope solution added to the high level solution calibration standard (g).	**0.4930**	g	Experimental	normal type B	0.00002	g	0.0046%
m_2	Mass of the analyte standard solution of high level calibration	**0.4932**	g	Experimental	normal type B	0.00002	g	0.0049%
m_x	Mass of sample to be measured (g).	**0.4550**	g	Experimental	normal type B	0.000035	g	0.0077%
m_{Ix}	Mass isotope of the solution added to the sample (g).	**0.4563**	g	Experimental	normal type B	0.000036	g	0.0080%
R_x	Instrument response relationship (GC or LC) between the analyte in the sample and its isotope added	**1.0816**		Experimental	normal type A	0.0015		0.1393%
	Mathematical model uncertainty	**0.0056**		**0.5%**				
	Repeatibility between subsamples	**0.0028**						
	Combined uncertainty	**0.006**						
	Expanded uncertainty	**0.020**		**1.8%**				

CCQM-K11.2 Uncertainty Information from HSA

The mass fraction of glucose in serum was calculated based on the IDMS calibration curve as follows:

$$C_X = (mR_B + b) \times \frac{W_Y}{M_X} = (mR_B + b) \times \frac{M_Y C_Y}{M_X} \quad (1)$$

where
C_X = mass fraction of glucose in the serum sample
M_X = mass of serum sample (determined by weighing)
M_Y = mass of isotope standard solution (determined by weighing)
W_Y = mass of the isotope labeled standard spiked into the serum sample (equals to $M_Y \times C_Y$)
R_B = peak area ratio of sample blend (determined by GC-MS or LC-MS/MS measurements)
C_Y = concentration of isotope labeled standard solution (determined by weighing and from purity of the isotope labeled standard)
m = gradient of the slope of linear regression plot (determined by the linear fit of the isotope mass ratio and the peak area ratio of the calibration blends)
b = intercept on y axis of the linear regression plot (determined by the linear fit of the isotope mass ratio and the peak area ratio of the calibration blends)

For the estimation of uncertainty, considering $R_M = mR_B + b$, and let $R_M = R_M' C_Y / C_Z$, Equation (1) is converted to:

$$C_X = R_M \times \frac{M_Y C_Z}{M_X} \quad (2)$$

where
R_M = isotope mass ratio in sample blend
C_Z = concentration of glucose in the calibration standard solution

A standard uncertainty was estimated for all components of the measurement in Equation (2), which were then combined using respective derived sensitivity coefficients to estimate a combined standard uncertainty in the reported result of glucose in serum samples. A coverage factor k with a value of 2 is used to expand the combined standard uncertainty at a 95 % confidence interval. Possible sources of biases [method precision (F_P), choice of different ion pair (F_I), and other factors during sample extraction (F_{C1}) and derivatisation (F_{C2})] are accounted for in the final uncertainty budget with the use of the measurement equation:

$$C_X = F_P \times F_I \times F_{C1} \times F_{C2} \times R_M' \times \frac{M_Y C_Z}{M_X} \quad (3)$$

The sensitivity coefficients of each component can be expressed as follows:

$$\frac{\partial C_X}{\partial R_M'} = \frac{C_X}{R_M'} \qquad \frac{\partial C_X}{\partial M_Y} = \frac{C_X}{M_Y} \qquad \frac{\partial C_X}{\partial M_X} = -\frac{C_X}{M_X} \qquad \frac{\partial C_X}{\partial C_Z} = \frac{C_X}{C_Z}$$

$$\frac{\partial C_X}{\partial F_P} = \frac{C_X}{F_P} \qquad \frac{\partial C_X}{\partial F_I} = \frac{C_X}{F_I} \qquad \frac{\partial C_X}{\partial F_{C1}} = \frac{C_X}{F_{C1}} \qquad \frac{\partial C_X}{\partial F_{C2}} = \frac{C_X}{F_{C2}}$$

The standard uncertainty of each component was calculated as follows:
(1) M_Y and M_X: The standard uncertainty was calculated based on the calibration report using the standard weights calibrated by the National Metrology Centre, A*STAR.
(2) F_P: The pooled standard deviation of the mean of the GC-MS and LC-MS/MS results for each sample was used as the the standard uncertainty of method precision.
(3) F_I: The standard deviation of the difference of the results using two ion pairs divided by the square root of the number of samples (for insignificant difference using t-test) or the average of the difference of the results using two ion pairs divided by 2 (for significant difference using t-test).
(4) F_{C1} and F_{C2}: A relatively standard uncertainty of 0.1% and 0.2% was employed for these two factors, respectively.
(5) C_Z: The certified purity and uncertainty of NIST SRM 917c in combination with the uncertainty of weighing for preparation of the calibration standard solution.
(6) R_M': Consider $R_M = R_M' \times C_Z / C_Y$, the conversion of equation $R_M = mR_B + b$ leads to:
$R_B = (C_Z \times R_M') / (C_Y \times m) - b/m$
Let $m' = C_Z/(C_Y \times m)$ and $b' = -b/m$, we have:
$R_B = m'R_M' + b'$
The standard uncertainty of R_M' was calculated using the following equation:

$$\mu_{R_M} = \frac{1}{m'} \times s_{y/x} \times \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(R_B - \overline{R_{Bc}})^2}{m'^2 \sum_{i=1}^{n} (R_{Mc} - \overline{R_{Mc}})^2}} \quad (4)$$

where

$s_{y/x}$ = standard deviation of the regression
R_B = peak area ratio of sample blend
= average peak area ratio of calibration blends
n = number of calibration blends used for the linear regression plot
N = injection time for each sample
R_{Mc} = isotope mass ratio in calibration blends
= average isotope mass ratio in calibration blends

The combined standard uncertainty was calculated using the equation below:

$$u = \sqrt{\sum_i c_i^2 u_{xi}^{\ 2}} \qquad (5)$$

where
u = combined standard uncertainty
c_i = sensitivity coefficient of each component
u_{xi} = standard uncertainty of each component

The expanded uncertainty (U) was calculated by mutiplying the combined standand uncertainty (u) with a coveragy factor (k = 2) for a confidence level of 95 %.

Table 1. Uncertainty Budget for Sample 1

Factor	Value x	Uncertainty u(x)	Relative Uncertainty u(x)/(x)	Sensitivity Coefficient (c) δCx/δx	c2 . u(x)2	% contribution
M_X (g)	0.1982	0.000099	0.050%	5827.04	0.3328	0.9%
M_Y (g)	0.2156	0.000099	0.046%	5357.90	0.2813	0.8%
C_Z (µg/g)	1410.8	3.9210	0.278%	0.82	10.3057	28.3%
R_M'	0.7436	0.0023	0.306%	1553.46	12.5252	34.4%
F_P (µg/g)	1155	2.1676	0.188%	1.00	4.6987	12.9%
F_I (µg/g)	1155	1.2607	0.109%	1.00	1.5894	4.4%
F_{C1} (µg/g)	1155	1.1551	0.100%	1.00	1.3343	3.7%
F_{C2} (µg/g)	1155	2.3102	0.200%	1.00	5.3371	14.7%

Table 2. Uncertainty Budget for Sample 2

Factor	Value x	Uncertainty u(x)	Relative Uncertainty u(x)/(x)	Sensitivity Coefficient (c) δCx/δx	c2 . u(x)2	% contribution
M_X (g)	0.1995	0.000099	0.050%	5795.42	0.3292	0.9%
M_Y (g)	0.2190	0.000099	0.045%	5277.96	0.2730	0.7%
C_Z (µg/g)	1410.8	3.9210	0.278%	0.82	10.3203	28.3%
R_M'	0.7436	0.0023	0.306%	1554.56	12.5429	34.4%
F_P (µg/g)	1156	2.1692	0.188%	1.00	4.7053	12.9%
F_I (µg/g)	1156	1.2616	0.109%	1.00	1.5917	4.4%
F_{C1} (µg/g)	1156	1.1559	0.100%	1.00	1.3362	3.7%
F_{C2} (µg/g)	1156	2.3119	0.200%	1.00	5.3447	14.7%

