CCQM-K109
High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum

Key Comparison
Track A

Final Report
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Coordinating institute:

Qinde Liu, Hong Liu, Yizhao Chen, Sharon Yong, Hui Ling Teo, Lingkai Wong and Tang Lin Teo
Health Sciences Authority (HSA), Singapore

With contributions from:

Veronica Vamathevan
National Measurement Institute, Australia (NMIA)

Eliane C. P. do Rego, Wagner Wollinger, Jane L. N. Fernandes, Tania M. Monteiro, Bruno C. Garrido and Fernando M. Violante
Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO), Brazil

Lian Hua Shi, Hai Hong He, Can Quan, Bei Xu, Hong Mei Li, Xin Hua Dai and Ya Juan He
National Institute of Metrology (NIM), China

Man-fung Lo and Yiu-chung Yip
Government Laboratory, Hong Kong (GLHK), Hong Kong SAR, China

Julie Cabillic and Vincent Delatour
Laboratoire National de Métrologie et d'Essais (LNE), France

Rüdiger Ohlendorf and Andre Henrion
Physikalisch-Technische Bundesanstalt (PTB), Germany

Migaku Kawaguchi
National Metrology Institute of Japan (NMIJ), Japan

Dukjin Kang and Hwashim Lee
Korea Research Institute of Standards and Science (KRISS), Republic of Korea

Mariana Arce Osuna, Victor Serrano and Claudia Marcela Salazar Arzate
Centro Nacional de Metrologia (CENAM), Mexico

Anatoliy Krylov and E. Lopushanskaya
D.I. Mendeleyev Institute for Metrology (VNIIM), Russian Federation

Jintana Nammoonnoy
National Institute of Metrology Thailand (NIMT), Thailand

Ahmet Ceyhan Gören, Simay Gündüz and Hasibe Yilmaz
TÜBİTAK Ulusal Metroloji Enstitüsü (UME), Turkey, (TÜBİTAK UME), Turkey

Christopher Mussell and John Warren
LGC, United Kingdom

Jeanita S. Pritchett, Katrice Lippa, Michael A. Nelson, Blaza Toman, Lorna T. Sniegoski and David Duewer
National Institute of Standards and Technology (NIST), United States of America

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1. INTRODUCTION

Urea serves an important role in the metabolism of nitrogen-containing compounds by humans and is the main nitrogen-containing substance in the urine of humans. The cycling and excretion of urea by the kidneys are vital parts of mammalian metabolism that remove unwanted waste from the body. High concentration of urea in the blood could be a symptom of kidney or renal failure. Likewise, uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal component of urine. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions including diabetes and the formation of kidney stones.

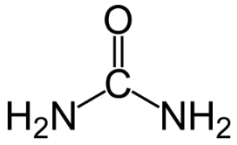
The OAWG has agreed on a list of Track A key comparisons to assess the core competencies of National Metrology Institutes/Designated Institutes (NMIs/DIs) for the delivery of measurement services to their customers. One of the Track A comparisons discussed and agreed upon under the matrix category was “Polar Organic in Biological Matrix”. In the OAWG meeting in November 2013 in Pretoria, South Africa, the meeting discussed the possible analytes and biological materials in this comparison, which could best cover current and future CMCs. The Health Sciences Authority (HSA), Singapore suggested urea and uric acid in human serum as a possible comparison for this category and presented a proposal at the meeting in April 2014 at BIPM. After considering the services offered by the NMIs/DIs[1] and that this would be the first Track A comparison for biological materials, the OAWG agreed on the HSA’s proposal for urea and uric acid in human serum as an appropriate comparison for this matrix category in April 2014. A key comparison and a parallel pilot study were thus organised.

2. OBJECTIVES

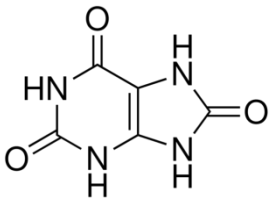
The comparison aimed to enable participating NMIs/DIs to demonstrate their competence in the determination of high polarity organic compounds in a biological matrix. As a model system for this comparison, two polar clinical biomarkers: urea and uric acid, in human serum were chosen.

[1] NMIs with existing CMCs for both urea and uric acid include KRISS (frozen human serum and lyophilised human serum), PTB (blood serum), and NIST (human serum). NIM, China has a CMC for uric acid in human serum.

3. MEASURANDS



Urea
CAS Number: 57-13-6
MW: 60.06 g/mol
pK_{OW}: 2.1[2]



Uric acid
CAS Number: 69-93-2
MW: 168.11 g/mol
pK_{OW}: 2.66[3]

Figure 1: Information on the measurands

4. THE COMPARISON MATERIALS

The comparison materials were frozen human sera. An experienced commercial human blood products supplier (Solomon Park Research Laboratories, Kirkland, WA, USA) was engaged by HSA to prepare the materials. Two pools of human serum materials with two different concentration levels of urea and uric acid were prepared, and pre-packed in 260 vials containing 1 mL of serum each.

The mass fractions of urea and uric acid in the comparison materials were in the range of 100 to 2,000 and 10 to 165 mg/kg, respectively. One of the concentration levels is within the normal biological range while the other is higher than normal range. The concentration levels are within the range of existing CMC claims for these types of analytes in the BIPM KCDB.

2 A.C. Moffat, M.D. Osselton, B. Widdap. Clarke's Analysis of Drugs and Poisons. Pharm. Press, Vol 2, 1690.

3 S.G. Machatha, S.H. Yalkowsky. Comparison of the octanol/water partition coefficients calculated by ClogP®, ACDlogP and KowWin® to experimentally determined values, 294 (2005), 185.

4.1. Homogeneity Studies

The homogeneity of the comparison materials was assessed by gas chromatography-isotope dilution mass spectrometry (GC-IDMS). A sample size of 0.10 g was taken for the study. Eleven vials were randomly and stratifically selected, and two subsamples were taken from each vial. Using ANOVA at 95% level of confidence, both materials were found to be sufficiently homogeneous. The plots showing the normalised concentrations of urea and uric acid in two subsamples taken from 11 vials are given in Figures 2 to 5. Summaries of the ANOVA for the homogeneity study are also given in Tables 1 to 4.

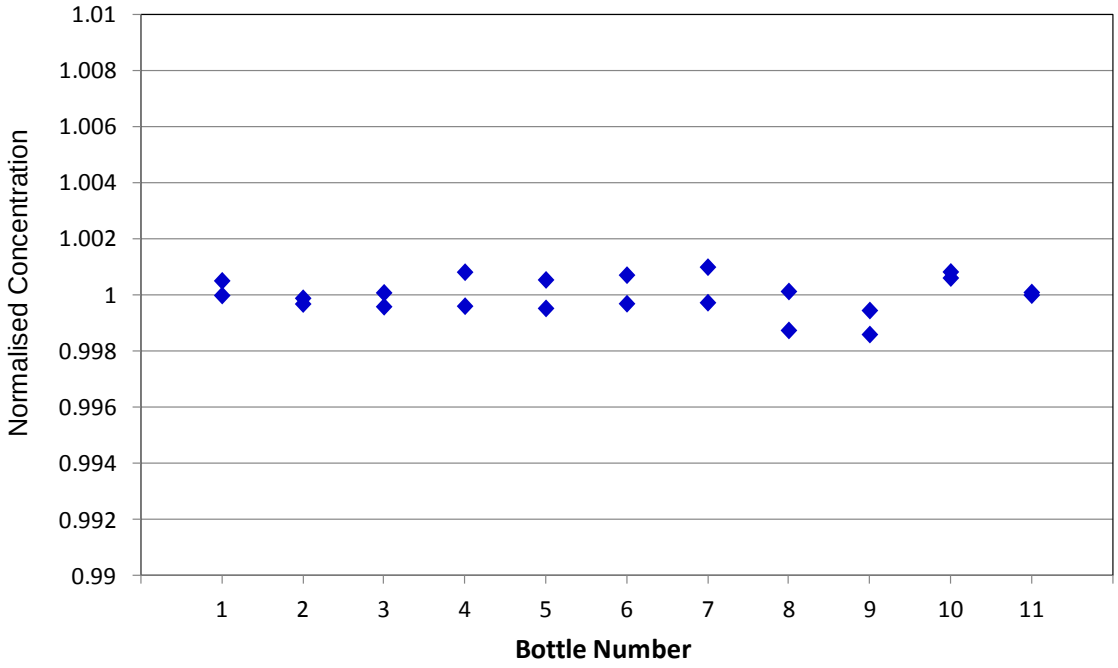


Figure 2: Homogeneity assessment of urea (Serum I)
Results are from two subsamples taken from 11 vials.

Table 1: Summary of ANOVA for urea (Serum I)

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between vials	9.46	10	0.946	1.12	42.5%	2.85
Within vials	9.29	11	0.844			
Total	18.8	21	0.893			

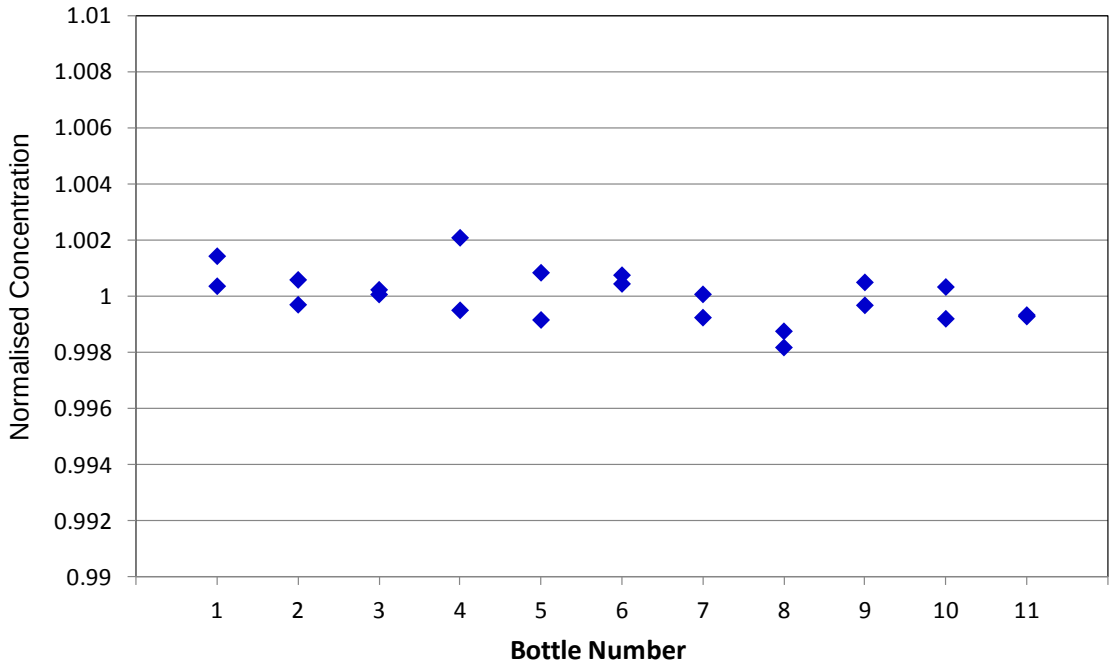


Figure 3: Homogeneity assessment of urea (Serum II)
Results are from two subsamples taken from 11 vials.

Table 2: Summary of ANOVA for urea (Serum II)

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between vials	1.09	10	0.109	1.47	26.9%	2.85
Within vials	0.817	11	0.0743			
Total	1.91	21	0.0908			

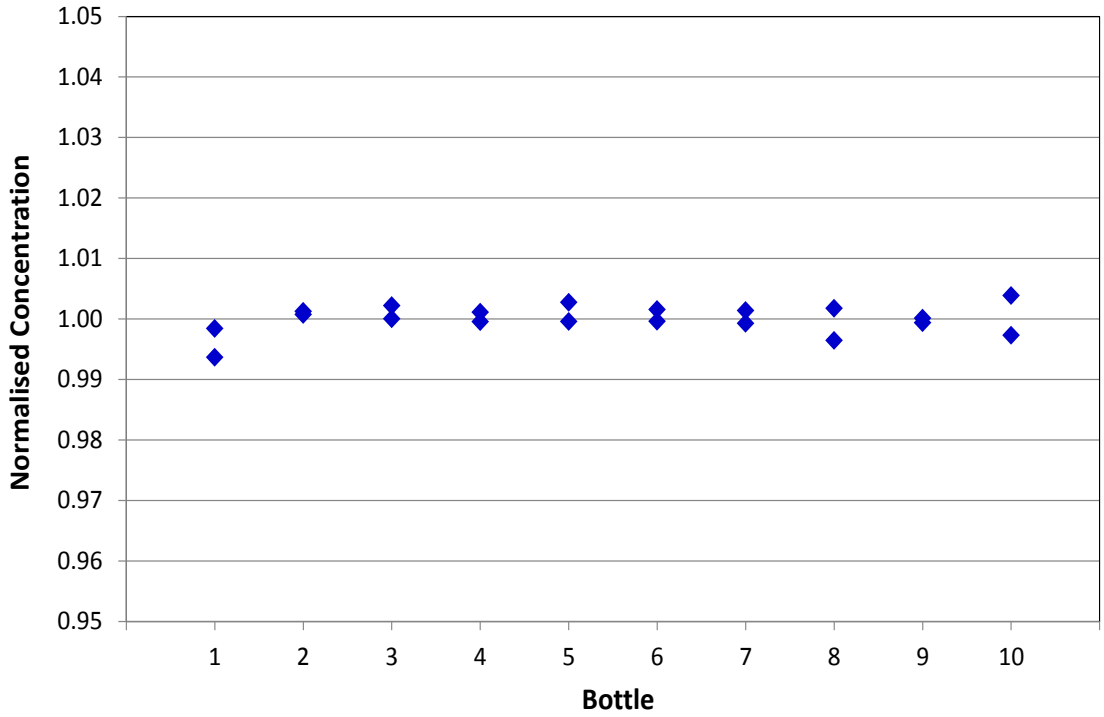


Figure 4: Homogeneity assessment of uric acid (Serum I)
Results are from two subsamples taken from 11 vials.

Table 3: Summary of ANOVA for uric acid (Serum I)

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between vials	0.486	9	0.0540	0.777	64.2%	3.02
Within vials	0.695	10	0.0695			
Total	1.18	19	0.0621			

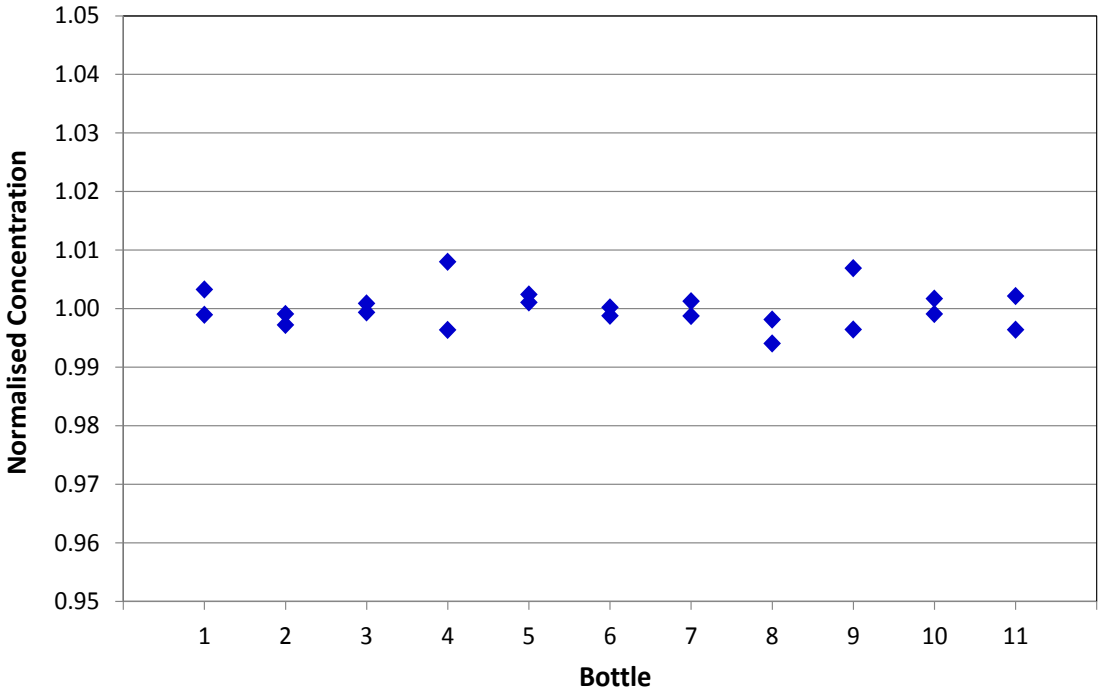


Figure 5: Homogeneity assessment of uric acid (Serum II)
Results are from two subsamples taken from 11 vials.

Table 4: Summary of ANOVA for uric acid (Serum II)

Source of variance	*SS*	DF	MS	F	p-Value	$F_{critical}$
Between vials	0.0606	10	0.00606	0.411	91.4%	2.85
Within vials	0.162	11	0.0147			
Total	0.223	21	0.0106			

The relative standard uncertainties of inhomogeneity were found to be below 0.04% and 0.18% for urea and uric acid (for both concentration levels), respectively.

4.2. Stability Studies

The stability of the comparison materials at -70 °C was assessed using GC-IDMS and liquid chromatography-isotope dilution mass spectrometry (LC-IDMS) for urea and uric acid, respectively. The same sample size as described in the homogeneity studies was also used. The study was carried out on four occasions over a period of about 230 days using classical design. On each occasion of the stability study, two vials were randomly selected, and two subsamples were taken from each vial.

The plots showing the normalised concentrations of urea and uric acid over the period of study are given in Figures 6 to 9. The effect of exposure time on the stability of the

measurands was determined by fitting linear regression lines to the data set. The slope (*b*) was tested for statistical significance using Student's *t* test at 95% confidence level, where the *t* value was calculating by dividing *b* by its standard deviation *s(b)* and compared against the critical *t* value. The statistical results given in Table 5 indicated that no significant trend at 95% confidence level was detected.

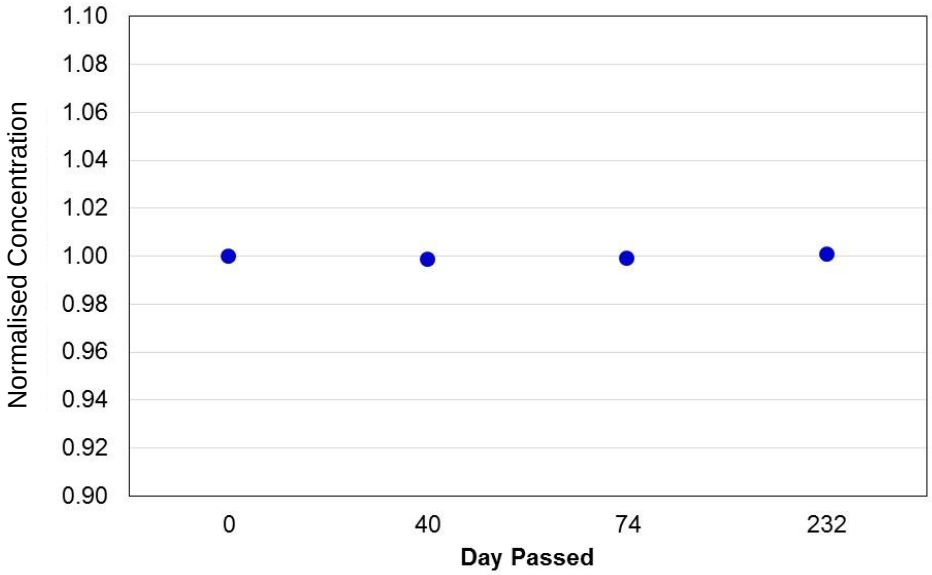


Figure 6: Stability assessment of urea (Serum I)
Results are from two subsamples taken from two vials on four occasions.

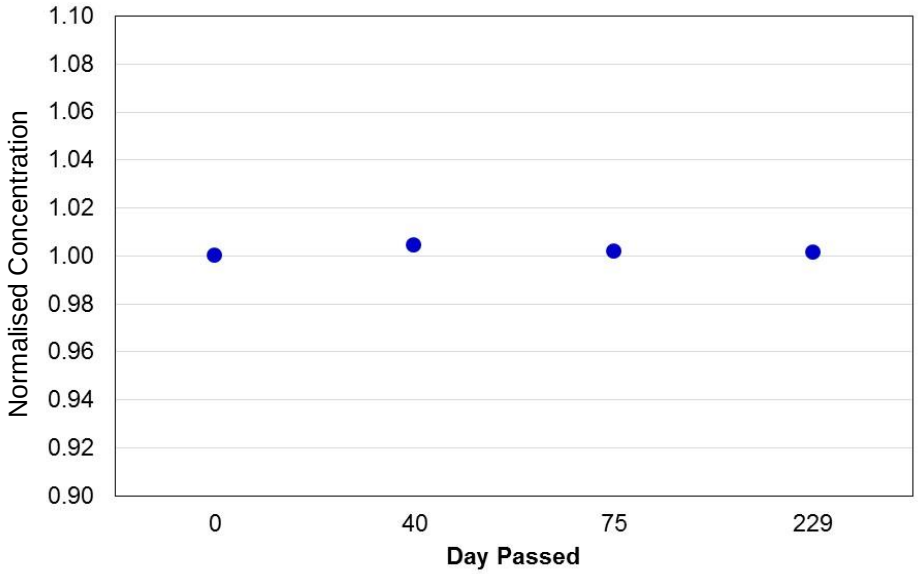


Figure 7: Stability assessment of urea (Serum II)
Results are from two subsamples taken from two vials on four occasions.