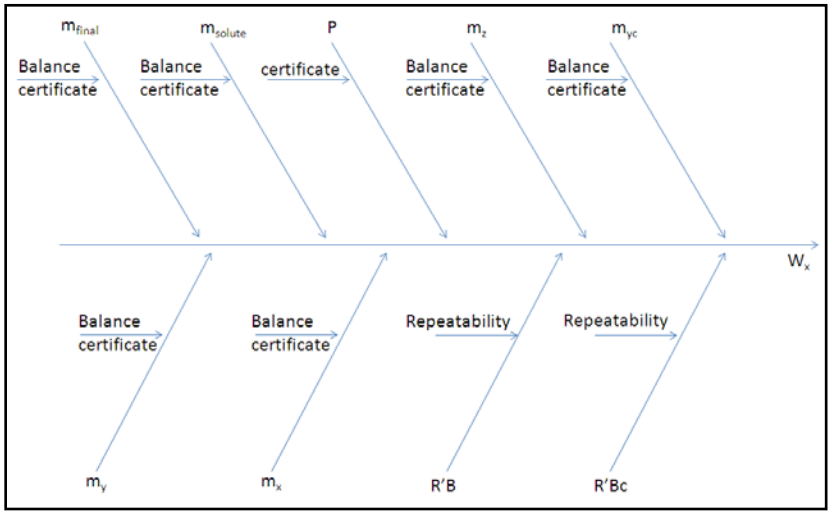
CCQM-K11.2 Uncertainty Information from INMETRO

$$W_x = \frac{m_{soluto}}{m_{final}} * P * \frac{m_z}{m_{yc}} * \frac{m_y}{m_x} * \frac{R'_B}{R'_{Bc}}$$

W_x = Sample mass fraction; m_{solute} = mass of the solute used to prepare the calibration solution; m_{final} = final mass (solute + solvent) of the calibration solution; P = purity of the calibrant; m_z = mass of the calibrant solution added to the standards; m_{yc} = mass of the internal standard solution added to the standards; m_y = mass of the internal standard solution added to the samples; m_x = mass of the sample; R'B = analyte/internal standard area ratio measured in the sample; R'Bc = analyte/internal standard area ratio measured in the standards

All factors from the measurement equation were considered in the uncertainty estimation. All of the evaluated uncertainties were of type B except for the R'B and R'Bc repeatabilities. Hence their standard uncertainties were obtained by dividing the expanded uncertainties by the coverage factors encountered in the certificates. For the repeatabilities, standard uncertainties were obtained by the standard errors of the means (s/√n). The standard uncertainties were multiplied by their sensitivity coefficients using the GUM methodology and then combined using the square root of the squared sum of the components. Effective degrees of freedom were calculated and the coverage factors for 95 % probability were taken for the expanded uncertainties. The full uncertainty budget is presented below as for sample 1:

Factor	% contribution
m_{final}	0,00005
m_{solute}	5,612384704
P	4,542992311
m_z	0,538251505
m_{yc}	0,540488392
m_y	0,550728997
m_x	0,078294985
R'B	72,01882778
R'Bc	16,11798144
Total	**100**



Method was validated by the preparation by two different analysts of the CRM from Nist 965b levels 2 and 3. These results showed that both analysts were capable of generating results equivalent to the certified property values for the CRM by comparison of the Δ_m (absolute difference between the mean measured value and the certified value) and the U_Δ (expanded uncertainty of the difference between the measurement result and the certified value), obtaining $\Delta_m < U_\Delta$ which means the measured value and the certified value have no significant differences according to ERM Application Note 1. These experiments demonstrated repeatability, intermediate precision and trueness (bias) evaluations of the method. Also, calibration curves were constructed in both water and human serum demonstrating the linearity of the method. The slopes of the curves were compared and shown to be equivalent, allowing control samples to be spiked in water instead of serum.

CCQM-K11.2 Uncertainty Information from KRISS

$$C = \frac{M_{is-glu,spiked} \cdot AR_{sample} M_{glu,std} \cdot C_{glu,std}}{W_s \cdot AR_{std} \cdot M_{is-glu,std}}$$

Here, $M_{is\text{-}sol,spiked}$ is the weight of the glucose-$^{13}C_6$ solution spiked in the sample, $C_{s\text{-}sol}$ is the concentration of the cholesterol standard solution (mg/kg), and W_s is the weight of the sample, . AR_{sample} is the observed area ratio of glucose/glucose-$^{13}C_6$ of the sample from the LC/MS/MS measurement, AR_i is the observed area ratio of glucose/glucose-$^{13}C_6$ of the calibration standard mixture i (i=1,2) from the LC/MS/MS measurement, and $MR_{mix,i}$ is the weight ratio of the glucose solution/glucose-$^{13}C_6$ solution in the calibration standard mixture i (i=1,2) from the LC/MS/MS measurement.

Uncertainty	CCQM sample2	CCQM sample3
1		
2	1.191	1.188
3	1.186	1.180
4	1.180	1.186
Avg	1.186	1.185
Stdev	0.006	0.004
Rel. stdev (%)	0.5	0.4
Standard unc. (%)	0.27	0.20
STD solution unc. (prep, %)	0.4	0.4
Purity unc. (%)	0.3	0.3
STD Mix (prep) unc of LC/MS/MS meas. For sample unc of LC/MS/MS meas. For STD Mix	included in std sol unc included in rel stdev included in rel stdev	
Combined standard unc. (%)	0.57	0.54
DOE	9	9
k	2.3	2.3
Urel%	1.3	1.2
Uexp (mg/g)	0.015	0.014

CCQM-K11.2 Uncertainty Information from LNE

C = (aR 314/319+ b) x ((mLabCLab)/ mser))

C = mass fraction of glucose in the serum sample (mg/g)
mLab = mass of labeled glucose solution (g)
CLab = concentration of labeled glucose solution (mg/g)
a = gradient of the slope for linear regression plot
b = intercept on y axis for the linear regression plot
R 314/319 = unlabeled/labeled ion peak area ratio of serum sample
mser = mass of serum sample (g)

sample1

Component	Type (A or B)	relative Uncertainty (%)
Purity of primary standard	B	10.76%
preparationof sample blends (weighings)	B	15.60%
Calibration model	B	11.76%
Preparation of calibration blend (weighings)	B	5.61%
Precision	B	56.26%

sample2

Component	Type (A or B)	relative Uncertainty (%)
Purity of primary standard	B	9.00%
preparationof sample blends (weighings)	B	13.39%
Calibration model	B	46.26%
Preparation of calibration blend (weighings)	B	4.70%
Precision	B	26.65%

CCQM-K11.2 Uncertainty Information from NIM

$$C_S = R_M \frac{M_L C_L}{M_S}$$

$R_M = aR_A + b$,

where:

R_M : isotope mass ratio in sample blend

R_A : isotope ratio in sample blend measured by LC-MS/MS

a: slope of the linear regression plot based on the 4 calibration blends

b :interception on y axis for the linear regression plot

C_S : mass fraction of D-glucose in serum sample

M_S : mass of serum sample

M_L : mass of ^{13}C6-D-glucose internal standard solution added to the serum sample

C_L : concentration of ^{13}C6-D-glucose internal standard solution added to the serum sample

The measurement equations can be converted to $C_S = R_M' \frac{M_L M_C P_C}{M_S M_{PC}}$

where

M_C : mass of calibrator

M_{PC} : mass of primary calibration solution

P_C : calibrator purity

R_M' : parameter only relevant to isotope ratio of calibration solution blends measured and weighing of calibration solution blends

Uncertainty budget

Uncertainty component	Sample1	Sample2	Source
u_l	0.27%	0.27%	Linear regression of calibration curve
u_{cal}	0.14%	0.14%	Calibration curve solution preparation
u_A	0.35%	0.18%	RSD of mean
u_{Ms}	0.05%	0.05%	Serum sample weighing
u_{Mc}	0.05%	0.05%	Calibrator weighing
u_{Ml}	0.05%	0.05%	weighing of Internal standard solution added to sample
u_{Pc}	0.25%	0.25%	Calibrator purity
u_c	0.53%	0.44%	Combined standard uncertainty
U_{rel}	1.1%	0.88%	Expanded uncertainty (k=2)
Obtained value (mg/g)	1.159	1.158	Mean of three aliquots measurement value (mg/g)
U (mg/g)	0.013	0.011	Expanded uncertainty(mg/g)

$u_c = \sqrt{\sum u_i^2}$ *Note: u* (M_{PC}) did not list in this table because it was too small.

CCQM-K11.2 Uncertainty Information from NIST

All results were used (no outliers were excluded). Single-factor ANOVA determinations found no significant between-vial differences, although the difference between the two aliquots of vial "1" was significant. Calculations of the measurement standard deviation of the mean used a value of n equal to the number of aliquots evaluated: $n = 4$. To determine the standard uncertainty, u_c, the measurement standard deviation was quadratically combined with the type B components, a 0.1 % uncertainty in the purity of the primary reference material, SRM 917c, and 0.5 % uncertainty related to possible incomplete equilibration. A factor of $k = 2$, was used to calculate the expanded uncertainty, $U_{k=2}$.

Vial	Aliquot	Replicate	mg/dL
1	1	1	120.32
1	1	2	120.71
1	2	1	123.13
1	2	2	123.21
2	1	1	121.42
2	1	2	119.90
3	1	1	121.92
3	1	2	121.05
		Mean:	121.46
		Standard Deviation (SD):	1.23
		SD of the Mean:	0.61
		SD purity of standard:	0.12
		SD equilibrium:	0.61
		Combined standard u_c:	0.87
		$U_{k=2}$(Mean) :	1.74

The density of the material was determined to be (102.26 ± 0.02) g/dL, giving a mass fraction result:

$$\frac{(121.46 \pm 0.87)\ \text{mg}}{\text{dL}} \times \frac{\text{dL}}{(102.26 \pm 0.02)\ \text{g}} = (1.188 \pm 0.017)\ \text{mg/g}.$$

CCQM-K11.2 Uncertainty Information from NIMT

Expanded measurement equation:

$$w_x = F_P.F_E.w_{z,c} \cdot \frac{m_y \cdot m_{zc}}{m_x \cdot m_{yc}} \cdot \frac{R'_b}{R'_{bc}}$$

$w_{z,c}$	is the mass fraction of analyte in the calibration solution used to prepare the calibration blend
m_y	is the mass of spike solution added to sample blend
$m_{y,c}$	is the mass of spike solution added to calibration blend
m_x	is the mass of sample added to sample blend
$m_{z,c}$	is the mass of standard solution added to calibration blend
R'_B and $R'_{B,C}$	are the observed isotope amount ratios in the sample blend and the calibration blend, respectively
F_E	is the extraction efficiency factor
F_P	is the method precision factor

$$\frac{u(w_x)}{w_x} = \sqrt{\left(\frac{u(w_{Z,C})}{w_{Z,C}}\right)^2 + \left(\frac{u(m_Y)}{m_Y}\right)^2 + \left(\frac{u(m_{Y,C})}{m_{Y,C}}\right)^2 + \left(\frac{u(m_X)}{m_X}\right)^2 + \left(\frac{u(m_{Z,C})}{m_{Z,C}}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2}$$

$u(w_{z,c})$ is the standard uncertainty of the mass fraction of analyte in the calibration solution used to prepare the calibration blend. The value was estimated from the certified mass fraction value of matrix-matched calibration standard, masses weighed for preparation of calibration standard and uncertainty using different standards (standard comparison).

$u(m_y)$, $u(m_{y,c})$, $u(m_x)$ and $u(m_{z,c})$ are standard uncertainties of the masses. These values were estimated from the bias and precison effect of the balance.

$u(F_P)$ is the standard uncertainty of the precision factor. This value was estimated from standard deviation of the multiple IDMS results.

$u(F_E)$ is the standard uncertainty of the extraction efficiency factor which was estimated from the extraction and protein precipitaion

Note: For the uncertainty contributing to the R'B and R'B,C ,the precision in measuring the isotope amount ratios of the analyte and the internal standard in the sample and calibration blends was assumed to be incorporated in the overall method precision.The effect of any biases on these ratios was assumed to be negligible as any systematic biases should cancel out since the calibration blends and sample blends were exact-matched for analyte concentration and isotope ratio. Other biases that may arise from extractions are captured in other factors.

Uncertainty budget of Glucose in serum (sample I)			
Factor	Values x	Uncertainties u(x)	u(x)/(x)
Parameter (unit)			
Method Precision, F_P(1)	1.0000	0.00508	0.508%
$m_{zc,}$ (g)	0.09986	0.000049	0.0496%
m_y, (g)	0.11834	0.000049	0.0418%
m_{yc}, (g)	0.11943	0.000049	0.0414%
m_x,(g)	0.10020	0.000049	0.0494%
$w_{z,c}$, (mg/g)	0.1165	0.0010	0.8269%
Additional Factors			
Extraction effects, F_E (1)	1.000	0.0100	1.000%

Uncertainty Analysis Results		
Cx=	**1.171**	mg/g
u(x) =	0.016	mg/g
u(x)/x =	1.40%	
Veff(total) =	26.718	
k=	2.06	(@ 95% level)
U(x) =	**0.034**	mg/g
%U(x) =	**2.87%**	

Uncertainty budget of Glucose in serum (sample II)			
Factor	Values x	Uncertainties u(x)	u(x)/(x)
Parameter (unit)			
Method Precision, F_P(1)	1.0000	0.00991	0.991%
$m_{zc,}$ (g)	0.09986	0.000049	0.0496%
m_y, (g)	0.11834	0.000049	0.0418%
m_{yc}, (g)	0.11943	0.000049	0.0414%
m_x,(g)	0.10020	0.000049	0.0494%
$w_{z,c}$, (mg/g)	0.1165	0.0010	0.8858%
Additional Factors			
Extraction effects, F_E (1)	1.000	0.0100	1.000%

Uncertainty Analysis Results		
Cx=	**1.184**	mg/g
u(x) =	0.020	mg/g
u(x)/x =	1.67%	
Veff(total) =	34.033	
k=	2.03	(@ 95% level)
U(x) =	**0.040**	mg/g
%U(x) =	**3.39%**	

CCQM-K11.2 Uncertainty Information from NMIJ

C_sample = mass_IS/mass_sample x (MS_sample/MS_IS – b)/a

a=(y2-y1)/(x2-x1)
b = y1-ax1 =y2-ax2

x1=C_std1 x (mass_std1/mass_std1_IS)
x2=C_std2 x (mass_std2/mass_std2_IS)
y1=MS_std1_cr/MS_std1_IS
y2=MS_std2_cr/MS_std2_IS

C_sample: glucose concentration in sample
C_std1: glucose concentration in std1
C_std2: glucose concentration in std2
mass_sample: mass fraction of glucose in sample
mass_IS: mass fraction of IS for sample
mass_std1: mass fraction of glucose in std1
mass_std1_IS: mass fraction of IS in std1
mass_std2: mass fraction of glucose in std2
mass_std2_IS: mass fraction of IS in std2
MS_sample: peak area of glucose in sample
MS_IS: peak area of IS in sample
MS_std1: peak area of glucose in std1
MS_std1_IS: peak area of IS in std1
MS_std2: peak area of glucose in std2
MS_std2_IS: peak area of IS in std2

Factor			Value	Unit	Std. Uncertainty	Rel. Std. Uncertainty (%)		Type
Standard material			0.993	kg/kg	0.0015	0.15		B
Balance								
	Calibration solution					0.071		B
	Sample		100	mg	0.05	0.05		B
	Add IS		100	mg	0.05	0.05		B
Calibration curve						0.15		A
Measurement			1172.2	ug/g				
	Between sample prep.					0.53		A
	Between measurement					0.38		A
	Combined	std. uncertainty		ug/g	8.102	0.69		
	Expanded	uncertainty (k = 2)		ug/g	16.20	1.38		

CCQM-K11.2 Uncertainty Information from PTB

Vial	Aliquot	Mass fraction (µg/g)
1	1	1.1431
1	2	1.1447
2	1	1.1410
2	2	1.1427
3	1	1.1334
3	2	1.1385

Mean:	1.1406
Standard uncertainty uc:	0.00626
Expanded Uncertainty U:	0.013 (1.1 %)
Coverage factor k (95%):	2

$$RF = \frac{A_{ABx} x C_{ISx}}{A_{ISx} x C_{ABx}}$$

RF : Response factor
CABx : Concentration of native compound (mg/g)
AABx : Peak area of native compound
AISx : Peak area of labelled compound
CISx : Concentration of labelled compound (mg/g)

Uncertainty Sources		
1-Mass of sample	Value	Standard Uncertainty
Mass of compound	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Compound}) = \sqrt{u_{CmCompound}^2 + u_{CmTare}^2}$$

2-Mass of Labelled STD	Value	Standard Uncertainty
Mass of labelled compound	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Compound\ ^{13}C3}) = \sqrt{u_{CmCompound\ ^{13}C3}^2 + u_{CmTare}^2}$$

3-Labelled Compounds Stock Solution	Value	Standard Uncertainty
Mass of Compound^{13}C3	m_{13C3}	
Calibration		uCm_{C13C3}
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}
Mass of Solvent	$m_{solvent}$	
Calibration		$uCm_{solvent}$

$$u(m_{stock\ ^{13}C3}) = \sqrt{u_{stock\ ^{13}C3}^2 + u_{CmSolvent}^2 + u_{CmTare}^2}$$