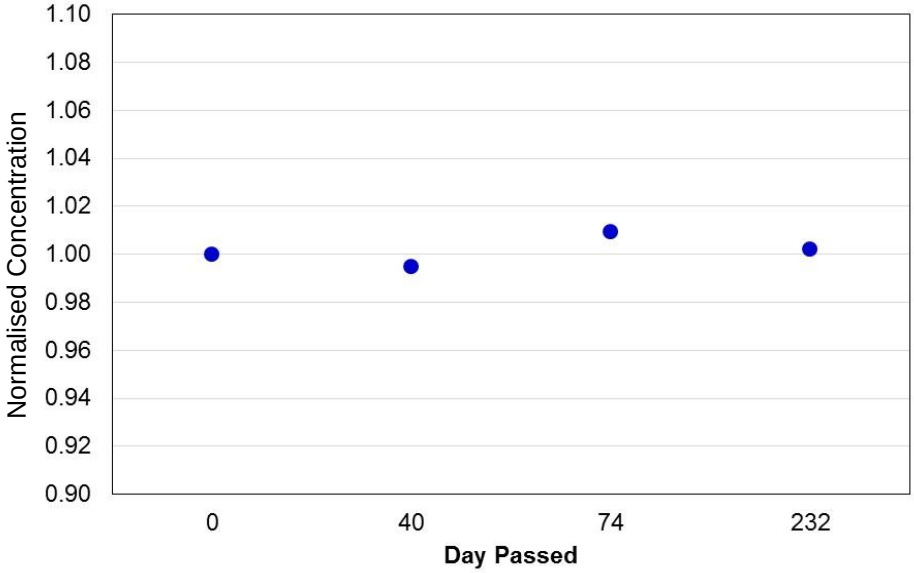


Figure 8: Stability assessment of uric acid (Serum I)
Results are from two subsamples taken from two vials on four occasions.

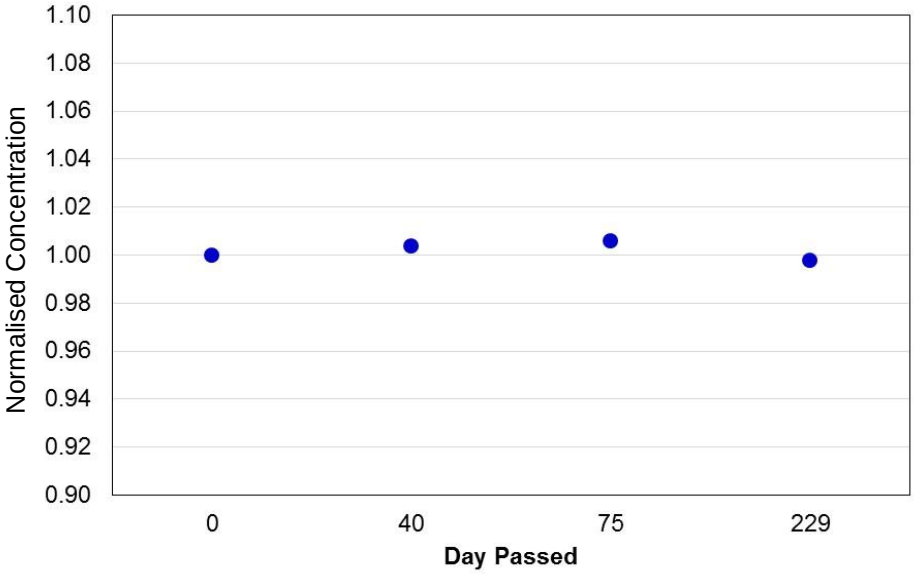


Figure 9: Stability assessment of uric acid (Serum II)
Results are from two subsamples taken from two vials on four occasions.

Table 5: Summary of statistics from stability study on urea and uric acid*

Descriptions	Urea		Uric acid	
	Serum I	Serum II	Serum I	Serum II
Slope of the regression line (*b*)	0.0081	-0.0005	0.0020	-0.0007
Intercept of the regression line	1484.4	335.9	136.0	39.22
Variance of the points (s^2)	2.073	0.627	0.952	0.022
Standard deviation of the points (*s*)	1.440	0.792	0.976	0.148
Uncertainty of slope [*s(b)*]	0.0082	0.0046	0.0055	0.0009
Calculated *t* ($\frac{\lvert b \rvert}{s(b)}$)	0.99	0.11	0.35	0.87
Critical *t* factor ($t_{0.95,n-2}$)	4.30	4.30	4.30	4.30

* Samples held at -70 °C for about 230 days

The relative standard uncertainties of instability were estimated to be below 0.21% and 0.46% for urea and uric acid (for both concentration levels), respectively.

The comparison samples were dispatched in dry ice to ensure their stability. The stability of the comparison samples under the conditions of analysis, i.e. at room temperature (18 to 25 °C), were not investigated. However, the participating institutes were requested to analyse the materials immediately after they had thawed.

5. SCHEDULE

Table 6: Schedule for the comparison

Date	Event
July 2015	Call for participation
February 2016	Distribution of study sample
September 2016	Deadline for submission of results
October 2016	Presentation of preliminary results at the OAWG meeting
February 2017	Preliminary Report
April 2017	2nd Presentation of results
September 2017	Draft A Report
March 2018	Draft B Report
July 2018	Final Report

6. INSTRUCTIONS TO PARTICIPATING INSTITUTES

In the Study Protocol (Appendix A), the participating institutes were pre-notified that comparison materials were tested non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV) by the supplier before distribution. However, the materials should be handled as biohazards materials capable of transmitting infectious diseases.

Upon receipt, the comparison materials should be immediately stored at a temperature below – 60 ^{o}C before measurement. The materials should be used immediately after they are thawed, as measurements on vials which have been previously thawed and opened, have not been conducted.

Each participating NMI/DI was provided with three vials of serum sample for each concentration level and measurand that it registered for, i.e. a NMI/DI would receive a total of 12 vials if it registered for both urea and uric acid. The participating NMIs/DIs could use one of the three vials as a practice sample and should report the results for the remaining two vials. At least two subsamples should be taken from each vial. The participating NMIs/DIs were free to decide on the number of times that each subsample was to be measured. Before sampling, the material should be allowed to thaw and warm to room temperature (18 – 25 ^{o}C), and homogenised by gentle swirling and inversing the vial several times. The subsamples taken from the same vial should be measured on the same day. The recommended minimum subsample size was 0.10 g.

The participating NMIs/DIs were requested to use their own methods for the determination. Metrologically traceable certified reference materials (CRMs) should be used as calibration standards.

7. REGISTRATION, SAMPLE RECEIPT AND REPORT SUBMISSION

A total of 15 NMIs/DIs registered to participate in the measurement of both urea and uric acid in the key comparison. Each participating NMI/DI was provided with three vials of comparison materials for each concentration level of each measurand, i.e. a total of 12 vials were provided. One of the three vials could be used as a practice sample, while the remaining two vials had to be used for reporting.

All the participating NMIs/DIs received the comparison materials intact and they were not exposed to temperature above -60 °C during transportation. Information on participating NMIs/DIs, contacts and sample receipts are summarised in Table 7. Upon request, CENAM was provided with two additional vials of Serum I for uric acid measurement as all the earlier provided comparison materials were used up due to technical issues.

Table 7: Information on participating NMIs/DIs, contacts and sample receipts

No.	Participating Institutes	Economy	Contact Person	Sample Receipt Date
1	NMIA National Measurement Institute Australia	Australia	Veronica Vamathevan	1 Mar 2016
2	INMETRO Instituto Nacional de Metrologia, Qualidade e Tecnologia	Brazil	Eliane C. P. do Rego	16 Mar 2016
3	NIM National Institute of Metrology	China	Can Quan	3 Mar 2016
4	GLHK Government Laboratory, Hong Kong	Hong Kong SAR, China	Man-fung Lo	1 Mar 2016
5	LNE Laboratoire National de Métrologie et d'Essais	France	Julie Cabillic / Vincent Delatour	1 Mar 2016
6	PTB Physikalisch-Technische Bundesanstalt	Germany	Rüdiger Ohlendorf and Andre Henrion	2 Mar 2016
7	NMIJ National Metrology Institute of Japan	Japan	Migaku Kawaguchi	2 Mar 2016
8	KRISS Korea Research Institute of Standards and Science	Republic of Korea	Hwashim Lee	1 Mar 2016
9	CENAM Centro Nacional de Metrologia	Mexico	Mariana Arce Osuna	4 Mar 2016
10	VNIIM D.I. Mendeleyev Institute for Metrology	Russian Federation	Anatoliy Krylov	28 Mar 2016
11	HSA Health Sciences Authority	Singapore	Tang Lin Teo / Qinde Liu	Not applicable
12	NIMT National Institute of Metrology Thailand	Thailand	Jintana Nammoonnoy	1 Mar 2016
13	UME TÜBİTAK Ulusal Metroloji Enstitüsü	Turkey	Ahmet Ceyhan Gören	3 Mar 2016
14	LGC	United Kingdom	Christopher Mussell / John Warren	1 Mar 2016
15	NIST National Institute of Standards and Technology	United States of America	Katrice Lippa	4 Mar 2016

8. REPORTING OF RESULTS

The participating institutes were requested to report their results based on at least four subsamples (two subsamples from each vial) for each level. The results were to be reported in the unit of mg/kg and should include standard and expanded uncertainties (95% level of confidence) for the mean of the replicate determinations. Information on measurement procedure, calibration standard, internal standard, quality control material, calculation of the results, and estimation of measurement uncertainty had to be provided as well.

9. RESULTS SUBMITTED BY PARTICIPATING INSTITUTES

For urea and uric acid in both Serum I and II comparison materials, 15 and 14 results were received, respectively. Table 8 and

Table 9 summarise the results for urea in Serum I and Serum II.

Table 8: Summary of results for urea (Serum I)

NMI/DI	Mean of Bottle 1 (mg/kg)	Mean of Bottle 2 (mg/kg)	Overall mean of results (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, k (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
NMIA	1473	1464	1469	8	10	1.97	20
INMETRO	1363	1434	1399	4	131	2	262
NIM	1475	1485	1481	8	16	2	32
GLHK	1516	1512	1514	9	22	2	44
LNE	1477	1468	1473	6	14	2	28
PTB	1487.3	1486.0	1486.7	6	7.5	2.0	15.0
NMIJ	1477	1473	1475	[#]	5	2	10
KRISS	1600.9	1607.5	1604.2	4	13.0	2.26	29.3
CENAM	1480.7	1490.8	1485.7	4	9.8	2	19.6
VNIIM	1506	1502	1504	6	37.6	2	75
HSA	1476.9	1477.5	1477	8	12.2	2	24
NIMT	1466	1446	1456	6	16	2.10	33
UME	1609.535	1618.622	1614.079	6	15.954	2	31.908
LGC	1552	1554	1553	6	10	2.571	26
NIST	1499.6	1515.6	1505.8	4	18.4	2	36.8

[#] Indicated as “Bottle × Sample prep. × Analysis = 2 × 3 × 3”

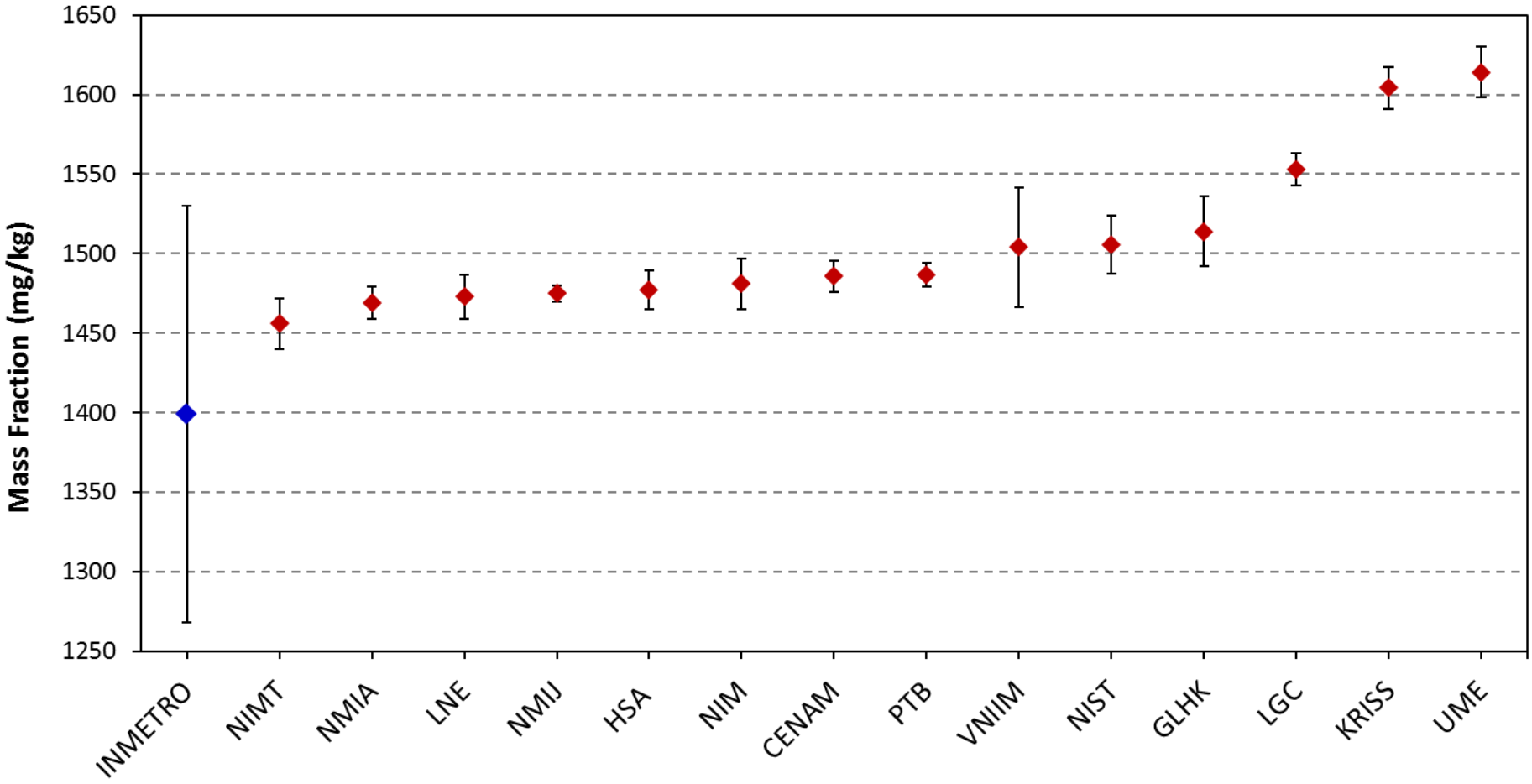


Figure 10: Reported results for urea (Serum I)

Bars represent standard uncertainties. INMETRO's result (blue diamond) was obtained using spectroscopic method (HPLC-DAD) rather than IDMS.

Table 9: Summary of results for urea (Serum II)

NMI/DI	Mean of Bottle 1 (mg/kg)	Mean of Bottle 2 (mg/kg)	Overall mean of results (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, k (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
NMIA	327.2	329.5	328.4	8	2.6	1.97	5.1
INMETRO	306	298	302	4	28	2	56
NIM	334.6	334.9	334.8	8	4.0	2	8.0
GLHK	329.8	331.3	330.6	8	4.8	2	9.7
LNE	330	343	337	6	8	2	16
PTB	334.3	334.1	334.2	6	1.7	2.0	3.4
NMIJ	333.6	333.8	333.7	[#]	1.9	2	3.7
KRISS	335.9	337.8	336.9	4	2.5	2.31	5.7
CENAM	339.9	337.0	338.4	4	3.86	2	7.7
VNIIM	336.2	336.47	336	6	5.04	2	10
HSA	334.15	332.59	333.4	8	2.72	2	5.4
NIMT	331.9	329.8	329.0	6	6.1	2.13	13
UME	357.872	355.569	356.72	6	3.526	2	7.052
LGC	342.8	342.8	342.8	6	2.5	2.571	6.4
NIST	331.5	332.4	331.8	4	4.4	2	8.8

[#] Indicated as “Bottle × Sample prep. × Analysis = 2 × 3 × 3”

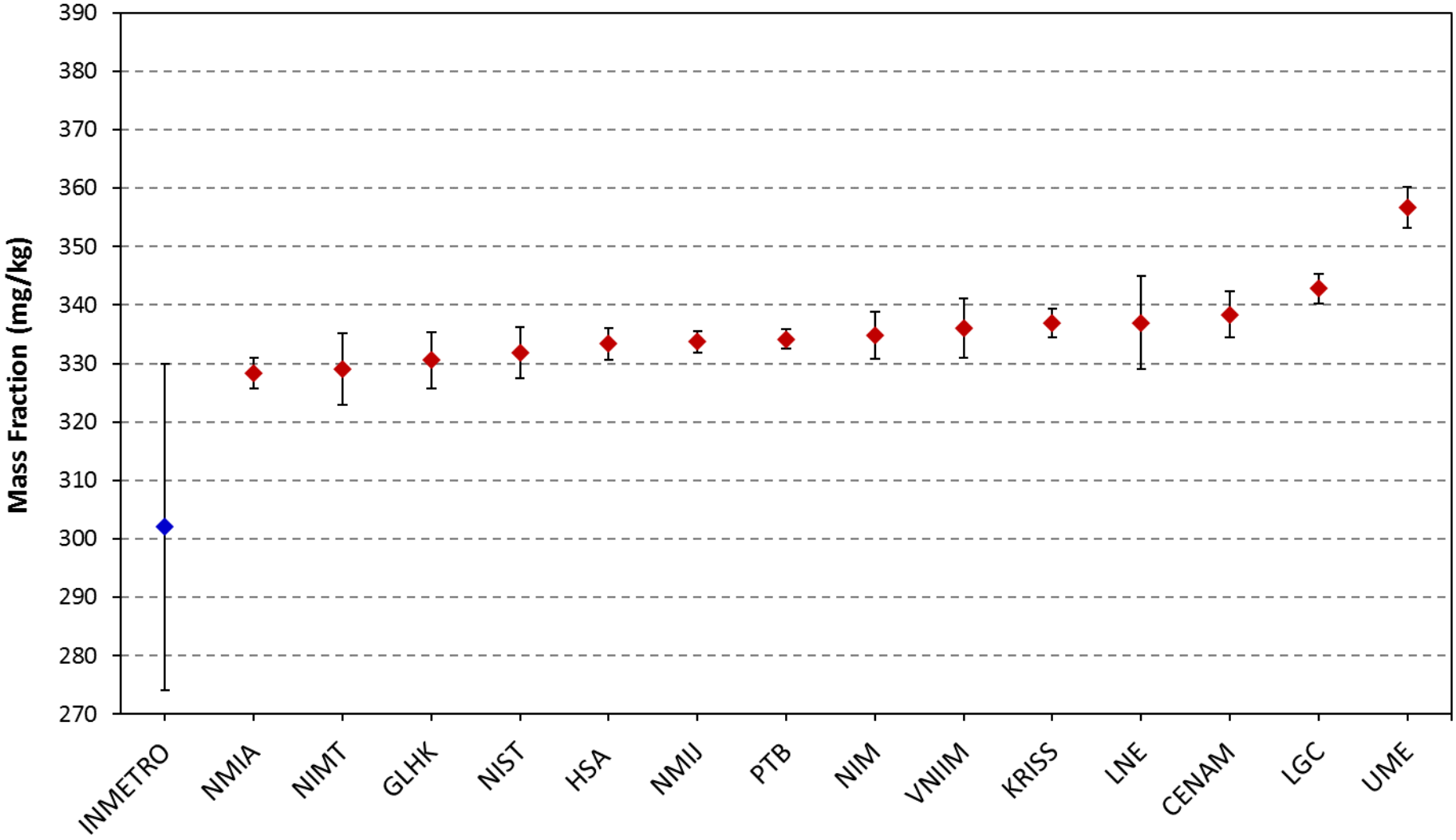


Figure 11: Reported results for urea (Serum II)

Bars represent standard uncertainties. INMETRO's result (blue diamond) was obtained using spectroscopic method (HPLC-DAD) rather than IDMS.