4- Uncertainty of calibration standard	Value	Standard Uncertainty
Mass of calib	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Calib}) = \sqrt{u_{CmCalib}^{2} + u_{CmTare}^{2}}$$

6-Method Precision

where,
$u(rep)$: Uncertainty of repeatability
SD: Standard deviation
n: Number of sample

$$u(rep) = \frac{SD}{\sqrt{n}}$$

7-Instrument Repeatability

where,
$u(rep)$: Uncertainty of repeatability
SD: Standard deviation
n: Number of sample

$$u(rep) = \frac{SD}{\sqrt{n}}$$

6-Calibration Graph

$$u(c_0) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{S_{xx}}} \qquad S\,xx = \sum_{i=1}^{n}(c_i - \bar{c})^2$$

S Residual standard deviation
B1 Slope
p number of measurement to determine c_0
n number of measurement for the calibration
c_0 determined concentration
$\bar{c}$ mean value of the different calibration standards (n number of measurement)
i index for the number of calibration standards

COMBINED STANDARD MEASUREMENT UNCERTAINTY

$$\frac{u_c}{c} = \sqrt{\left(\frac{u\ (m_{Compound})}{mcompound}\right)^2 + \left(\frac{u(m_{13C3})}{m13C3}\right)^2 + \left(\frac{u(m_{13C3})}{mstock13C3}\right)^2 + \left(\frac{u(m_{calib})}{m_{calib}}\right)^2 + \left(\frac{u(CG)}{CG}\right)^2 + u(rep)^2 + u(p)^2}$$

CCQM SAMPLE 2

Parameter	***Value(X)***	**u(x)**	**u(x)/X**
Mass of sample (mg)	3.017E+02	6.218E-05	2.061E-07
Mass of labelled std(mg)	*9.795E+01*	6.553E-06	6.691E-08
Labelled stock solution (mg/kg)	*1.000E+03*	9.719E-06	9.719E-09
Uncertainty of calibration standard level 2 (mg)	*3.010E+02*	6.192E-05	2.057E-07
Uncertainty of calibration standard level 3 (mg)	3.028E+02	6.265E-05	2.069E-07
Uncertainty of calibration standard level 4 (mg)	3.027E+02	6.259E-05	2.068E-07
Method Precision	*1.000E+02*	1.387E-01	1.387E-03
Instrument repeatability	1.000E+02	1.729E-01	1.729E-03
Calibration curve	1.159E+00	6.445E-03	5.562E-03
Relative Combined Uncertainty			5.988E-03
Result (mg/g)	1.159E+00		
Combined Standard Measurement Uncertainty		6.938E-03	
Expanded Uncertainty (k=2)		1.388E-02	
Relative Uncertainty		1.198E+00	

CCQM SAMPLE 3

Parameter	***Value(X)***	**u(x)**	**u(x)/X**
Mass of sample (mg)	3.024E+02	6.246E-05	2.066E-07
Mass of labelled std(mg)	*9.985E+01*	6.810E-06	6.821E-08
Labelled stock solution (mg/kg)	*1.000E+03*	9.719E-06	9.719E-09
Uncertainty of calibration standard level 2 (mg)	*3.010E+02*	6.192E-05	2.057E-07
Uncertainty of calibration standard level 3 (mg)	3.028E+02	6.265E-05	2.069E-07
Uncertainty of calibration standard level 4 (mg)	3.027E+02	6.259E-05	2.068E-07
Method Precision	1.000E+02	1.387E-01	1.387E-03
Instrument repeatability	1.000E+02	1.459E-01	1.459E-03
Calibration curve	1.149E+00	5.696E-03	4.958E-03
Relative Combined Uncertainty			5.351E-03
Result (mg/g)	1.149E+00		
Combined Standard Measurement Uncertainty		6.147E-03	
Expanded Uncertainty (k=2)		1.229E-02	
Relative Uncertainty		1.070E+00	

CCQM-K11.2 Uncertainty Information from VNIIM

W=(San*mis)/(Sis*m*F)
W - mass fraction of the glucose in the sample, mg/g;
mis - mass of internal standard added to sample before sample preparation, mg;
m - mass of sample, g;
F - response factor; $F=(San_{cal}*Cis)/(Sis_{cal}*Can)$
Can- concentration of glucose in calibration solution;
Cis - concentration of internal standard in calibration solution
San_{cal} - peak area for the glucose; Sis_{cal} - peak area for the internal standard

Source of uncertainty	u, %
mass of sample (m)	0.29
mass of internal standard added to sample before extraction (m_{IS})	0.58
response factor (F)	0.86
preparation of calibration solution	*0.82*
RSD of F determination	*0.27*
purity of reference standard	*0.087*
RSD of results, %	0.28
comb.std uncertainty	1.11
expanded uncertainty (k=2)	2.22

APPENDIX C: Analytical Methods Used in K12.2

Table C1. Summary of Extraction and Chromatographic Techniques in CCQM-K12.2

NMI/DI	Sample Size (g)	Extraction Method	Post Extraction Cleanup	Analytical Technique	Chromatographic Column
CENAM	0.5	Protein precipitation with ethanol; acetone and water added with agitation 30 s; centrifugation for 30 min	After centrifugation, supernatant transferred to vial and evaporated to dryness under N_2 flow; residue reconstituted in mobile phase and passed through cartridge for purification	LC-MS	Column: X-Terra RP-18 (250 mm × 4.6 mm)
HSA	0.1	Protein precipitation: sample allowed to equilibrate for 2 h then proteins precipitated with 3 equivalent volumes of acetonitrile; centrifuge and remove supernatant	Supernatant evaporated to dryness under N_2 at 40 ºC; residue reconstituted with 500 µL H_2O and filter through 0.22 µm syringe filter. Dilute and inject in LC-MS/MS	LC-MS/MS	Column: Agilent Zorbax SB-Aq, 100 mm × 2.1 mm, 3.5 µm particles
INMETRO	0.0015	Protein precipitation: sample weighed and IS added; H_2O added and MeOH added to precipitate proteins	Centrifuge at 4 ºC; remove 100 µL dilute 10x with H_2O; inject 2 µL into LC-MS/MS	LC-MS/MS	Column: Waters Acquity HSS C18 SB 1.8 µm particles, 50 mm × 2.1 mm
KRISS	0.015	Spike with IS; equilibrate for 3 h; filtration	None	LC-MS/MS	Hypersil Gold 3, 50 mm × 2.1 mm, 3 µm particles
LNE	0.1	Protein precipitation with EtOH for 5 min	No further cleanup	UPLC-MS/MS	Column: Acquity BEH C18, 50 mm × 2.1 mm; 1.7 µm particles
NIMT	0.1	Protein precipitation with acetonitrile for 5 min; centrifugation	No further cleanup	LC-MS/MS	Luna C18, 150 mm × 3.0 mm, 3 µm particles

NMI/DI	Sample Size (g)	Extraction Method	Post Extraction Cleanup	Analytical Technique	Chromatographic Column
NIST	0.45	Spike with IS and equilibrate overnight (20 h); EtOH to precipitate proteins	Centrifuge; supernatant evaporated to dryness and reconstituted in water	LC-MS	Luna C18, 150 mm × 2.0 mm, 3µm particles
NMIJ	0.1	Protein precipitation with acetonitrile; vortex for 5 min	Centrifugal separation (5000 × g for 10 min)	LC-MS	Column: ZIC HILIC, 250 mm × 2.1 mm
PTB	1.0	Spike with IS; equilibrate for 20 h	Separate Creatinine and creatine by cation-exchange clean up; lyophilize and convert creatinine to trimethylsilyl derivative with MSTFA	GC-MS	5% phenyl/95% methylpolysiloxane, 30 m × 0.25 mm; 0.25 µm thickness
UME	0.05	Add IS, vortex mixing; MeOH added to precipitate proteins, vortex 15 s then centrifuged (11000 × g for 5 min); supernatant filtered through 0.22 µm syringe filter	No further cleanup	LC-MS/MS	Reprosil-Por RP 18-NE, 75 mm × 4 mm, 3 µm particles
VNIIM	0.1	Protein precipitation with acetonitrile for 15 min; centrifugation	No further cleanup	LC-MS/MS	Eclipse Plus C18, 100 mm × 2.1 mm, 3.5 µm particles

Table C2: Summary of Detection and Quantification Techniques in CCQM-K12.2

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
CENAM	Mobile phase: Isocratic at 0.02 mol/L ammonium acetate at flow rate of 0.7 mL/min at 25 ºC Mass Selective Detector: positive – mode electrospray ionization. Ions monitored: *m/z* 114 and *m/z* 117 (IS)	IDMS	Bracketing	Creatinine-d_3 (Cambridge Isotopes)	Purity assessed at CENAM using LC, DSC, and Karl Fischer (moisture); DMR-263a Human Serum (CENAM) used as control
HSA	Mobile phase (A) 5 mmol/L ammonium formate with 0.05% formic acid and (B) acetonitrile, isocratic A at 0.3 mL/min; post-injection wash with 90% B. Ions Monitored: *m/z* 114/86 and *m/z* 117/89 (IS) (quantifying ions) and *m/z* 114/44 and *m/z* 117/47 (IS) (confirmatory ions)	IDMS	6-point calibration	Creatinine-d_3 (CDN Isotopes) >99% purity with 99.9% isotope enrichment	NIST SRM 914a
INMETRO	Mobile phase: (A) 10 mmol/L ammonium acetate and (B) acetonitrile; isocratic at 98% A at flow rate 0.35 mL/min MRM transitions monitored: *m/z* 114.2/44.1 for creatinine and *m/z* 117.1/47.1 (IS)	IDMS	Bracketing	Creatinine-d_3 added before sample preparation	NIST SRM 914a
LNE	Mobile phase: Isocratic (A) H_2O with 10 mmol/L ammonium acetate (B) acetonitrile Ions monitored: *m/z* 114 and *m/z* 117 (IS)	IDMS	5-point calibration curve	Creatinine ^{13}C; ^{15}N (ICON Isotopes)	NIST SRM 914a

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
KRISS	Mobile phase: 100% 10 mmol/L ammonium acetate in water at 0.3 mL/min; run time – 5 min. Ions monitored: *m/z* 225/89 and *m/z* 231/92 (IS)	ID LC-MS/MS	Bracketing	Creatinine-d_3 (Cambridge Isotopes)	NIST SRM 914a KRISS CRM 111-01-001 as control
NIMT	Mobile phase: Isocratic 5% 10 mmol/L ammonium acetate in MeOH and 95% 10 mmol/L ammonium acetate in H_2O at flow rate of 0.25 mL/min.	IDMS	Exact matching IDMS; one-point calibration for bracketing	Creatinine-d_3 (methyl d_3) added prior to protein precipitation; equilibration for 1 h with mechanical shaker	NIST SRM 967a used for matrix-matched calibration blends
NMIJ	Ions monitored: *m/z* 114 and *m/z* 117 (IS)	IDMS	2-point calibration curve	Creatinine-d_3 (methyl-d_3)	NMIJ CRM 6005-a
NIST	Mobile phase: 10 mmol/L ammonium acetate in water isocratic at 0.20 mL/min; temperature at 22 ºC. MS positive mode electrospray ionization. Ions monitored: *m/z* 114 and *m/z* 117 (IS)	ID LC-MS	6-point calibration curve	Creatinine-d_3 (Isotec, Miamisburg, OH)	NIST SRM 914a NIST SRM 967a as control
PTB	Ions monitored: *m/z* 329 and *m/z* 332 (IS)	ID GC-MS	Single point	$^{13}C,^{15}N$-Creatinine	NIST SRM 914a
UME	Mobile phase: Isocratic at 75% MeOH and 25% 10 mmol ammonium acetate with 0.4% formic acid (v/v) at flow rate of 0.5 mL/min Ions monitored: *m/z* 114/44 and *m/z* 117/47 (IS)	IDMS	Single point	Creatinine-d_3 (Medical Isotopes) added at beginning of extraction process	NIST SRM 967a

NMI/DI	Chromatographic and Mass Spectrometry Conditions	Quantification Method	Type of Calibration	Internal Standard Used	Sources, Purity, and Traceability of Calibrants
VNIIM	Mobile phase: Isocratic water with 10 mmol/L ammonium acetate Ions monitored: *m/z* 114/44 and *m/z* 115/45 (IS)	IDMS	Single point	Creatinine (methyl ^{13}C) (Sigma-Aldrich) added prior to sample preparation	NIST SRM 914a

Table C3. Comparison of Methods Potentially Critical Parameters in CCQM-K12.2

NMI/DI	Equilibration Time	Internal Standard	Calibrant	CRMs Used as Control	Analytical Technique
CENAM	NR	Creatinine-d_3	CENAM	DMR-263a	LC-MS
HSA	2 h	Creatinine-d_3	SRM 914a		LC-MS/MS
INMETRO	None	Creatinine-d_3	SRM 914a	SRM 909c	UPLC-MS/MS
KRISS	3 h	Creatinine-d_3	SRM 914a	KRISS CRM 111-01-001	ID LC-MS/MS
LNE	24 h	^{13}C,^{15}N-Creatinine	SRM 914a		UPLC-MS/MS
NIMT	NR	Creatinine-d_3	CRM 6005a		LC-MS/MS
NIST		Creatinine-d_3	SRM 914a	SRM 967a	LC-MS
NMIJ	NR	Creatinine-d_3	CRM 6005a		LC-MS
PTB	20 h	^{13}C,^{15}N-Creatinine	SRM 914a		ID GC-MS
UME	NR	Creatinine-d_3	SRM 967a*		LC-MS/MS
VNIIM	NR	^{13}C-Creatinine	SRM 914a		LC-MS/MS

NR Not Reported
* Matrix CRM used as calibrant

APPENDIX D: Summary of Uncertainty Estimation Methods in CCQM-K12.2

The following are pictures of the uncertainty-related information provided by the participants in the "Analytical Information" worksheet of the "Reporting Form" Excel workbook. Information is grouped by participant and presented in alphabetized acronym order.

CCQM-K12.2 Uncertainty Information from CENAM

$$w_x = \left(\frac{\left(\frac{m_2}{m_{I2}}\right) \cdot w_2 \cdot (\overline{R_x} - \overline{R_1}) - \left(\frac{m_1}{m_{I1}}\right) \cdot w_1 \cdot (\overline{R_x} - \overline{R_2})}{\overline{R_2} - \overline{R_1}} \right) \cdot \frac{m_{Ix}}{m_x}$$

Symbol	Description
w_1	Mass fraction of the solution calibration standard (low level) (mg/g)
w_2	Mass fraction of the solution calibration standard (high level) (mg/g)
R_1	Response relationship of low level solution
R_2	Response relationship of high level solution
m_{I1}	Mass of the isotope solution added to the low level solution calibration standard (g)
m_1	Mass of the analyte standard solution of low level calibration (g)
m_{I2}	Mass of the isotope solution added to the high level solution calibration standard (g)
m_2	Mass of the analyte standard solution of high level calibration (g)
m_x	Mass of sample to be measured (g)
m_{Ix}	Mass isotope of the solution added to the sample (g)
R_x	Instrument response relationship (GC or LC) between the analyte in the sample and its isotope added (dimensionless)

Symbol	Description	Value	Units	Uncertainty source	Type of distribution	Standard uncertainty
w_1	Mass fraction of the solution calibration standard (low level)	**5.0139**	µg/g	Experimental	normal type A	0.0342
w_2	Mass fraction of the solution calibration standard (high level)	**8.9998**	µg/g	Experimental	normal type A	0.0371
R_1	Response relationship of low level solution	**0.7160**		Experimental	normal type A	0.0058
R_2	Response relationship of high level solution	**1.2632**		Experimental	normal type A	0.0029
m_{I1}	Mass of the isotope solution added to the low level solution	**0.5671**	g	Experimental	normal type B	0.00003
m_1	Mass of the analyte standard solution of low level calibration (g)	**0.5895**	g	Experimental	normal type B	0.00003
m_{I2}	Mass of the isotope solution added to the high level solution	**0.4808**	g	Experimental	normal type B	0.00002
m_2	Mass of the analyte standard solution of high level calibration	**0.4765**	g	Experimental	normal type B	0.00002
m_x	Mass of sample to be measured (g)	**0.4875**	g	Experimental	normal type B	0.001725
m_{Ix}	Mass isotope of the solution added to the sample (g)	**0.4914**	g	Experimental	normal type B	0.001726
R_x	Instrument response relationship (GC or LC) between the analyte in	**0.9869**		Experimental	normal type A	0.0061
	Mathematical model uncertainty	**0.065288**		**1.4%**		
	Repeatibility between subsamples	**0.0627**				
	Combined uncertainty	**0.09052**				
	Expanded uncertainty	**0.19017**		**2.62%**		
k(95%)	2.10					

CCQM-K12.2 Uncertainty Information from HSA

The mass fraction of creatinine in serum was calculated based on the IDMS calibration curve as follows:

$$C_X = (mR_B + b) \times \frac{W_Y}{M_X} = (mR_B + b) \times \frac{M_Y C_Y}{M_X} \quad (1)$$

where

C_X = mass fraction of creatinine in the serum sample

M_X = mass of serum sample (determined by weighing)