Table 10: Summary of results for uric acid (Serum I)

NMI/DI	Mean of Bottle 1 (mg/kg)	Mean of Bottle 2 (mg/kg)	Overall mean of results (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, k (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
NMIA	137.4	135.9	136.4	12	2	2.05	4.1
INMETRO	162	142	152	4	10	2	21
NIM	137.0	136.1	136.5	6	1.5	2	3.0
LNE	137.4	135.3	136.4	6	1.4	2	2.8
PTB	134.18	134.86	134.52	6	0.72	2.03	1.5
GLHK	136.0	136.4	136.2	9	2.5	2	5.0
NMIJ	137.8	138.9	138.3	[#]	0.9	2	1.9
KRISS*	145.9	146.7	146.4	6	1.5	3.18	4.9
KRISS♦[a]	*139.2*	140.9	140.0	*6*	*0.7*	2.12	1.4
CENAM	149.82	150.87	150.42	4	5.95	2	11.91
CENAM-1[a]			133.9	*9*	*1.65*	2	3.3
CENAM-2[a]			139.9	*4*	*1.3*	2	2.6
VNIIM	122.19	125.36	123.8	6	3.34	2	6.7
VNIIM[b]	*129.79*	133.16	131.5	*6*	*3.34*	2	6.7
HSA	136.8	136.81	136.8	8	1.41	2	2.8
NIMT	135.0	134.9	134.6	8	1.5	2.16	3.2
UME	148.706	148.355	148.530	6	2.105	2	4.211
NIST	135.5	136.2	135.8	4	1.7	2	3.4

[#] Indicated as “Bottle × Sample prep. × Analysis = 2 × 3 × 6”; * Mean of Bottle 3: 146.6 mg/kg; ♦ Mean of Bottle 3: 140.0 mg/kg;
[a] Investigational study, result not used in analysis; [b] Re-calculated value, not used in analysis

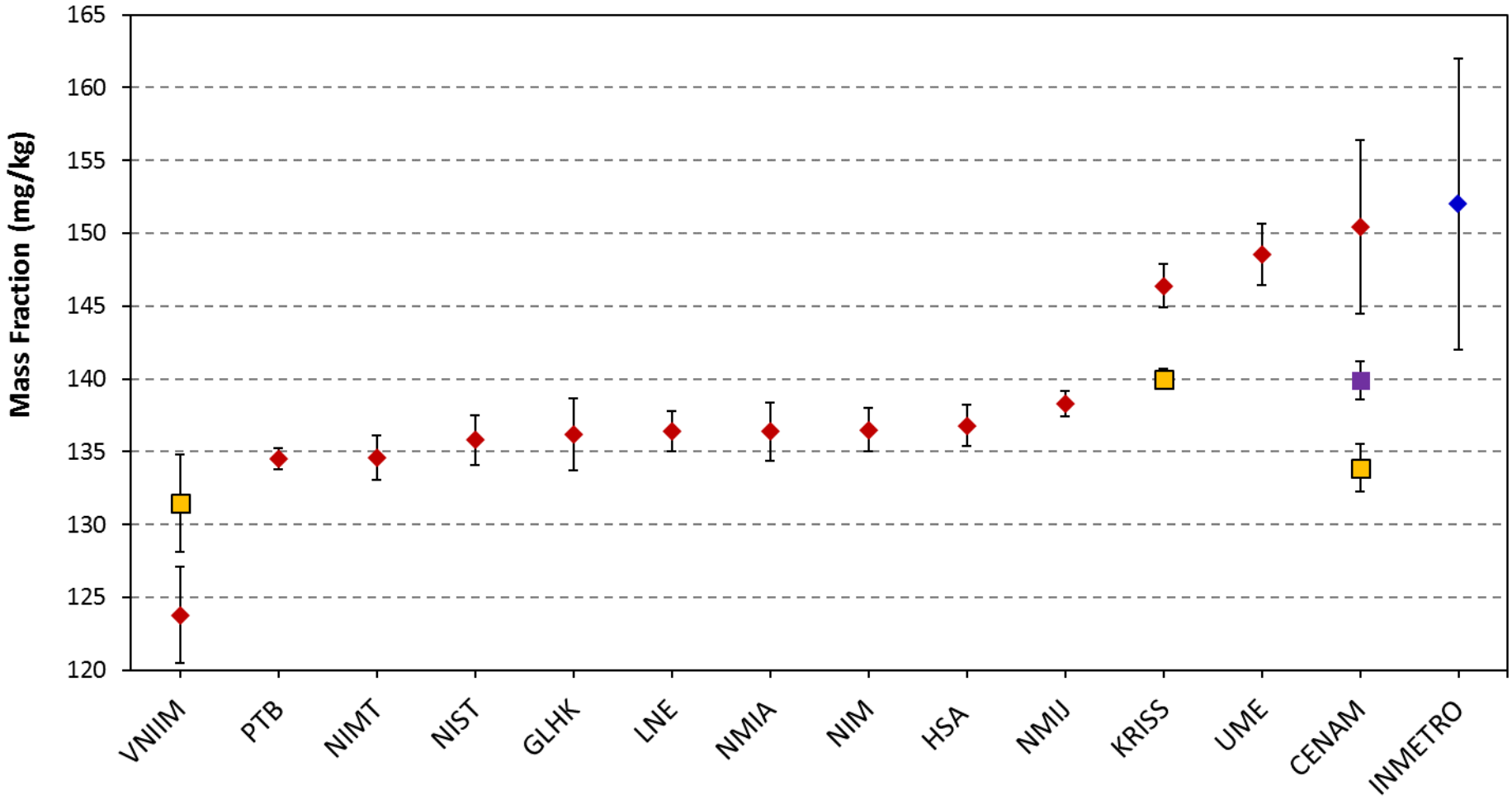


Figure 12: Reported results for uric acid (Serum I)

Bars represent standard uncertainties. INMETRO's result (blue diamond) was obtained using standard addition with LC-MS/MS. The results from re-calculation and investigation (yellow boxes) by VNIIM and KRISS are included for information, as are CENAM's 1st (yellow box) and 2nd (purple box) investigational results.

Table 11: Summary of results for uric acid (Serum II)

NMI/DI	Mean of Bottle 1 (mg/kg)	Mean of Bottle 2 (mg/kg)	Overall mean of results (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, *k* (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
NMIA	39.4	39.2	39.2	12	0.6	2.03	1.2
INMETRO	43	43	43	4	3	2	5
NIM	39.6	39.78	39.67	5	0.65	2	1.3
LNE	39.1	40.0	39.6	6	0.61	2	1.2
PTB	39.36	39.38	39.37	6	0.20	2.0	0.41
GLHK	38.6	39.0	38.8	10	0.8	2	1.6
NMIJ	39.38	39.37	39.37	[#]	0.17	2	0.34
KRISS*	42.4	42.3	42.3	6	0.5	2.57	1.4
KRISS♦[a]	*40.2*	*40.5*	*40.3*	*6*	*0.2*	*2.12*	*0.4*
CENAM	45.14	45.36	45.25	4	0.68	2	1.37
CENAM-1[a]			*38.7*	*9*	*0.60*	*2*	*1.2*
CENAM-2[a]			*40.3*	*4*	*0.57*	*2*	*1.1*
VNIIM	38.94	39.01	39.0	6	1.01	2	2.0
HSA	39.281	39.306	39.29	8	0.407	2	0.81
NIMT	38.76	38.35	38.66	6	0.52	2.04	1.07
UME	40.150	39.731	39.940	6	0.566	2	1.132
NIST	38.9	39.2	39.1	4	0.5	2	1.0

[#] Indicated as “Bottle × Sample prep. × Analysis = 2 × 3 × 6”; * Mean of Bottle 3: 42.3 mg/kg; ♦ Mean of Bottle 3: 40.1 mg/kg;
[a] Investigational study, result not used in analysis

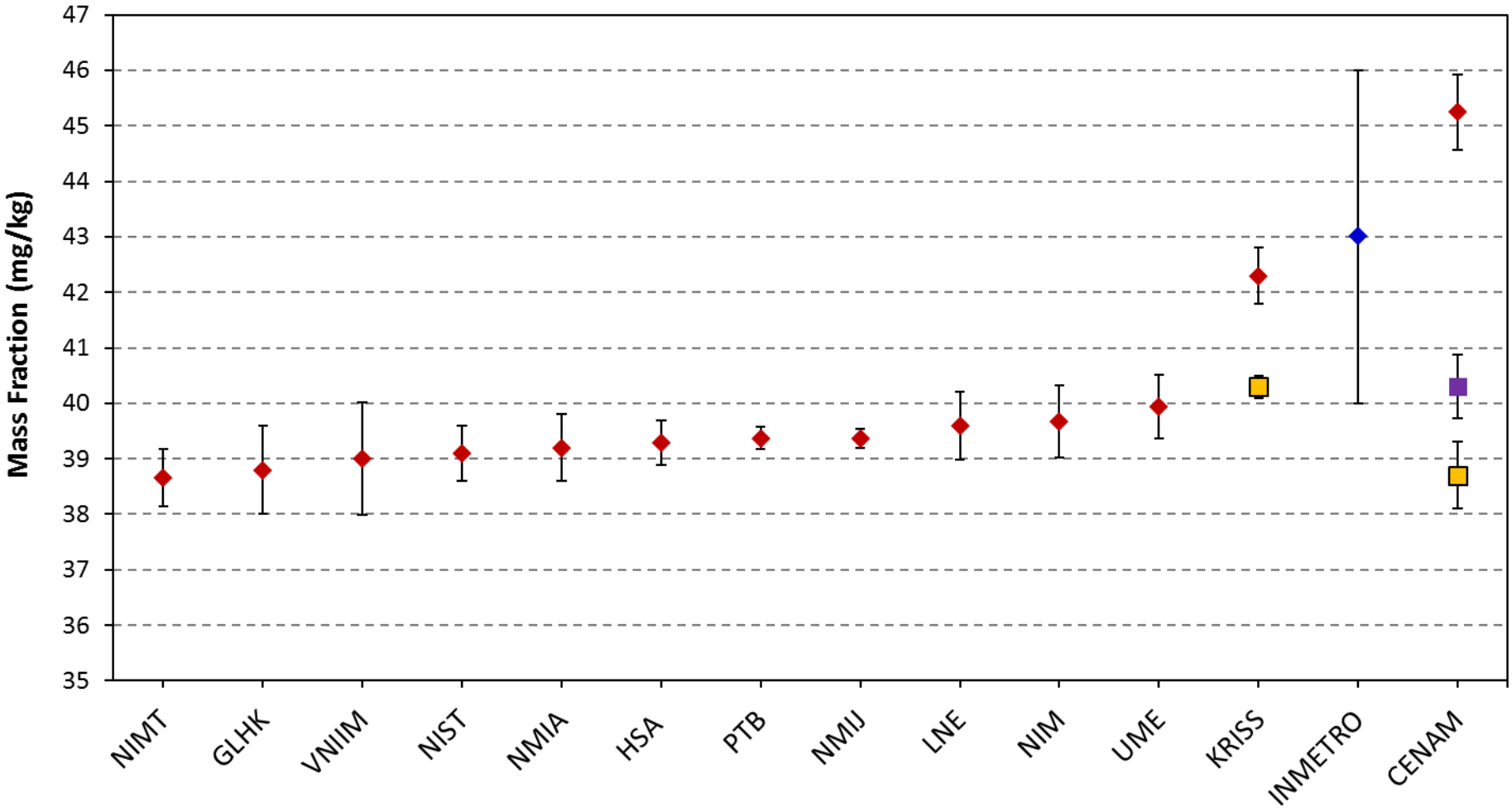


Figure 13: Reported results for uric acid (Serum II)

Bars represent standard uncertainties. INMETRO's result (blue diamond) was obtained using standard addition with LC-MS/MS. The result from investigation (yellow box) by KRISS is included for information, as are CENAM's 1st (yellow box) and 2nd (purple box) investigational results.

10. SUMMARY OF TECHNICAL INFORMATION

The following Tables summarise the technical information provided in the Report of Results Forms from participating NMIs/DIs. The participating institutes' measurement uncertainty statements are provided in Appendix B.

Table 12: Summary of sample size and pre-treatment for urea

NMI/DI	Sample Size (g)	Pre-treatment
NMIA	0.1 g	Protein precipitation with 3 mL ethanol followed by derivatisation with 0.3 mol/L malonaldehyde bis(dimethylacetal) in the presence of hydrochloric acid. Derivatised samples were cleaned-up using HILIC HPLC using an amino phase (Alltech Alltima, 4.6 × 250 mm, 5 μm) and an acetonitrile/water mobile phase. A fraction containing the 2-hydroxypyrimidine derivative of urea was collected for analysis.
INMETRO	0.1 g	Protein precipitation with 2.5 volumes acetonitrile, centrifugation.
NIM	0.2 mL	Protein precipitation was used. After spiking the labeled $^{13}C,^{15}N_2$-Urea as internal stand, the sample was added acetonitrile for protein precipitation. The mixture was shaken gently for 30 min using an orbital shaker, and was centrifuged at 8000 rpm for 15min. The upper supernatant was dried under nitrogen at 40℃, and the residue was diluted with a mobile phase to a urea concentration of ~ 10 mg/kg, and was filtered with a 0.22μm filter for LC/MS.
GLHK	0.1 g	Appropriate amount isotopic internal standard solution was gravimetrically added to the sample. Acetonitrile was added for protein precipitation. The sample was then centrifuged, filtered and dried under gentle nitrogen flow. 2 mmol/L NH_4OH solution was added for reconstitution.
LNE	0.1 g	Add 3 mL ethanol. Precipitation of proteins was done by intensive shaking with vortex mixer. Centrifugation 10°C, 10 min, 2800 g for phase separation.
PTB	0.25 g	Protein precipitation with ethanol
NMIJ	0.1 g	Protein precipitation
KRISS	>0.1 g	Precipitation of protein with acetonitrile corresponding to 10 times of sample volume – Centrifugation – Drying of supernatant – Dissolving with water - Filtration with 0.2 μm filter – Analysis by LC/MS/MS.
CENAM	0.3 g	Protein precipitation
VNIIM	0.1 g	Protein precipitation by acetonitrile

HSA	0.1 g	Protein precipitation was used for clean-up. The details are as follows: After spiking the isotope labelled internal standard solution, the sample was vortexed, and allowed to equilibrate at ambient temperature for 2 h. Acetonitrile (3 fold of aqueous volume) was then added for protein precipitation. The sample was vortexed vigorously and centrifuged for 5 min at 4000 rpm. The supernatant was filtered through 0.22 µm syringe filter. For LC-MS/MS analysis, the filtrate was diluted to approximately 2000 ng/g with acetonitrile.
NIMT	0.1 g	Ethanol was used for protein precipitation
UME	0.1 g	Protein precipitation with acetonitrile, sample dilution, vortex, centrifuge, filtration
LGC	0.2 g	Protein precipitation with 10 mL of 10 mmol/L ammonium acetate solution: acetonitrile (10:90 v/v) followed by centrifugation.
NIST	0.09297 g – 0.09730 g (Serum I) & 0.32316 g – 0.32506 g (Serum II)	Solid phase extraction: Sep-Pak Vac RC (500 mg) C18 cartridges

Table 13: Summary of sample size and pre-treatment for uric acid

NMI/DI	Sample Size (g)	Pre-treatment
NMIA	0.1 g	Protein precipitation using 200 mL acetonitrile. Sample extracts were cleaned-up using reversed-phase HPLC (Grace Platinum C18-EPS, 4.6 × 250 mm, 5 mm, acetonitrile/0.2% acetic acid (aqueous) mobile phase). A fraction containing uric acid was collected for analysis.
INMETRO	0.1 g	Protein precipitation with 2.5 volumes acetonitrile, centrifugation.
NIM	0.2 mL	Protein precipitation was used. After spiking the labeled $^{13}C,^{15}N_2$-Urea as internal stand, the sample was added acetonitrile for protein precipitation. The mixture was shaken gently for 30 min using an orbital shaker, and was centrifuged at 8000 rpm for 15 min. The upper supernatant was dried under nitrogen at 40℃, and the residue was diluted with a mobile phase to a uric acid concentration of ~ 10 mg/kg, and was filtered with a 0.22 µm filter for LC/MS.
GLHK	0.1 g	Appropriate amount isotopic internal standard solution was gravimetrically added to the sample. Acetonitrile was added for protein precipitation. The sample was then centrifuged, filtered and dried under gentle nitrogen flow. 2 mmol/L NH_4OH solution was added for reconstitution.
LNE	0.1 g	Add of acetonitrile. Precipitation of proteins was done by intensive shaking with vortex mixer. Centrifugation 10°C, 5 min, 1700 g for phase separation
PTB	0.25 g	Ion exchange chromatography, AG1-X2 resin
NMIJ	0.1 g	Protein precipitation
KRISS	>0.1 g	1. The sample clean-up was carried out by the protein precipitation with acetonitrile and followed by a heating at 60°C for 2 hours. 2. After centrifugation, the supernatant was lyophilized with a speed-vac and then reconstituted with 1 mmol/L NH_4OH. 3. The sample was purified with 0.2 µm membrane for next LC-MRM analysis
CENAM	0.3 g (Serum I) 0.2 g (Serum II)	Liquid-liquid, protein precipitation
VNIIM	0.1 g	Protein precipitation by acetonitrile
HSA	0.1 – 0.15 g	SPE was used for clean-up. The details are as follows: After spiking the isotope labelled internal standards, the sample was vortexed, and allowed to equilibrate at ambient temperature for 2 h. The sample was then vortexed, and SPE was conducted using Waters Oasis MAX cartridge (30 µm, 1 cc, 30 mg). The eluent was dried under nitrogen at 45 °C, and was then reconstituted with 2 mmol/L ammonia solution (concentration about 500 ng/g) for LC-MS/MS analysis.
NIMT	0.1 g	Acetonitrile was used for protein precipitation

NMI/DI	Sample Size (g)	Pre-treatment
UME	0.1 g	Protein precipitation with acetonitrile, sample dilution, vortex, centrifuge, filtration
NIST	0.14735 g – 0.15777 g (Serum I) & 0.45288 g – 0.46282 g (Serum II)	Solid phase extraction: Bio-Rad Poly-Pre prefilled chromatography columns, AG 1-X8 resin, 100-200 Mesh, chloride form, 0.8 X 4 cm.

Table 14: Summary of analytical techniques for the measurement of urea

NMI/DI	Analytical Technique	Chromatographic Conditions	Ion/MRM monitored
NMIA	GC-HRMS (EI)	Injection mode: PTV Constant Temperature Split at 250 °C, injection volume: 1 µL, carrier gas: helium, column: Agilent J&W VF-17MS (0.25 mm × 30 m, 0.25 µm), carrier flow rate: 0.8 mL/min Temperature program: 75 °C hold for 6 minutes, ramp at 5 °C/min to 120 oC, ramp at 30 °C/min to 325 °C, hold for 5 minutes	153.04787 / 156.04529
INMETRO	HPLC-DAD	Column: Luna C18(2) column (250 mm × 4.6 mm, 5 µm) Temperature: 25 ºC. Flow rate of 1.0 mL/min LC run mode: isocratic mode Mobile phase: water : methanol (90:10, v/v).	-
NIM	LC-MS (ESI+)	Column: Agilent Zorbax SB-CN (4.6 mm × 250 mm × 5 µm); Flow rate : 0.5 mL/ min Mobile phase: 50:50 (Methanol: H_2O with 0.1% formic acid) Temperature: Room temperature	61.1 / 64.1
GLHK	GC-MS (EI)	Column: DB-5MS (30 m × 0.25 mm, 0.25 µm) Temperature Programme: 70 °C for 1 min, then 10 °C/min to 150 °C and then 10 °C/min to 240 °C and hold at temperature for 1 min. (Injection Mode: Split)	153 / 158
LNE	GC-MS (EI)	Column: DB-5 MS, (30 m × 0.25 mm × 0.25 µm) ; Oven : 70°C 10°C/min to 150°C followed by 20°C/min to 240°C. Inlet : 300°C, split, Flow rate: 1.3 mL/min	153 / 156
PTB	GC-MS (EI)	Column: OPTIMA-5-MS 5%Phenyl-95%Methylpolysiloxane (30 m × 0.25 mm) Temperature programme: 60°, 1 min …10°/min →100°, 0 min ... 20°/min →150°, 0 min … 40°/min →250°, 5min Injector: splitless. Injection volume: 1 µl	153.0484 / 156.0458
NMIJ	GC-MS (EI)	Column: DB-5MS (30 m × 0.25 mm × 0.25 µm). Mobile phase: He (1 mL/min). Oven: 60 °C (hold 1 min) → 15 °C/min → 300 °C (hold 3 min). Injection: split (50:1)	153 / 156

KRISS	LC-MS (ESI+)	Column: Thermo Hypersil Gold AQ (150 mm × 4 mm, 5 µm). Mobile phase: Water 100%.; Column temperature: Room temp. LC run mode: Isocratic. Flow rate: 0.7 mL/min. Injection mode: full loop mode	61.1 → 44.1, 63.1 → 45.1
CENAM	GC-MS (EI)	Column: HP-1MS (30 m × 320 µm × 0.25 µm) Flow: 2 mL/min. Run: 70 °C for 1 min; 10 °C/min to 150°C; Post run: 240 °C for 5 min.	168 / 171
VNIIM	LC-MS/MS (ESI+)	Column: Kinetex HILIC (2.6 µm × 150 mm × 4.6 mm). Mobile phase A: Water +5 mmol Ammonium acetate + 0.1% acetic acid - 10%. Mobile phase B: Acetonitrile - 90%. isocratic	61.1 → 44.1, 64.1 → 47.1
HSA	LC-MS/MS (ESI+) & GC-MS (EI)	LC-MS/MS conditions: Column: Agilent RX-SIL (2.1 mm × 150 mm, 5 µm). Mobile phase A: 0.1% formic acid in water (v/v). Mobile phase B: 0.1 formic acid in acetonitrile (v/v). Binary setting: 95% mobile phase B (isocratic, post wash with 40% mobile phase B after each injection). Injection volume: 10 µL. Flow rate: 0.5 mL/min. GC-MS conditions: Column: Agilent DB-5MS (30 m, 0.25 µm, 0.25 mm). Oven programme: 80 °C for 1 min, then 10 °C/min to 120 °C for 2 min. Inlet temperature: 250 °C. Transfer line: 270 °C. Inject volume: 1 µL	LC-MS/MS: 61→44, 64→46 GC-MS: 153 / 156
NIMT	LC-MS/MS (ESI+)	Column: Zorbax RX-Sil Narrow-bore (2.1 m × 150 mm × 5 µm) Mobile Phase: Isocratic, 90% of MeOH (0.1% formic acid) and 10% of H_2O (0.1% formic acid). Flow rate: 0.4 mL/min. Injection Volume: 5 µL	61.00 → 44.05, 64.00 → 46.05
UME	LC-HRMS (ESI+)	Column: Synergy Max (150 mm × 2 mm × 5 µm). Mobile phase 10 mmol/L ammonium acetate (aq) : Acetonitrile, 30:70 Flow rate: 0.250 mL/min, column temperature: 30 °C. Injection volume: 2 µL.	61.0406 / 63.0346
LGC	LC-MS/MS (ESI+)	Column: SeQuant ZIC-HILIC (5 µm × 150 mm × 2.1 mm, 200Å). Mobile phase: 10 mmol/L ammonium acetate solution and acetonitrile. Column temperature: 30 ºC. Injection volume: 20 µL	61.2 → 44.5, 64.2 → 46.5

NIST	GC-MS (EI)	Column: DB-5 MS (30 m × 0.25 mm × 0.25 µm); Injector temperature: 220 °C, MS source: 230 °C, MS quad: 150 °C, Auxiliary heater: 200 °C, Column pressure: 18.7 psi (constant pressure), and the initial flow rate 1.6 mL/min. The temperature program was 80 °C (initial temperature), 1 min (hold time), 7 °C/min (heating rate), to 120 °C, 30 °C/min to 250 °C (final temperature), 1 min (hold time). The EMV was set to the tune voltage. The amount injected was 1 µL. The split injection mode was used with a split ratio of 25:1.	168 / 170

Table 15: Summary of analytical techniques for uric acid

NMI/DI	Analytical Technique	Chromatographic Conditions	Ion/MRM monitored
NMIA	GC-MS/MS (EI)	Injection mode: Split mode at 250 °C, injection volume: 1 µL, carrier gas: helium Column: Agilent J&W DB-5MS (0.25 mm × 30 m, 0.25 µm) Carrier flow rate: 0.8 mL/min. Temperature program: 120 °C hold for 2 minutes, ramp at 5 °C/min to 180 °C, ramp at 20 °C/min to 300 °C, ramp at 60 °C/min to 320 °C, hold for 5 minutes	456→367, 458→369
INMETRO	LC-MS/MS	Column: HILIC column (100 mm × 2.1 mm, 1.7 µm). Temp: 25 ºC. Flow rate: 0.3 mL/min Mobile phase A: Acetic acid pH=3.0; B: acetonitrile Gradient ranging from 50% to 90%B.	167 → 124
NIM	LC-MS (ESI-)	Column: ACQUITY UPLC BEH C18 (2.1 mm × 150 mm, 1.7 µm) Mobile phase: 20 mmol/L NH4OAc pH 4.6. Flow rate: 0.2 mL/min. Temperature: Room temperature. Injection volume: 5µL	167.1/ 169.1
GLHK	LC-MS (ESI+)	Column: SIELC Obelisc R (150 mm × 2.1 mm, 5µm) Mobile Phase: (A) 0.1% formic acid in water; (B) Acetonitrile Mobile Phase Programme: Time (min) Flow Rate (mL/min) %A %B 0 0.2 98 2 6.5 0.2 98 2 6.6 0.5 30 70 11.5 0.5 30 70 11.6 0.2 98 2 18.5 0.2 98 2	169 → 141, 171 → 143
LNE	LC-MS (ESI-)	Column: C18. Mobile phase: A: 95% water/ 5% Acetonitrile; B: Acetonitrile; C: 5 mmol/L ammonium acetate + 0.05% Formic acid. Flow rate: 0.2 mL/min. 1% A and 99% C	167/ 169

NMI/DI	Analytical Technique	Chromatographic Conditions	Ion/MRM monitored
PTB	GC-MS (EI)	Column: OPTIMA-5-MS 5%Phenyl-95%Methylpolysiloxane (30 m × 0.25 mm) Temp. programme: 80°C,1min…15°/min followed by 300°C, 5min. Injector: splitless, Injection volume: 1 µL	567.3038/ 569.2979
NMIJ	LC-MS/MS (ESI-)	Column: HILIC COSMOSIL (2.0 mm × 150 mm) Mobile phase: 10 mmol/L ammonium acetate/ acetonitrile (60/40). Flow rate: 0.2 mL/min. Column temp.: 40 ℃	167/96, 169/97
KRISS	LC-MS (ESI+)	Column: ACQUITY UPLC HSS C18 (2.5 mm × 100 mm, 1.8 µm). Isocratic elution with a mobile phase [20 mmol/L ammonium acetate in water/MeOH (v/v, 10/90)]. Flow rate: 0.2 mL/min. Temp. of LC run: room temp. Injection mode: full loop mode	168.9 → 141.0, 170.9 → 143.0
CENAM	LC-MS (ESI+)	Column: Waters Xterra RP18 (3.0 mm × 250 mm, 5µm). Mobile phase: ammonium acetate 20 mmol/L flow: 0.45 mL/min. Room temperature: 20°C	169/ 171
VNIIM	LC-MS/MS (ESI-)	Column: Discovery HS F5 (150 mm × 4.6mm, 5 µm) Mobile phase A: Water + 0.05% Acetic Acid - 10%. Mobile phase B: Acetonitrile - 90% isocratic.	167.1→ 124, 167.1→ 125
HSA	LC-MS/MS (ESI-) & GC-MS (EI)	LC-MS/MS conditions: Column: Agilent Zorbax SB-Aq (2.1 mm × 100 mm, 3.5 µm) Mobile phase A: 5 mmol/L ammonium formate with 0.05% formic acid in water (v/v). Mobile phase B: acetonitrile. Binary setting: 2% mobile phase B (isocratic, post wash with 90% mobile phase B after each injection). Injection volume: 10 µL. Flow rate: 0.3 mL/min. GC-MS conditions: Column: Agilent DB-5MS (30 m, 0.25 µm, 0.25 mm). Oven programme: 200 °C for 1 min, then 30 °C/min to 280 °C for 6.3 min. Inlet temperature: 280 °C. Transfer line: 270 °C. Inject volume: 1 µL.	LC-MS/MS: 167 → 124, 169 → 125 GC-MS: 567.3/ 569.3

NMI/DI	Analytical Technique	Chromatographic Conditions	Ion/MRM monitored
NIMT	LC-MS/MS (ESI-)	Column: Inertsil® ODS-SP (2.1 mm × 150 mm, 5 µm). Mobile Phase: Isocratic, 10% of MeOH (0.1% formic acid) and 90% of H_2O (0.1% formic acid). Flow rate: 0.2 mL/min. Injection Volume: 5 µL.	166.89 →123.95, 168.83 →124.95
UME	LC-HRMS (ESI+)	Column: Synergy Max (150 mm × 2 mm, 5 µm) Mobile phase:10 mmol/L ammonium acetate (aq) Acetonitrile (30:70). Flow rate: 0.250 mL/min, column temperature: 30 °C. Injection volume was 2 µL.	169.0356/ 171.0294
NIST	GC-MS (EI)	Column: DB-5 MS (30 m × 0.25 mm × 0.25 µm); Injector temperature was 280 °C, the MS source 200 °C, the MS quad 200°C, the auxiliary heater 280 °C, the column pressure 18.7 psi (constant pressure), and the initial flow rate 1.6 mL/min. Spilt ratio: 25:1. The temperature program was 200 °C (initial temperature), 1 min (hold time), 16 °C/min (heating rate), to 300 °C, (final temperature), 2 min (hold time), for a total time of 9.25 min.	567/ 569

Table 16: Type of calibration, method of quantification, calibrants and internal standards used for measurement of urea

NMI/DI	Type of Calibration	Method of Quantification	Calibrants	Internal Standards
NMIA	Isotope dilution	Single-point calibration / Bracketing calibration	NMIJ CRM 6006-a	$^{13}C,^{15}N_2$-Urea
INMETRO	Standard addition	-	NIST SRM 912a	-
NIM	Isotope dilution	Single-point calibration	GBW09201	$^{13}C,^{15}N_2$-Urea
GLHK	Isotope dilution	Bracketing calibration	NIST SRM 912a	$^{13}C,^{15}N_2{}^{18}O$-Urea
LNE	Isotope dilution	Multi-level calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea
PTB	Isotope dilution	Single-point calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea
NMIJ	Isotope dilution	Bracketing calibration	NMIJ CRM 6006-a	$^{13}C,^{15}N_2$-Urea
KRISS	Isotope dilution	Single-point calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea
CENAM	Isotope dilution	Single-point calibration	NMIJ CRM 6006-a	$^{13}C,^{15}N_2$-Urea
VNIIM	Isotope dilution	Single-point calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea
HSA	Isotope dilution	Multi-level calibration	NIST SRM 912a	$^{13}C,^{18}O$-Urea
LGC	Isotope dilution	Single-point calibration / Bracketing calibration / Multi-level calibration	NIST	$^{13}C,^{15}N_2$-Urea
NIMT	Isotope dilution	Single-point calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea
UME	Isotope dilution	Multi-level calibration	Sigma Aldrich, purity assessed with mass balance via TGA/DSC and LC-MS. Capability demonstrated in the K55 series.	$^{13}C,^{15}N_2$-Urea
NIST	Isotope dilution	Multi-level calibration	NIST SRM 912a	$^{13}C,^{15}N_2$-Urea

Table 17: Type of calibration, method of quantification, calibrants and internal standards used for measurement of uric acid

NMI/DI	Type of Calibration	Method of Quantification	Calibrants	Internal Standards
NMIA	Isotope dilution	Single-point calibration Bracketing calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
INMETRO	Standard addition	-	NIST SRM 913b	-
NIM	Isotope dilution	Single-point calibration	GBW09202	1,3-$^{15}N_2$-Uric acid
GLHK	Isotope dilution	Bracketing calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
LNE	Isotope dilution	Multi-level calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
PTB	Isotope dilution	Single-point calibration	NIST SRM 913a	1,3-$^{15}N_2$-Uric acid
NMIJ	Isotope dilution	Bracketing calibration	NMIJ CRM 6008-a	1,3-$^{15}N_2$-Uric acid
KRISS	Isotope dilution	Single-point calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
CENAM	Isotope dilution	Single-point calibration	NMIJ CRM 6008-a	1,3-$^{15}N_2$-Uric acid
VNIIM	Isotope dilution	Single-point calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
HSA	Isotope dilution	Multi-level calibration	NIST SRM 913a	1,3-$^{15}N_2$-Uric acid
NIMT	Isotope dilution	Single-point calibration	NIST SRM 913b	1,3-$^{15}N_2$-Uric acid
UME	Isotope dilution	Multi-level calibration	Sigma Aldrich, purity assessed with mass balance via TGA/DSC and LC-MS. Capability demonstrated in the K55 series.	1,3-$^{15}N_2$-Uric acid
NIST	Isotope dilution	Multi-level calibration	NIST SRM 913a	1,3-$^{15}N_2$-Uric acid

11. DISCUSSION OF RESULTS

11.1. Urea

The result set for urea had an overall relative standard deviation (RSD) of 3.7 % and 3.3 % relative to the arithmetic mean for all 15 participating institutes of the key comparison for Serum I and II, respectively.

The lowest results from INMETRO were obtained using spectroscopy rather than IDMS. The higher results from LGC, KRISS and UME were discussed. During the meeting in October 2016, LGC shared that they achieved excellent precision for both pools of serum and excellent agreement with the NIST serum SRM but one of their results for Serum I was higher than the main set of results. They could not determine a reason for this.

The results from UME for both serum pools were high, but no further comments were provided by UME for these observations.

Following the meeting in April 2017, KRISS acknowledged that the comparison materials were not treated in accordance with the Study Protocol. The results were reported from measurements of aliquoted comparison materials, which had been subjected to more than one round of freeze-thaw cycle. Following a precedence in CCQM-K102, KRISS agreed that the results on urea would not be included for the calculation of the KCRV.

11.2. Uric Acid

The result set for uric acid had an overall RSD of 5.5 % and 4.8 % relative to the arithmetic mean for all 14 participating institutes of the key comparison for Serum I and II, respectively.

For INMETRO, results for both Serum I and II were high and these were obtained using standard addition technique rather than IDMS.

The result for Serum I from UME was significantly higher, but no comment was provided on this by UME.

VNIIM's result for Serum I was 9.2 % lower than the main set of data[4]. Following the meeting in October 2016, VNIIM informed HSA regarding a calculation error, leading to the low reported result for this serum pool. Hence, this result would not be included in the

4 Relative to the median for uric acid in Serum I given in Table 24.

calculation of the KCRV. The Table below summarises VNIIM's original reported and corrected results.

Table 18: VNIIM original and revised data for uric acid in Serum I*

	Original data	Revised data
Mean of results of Bottle 1 (mg/kg)	122.19	129.79
Mean of results of Bottle 2 (mg/kg)	125.36	133.16
Overall mean of results (mg/kg)	123.8	131.5

* No change to VNIIM's other results.

During the meeting in October 2016, CENAM shared its problems with the calibrant and solubility, as well as difficulties in optimising the equilibration. After the meeting, CENAM was provided with additional samples for a re-measurement using their GC-IDMS method (LC-IDMS was employed during the comparison). During the meeting in April 2017, CENAM presented the results of its investigation. The GC-IDMS[5] results agreed well with the majority of the reported results. After the meeting in April 2017, CENAM was provided with further samples for a re-measurement using the GC-IDMS method[6] and confirmed the findings of its first investigation. CENAM agreed to exclude its results for uric acid in the calculation of the KCRV for both Serum I and II. The Tables below summarises CENAM's original reported results and those from the investigative studies.

[5] Re-measurement was not conducted using LC-IDMS.

[6] The investigative study was conducted at NIST as the Analyst who performed the original work was under secondment.

Table 19: CENAM's original and revised data for uric acid in Serum I

Method	Overall mean (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, k (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
LC-IDMS[a]	150.42	4	5.95	2	11.91
GC-IDMS[b]	133.9	9	1.65	2	3.3
GC-IDMS[c]	139.9	4	1.3	2	2.6

a Employed for CCQM-K109
b Obtained using GC-IDMS method during 1st post comparison investigative study
c Obtained using GC-IDMS method during 2nd post comparison investigative study. Measurements were made at NIST by CENAM analyst.

Table 20: CENAM's original and revised data for uric acid in Serum II

Method	Overall mean (mg/kg)	Total no. of subsamples for calculation of the overall mean	Combined standard uncertainty (mg/kg)	Coverage factor, k (95% confidence level)	Expanded uncertainty at approximately 95% confidence level (mg/kg)
LC-IDMS[a]	45.25	4	0.68	2	1.37
GC-IDMS[b]	38.7	9	0.60	2	1.2
GC-IDMS[c]	40.3	4	0.57	2	1.1

a Employed for CCQM-K109
b Obtained using GC-IDMS method during 1st post comparison investigative study
c Obtained using GC-IDMS method during 2nd post comparison investigative study. Measurements were made at NIST by CENAM analyst.

During the meeting in October 2016, KRISS shared that they had applied a 2 h equilibration time at 60°C after protein precipitation and attributed their high results for uric acid to improved extraction efficiency at elevated temperatures. Following the meeting, HSA conducted a parallel investigation with KRISS. The investigation by HSA confirmed that heating did not significantly affect the uric acid results. KRISS also confirmed this in their investigative study using additional samples provided. The high results for uric acid were attributed to the degradation of the standard solutions, which were stored at room temperature. Hence, KRISS agreed that their results on uric acid would not be included for the calculation of the KCRVs. The Table below summarises KRISS's original reported results and the corrected results from the investigative studies.

Table 21: KRISS's original and revised data for uric acid in Serum I

	Original data	Revised data
Mean of results of Bottle 1 (mg/kg)	145.9	139.2
Mean of results of Bottle 2 (mg/kg)	146.7	140.9
Mean of results of Bottle 3 (mg/kg)	146.6	140.0
Overall mean of results (mg/kg)	146.4	140.0
Total no. of subsamples for calculation of the overall mean	6	6
Combined standard uncertainty (mg/kg)	1.5	0.7
Coverage factor, k (95% confidence level)	3.18	2.12
Expanded uncertainty at approximately 95% confidence level (mg/kg)	4.9	1.4

Table 22: Original and revised data for uric acid in Serum II from KRISS

	Original data	Revised data
Mean of results of Bottle 1 (mg/kg)	42.4	40.2
Mean of results of Bottle 2 (mg/kg)	42.3	40.5
Mean of results of Bottle 3 (mg/kg)	42.3	40.1
Overall mean of results (mg/kg)	42.3	40.3
Total no. of subsamples for calculation of the overall mean	6	6
Combined standard uncertainty (mg/kg)	0.5	0.2
Coverage factor, k (95% confidence level)	2.57	2.12
Expanded uncertainty at approximately 95% confidence level (mg/kg)	1.4	0.4

12. EVALUATION OF RESULTS FOR KCRVs

The OAWG agreed that the Key Comparison Reference Value (KCRV) and associated uncertainty would be determined from results of NMIs/DIs that participate in the key comparison using IDMS methods that are valid and have demonstrated metrological traceability. All participants used appropriate traceable calibrants except for UME who used materials from a commercial source and carried out an in-house purity assessment. INMETRO used non-IDMS methods and thus none of its results were considered for inclusion in KCRV calculations. The results of VNIIM, LGC, CENAM and KRISS were reviewed as described in the section on Discussion of Results.

For urea (both Serum I and II), the KCRVs were calculated from results of all NMIs/DIs except for INMETRO and KRISS. For uric acid (Serum I), the KCRV was calculated from results of all NMIs/DIs except for VNIIM, INMETRO, KRISS and CENAM. For uric acid (Serum II), the KCRV was calculated from results of all NMIs/DIs except for INMETRO, KRISS and CENAM. In all cases, the participating institutes chose to withdraw their results from the KCRV calculation following their further investigation. The RSDs of the 13 results included in the KCRV calculations for urea in Serum I and Serum II were 2.8 % and 2.2 %, rather than the 3.7 % and 3.3 % of all 15 participants. The RSDs of the 10 and 11 results included in the calculations for uric acid were 2.9 % and 1.0 % rather than the 5.5 % and 4.8 % of all 14 participants.

Potential candidate KCRVs were presented at the meeting in April 2017. In the meeting, although the reported measurement uncertainties were regarded as generally valid it was agreed that the median was an appropriate estimator of consensus given potential non-technical “outliers” (values distant from the majority) and apparent lack of excess variance. In accordance with CCQM guidance[7], the normality-adjusted median absolute median (MAD_E) was regarded as an appropriate estimator for the uncertainty of these medians.

Recognising that use of the uncertainty-ignoring median and MAD_E estimators can lead to underestimation of the KCRV uncertainties, at the meeting in September 2017 it was suggested that HSA work with NIST to evaluate the results with an estimator that is robust, uses the reported measurement uncertainties, and accommodates excess variance when present. While the DerSimmonian-Laird weighted mean uses uncertainties

7 CCQM/13-22. Guidance note: Estimation of a consensus KCRV and associated Degrees of Equivalence, 11-Apr-2013. http:\\www.bipm.org/cc/CCQM/Allowed/19/CCQM13-22_Consensus_KCRV_v10.pdf

and accommodates excess variance, it is not robust to outliers. None of the estimators described in the current CCQM guidance document address all three issues.

However, a random effects model that is robust, uses the reported measurement uncertainties, and accommodates excess variance has recently been fully characterised.[8] This method differs from the usual random effects model in that it models laboratory effects as following the double exponential (Laplacian) distribution rather than the normal (Gaussian) distribution:

$$X_i = \mu + \lambda_i + E_i$$

where *i* indexes the participating laboratories, X_i are the lab means, μ is the consensus value, λ_i are the laboratory effects distributed as Laplacian with mean 0 and variance σ_λ^2, and E_i are the lab specific measurement errors distributed as Gaussian with mean 0 and variance u_i^2. The σ_λ^2 parameter directly estimates excess variance. The μ estimate provided by this model can be regarded as a weighted median.

Unlike the closed-form estimators described in the CCQM guidance document, these Laplacian weighted median results are evaluated via Markov Chain Monte Carlo (MCMC) resampling. Rather than producing single analytic location and uncertainty estimates, MCMC techniques generate large numbers of realisations (draws) of the model parameters. With a suitably large number of draws (typically a few tens of thousands), the likely value of a parameter can be estimated as the arithmetic mean of the draws, the standard uncertainty of this mean as the standard deviation of the draws, and the expanded uncertainty as the 95% credible interval between the 2.5th percentile and the 97.5th percentiles of the draws. When the distribution of a model parameter is approximately symmetrical about its mean, the expanded uncertainty can be estimated as one-half of the interval between these two percentiles.

8 Rukhin A, Possolo A, (2011) Laplace random effects models for interlaboratory studies. *Computational Statistics and Data Analysis* 55, 1815 – 1825. https:\\doi.org\10.1016/j.csda.2010.11.016

Table 23 lists candidate KCRV values for urea in Serum I and Serum II as estimated with the 1) arithmetic mean and standard deviation (non-robust, uncertainty-ignoring), 2) median and MAD_E (robust, uncertainty-ignoring), and 3) Laplacian weighted median (robust, uncertainty-using). Table 24 similarly lists the candidate values for uric acid. Appendix C details the Laplacian weighted median results and an example of the computer code used to calculate the weighted medians and associated parameters of interest.

Table 23: Candidate KCRVs and uncertainties for urea in Serum I & II

	Urea	
	Serum I	Serum II
Number of results (*N*) used to calculate KCRV	13	13
Approach 1 (Arithmetic Mean):		
Arithmetic mean (mg/kg)	1,500	335.9
Standard deviation, SD (mg/kg)	42.4	7.37
Standard uncertainty, SD/√*N* (mg/kg)	12	2.0
Approach 2 (Median):		
Median (mg/kg)	1,485.7	334.20
Median absolute deviation, MAD (mg/kg)	16.7	2.80
1.483×MAD, MAD_e (mg/kg)	24.8	4.15
Standard uncertainty, 1.25×MAD_e/√N (mg/kg)	8.6	1.4
Approach 3 (Laplacian Weighted Median):		
Weighted median (mg/kg)	1,486.0	334.7
Standard uncertainty (mg/kg)	9.0	1.8

Table 24: Candidate KCRVs and uncertainties for uric acid in Serum I & II

	Uric Acid	
	Serum I	Serum II
Number of results (*N*) used to calculate KCRV	10	11
Approach 1 (Arithmetic Mean):		
Arithmetic mean (mg/kg)	137.4	39.27
Standard deviation, SD (mg/kg)	4.06	0.38
Standard uncertainty, SD/√*N* (mg/kg)	1.3	0.11
Approach 2 (Median):		
Median (mg/kg)	136.40	39.29
Median absolute deviation, MAD (mg/kg)	0.500	0.290
1.483×MAD, MAD_e (mg/kg)	0.742	0.430
Standard uncertainty, 1.25×MAD_e/√N (mg/kg)	0.29	0.16
Approach 3 (Laplacian Weighted Median):		
Weighted median (mg/kg)	136.50	39.39
Standard uncertainty (mg/kg)	0.98	0.11

The median and weighted median results agree well for urea and uric acid in both Serum I and Serum II. This suggests that the weighted median location values are as appropriate as the simple medians.