M_Y = mass of isotope standard solution (determined by weighing)

W_Y = mass of the isotope labeled standard spiked into the serum sample (equals to $M_Y \times C_Y$)

R_B = peak area ratio of sample blend (determined by LC-MS/MS measurements)

C_Y = concentration of isotope labeled standard solution (determined by weighing and from purity of the isotope labeled standard)

m = gradient of the slope of linear regression plot (determined by the linear fit of the isotope mass ratio and the peak area ratio of the calibration blends)

b = intercept on y axis of the linear regression plot (determined by the linear fit of the isotope mass ratio and the peak area ratio of the calibration blends)

For the estimation of uncertainty, considering $R_M = mR_B + b$, and let $R_M = R_M' C_Y / C_Z$, Equation (1) is converted to:

$$C_X = R_M \times \frac{M_Y C_Z}{M_X} \quad (2)$$

where

R_M = isotope mass ratio in sample blend

C_Z = concentration of creatinine in the calibration standard solution

A standard uncertainty was estimated for all components of the measurement in Equation (2), which were then combined using respective derived sensitivity coefficients to estimate a combined standard uncertainty in the reported result of creatinine in serum samples. A coverage factor k with a value of 2 is used to expand the combined standard uncertainty at a 95 % confidence interval. Possible sources of biases [method precision (F_P), choice of different ion pair (F_I), and other factors during sample extraction (F_C)] are accounted for in the final uncertainty budget with the use of the measurement equation:

$$C_X = F_P \times F_I \times F_C \times R_M' \times \frac{M_Y C_Z}{M_X} \quad (3)$$

The sensitivity coefficients of each component can be expressed as follows:

$$\frac{\partial C_X}{\partial R_M'} = \frac{C_X}{R_M'} \qquad \frac{\partial C_X}{\partial M_Y} = \frac{C_X}{M_Y} \qquad \frac{\partial C_X}{\partial M_X} = -\frac{C_X}{M_X} \qquad \frac{\partial C_X}{\partial C_Z} = \frac{C_X}{C_Z}$$

$$\frac{\partial C_X}{\partial F_P} = \frac{C_X}{F_P} \qquad \frac{\partial C_X}{\partial F_I} = \frac{C_X}{F_I} \qquad \frac{\partial C_X}{\partial F_C} = \frac{C_X}{F_C}$$

The standard uncertainty of each component was calculated as follows:

(1) M_Y and M_X: The standard uncertainty was calculated based on the calibration report using the standard weights calibrated by the National Metrology Centre, A*STAR.

(2) F_P: The standard deviation of the mean of the results was used as the the standard uncertainty of method precision.

(3) F_I: The standard deviation of the difference of the results using two ion pairs divided by the square root of the number of samples (for insignificant difference using t-test) or the average of the difference of the results using two ion pairs divided by 2 (for significant difference using t-test).

(4) F_C : A relatively standard uncertainty of 0.1 % was employed for this factor.

(5) C_Z: The certified purity and uncertainty of NIST SRM 914a in combination with the uncertainty of weighing for preparation of the calibration standard solution.

(6) R_M' : Consider $R_M = R_M' \times C_Z / C_Y$, the conversion of equation $R_M = mR_B + b$ leads to:

$R_B = (C_Z \times R_M') / (C_Y \times m) - b/m$

Let $m' = C_Z/(C_Y \times m)$ and $b' = -b/m$, we have:

$R_B = m'R_M' + b'$

The standard uncertainty of R_M' was calculated using the following equation:

$$\mu_{R_M} = \frac{1}{m'} \times s_{y/x} \times \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(R_B - \overline{R_{Bc}})^2}{m'^2 \sum_{i=1}^{n} (R_{Mc} - \overline{R_{Mc}})^2}} \quad (4)$$

where

$s_{y/x}$ = standard deviation of the regression
R_B = peak area ratio of sample blend
$\overline{R_{Bc}}$ = average peak area ratio of calibration blends
n = number of calibration blends used for the linear regression plot
N = injection time for each sample
R_{Mc} = isotope mass ratio in calibration blends
$\overline{R_{Mc}}$ = average isotope mass ratio in calibration blends
The combined standard uncertainty was calculated using the equation below:

$$u = \sqrt{\sum_i c_i^2 u_{xi}^{\ 2}} \qquad (5)$$

where
u = combined standard uncertainty
c_i = sensitivity coefficient of each component
u_{xi} = standard uncertainty of each component
The expanded uncertainty (U) was calculated by mutiplying the combined standand uncertainty (u) with a coveragy factor (k = 2) for 95% confidence level.

Table 1. Uncertainty Budget for Sample 1

Factor	Value x	Uncertainty u(x)	Relative Uncertainty u(x)/(x)	Sensitivity Coefficient (c) δCx/δx	c2 . u(x)2	% Contribution
M_X (g)	0.0998	0.000099	0.099%	73.57	0.000053	1.7%
M_Y (g)	0.0743	0.000099	0.133%	98.86	0.000096	3.1%
C_Z (µg/g)	1067.7	4.1438	0.388%	0.0069	0.000812	26.5%
R_M '	0.00918	0.00004	0.432%	799.35	0.001006	32.8%
F_P (µg/g)	7.34	0.0090	0.123%	1.00	0.000081	2.6%
F_I (µg/g)	7.34	0.0310	0.422%	1.00	0.000962	31.4%
F_C (µg/g)	7.34	0.0073	0.100%	1.00	0.000054	1.8%

Table 2. Uncertainty Budget for Sample 2

Factor	Value x	Uncertainty u(x)	Relative Uncertainty u(x)/(x)	Sensitivity Coefficient (c) δCx/δx	c2 . u(x)2	% Contribution
M_X (g)	0.0989	0.000099	0.100%	74.38	0.000054	1.8%
M_Y (g)	0.0738	0.000099	0.134%	99.73	0.000097	3.2%
C_Z (µg/g)	1067.7	4.1438	0.388%	0.0069	0.000815	26.5%
R_M '	0.00918	0.00004	0.432%	800.82	0.001010	32.8%
F_P (µg/g)	7.36	0.0090	0.123%	1.00	0.000081	2.6%
F_I (µg/g)	7.36	0.0311	0.422%	1.00	0.000966	31.4%
F_{C2} (µg/g)	7.36	0.0074	0.100%	1.00	0.000054	1.8%

CCQM-K12.2 Uncertainty Information from INMETRO

$$W_x = \left(\frac{m_{solute}}{m_{final}}\right)_{stock\ sol} * \left(\frac{m_{conc\ sol}}{m_{final'}}\right)_{use\ sol} * \frac{m_z}{m_{yc}} * \frac{m_y}{m_x} * \frac{R'_B}{R'_{Bc}} * P$$

Wx = Sample mass fraction; msolute = mass of the solute used to prepare the stock solution; mfinal = final mass (solute + solvent) of the stock solution; mconc sol = mass of the stock solution used to prepare the use solution; mfinal' = final mass (solute + solvent) of the use solution; P = purity of the calibrant; mz = mass of the calibrant solution added to the standards; myc = mass of the internal standard solution added to the standards; my = mass of the internal standard solution added to the samples; mx = mass of the sample; R'B = analyte/internal standard area ratio measured in the sample; R'Bc = analyte/internal standard area ratio measured in the standards

All factors from the measurement equation were considered in the uncertainty estimation. All of the evaluated uncertainties were of type B except for the R'B and R'Bc repeatabilities. Hence their standard uncertainties were obtained by dividing the expanded uncertainties by the coverage factors found in the certificates. For the repeatabilities, standard uncertainties were obtained by the standard errors of the means (s/√n). The standard uncertainties were multiplied by their sensitivity coefficients using the GUM methodology and then combined using the square root of the squared sum of the components. Effective degrees of freedom were calculated and the coverage factors for 95 % probability were taken for the expanded uncertainties. The full uncertainty budget is presented below as for sample 1:

Factor	% contribution
m_{solute}	16,5299965
m_{final}	0,0000005
$m_{conc\ sol}$	0,0007036
$m_{final'}$	0,0000116
P	0,4749587
m_z	0,7222476
m_{yc}	1,0838228
m_y	1,0838228
m_x	0,0899882
R'B	12,6658313
R'Bc	67,3486163

Method was validated by the preparation in two different days of the CRM from Nist 909c. Both preparations were equivalent to the certified property values for the CRM by comparison of the Δm (absolute difference between the mean measured value and the certified value) and the UΔ (expanded uncertainty of the difference between the measurement result and the certified value), obtaining Δm < UΔ which means the measured value and the certified value have no significant differences according to ERM Application Note 1. These experiments demonstrated repeatability, intermediate precision and trueness (bias) evaluations of the method.