The MAD_E-based relative uncertainties for the medians are, as expected, slightly smaller than the uncertainties associated with weighted medians for urea in Serum I (100×8.6/1485.7 = 0.58 % vs. 100×9.0/1486.0 = 0.61 %) and Serum II (100×1.4/334.2 = 0.42 % vs. 100×1.8/334.7 = 0.54 %). For uric acid in Serum I, the MAD_E-based relative uncertainty (100×0.29/136.40 = 0.21 %) is considerably smaller than that of the weighted median (100×0.98/136.50 = 0.72 %), while for uric acid in Serum II, the MAD_E-based relative uncertainty (100×0.16/39.29 = 0.41 %) is somewhat larger than that of the weighted median (100×0.11/39.39 = 0.28 %). Regardless of this one reversal, since the uncertainty of a weighted median explicitly includes the reported measurement uncertainties and accounts for potential "dark" uncertainty, it may be less prone to underestimation than is MAD_E.

Recognising the need for clarity on the choice of estimators, weighted medians are used as the KCRVs for all four measurands for the following considerations:

- the OAWG regards the reported measurement uncertainties as mostly credible,
- the measurement distributions are potentially somewhat non-normal,
- the measurement distribution for at least one of the measurands contains an influential value (potential outlier) that cannot be excluded on technical grounds, and
- the Laplacian weighted median has been well-characterised in an appropriate peer-reviewed publication.

The following Figures display the reported measurement values and uncertainties relative to the weighted medians and their standard uncertainties.

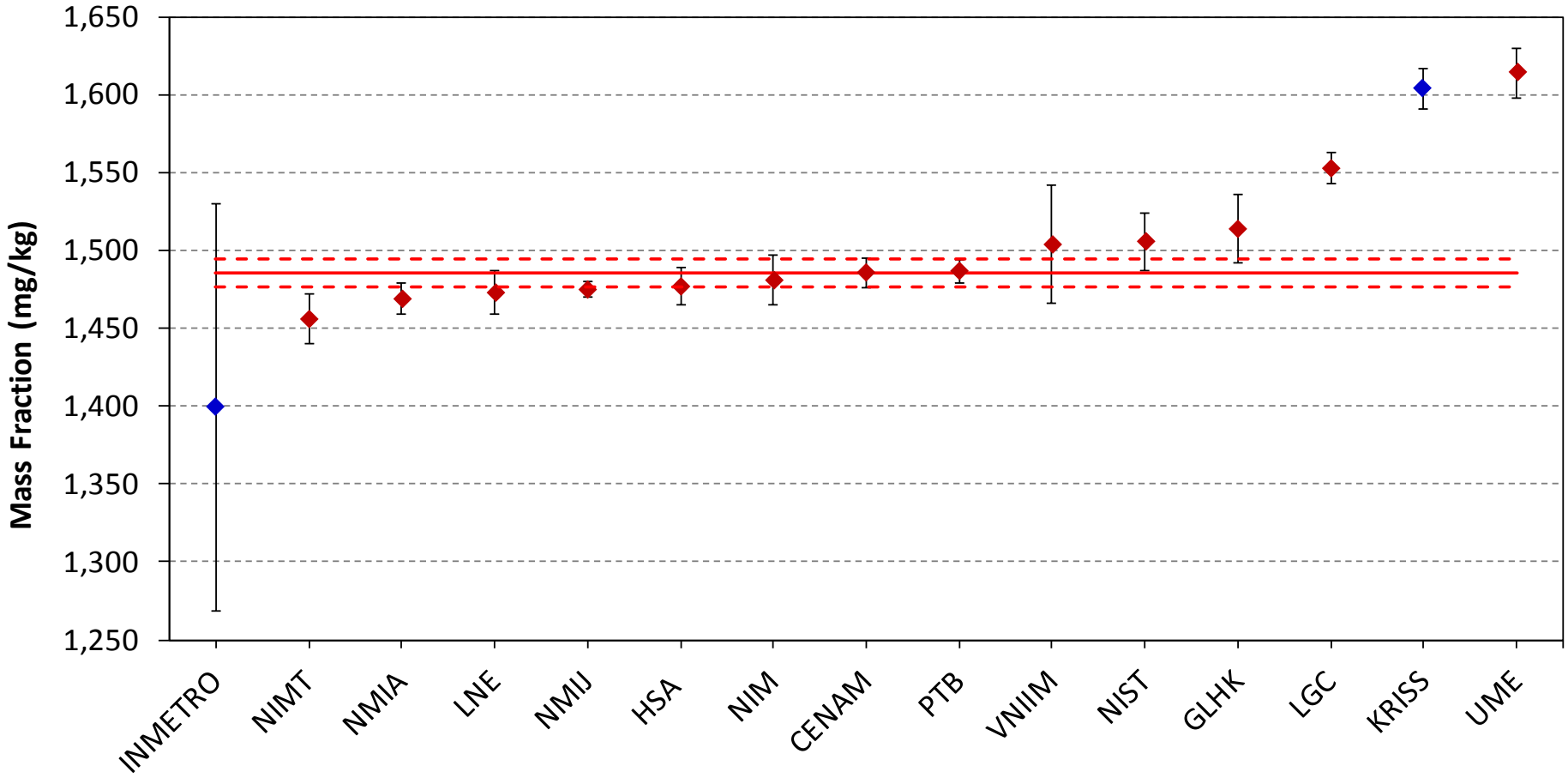


Figure 14: Reported results for urea (Serum I) relative to the KCRV

The KCRV (solid red line) with a value of 1,486.0 mg/kg was calculated from the weighted median of 13 results and has a standard uncertainty of 9.0 mg/kg (red dotted line). Bars represent standard uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

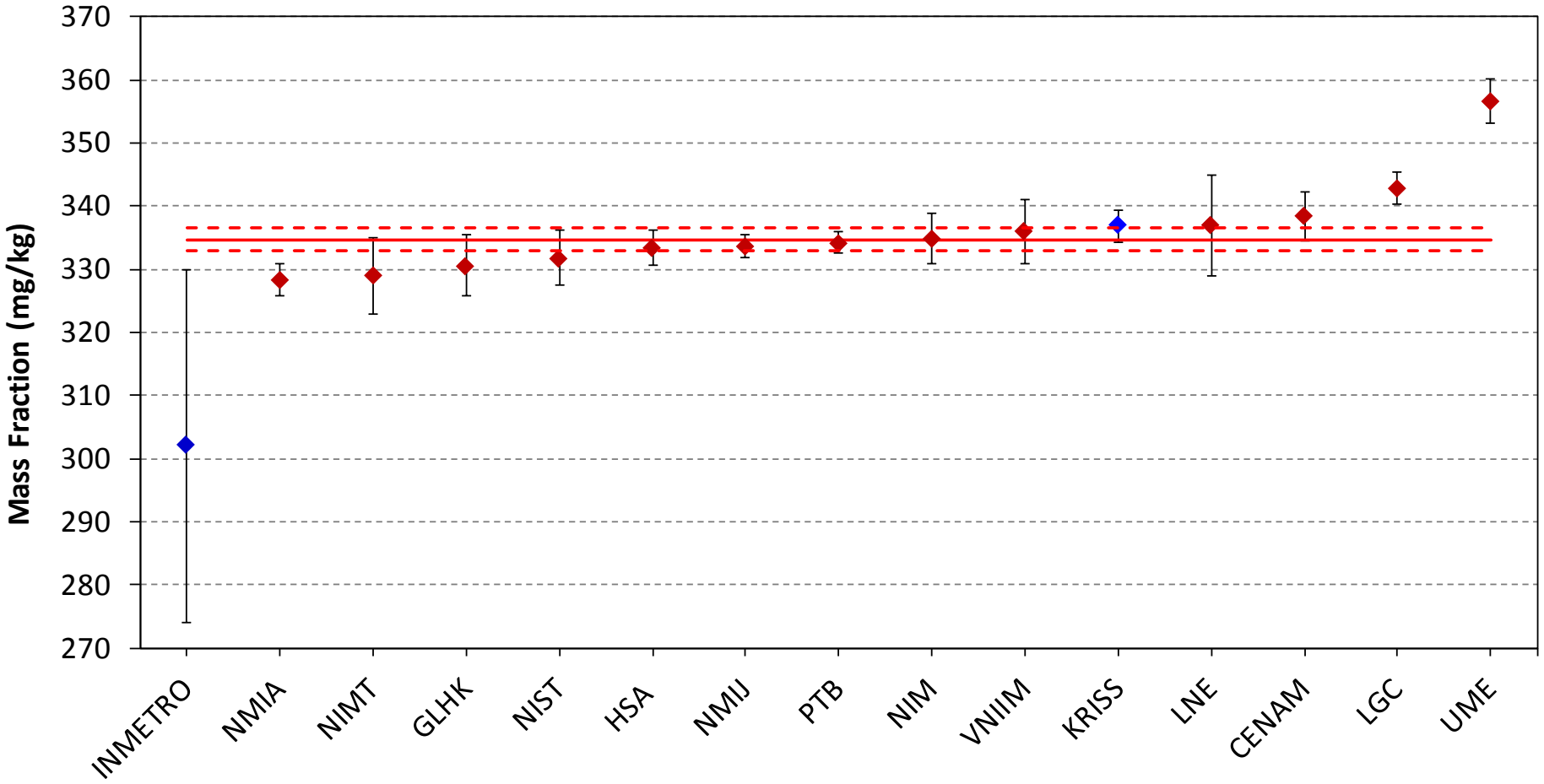


Figure 15: Reported results for urea (Serum II) relative to the KCRV

The KCRV (solid red line) with a value of 334.7 mg/kg was calculated from the weighted median of 13 results and has a standard uncertainty of 1.8 mg/kg. Bars represent standard uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

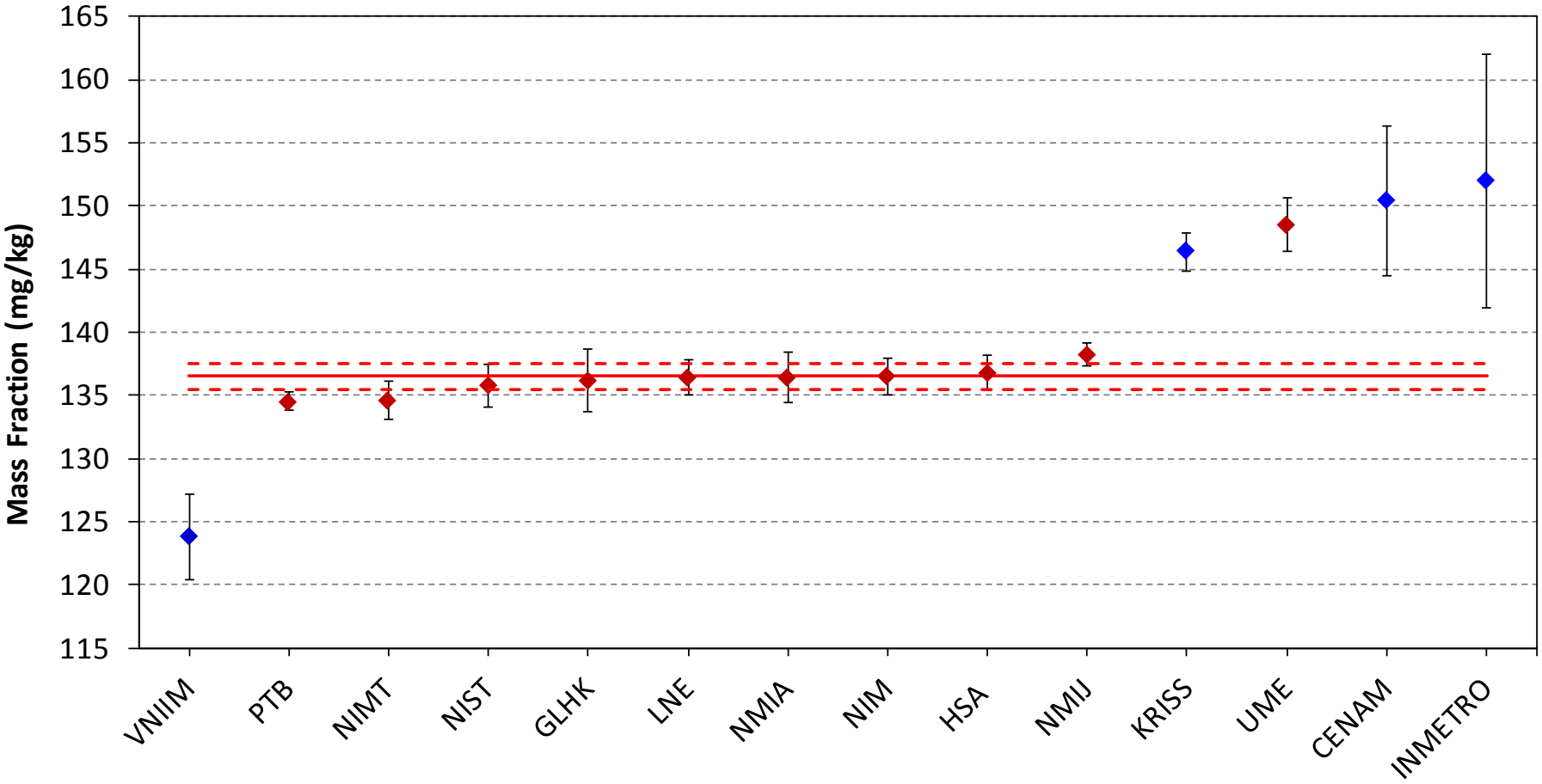


Figure 16: Reported results for uric acid (Serum I) relative to the KCRV

The KCRV (solid red line) with a value of 136.50 mg/kg was calculated from the weighted median of 10 results and has a standard uncertainty of 0.98 mg/kg. Bars represent standard uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

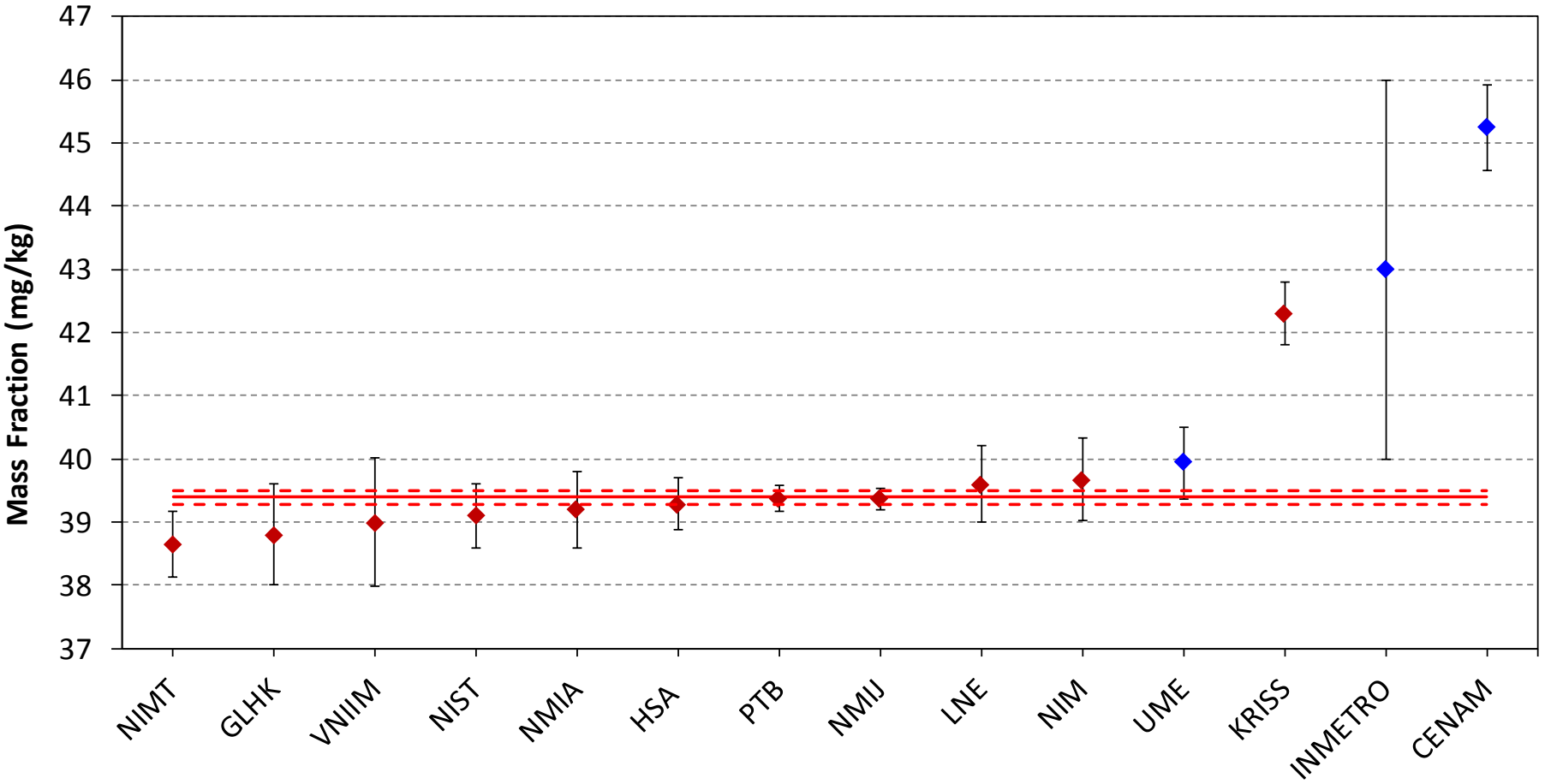


Figure 17: Reported results for uric acid (Serum II) relative to the KCRV

The KCRV (solid red line) with a value of 39.39 mg/kg was calculated from the weighted median of 11 results and has a standard uncertainty of 0.11 mg/kg. Bars represent standard uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

13. DEGREES OF EQUIVALENCE (DOE) CALCULATION

The degrees of equivalence (D_i) for each participating NMI/DI is defined as

$$D_i = X_i - X_{\mathrm{KCRV}}$$

where X_i is the result reported by participant i and X_{KCRV} is the KCRV. With the KCRVs estimated using any Monte Carlo technique, these D_i and their 95 % level of confidence expanded uncertainties, $U(D_i)$, can readily be estimated along with the KCRV. The distributions of the D_i as estimated along with the weighted medians were examined and determined to be essentially symmetric, allowing the $U(D_i)$ to be estimated as the half-width of the interval between the 2.5th and 97.5th percentiles of the MC draws. See Appendix C for an example of the code and its summary output. Appendix D compares the $D_i \pm U(D_i)$ for the median and weighted median candidate KCRVs.

The percentage relative D_i, $\%D_i$, were calculated as:

$$\%D_i = 100 \times D_i / X_{\mathrm{KCRV}}.$$

The expanded uncertainties for these $\%D_i$, $U(\%D_i)$, were calculated as:

$$U(\%D_i) = 100 \times U(D_i) / X_{\mathrm{KCRV}}.$$

The following Tables and Figures summarise the CCQM-K109 D_i (mg/kg) and $\%D_i$ (%) estimates for the four measurands.

Table 25: Degrees of equivalence for urea (Serum I)
The KCRV is 1,486.0 mg/kg, standard uncertainty 9.0 mg/kg, and relative standard uncertainty 0.61 %

NMI/DI	D_i (mg/kg)	$U(D_i)$ (mg/kg)	$D_i / U(D_i)$	$\%D_i$ (%)	$\%U(D_i)$ (%)
NMIA	-16.85	26.76	-0.63	-1.13%	1.80%
INMETRO[*]	-88.71	260.40	-0.34	-5.97%	17.52%
NIM	-4.81	36.56	-0.13	-0.32%	2.46%
GLHK	27.79	47.40	0.59	1.87%	3.19%
LNE	-13.07	33.08	-0.40	-0.88%	2.23%
PTB	0.91	23.58	0.04	0.06%	1.59%
NMIJ	-10.97	20.74	-0.53	-0.74%	1.40%
KRISS[*]	118.20	31.88	3.71	7.95%	2.15%
CENAM	-0.04	26.86	0.00	0.00%	1.81%
VNIIM	18.28	78.88	0.23	1.23%	5.31%
HSA	-9.02	30.28	-0.30	-0.61%	2.04%
NIMT	-29.75	37.20	-0.80	-2.00%	2.50%
UME	128.40	36.74	3.49	8.64%	2.47%
LGC	66.97	26.98	2.48	4.51%	1.82%
NIST	20.13	41.10	0.49	1.35%	2.77%

* The measurement result reported by this institute was not used to estimate the KCRV.