CCQM-K12.2 Uncertainty Information from LNE

C = (aR 114/117+ b) x ((mLabCLab)/ mser))

C = mass fraction of creatinine in the serum sample (µg/g)
mLab = mass of labeled creatinine solution
CLab = concentration of labeled creatinine solution
a = gradient of the slope for linear regression plot
b = intercept on y axis for the linear regression plot
R 114/117 = unlabeled/labeled ion peak area ratio of serum sample
mser = mass of serum sample

sample1

Component	Type (A or B)	relative Uncertainty (%)
Purity of primary standard	B	2.87%
preparationof sample blends (weighings)	B	14.27%
Calibration model	B	0.95%
Preparation of calibration blend (weighings)	B	12.74%
Precision	B	69.16%

sample2

Component	Type (A or B)	relative Uncertainty (%)
Purity of primary standard	B	5.14%
preparationof sample blends (weighings)	B	6.24%
Calibration model	B	1.28%
Preparation of calibration blend (weighings)	B	15.74%
Precision	B	71.54%

Expanded measurement equation:

$$w_x = F_P . F_E . F_I . w_{z,c} \cdot \frac{m_y \cdot m_{zc}}{m_x \cdot m_{yc}} \cdot \frac{R'_b}{R'_{bc}}$$

$w_{z,c}$	is the mass fraction of analyte in the calibration solution used to prepare the calibration blend
m_y	is the mass of spike solution added to sample blend
$m_{y,c}$	is the mass of spike solution added to calibration blend
m_x	is the mass of sample added to sample blend
$m_{z,c}$	is the mass of standard solution added to calibration blend
R'_B and $R'_{B,C}$	are the observed isotope amount ratios in the sample blend and the calibration blend, respectively
F_E	is the extraction efficiency factor
F_P	is the method precision factor
F_I	is the interference effect factor

$$\frac{u(w_x)}{w_x} = \sqrt{\left(\frac{u(w_{Z,C})}{w_{Z,C}}\right)^2 + \left(\frac{u(m_Y)}{m_Y}\right)^2 + \left(\frac{u(m_{Y,C})}{m_{Y,C}}\right)^2 + \left(\frac{u(m_X)}{m_X}\right)^2 + \left(\frac{u(m_{Z,C})}{m_{Z,C}}\right)^2 + \left(\frac{u(F_P)}{F_P}\right)^2 + \left(\frac{u(F_E)}{F_E}\right)^2 + \left(\frac{u(F_I)}{F_I}\right)^2}$$

$u(w_{z,c})$ is the standard uncertainty of the mass fraction of analyte in the calibration solution used to prepare the calibration blend. The value was estimated from the certified mass fraction value of matrix-matched calibration standard, masses weighed for preparation of calibration standard and uncertainty using different standards (standard comparison).

$u(m_y)$, $u(m_{y,c})$, $u(m_x)$ and $u(m_{z,c})$ are standard uncertainties of the masses. These values were estimated from the bias and precison effect of the balance.

$u(F_P)$ is the standard uncertainty of the precision factor. This value was estimated from standard deviation of the multiple IDMS results.

$u(F_E)$ is the standard uncertainty of the extraction efficiency factor which was estimated from the extraction and protein precipitaion

$u(F_I)$ is the standard uncertainty of the interference effect. This value was estimated from potential bias between primary ion pair and secondary ion pair of the MRM program.

Note: For the uncertainty contributing to the R'_B and $R'_{B,C}$,the precision in measuring the isotope amount ratios of the analyte and the internal standard in the sample and calibration blends was assumed to be incorporated in the overall method precision.The effect of any biases on these ratios was assumed to be negligible as any systematic biases should cancel out since the calibration blends and sample blends were exact-matched for analyte concentration and isotope ratio. Other biases that may arise from extractions are captured in other factors.

Uncertainty budget of creatinine (sample I)			
Factor	Values x	Uncertainties u(x)	u(x)/(x)
Parameter (unit)			
Method Precision, F_P(1)	1.0000	0.00702	0.702%
$m_{z,c}$ (g)	0.08372	0.000049	0.0591%
m_y (g)	0.07478	0.000049	0.0662%
$m_{y,c}$ (g)	0.07489	0.000049	0.0661%
m_x (g)	0.10035	0.000049	0.0493%
$w_{z,c}$ (ug/g)	0.7557	0.0088	1.1680%
Additional Factors			
Extraction effects, F_E (1)	1.000	0.0100	1.000%
Interference from two different ion pairs, F_I (1)	1.000	0.0029	0.293%

Uncertainty Analysis Results		
wx=	**7.451**	ug/g
u(x) =	0.128	ug/g
u(x)/x =	1.72%	
Veff(total) =	41.989	
k=	2.02	(@ 95% level)
U(x) =	**0.259**	ug/g
%U(x) =	**3.47%**	

Uncertainty budget of creatinine (sample II)			
Factor	Values x	Uncertainties u(x)	u(x)/(x)
Parameter (unit)			
Method Precision, F_P(1)	1.0000	0.00761	0.761%
$m_{z,c}$ (g)	0.08372	0.000049	0.0591%
m_y (g)	0.07478	0.000049	0.0662%
$m_{y,c}$ (g)	0.07489	0.000049	0.0661%
m_x (g)	0.10035	0.000049	0.0493%
$w_{z,c}$ (ug/g)	0.7557	0.0091	1.2028%
Additional Factors			
Extraction effects, F_E (1)	1.000	0.0100	1.000%
Interference from two different ion pairs, F_I (1)	1.000	0.0031	0.312%

Uncertainty Analysis Results		
wx=	**7.435**	ug/g
u(x) =	0.132	ug/g
u(x)/x =	1.77%	
Veff(total) =	43.585	
k=	2.02	(@ 95% level)
U(x) =	**0.266**	ug/g
%U(x) =	**3.57%**	

CCQM-K12.2 Uncertainty Information from NIST

For sample preparation of 1951c, Level 2, duplicate preparations were made from three different vials chosen at random from three different boxes stored at -80 °C. Creatinine values were based on duplicate LC-MS injections of all calibrants, controls, and SRM 1951c, Level 2 and were calculated using an exact matching stable-isotope labeled standards. The run order was as follows: calibrant solutions, SRM 1951c, SRM 909c, SRM 967a followed by SRM 976a, SRM 909c, SRM 1951c, and calibrant solutions in reverse order. The measured ratios for each calibration curve standard were subjected to linear regression analysis, and the least squares fit then used to calculate the weight ratios for the samples from the measured intensity ratios. A mix of standards from the independently prepared solutions of SRM 914a was used, and the excellent R^2 (0.995) value obtained showed that the two solutions were equivalent. To determine creatinine values, the relative response factor (RRF) was calculated using the following equation:

$$\mathbf{RRF} = \frac{\mathbf{(Area\ Creatinine_{Calibrant})(Mass\ Internal\ Standard_{Calibrant})}}{\mathbf{(Area\ Internal\ Standard_{Calibrant})(Mass\ Creatinine_{Calibrant})}}$$

The RRF was averaged from duplicate injections of each of four independently prepared calibrant solutions on the day of analysis. The RRF was then applied to the control (SRM 909c and 967a) and SRM 1951c samples to determine the mass fraction of creatinine according to the following equation:

$$\mathbf{Mass\ Fraction,\ \mu g/g} = \frac{\mathbf{(Area\ Creatinine_{Sample})(Mass\ Internal\ Standard_{Sample},\ \mu g)}}{\mathbf{(Area\ Internal\ Standard_{Sample})(RRF)(Mass\ Sample,\ g)}}$$

The uncertainty in the result was estimated from the variability of all measurements. The standard uncertainty of the mean was conservatively estimated as the standard deviation divided by √3.

	Mass Fraction, µg/g	
Vial	Injection 1	Injection 2
1	7.7094	7.5691
2	7.5240	7.8790
3	7.6292	7.7132

Parameter	µg/g
Mean:	7.671
SD:	0.127 (1.65 %)
u:	0.073
$U_{k=2}$:	0.146

As a check on the homogeneity assessment and to confirm creatinine stability, in Feb. 2014 ten units of SRM 1951c, Level 2 were evaluated using the approach described above. Two independent aliquots were analyzed for each vial. Each aliquot was injected twice. The results were very similar, confirming stability and homogeneity of 2 % or better.