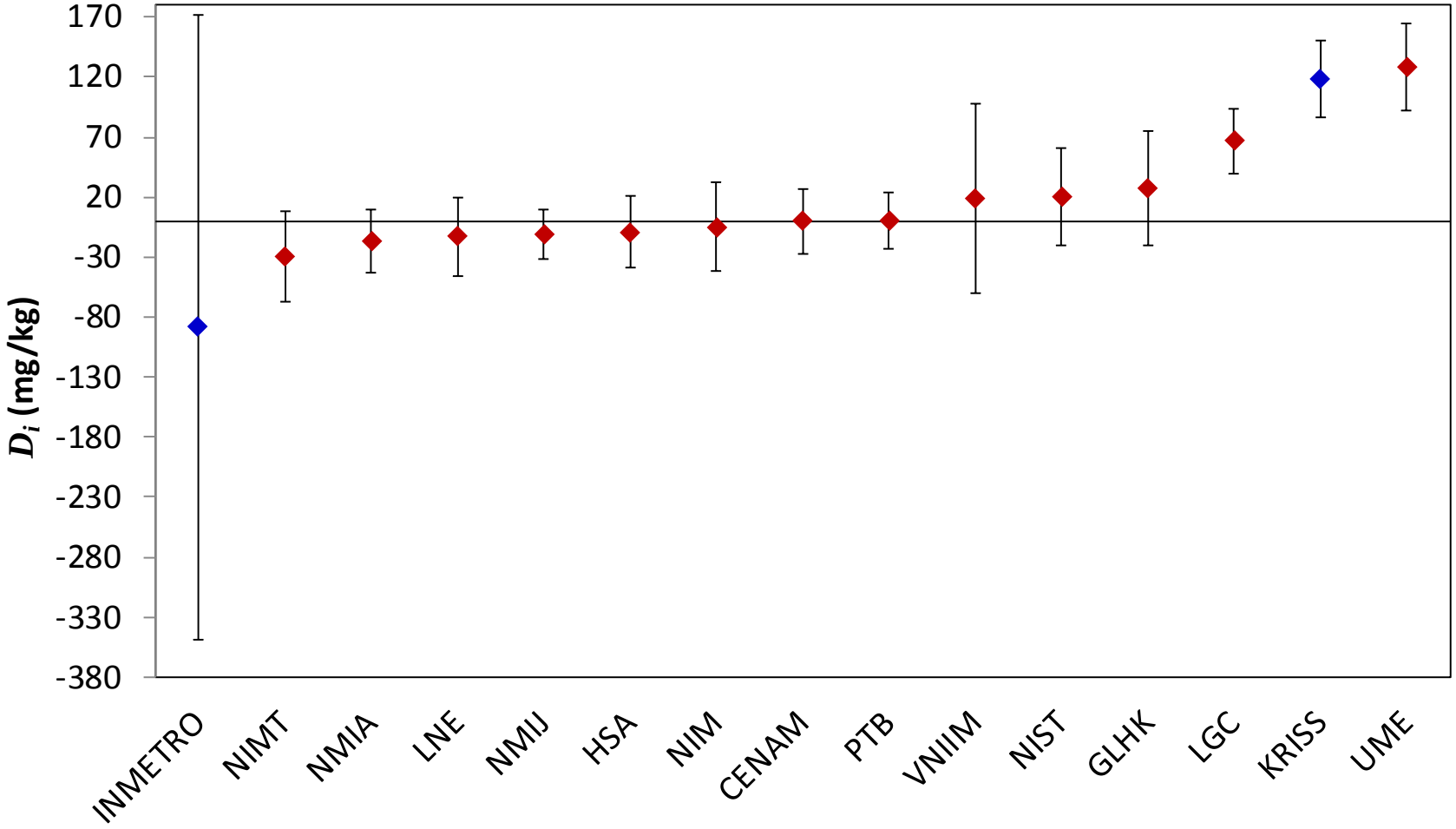


Figure 18: Degrees of equivalence, $D_i \pm U(D_i)$, for urea (Serum I)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

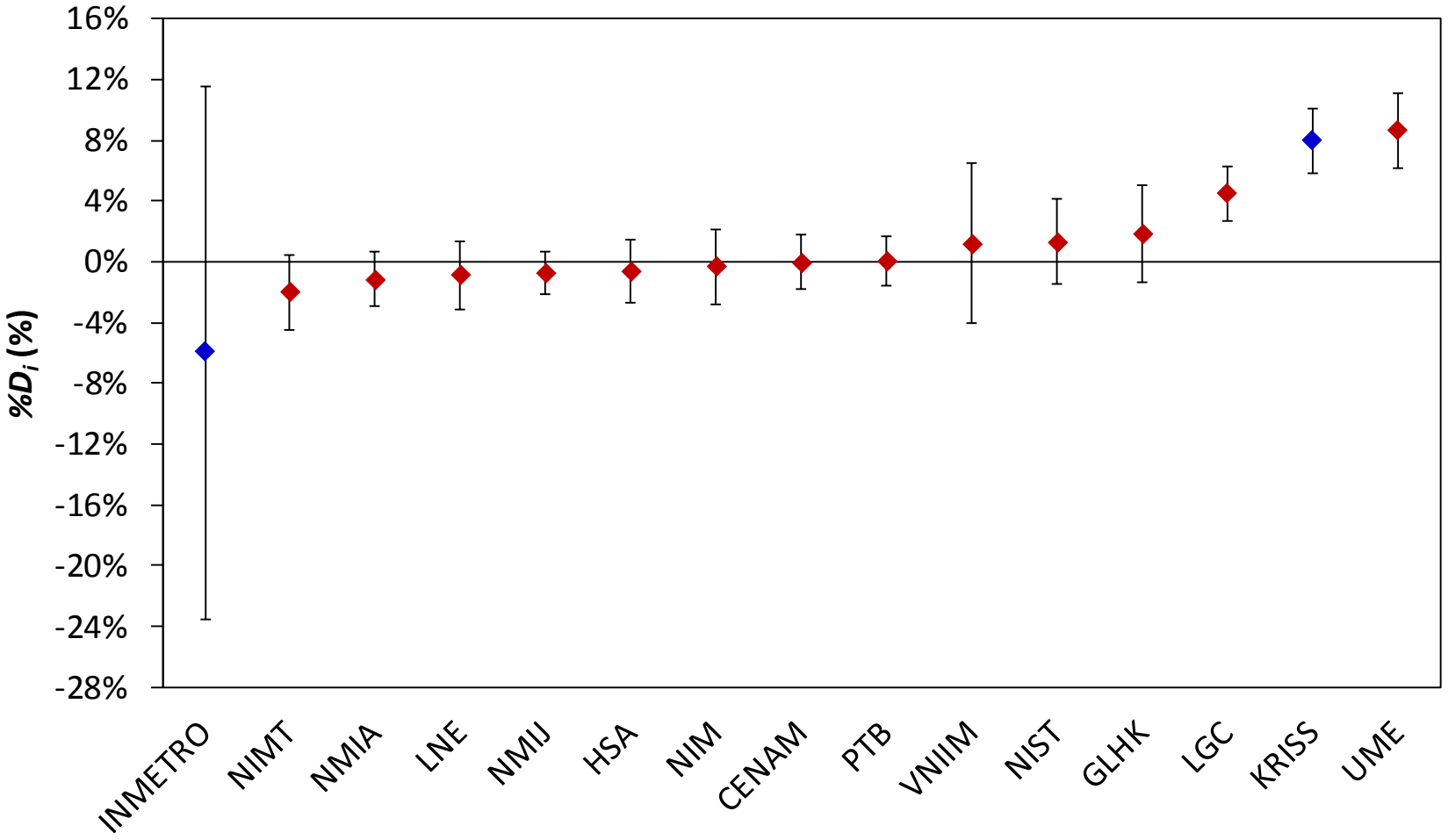


Figure 19: Relative degrees of equivalence, $\%D_i \pm U(\%D_i)$, for urea (Serum I)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

Table 26: Degrees of equivalence for urea (Serum II)

The KCRV is 334.7 mg/kg, standard uncertainty 1.8 mg/kg, and relative standard uncertainty 0.54 %

NMI/DI	D_i	$U(D_i)$	$D_i/ U(D_i)$	$\%D_i$ (%)	$\%U(D_i)$ (%)
NMIA	-6.26	6.33	-0.99	-1.87%	1.89%
*INMETRO**	-33.06	55.62	-0.59	-9.88%	16.62%
NIM	0.15	8.70	0.02	0.04%	2.60%
GLHK	-4.14	10.24	-0.40	-1.24%	3.06%
LNE	2.24	16.34	0.14	0.67%	4.88%
PTB	-0.45	4.95	-0.09	-0.13%	1.48%
NMIJ	-1.00	5.25	-0.19	-0.30%	1.57%
*KRISS**	2.21	6.13	0.36	0.66%	1.83%
CENAM	3.80	8.55	0.44	1.14%	2.55%
VNIIM	1.35	10.87	0.12	0.40%	3.25%
HSA	-1.30	6.52	-0.20	-0.39%	1.95%
NIMT	-5.61	12.89	-0.44	-1.68%	3.85%
UME	22.09	7.91	2.79	6.60%	2.36%
LGC	8.09	6.18	1.31	2.42%	1.85%
NIST	-2.82	9.58	-0.29	-0.84%	2.86%

* The measurement result reported by this institute was not used to estimate the KCRV.

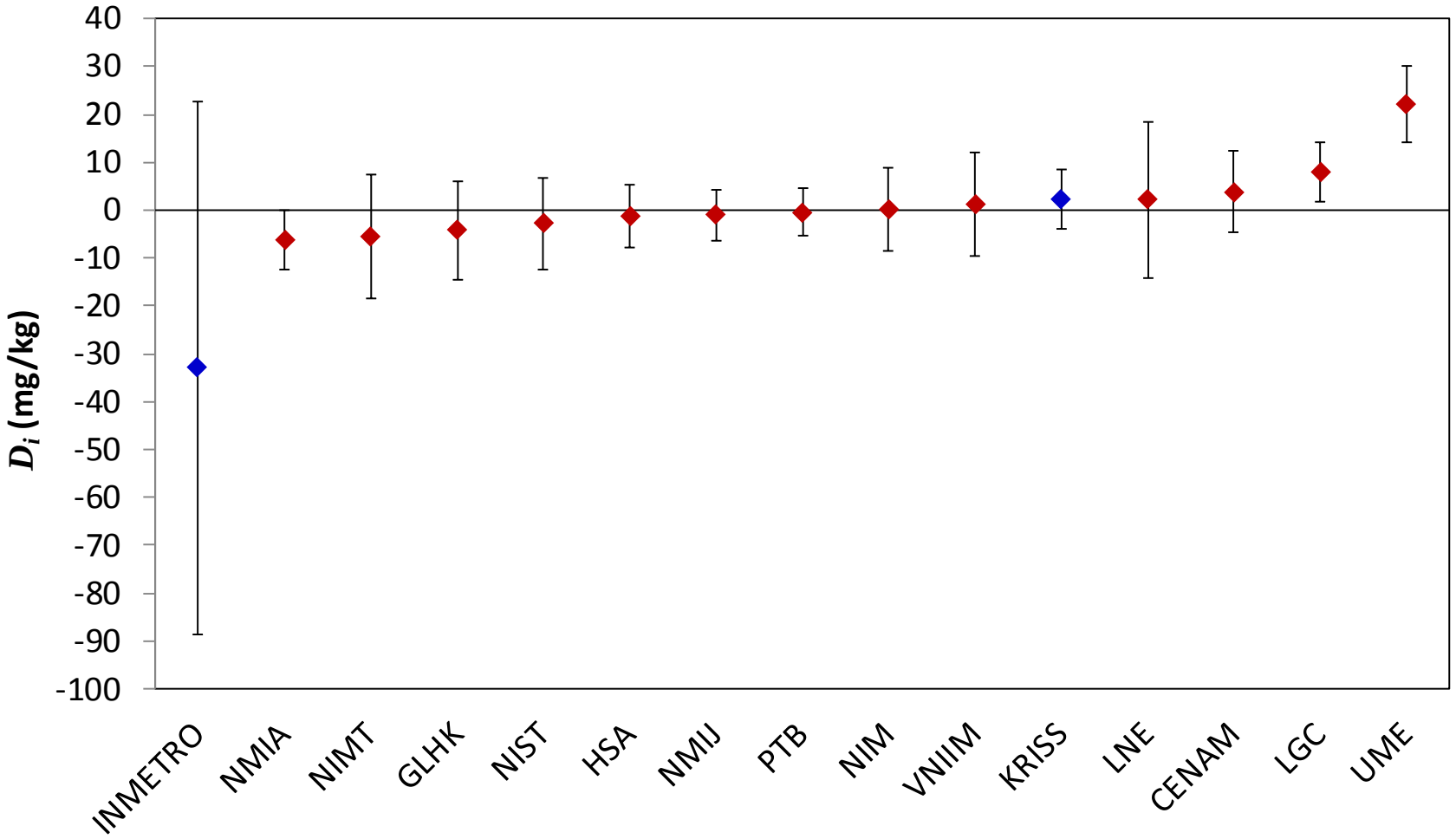


Figure 20: Degrees of equivalence, $D_i \pm U(D_i)$, for urea (Serum II)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

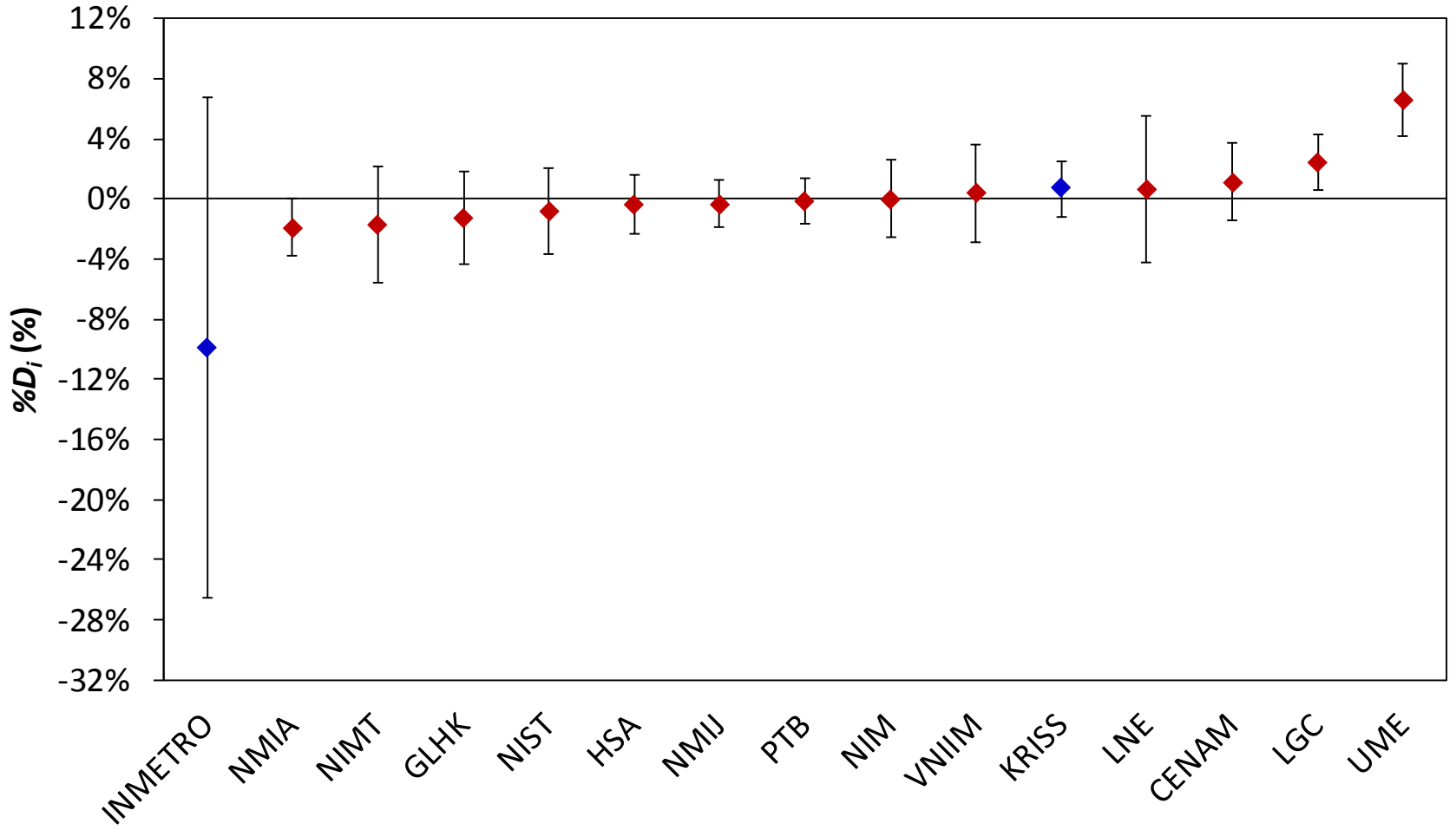


Figure 21: Relative degrees of equivalence, %Di ± U(%Di), for urea (Serum II)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

Table 27: Degrees of equivalence for uric acid (Serum I)

The KCRV is 136.50 mg/kg, standard uncertainty 0.98 mg/kg, and relative standard uncertainty 0.72 %

NMI/DI	D_i	$U(D_i)$	$D_i/U(D_i)$	$\%D_i$ (%)	$\%U(D_i)$ (%)
NMIA	-0.12	4.45	-0.03	-0.09	3.26
*INMETRO**	15.48	20.26	0.76	11.34	14.84
NIM	-0.03	3.56	-0.01	-0.02	2.61
GLHK	-0.34	5.39	-0.06	-0.25	3.95
LNE	-0.10	3.44	-0.03	-0.07	2.52
PTB	-2.02	2.41	-0.84	-1.48	1.77
NMIJ	1.78	2.66	0.67	1.30	1.95
*KRISS**	9.89	3.61	2.74	7.25	2.64
*CENAM**	13.9	12.01	1.16	10.18	8.80
*VNIIM**	-12.74	6.98	-1.82	-9.33	5.11
HSA	0.28	3.42	0.08	0.21	2.51
NIMT	-1.93	3.61	-0.53	-1.41	2.64
UME	12.04	4.61	2.61	8.82	3.38
NIST	-0.72	3.89	-0.19	-0.53	2.85

* The measurement result reported by this institute was not used to estimate the KCRV.

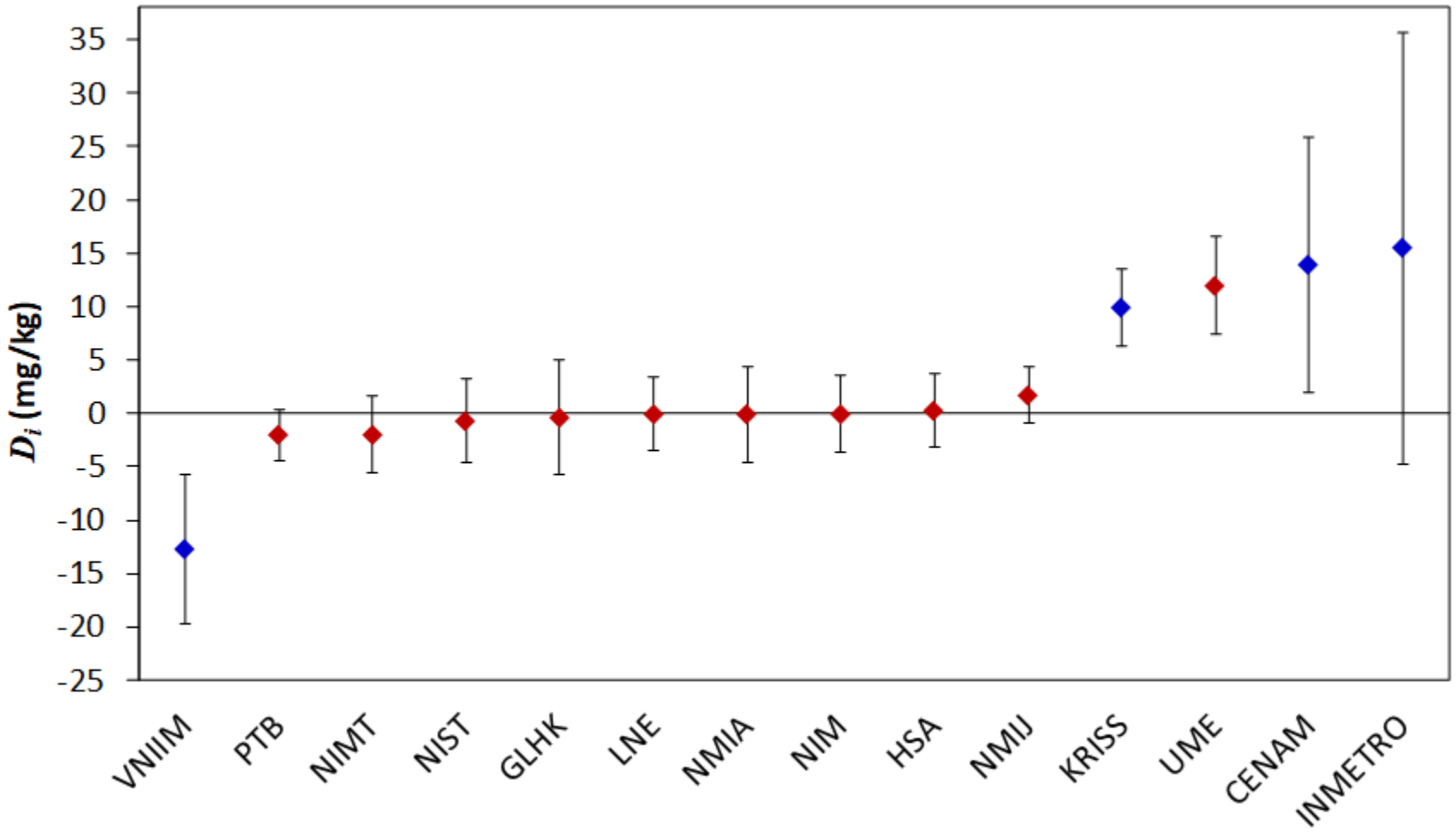


Figure 22: Degrees of equivalence, $D_i \pm U(D_i)$, for uric acid (Serum I)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

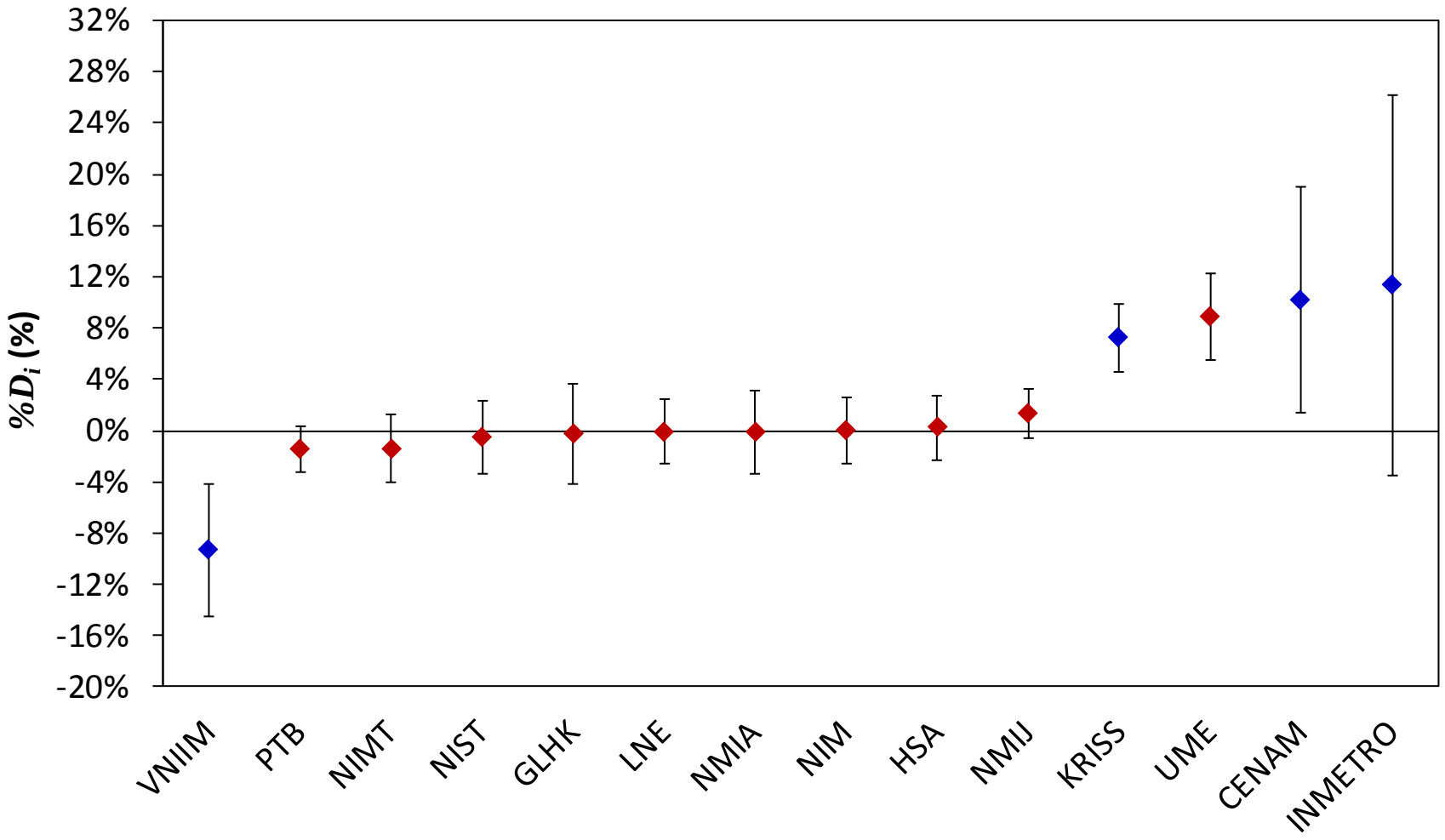


Figure 23: Relative degrees of equivalence, $\%D_i \pm U(\%D_i)$, for uric acid (Serum I)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

Table 28: Degrees of equivalence for uric acid (Serum II).

The KCRV is 39.39 mg/kg, standard uncertainty 0.11 mg/kg, and relative standard uncertainty 0.28 %

NMI/DI	D_i	$U(D_i)$	$D_i/ U(D_i)$	$\%D_i$ (%)	$\%U(D_i)$ (%)
NMIA	-0.18	1.21	-0.15	-0.46%	3.07%
*INMETRO**	3.67	6.06	0.61	9.32%	15.38%
NIM	0.28	1.31	0.21	0.71%	3.33%
GLHK	-0.59	1.62	-0.36	-1.50%	4.11%
LNE	0.20	1.25	0.16	0.51%	3.17%
PTB	-0.02	0.46	-0.04	-0.05%	1.17%
NMIJ	-0.02	0.4	-0.05	-0.05%	1.02%
*KRISS**	2.92	1.03	2.84	7.41%	2.61%
*CENAM**	5.88	1.37	4.3	14.93%	3.48%
VNIIM	-0.39	2.01	-0.19	-0.99%	5.10%
HSA	-0.1	0.85	-0.12	-0.25%	2.16%
NIMT	-0.73	1.06	-0.69	-1.85%	2.69%
UME	0.55	1.16	0.48	1.40%	2.94%
NIST	-0.28	1.02	-0.28	-0.71%	2.59%

* The measurement result reported by this institute was not used to estimate the KCRV.

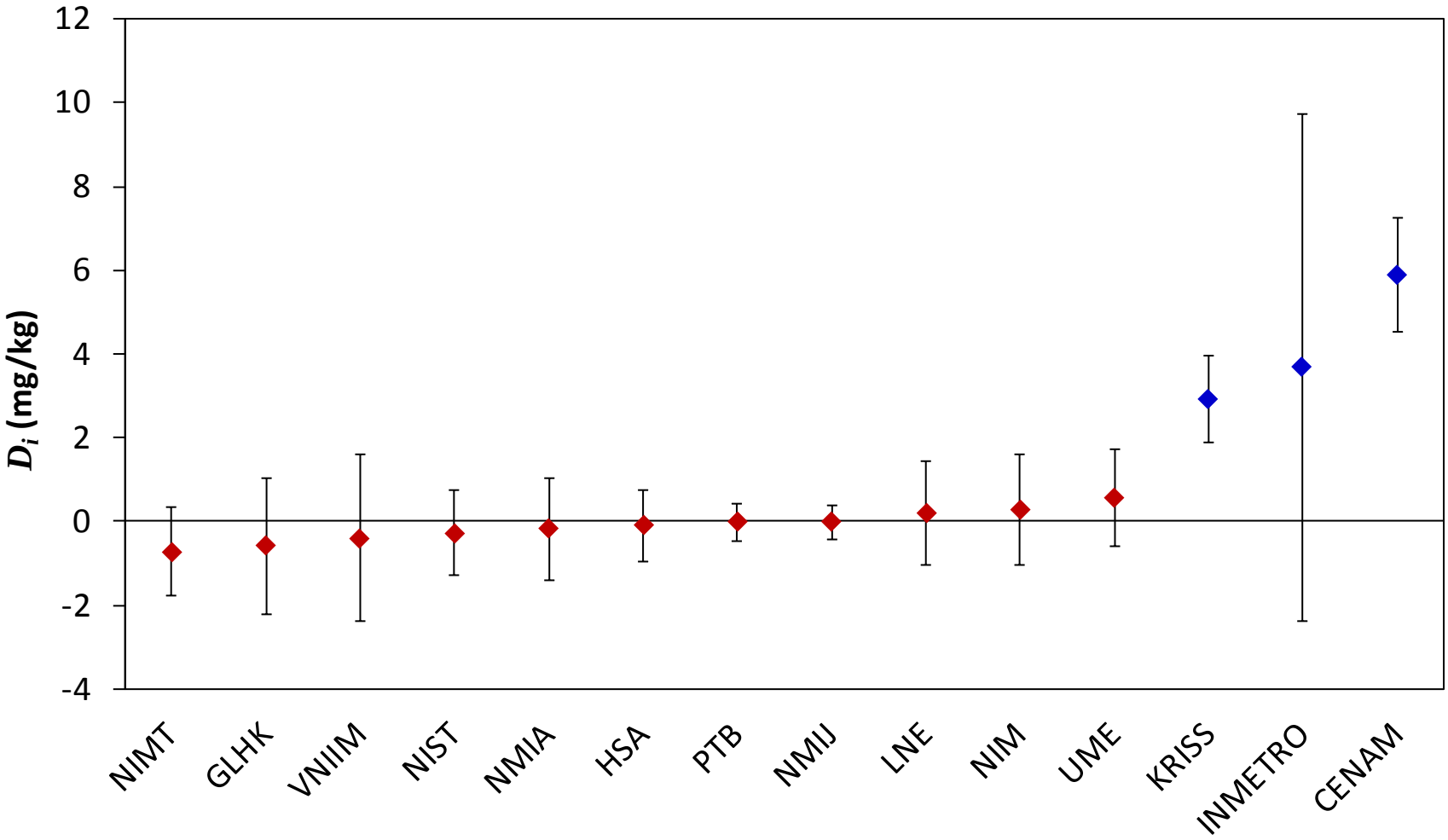


Figure 24: Degrees of equivalence, $D_i \pm U(D_i)$, for uric acid (Serum II)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

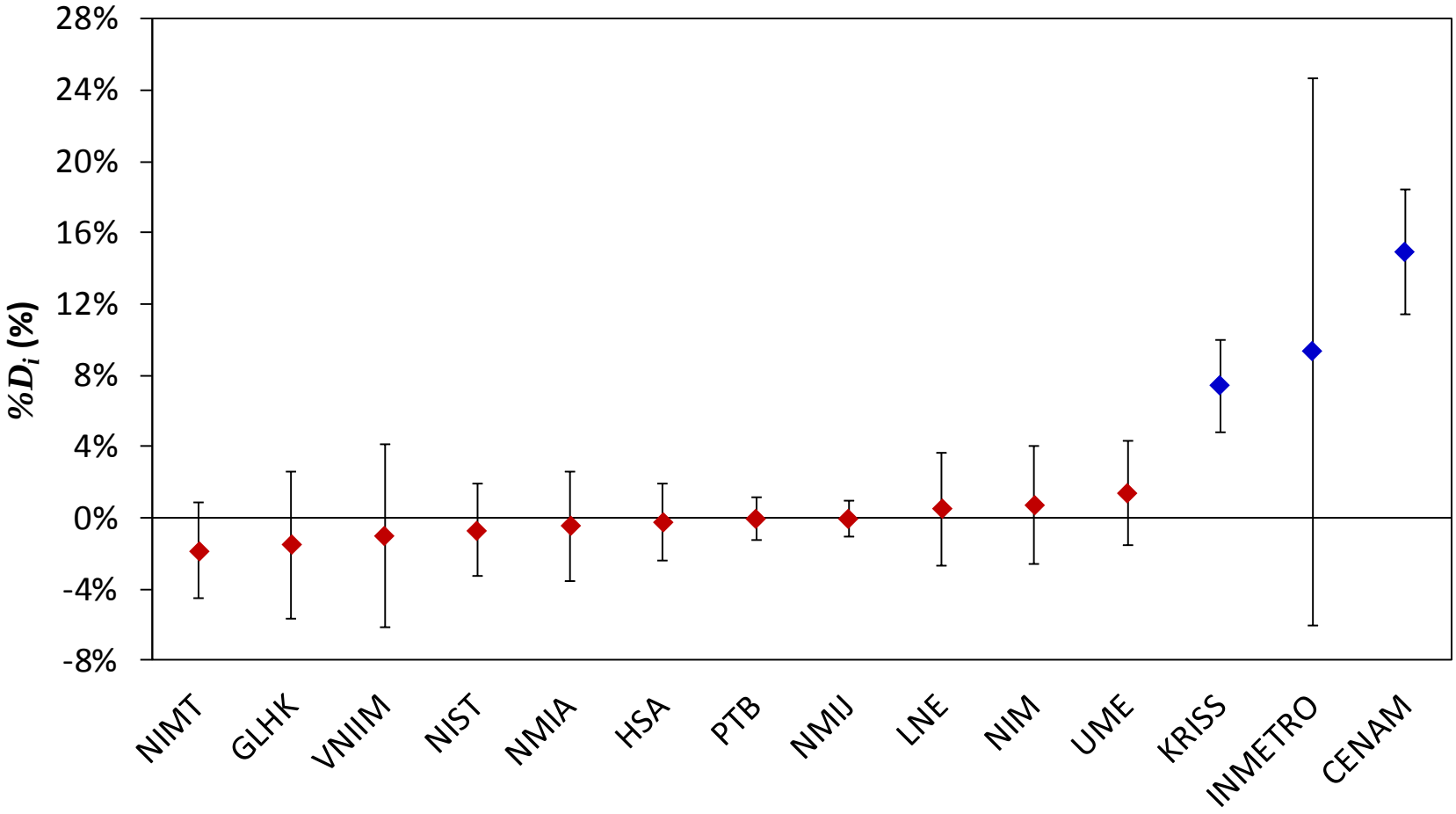


Figure 25: Relative degrees of equivalence, $\%D_i \pm U(\%D_i)$, for uric acid (Serum II)
Bars represent 95 % expanded uncertainties. The results represented by blue diamonds are not included in the calculation of the KCRV.

14. POLARITY vs LOG(KCRV) PLOT

The polarities and concentrations of the analytes in this key comparison are plotted in the Figure below. Both urea and uric acid are represented in the upper right quadrant, together with analytes of high polarity and concentration ranges.

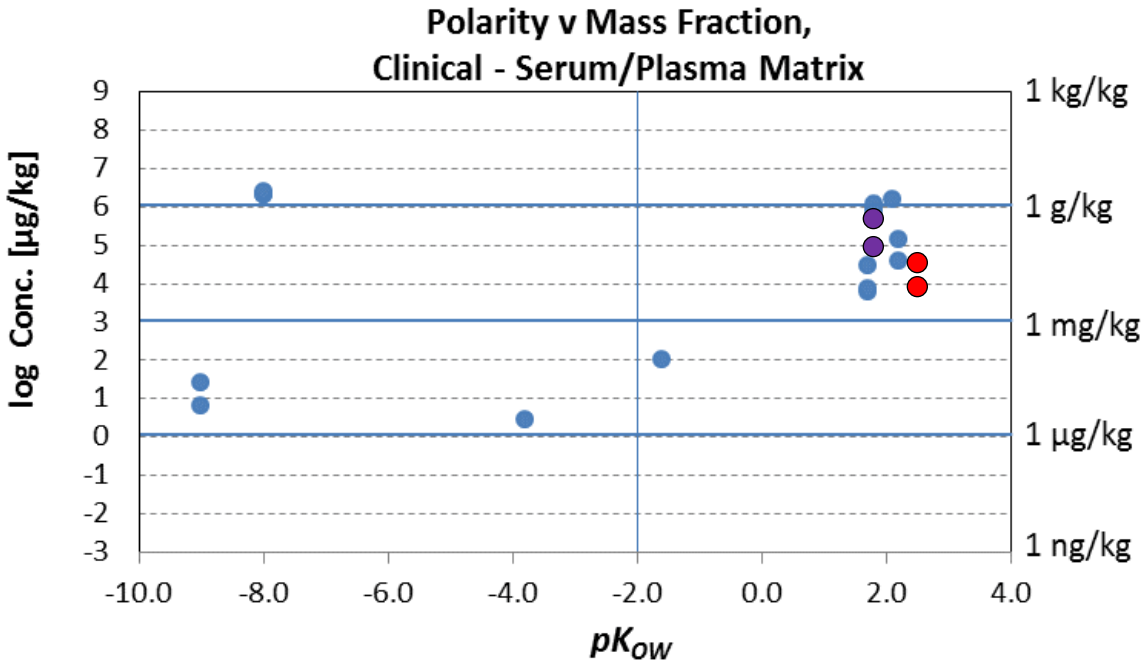


Figure 26: Polarity vs log(KCRV) for OAWG KC of clinical analytes in serum/plasma.

The blue dots are sourced from: A revised model for core competency key comparisons in organic analysis supporting and assessing all calibration and accuracy control CMCs; Steven Westwood, Ralf Joseph & Robert Wielgosz, BIPM, April 2017. The purple and red dots show the polarity and log(KCRV) for urea and uric acid in this comparison.

15. CORE COMPETENCY AND HOW FAR DOES THE LIGHT SHINE?

This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity $pK_{OW} > 2$ in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.

Through this comparison, majority of the participating institutes in CCQM-K109 demonstrated their capabilities in the measurement of the clinical markers in the biological matrix using IDMS. The Core Competency Tables of the participating NMIs/DIs are presented in Appendix E.

16. USE OF REPORT

This report is intended to be used as an internal reference for the participating NMIs/DIs and CCQM OAWG. Its content shall not be disclosed to other parties or used for other purposes.

17. ACKNOWLEDGEMENT

The Coordinators of this comparison would like to express their sincere thanks to all the participating institutes for their contributions, as well as for the support of Dr. Lindsey Mackay, Chair, CCQM OAWG and Dr. Della Sin, Chair, CCQM KCWG. The Coordinators would also like to express their special thanks to Drs. Blaza Toman, Michael Nelson and David Duewer from NIST for their advice and contributions to the evaluation of the results.

18. APPENDIX A: STUDY PROTOCOL

Background

The OAWG has agreed on a list of Track A key comparisons to assess the core competencies of National Metrology Institutes/Designated Institutes (NMIs/DIs) for the delivery of measurement services to their customers. One of the Track A comparisons discussed and agreed upon under the matrix category was “Polar Organic in Biological Matrix”. In the OAWG meeting in November 2013 in Pretoria, South Africa, the meeting discussed the possible analytes and biological materials in this comparison which could best cover current and future CMCs. The Health Sciences Authority (HSA), Singapore suggested urea and uric acid in human serum as a possible comparison for this category and would present a proposal at the meeting in April 2014 in BIPM. At the April 2014 OAWG meeting, a proposal was presented by HSA. After considering the services offered by the NMIs/DIs and that this being the first Track A comparison for biological materials, the OAWG agreed on the HSA’s proposal on urea and uric acid in human serum as an appropriate comparison for this matrix category. A key comparison and a parallel pilot study will be organised.

Objectives

The comparison aims to enable participating NMIs/DIs to demonstrate their competence in the determination of high polarity organic compounds in a biological matrix. As a model system for this comparison, two polar clinical biomarkers: urea and uric acid, in human serum are chosen.

Preparation of the Comparison Materials

The comparison materials are frozen human sera. An experienced commercial human blood products supplier (Solomon Park Research Laboratories, Kirkland, WA, USA) was engaged by the Health Sciences Authority (HSA) to prepare the materials. Two pools of human serum materials with two different concentration levels of urea and uric acid were prepared, and pre-packed in 260 vials containing 1 mL of serum each.

The homogeneity of the comparison materials was assessed by gas chromatography-isotope dilution mass spectrometry (GC-IDMS). A sample size of 0.10 g was used in the assessment of homogeneity for both urea and uric acid. Eleven bottles were randomly and stratifically selected, and two subsamples were taken from each bottle. Using ANOVA at 95 % level of confidence, both materials were found to be sufficiently homogeneous.

The relative standard uncertainties[9] of inhomogeneity were found to be below 0.04 % and 0.18 % for urea and uric acid (for both concentration levels), respectively.

The stability of the comparison materials at – 70 ^{o}C was assessed using the GC-IDMS and liquid chromatography-isotope dilution mass spectrometry (LC-IDMS) for urea and uric acid, respectively. The same sample size as described in the homogeneity testing was also used. The testing was carried out on four occasions over a period of about 230 days using classical design. For each occasion of the stability testing, two bottles were randomly selected, and two subsamples were taken from each bottle. Using Student's *t*-test at 95 % level of confidence, no significant instability of the comparison materials was observed. The relative standard uncertainties of instability were estimated to be below 0.21 % and 0.46 % for urea and uric acid (for both concentration levels), respectively.

A study on the effect of different equilibration times (2 h to 29.5 h) on the measurement results of urea and uric acid for serum materials containing a high and low level of lipid was carried out. The results showed that equilibration times do not have a significant effect on the measurement results of urea and uric acid for serum materials with either high or low level of lipid.

The Measurands

The mass fractions of urea and uric acid in the comparison materials are in the range of 100 to 2,000 and 10 to 165 mg/kg, respectively. One of the concentration levels is within the normal biological range while the other is higher than normal range. The concentration levels are within the range of existing CMC claims in the BIPM KCDB.

Registration

Interested institutes should complete the Registration Form and return to HSA before the deadline and an email will be sent to confirm the registration. The institutes may choose to register for one or both the measurands. Potential DIs that are nominated by their respective NMIs are welcome to participate in the pilot study.

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[9] As the between-bottle mean squares were found to be smaller than the within-bottle mean squares, the uncertainties for inhomogeneity were estimated using the following equation:

$$\sqrt{\frac{MS_w}{n}} \sqrt[4]{\frac{2}{v_{MS_w}}}$$

where MS_w is the within-bottle mean square, n is the number of observations, v_{MSw} is the number of degrees of freedom

Instructions for Participating Institutes

The materials used for this comparison were tested non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV) by the supplier before distribution. However, the materials should be handled as biohazards materials capable of transmitting infectious diseases.

The materials will be transported using dry ice. Upon receipt, the materials should be immediately stored at a temperature below – 60 ^{o}C before measurement. The materials should be used immediately after they are thawed, as measurements on vials which have been previously thawed and opened, have not been conducted.

Each participating NMI/DI will receive three vials of serum sample for each concentration level and measurand that it registers for, i.e. the NMI/DI will receive a total of 12 vials if it registers for both urea and uric acid. The participating NMIs/DIs may use one of the three vials as a practice sample and should report the results for the remaining two vials. At least two subsamples should be taken from each vial. The participating NMIs/DIs may decide on the number of times that each subsample is to be measured. Before sampling, the material should be allowed to thaw and warm to room temperature (18 – 25 ^{o}C), and homogenised by gentle swirling and inversing the vial several times. The subsamples taken from the same vial should be measured on the same day. The recommended minimum subsample size is 0.10 g.