CCQM-K12.2 Uncertainty Information from NIST (Continued)

	Mass Fraction, µg/g			
	Aliquot1		Aliquot2	
Vial	Inj1	Inj2	Inj1	Inj2
1	7.6718	7.5618	7.2976	7.6469
2	7.9061	7.7614	7.4891	7.6090
3	7.6123	7.7608	7.5475	7.5784
4	7.4267	7.5260	7.5838	7.5783
5	7.7660	7.7245	8.0854	7.9162
6	7.7327	7.7512	7.5946	7.6102
7	7.7016	7.6480	7.5837	7.5604
8	7.6714	7.7502	7.6620	7.7043
9	7.5501	7.3022	7.5573	7.6682
10	7.3932	7.5247	7.6803	7.5951

Parameter	µg/g
Mean:	7.631
SD:	0.152 (2.0 %)
u:	0.048
$U_{k=2}$:	0.096

CCQM-K12.2 Uncertainty Information from NMIJ

C_sample = mass_IS/mass_sample x (MS_sample/MS_IS – b)/a

a=(y2-y1)/(x2-x1)
b = y1-ax1 =y2-ax2

x1=C_std1 x (mass_std1/mass_std1_IS)
x2=C_std2 x (mass_std2/mass_std2_IS)
y1=MS_std1_cr/MS_std1_IS
y2=MS_std2_cr/MS_std2_IS

C_sample: creatinine concentration in sample
C_std1: creatinine concentration in std1
C_std2: creatinine concentration in std2
mass_sample: mass fraction of creatinine in sample
mass_IS: mass fraction of IS for sample
mass_std1: mass fraction of creatinine in std1
mass_std1_IS: mass fraction of IS in std1
mass_std2: mass fraction of creatinine in std2
mass_std2_IS: mass fraction of IS in std2
MS_sample: peak area of creatinine in sample
MS_IS: peak area of IS in sample
MS_std1: peak area of creatinine in std1
MS_std1_IS: peak area of IS in std1
MS_std2: peak area of creatinine in std2
MS_std2_IS: peak area of IS in std2

Factor			Value	Unit	Std. Uncertainty	Rel. Std. Uncertainty (%)		Type
Standard material			0.999	kg/kg	0.001	0.10		B
Balance								
	Calibration solution					0.07		B
	Add sample		100	mg	0.05	0.05		B
	Add IS		100	mg	0.05	0.05		B
Calibration curve						0.75		A
Measurement			7.431	ug/g				
	Between sample prep.					0.49		A
	Between measurement					0.28		A
	Combined	std. uncertainty		ug/g	0.071	0.95		
	Expanded	uncertainty (k = 2)		ug/g	0.141	1.90		

CCQM-K12.2 Uncertainty Information from PTB

Vial	Aliquot	Mass fraction (µg/g)
1	1	7.6727
2	1	7.7278
3	1	7.4599
4	1	7.5842
5	1	7.7316
6	1	7.6623

Mean:	7.6398
Standard uncertainty uc:	0.0584
Expanded Uncertainty U:	0.1168 (1.50 %)
Coverage factor k (95%):	2

$$RF = \frac{A_{ABx} x C_{ISx}}{A_{ISx} x C_{ABx}}$$

RF : Response factor
CABx : Concentration of native compound (mg/g)
AABx : Peak area of native compound
AISx : Peak area of labelled compound
CISx : Concentration of labelled compound (mg/g)

Uncertainty Sources		
1-Mass of sample	Value	Standard Uncertainty
Mass of compound	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Compound}) = \sqrt{u_{CmCompound}^2 + u_{CmTare}^2}$$

2-Mass of Labelled STD	Value	Standard Uncertainty
Mass of labelled compound	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Compound\ ^{13}C3}) = \sqrt{u_{CmCompound\ ^{13}C3}^2 + u_{CmTare}^2}$$

3-Labelled Compounds Stock Solution	Value	Standard Uncertainty
Mass of Compound^{13}C3	m_{13C3}	
Calibration		uCm_{C13C3}
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}
Mass of Solvent	$m_{solvent}$	
Calibration		$uCm_{solvent}$

$$u(m_{stock\ ^{13}C3}) = \sqrt{u_{stock\ ^{13}C3}^2 + u_{CmSolvent}^2 + u_{CmTare}^2}$$

4- Uncertainty of calibration standard	Value	Standard Uncertainty
Mass of calib	$m_{Compound}$	
Calibration		$uCm_{Compound}$
Mass of Tare	m_{tare}	
Calibration		uCm_{tare}

$$u(m_{Calib}) = \sqrt{u_{CmCalib}^{2} + u_{CmTare}^{2}}$$

6-Method Precision

where,
$u(rep)$: Uncertainty of repeatability
SD: Standard deviation
n: Number of sample

$$u(rep) = \frac{SD}{\sqrt{n}}$$

7-Instrument Repeatability

where,
$u(rep)$: Uncertainty of repeatability
SD: Standard deviation
n: Number of sample

$$u(rep) = \frac{SD}{\sqrt{n}}$$

6-Calibration Graph

$$u(c_0) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{S_{xx}}} \qquad S_{xx} = \sum_{i=1}^{n}(c_i - \bar{c})^2$$

S Residual standard deviation
B1 Slope
p number of measurement to determine c_0
n number of measurement for the calibration
c_0 determined concentration
$\bar{c}$ mean value of the different calibration standards (n number of measurement)
i index for the number of calibration standards

COMBINED STANDARD MEASUREMENT UNCERTAINTY

$$\frac{u_c}{c} = \sqrt{\left(\frac{u(m_{Compound})}{mcompound}\right)^2 + \left(\frac{u(m_{13C3})}{m13C3}\right)^2 + \left(\frac{u(m_{13C3})}{mstock13C3}\right)^2 + \left(\frac{u(m_{calib})}{m_{calib}}\right)^2 + \left(\frac{u(CG)}{CG}\right)^2 + u(rep)^2 + u(p)^2}$$

CCQM-K11.2 Uncertainty Information from UME (Continued)

CCQM SAMPLE 1

Parameter	***Value(X)***	**u(x)**	**u(x)/X**
Mass of sample (mg)	5.014E+01	1.717E-06	3.425E-08
Mass of labelled std	*9.252E+00*	5.921E-08	6.400E-09
Labelled stock solution (mg/kg)	*5.000E+01*	3.093E-02	6.186E-04
Mass of calibration standard level 1 (mg)	*4.890E+01*	1.666E-06	3.406E-08
Method Precision	*1.000E+02*	4.191E-01	4.191E-03
Instrument repeatability	1.000E+02	2.630E-01	2.630E-03
Relative Combined Uncertainty			4.986E-03
Result (µg/g)	7.472E+00		
Combined Standard Measurement Uncertainty		3.725E-02	
Expanded Uncertainty (k=2)		7.451E-02	
Relative Uncertainty		9.972E-01	

CCQM SAMPLE 3

Parameter	***Value(X)***	**u(x)**	**u(x)/X**
Mass of sample (mg)	4.987E+01	1.699E-06	3.407E-08
Mass of labelled std	*9.755E+00*	6.502E-08	6.666E-09
Labelled stock solution (mg/kg)	*5.000E+01*	3.093E-02	6.186E-04
Mass of calibration standard level 1 (mg)	*4.890E+01*	1.666E-06	3.406E-08
Method Precision	*1.000E+02*	4.191E-01	4.191E-03
Instrument repeatability	1.000E+02	2.968E-01	2.968E-03
Relative Combined Uncertainty			5.172E-03
Result (µg/g)	7.481E+00		
Combined Standard Measurement Uncertainty		3.869E-02	
Expanded Uncertainty (k=2)		7.739E-02	
Relative Uncertainty		1.034E+00	

CCQM-K12.2 Uncertainty Information from VNIIM

W=(San*mis)/(Sis*m*F)
W - mass fraction of the creatinine in the sample, mkg/g;
mis - mass of internal standard added to sample before sample preparation, mkg;
m - mass of sample, g;
F - response factor; $F=(San_{cal}*Cis)/(Sis_{cal}*Can)$
Can- concentration of creatinine in calibration solution;
Cis - concentration of internal standard in calibration solution
San_{cal} - peak area for the creatinine; Sis_{cal} - peak area for the internal standard

Source of uncertainty	u, %
mass of sample (m)	0.29
mass of internal standard added to sample before extraction (m_{IS})	0.58
response factor (F)	0.84
preparation of calibration solution	*0.82*
RSD of F determination	*0.18*
purity of reference standard	*0.087*
RSD of results, %	0.38
comb.std uncertainty	1.12
expanded uncertainty (k=2)	2.24