The participating NMIs/DIs should use their own methods for the determination. Metrologically traceable certified reference materials (CRMs) should be used as calibration standards. CRMs of urea and uric acid are available from the National Institute of Science and Technology (NIST), the National Metrology Institute of Japan (NMIJ), and the National Institute of Metrology (NIM), China. Other sources of reference materials may be used, provided that they are purity assessed adequately to demonstrate the metrological traceability. It is recommended to use matrix CRMs as quality controls. CRMs for urea and uric acid in human serum are available from the Korea Research Institute of Standards and Science (KRISS), NIST, HSA and NIM, China. These CRMs are listed in the following Table.[10]

[10] The Appendix may not contain an exhaustive list of all CRMs that may be used as calibration standards and quality control materials in this comparison.

CRMs of Urea

CRM Code	Source of CRM
SRM 912a (clinical standard)	NIST
CRM 6006-a	NMIJ
GBW09201	NIM, China

CRMs of Uric Acid

CRM Code	Source of CRM
SRM 913a	NIST
CRM 6008-a	NMIJ
GBW09202	NIM, China

Matrix CRMs Containing Urea and Uric Acid

CRM Code	Matrix Type	Source of CRM
SRM 909c	Frozen Human Serum	NIST
HRM-3002A	Frozen Human Serum	HSA
CRM 111-1-001 and CRM 111-01-002	Frozen Human Serum	KRISS
CRM 111-1-003 and CRM 111-01-004	Lyophilized Human Serum	KRISS

Matrix CRMs Containing Uric Acid

CRM Code	Matrix Type	Source of CRM
GBW09157	Frozen Human Serum	NIM, China
GBW09169	Frozen Human Serum	NIM, China

Internal Standards

Isotopic labelled urea is available from Cambridge Isotopes Laboratories, and isotopic labelled uric acid is available from Sigma Aldrich and Cambridge Isotopes Laboratories.

Reporting of Results

A Report of Results Form will be provided to the participating NMIs/DIs for completion. The participating NMIs/DIs are expected to report their results based on at least four subsamples (two subsamples from each vial) for each level. The results should be reported in the unit of mg/kg, and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure, the calibration standard, the internal standard, the quality control material, the calculation of the results, and the estimation of measurement uncertainty should be included. The completed form should be sent to HSA on or before the scheduled deadline. The submitted results will be considered as final.

Evaluation of Results

Results of all participating NMIs/DIs will be evaluated against the key comparison reference value (KCRV). The KCRV and associated uncertainty will be determined from results of NMIs/DIs that participate in the key comparison using IDMS method with demonstrated metrological traceability. However, other techniques may also be used for comparison purposes. Results from NMIs/DIs that participated in the pilot study will not be included in the calculation of the KCRV.

Core Competency and How Far Does the Light Shine?

This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity $pK_{OW} > 2$ in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.

Schedule

Official call for participation:	13 July 2015
Deadline for registration:	1 December 2015
Distribution of comparison samples:	by 29 February 2016
Deadline for submission of results:	1 September 2016

Coordinating Laboratory and Contact Person

Dr Tang Lin TEO & Dr Qinde LIU
Health Sciences Authority
Applied Sciences Group
Chemical Metrology Laboratory
1 Science Park Road
#01-05/06, The Capricorn
Singapore Science Park II
Singapore 117528
Tel:+65 6775 1605
Fax:+65 6775 1398
E-mail: HSA_CML@hsa.gov.sg

Note: A potential DI may also register to participate in the CCQM pilot study upon a written agreement from the NMI.

19. APPENDIX B: SUMMARY OF PARTICIPATING INSTITUTES' UNCERTAINTY ESTIMATION APPROACHES

NMIA

All masses and mass fractions used to calculate ω_x were determined using balances calibrated with metrological traceability to the SI unit of the kilogram through Australian national standards for mass. Peak areas were determined from chromatographic traces generated for characteristic ions corresponding to analytes and internal standards.

A standard uncertainty was estimated for all components in the measurement equation. These were combined using derived sensitivity coefficients to estimate a combined standard uncertainty in the reported result for each analyte in the study sample. The total effective degrees of freedom was determined using the Welch-Satterthwaite equation to calculate the appropriate coverage (k) factor to expand the combined standard uncertainty to a 95% confidence interval for reporting. The method precision term was calculated as the standard deviation of the mean of all the measurements used in the calculation of the reference value.

To ensure that all likely sources of bias were accounted for in the final uncertainty budget a trueness factor was also included. This factor was assigned a nominal value of one with an uncertainty representing the potential magnitude of undetected bias due to factors affecting the measured peak area ratios such as matrix interferences and matrix effects. The magnitude of the standard uncertainty in the trueness factor was estimated as the standard deviation of the various average analyte mass fractions determined in multiple serum sub-samples analysed by the primary method of analysis and different confirmatory methods of analysis used to assess bias due to matrix interferences/effects.

Urea Serum Pool I

Details	
\\PINS4VFI05\Home\w1985\Profile\Desktop\CCQM-K109 Urea and Uric Acid in Human Serum\Uncertainty Budgets\Budgets\[Urea Pool I Uncertainty Budget.xlsm]GUM Budget	
Project	CCQM K109
Sample Name	K109
Analyte	Urea Pool I
Matrix	Human Serum
Measurand	Mass Fraction
Measurand Symbol	Wx
Reporting units	mg/kg

Summary of Contributions to Total Combined Measurement Uncertainty							
Number	*Name of Component*	*Symbol*	*Units*	*Value*	*Standard Uncertainty*	*Relative Standard Uncertainty*	*Degrees of Freedom*
i	Xi			xi	u(xi)	u(xi)/xi (%)	vi
1	Method Precision	F(MP)	dimensionless	1.0000	0.0016	0.161%	7.0
2	Method Trueness	F(MT)	dimensionless	1.0000	0.0030	0.302%	30.0
3	Standard	Wz	ng/g	995.6679	2.1023	0.211%	57.6
4	Moisture Content	MC	n/a	1.0000	0.0000	0.000%	1.0
5	Gravimetry	Mx	g	0.1013	0.00035	0.35%	100.0
6	Gravimetry	My(SB)	g	0.1406	0.00035	0.25%	100.0
7	Gravimetry	Mz	g	0.1456	0.00035	0.24%	100.0
8	Gravimetry	My(CB)	g	0.1423	0.00035	0.25%	100.0
9	Isotope Amount Ratio	Rx,Rz	mol/mol	335	14.6	4.36%	2.0
10	Isotope Amount Ratio	Ry	mol/mol	0.0077853	0.000333	4.28%	2.0
11	Blend Isotope Amount Ratio	R(SB)	mol/mol	1.1364			7.0
12	Blend Isotope Amount Ratio	R(CB)	mol/mol	1.0940			7.0

Urea Serum Pool II

Details	
\\PINS4VFI05\Home\w1985\Profile\Desktop\CCQM-K109 Urea and Uric Acid in Human Serum\Uncertainty Budgets\Budgets\[Urea Pool II Uncertainty Budget.xlsm]GUM Budge	
Project	CCQM K109
Sample Name	K109
Analyte	Urea Pool II
Matrix	Human Serum
Measurand	Mass Fraction
Measurand Symbol	Wx
Reporting units	mg/kg

Summary of Contributions to Total Combined Measurement Uncertainty							
Number	*Name of Component*	*Symbol*	*Units*	*Value*	*Standard Uncertainty*	*Relative Standard Uncertainty*	*Degrees of Freedom*
i	Xi			xi	u(xi)	u(xi)/xi (%)	vi
1	Method Precision	F(MP)	dimensionless	1.0000	0.0022	0.218%	7.0
2	Method Trueness	F(MT)	dimensionless	1.0000	0.0038	0.381%	30.0
3	Standard	Wz	ng/g	249.2115	0.5285	0.212%	58.6
4	Moisture Content	MC	n/a	1.0000	0.0000	0.000%	1.0
5	Gravimetry	Mx	g	0.1015	0.00035	0.34%	100.0
6	Gravimetry	My(SB)	g	0.1314	0.00035	0.27%	100.0
7	Gravimetry	Mz	g	0.1203	0.00035	0.29%	100.0
8	Gravimetry	My(CB)	g	0.1322	0.00035	0.26%	100.0
9	Isotope Amount Ratio	Rx,Rz	mol/mol	335	14.6	4.36%	2.0
10	Isotope Amount Ratio	Ry	mol/mol	0.0077853	0.0003331	4.28%	2.0
11	Blend Isotope Amount Ratio	R(SB)	mol/mol	1.1271			7.0
12	Blend Isotope Amount Ratio	R(CB)	mol/mol	1.0103			7.0

Uric Acid Serum Pool I

Details	
\\PINS4VFI05\Home\w1985\Profile\Desktop\CCQM-K109 Urea and Uric Acid in Human Serum\Uncertainty Budgets\Budgets\[Uric Acid Pool I Uncertainty Budget.xlsm]GUM Budget	
Project	CCQM K109
Sample Name	K109
Analyte	Uric Acid Pool I
Matrix	Human Serum
Measurand	Mass Fraction
Measurand Symbol	Wx
Reporting units	mg/kg

Summary of Contributions to Total Combined Measurement Uncertainty							
Number	*Name of Component*	*Symbol*	*Units*	*Value*	*Standard Uncertainty*	*Relative Standard Uncertainty*	*Degrees of Freedom*
i	Xi			xi	u(xi)	u(xi)/xi (%)	vi
1	Method Precision	F(MP)	dimensionless	1.0000	0.0022	0.220%	11.0
2	Method Trueness	F(MT)	dimensionless	1.0000	0.0063	0.629%	30.0
3	Standard	Wz	ng/g	100.5920	1.1225	1.12%	11.2
4	Moisture Content	MC	n/a	1.0000	0.0000	0.000%	1.0
5	Gravimetry	Mx	g	0.1014	0.00035	0.35%	100.0
6	Gravimetry	My(SB)	g	0.1337	0.00035	0.26%	100.0
7	Gravimetry	Mz	g	0.1342	0.00035	0.26%	100.0
8	Gravimetry	My(CB)	g	0.1339	0.00035	0.26%	100.0
9	Isotope Amount Ratio	Rx,Rz	mol/mol	4.8539	0.02543	0.52%	2.0
10	Isotope Amount Ratio	Ry	mol/mol	0.00766	0.00136	17.72%	2.0
11	Blend Isotope Amount Ratio	R(SB)	mol/mol	0.8490			11.0
12	Blend Isotope Amount Ratio	R(CB)	mol/mol	0.8245			11.0

Uric Acid Serum Pool II

Details	
\\PINS4VFI05\Home\w1985\Profile\Desktop\CCQM-K109 Urea and Uric Acid in Human Serum\Uncertainty Budgets\Budgets\[Uric Acid Pool II Uncertainty Budget.xlsm]GUM Budget	
Project	CCQM K109
Sample Name	K109
Analyte	Uric Acid Pool II
Matrix	Human Serum
Measurand	Mass Fraction
Measurand Symbol	Wx
Reporting units	mg/kg

Summary of Contributions to Total Combined Measurement Uncertainty							
Number	*Name of Component*	*Symbol*	*Units*	*Value*	*Standard Uncertainty*	*Relative Standard Uncertainty*	*Degrees of Freedom*
i	Xi			xi	u(xi)	u(xi)/xi (%)	vi
1	Method Precision	F(MP)	dimensionless	1.0000	0.0021	0.206%	11.0
2	Method Trueness	F(MT)	dimensionless	1.0000	0.0074	0.740%	30.0
3	Standard	Wz	ng/g	30.0019	0.3348	1.12%	11.2
4	Moisture Content	MC	n/a	1.0000	0.0000	0.000%	1.0
5	Gravimetry	Mx	g	0.1016	0.00035	0.34%	100.0
6	Gravimetry	My(SB)	g	0.1188	0.00035	0.29%	100.0
7	Gravimetry	Mz	g	0.0984	0.00035	0.36%	100.0
8	Gravimetry	My(CB)	g	0.1155	0.00035	0.30%	100.0
9	Isotope Amount Ratio	Rx,Rz	mol/mol	4.7246	1.37025	29.00%	3.0
10	Isotope Amount Ratio	Ry	mol/mol	0.00397	0.00037	9.43%	3.0
11	Blend Isotope Amount Ratio	R(SB)	mol/mol	0.8593			11.0
12	Blend Isotope Amount Ratio	R(CB)	mol/mol	0.8379			11.0

INMETRO

Uncertainty was firstly estimated for each set of 5 subsamples taken to produce one standard addition curve. The stated standard uncertainty was the highest standard uncertainty of the 4 results (2 results for each bottle). The repeatability of the four results was combined as a component as shown in the tables bellow.

The tables below show the components from the determination that presented the highest standard uncertainty.

Urea (STY-0049-001):

Component	type	u component (mg/kg)	relative contribution (%)
repeatability	A	3.8×10^{-2}	1.61×10^{1}
calibrant purity value	B	5.0×10^{-4}	2.87×10^{-5}
calibration curve	A	8.6×10^{-2}	0.84×10^{2}
sample mass	B	2.2×10^{-6}	5.55×10^{-10}
mass after first sample dilution	B	3.7×10^{-7}	1.58×10^{-11}
mass after second sample dilution	B	8.4×10^{-8}	8.00×10^{-13}
aliquot from first dilution	B	9.0×10^{-6}	9.29×10^{-9}
mass of standard solution added to the sample	B	2.4×10^{-6}	6.63×10^{-10}
Standard mass	B	8.6×10^{-6}	8.51×10^{-9}
Standard solution mass	B	3.8×10^{-8}	1.65×10^{-13}

Urea (STY-0049-002):

Component	type	u component (mg/kg)	relative contribution (%)
repeatability	A	4.0×10^{-2}	1.85×10^{1}
calibrant purity value	B	5.0×10^{-4}	2.94×10^{-5}
calibration curve	A	8.3×10^{-2}	8.15×10^{1}
sample mass	B	2.2×10^{-6}	5.69×10^{-10}
mass after first sample dilution	B	3.7×10^{-7}	1.58×10^{-11}
mass after second sample dilution	B	3.5×10^{-7}	1.40×10^{-11}
aliquot from first dilution	B	4.2×10^{-6}	2.09×10^{-9}
mass of standard solution added to the sample	B	2.1×10^{-6}	5.37×10^{-10}
standard mass	B	8.6×10^{-6}	8.73×10^{-9}
standard solution mass	B	3.8×10^{-8}	1.70×10^{-13}

Uric acid (STY-0049-001):

Component	type	u component (mg/kg)	relative contribution (%)
repeatability	A	4.1×10^{-2}	3.60×10^{1}
calibrant purity value	B	1.0×10^{-3}	2.11×10^{-2}
calibration curve	A	5.5×10^{-2}	6.37×10^{1}
sample mass	B	2.7×10^{-3}	1.55×10^{-1}
mass after first sample dilution	B	1.2×10^{-3}	2.85×10^{-2}
mass after second sample dilution	B	5.8×10^{-5}	6.96×10^{-5}
aliquot from first dilution	B	7.1×10^{-4}	1.05×10^{-2}
mass of standard solution added to the sample	B	2.5×10^{-3}	1.35×10^{-1}
standard mass	B	1.3×10^{-5}	3.73×10^{-6}
standard solution mass	B	4.7×10^{-6}	4.55×10^{-7}

Uric acid (STY-0049-002):

Component	type	u component (mg/kg)	relative contribution (%)
repeatability	A	2.0×10^{-2}	1.02×10^{1}
calibrant purity value	B	1.0×10^{-3}	2.49×10^{-2}
calibration curve	A	6.0×10^{-2}	8.93×10^{1}
sample mass	B	2.5×10^{-3}	1.65×10^{-1}
mass after first sample dilution	B	1.1×10^{-3}	3.23×10^{-2}
mass after second sample dilution	B	2.0×10^{-4}	1.03×10^{-3}
aliquot from first dilution	B	6.9×10^{-4}	1.17×10^{-2}
mass of standard solution added to the sample	B	2.6×10^{-3}	1.64×10^{-1}
standard mass	B	1.7×10^{-5}	7.2×10^{-6}
standard solution mass	B	4.2×10^{-6}	4.4×10^{-7}

Duewer, D.L.; Parris, R.M.; White V, E.; May, W.E.; Elbaum, H. NIST Special Publication 1012. National Institute of Standards and Technology, 2004

NIM

Uncertainty Information of Serum Urea from NIMC

Parameter	STY-0049-001	STY-0049-002	Source of uncertainty
$u_{w\text{-sam}}$	0.01%	0.01%	Serum Sample weight
u_{p}	0.10%	0.10%	Purity of calibrant
u_{cs}	0.14%	0.14%	Calibration solutions
u_{cb}	0.02%	0.02%	Calibration blends
$u_{r\text{-}std}$	0.66%	0.66%	Ratio area of Standard
$u_{r\text{-}sam}$	0.72%	0.93%	Ratio area of Sample
u_{IS}	0.02%	0.02%	Internal Standard spiked into Sample
u_{sam}	0.43%	0.28%	RSD of samples measured
u_{c}	1.08%	1.19%	combined uncertainty
k	2	2	Coverage factor
U_{rel}	2.16%	2.38%	Expanded uncertainty
Concentration ±combined uncertainty(mg/kg)	1481±16	334.8±4.0	

Uncertainty Information of Serum Uric Acid from NIMC

Parameter	STY-0049-001	STY-0049-002	Source of uncertainty
$u_{w\text{-sam}}$	0.01%	0.01%	Serum Sample weight
u_{p}	0.15%	0.15%	Purity of calibrant
u_{cs}	0.22%	0.22%	Calibration solutions
u_{cb}	0.01%	0.01%	Calibration blends
$u_{r\text{-}std}$	0.42%	0.42%	Ratio area of Standard
$u_{r\text{-}sam}$	0.40%	0.65%	Ratio area of Sample
u_{IS}	0.02%	0.02%	Internal Standard spiked into Sample
u_{sam}	0.85%	1.41%	RSD of samples measured
u_{c}	1.07%	1.63%	combined uncertainty
k	2	2	Coverage factor
U_{rel}	2.14%	3.26%	Expanded uncertainty
Concentration ±combined uncertainty(mg/kg)	136.5±1. 5	39.67±0.65	

GLHK

Uncertainty budget of Urea in STY-0049-001 (Green Cap)

Urea	Value *x*	Standard uncertainty *u*(*xi*)	Relative standard uncertainty *u*(*xi*)/*xi*	Contribution to total *uc* (%)	Uncertainty evaluation
cz (mg/kg)	508.97	5.101	0.01002	47.47	B
m_Y (mg)	149.86	0.0375	0.00025	0.03	B
m_X (mg)	101.43	0.0375	0.00037	0.06	B
m_{YC} (mg)	354.30	0.0375	0.00011	0.01	B
m_{ZC} (mg)	353.87	0.0375	0.00011	0.01	B
R_b	1.12	0.0041	0.00363	6.22	A
R_{bc}	1.09	0.0048	0.00442	9.16	A
D_{is}	1.96	0.0001	0.00006	0.00	B
R	1.00	0.0089	0.00885	37.04	A

Uncertainty budget of Urea in STY-0049-002 (Red Cap)

Urea	Value *x*	Standard uncertainty *u*(*xi*)	Relative standard uncertainty *u*(*xi*)/*xi*	Contribution to total *uc* (%)	Uncertainty evaluation
cz (mg/kg)	1000.57	10.419	0.01041	56.93	B
m_Y (mg)	111.19	0.0375	0.00034	0.06	B
m_X (mg)	100.50	0.0375	0.00037	0.07	B
m_{YC} (mg)	180.66	0.0375	0.00021	0.02	B
m_{ZC} (mg)	186.63	0.0375	0.00020	0.02	B
R_b	1.14	0.0039	0.00341	6.11	A
R_{bc}	1.12	0.0039	0.00347	6.27	A
D_{is}	3.31	0.0001	0.00002	0.00	B
R	1.00	0.0076	0.00762	30.52	A

Uncertainty budget of Uric acid in STY-0049-001 (Green Cap)

Uric acid	Value *x*	Standard uncertainty *u*(*xi*)	Relative standard uncertainty *u*(*xi*)/*xi*	Contribution to total *uc* (%)	Uncertainty evaluation
c_z (mg/kg)	136.88	1.787	0.01306	49.47	B
m_y (mg)	48.69	0.0375	0.00077	0.17	B
m_X (mg)	99.31	0.0375	0.00038	0.04	B
m_{yc} (mg)	367.60	0.0375	0.00010	0.00	B
m_{zc} (mg)	145.35	0.0375	0.00026	0.02	B
R_b	0.98	0.0059	0.00601	10.49	A
R_{bc}	0.96	0.0103	0.01074	32.73	A
D_{is}	4.98	0.0001	0.00001	0.00	B
R	1.00	0.0049	0.00494	7.08	A

Uncertainty budget of Uric acid in STY-0049-002 (Red Cap)

Uric acid	Value *x*	Standard uncertainty *u*(*xi*)	Relative standard uncertainty *u*(*xi*)/*xi*	Contribution to total *uc* (%)	Uncertainty evaluation
c_z (mg/kg)	125.92	2.102	0.01669	63.83	B
m_y (mg)	38.96	0.0375	0.00096	0.21	B
m_X (mg)	97.11	0.0375	0.00039	0.03	B
m_{yc} (mg)	205.38	0.0375	0.00018	0.01	B
m_{zc} (mg)	160.12	0.0375	0.00023	0.01	B
R_b	1.01	0.0080	0.00793	14.39	A
R_{bc}	1.02	0.0092	0.00900	18.24	A
R	1.00	0.0038	0.00378	3.27	A

LNE

Urea

Sample 1

	Type (A or B)	Final uncertainty budget (%)
Preparation of sample blends (weighings)	B	1
Calibration model	B	1
Preparation of calibration blends (weighings)	B	4
Precision	B	94

Sample 2

	Type (A or B)	Final uncertainty budget (%)
Preparation of sample blends (weighings)	B	0.5
Calibration model	B	0.5
Preparation of calibration blends (weighings)	B	1
Precision	B	98

Uric Acid

Sample 1

	Type (A or B)	Final uncertainty budget (%)
Preparation of sample blends (weighings)	B	4
Calibration model	B	21
Preparation of calibration blends (weighings)	B	9
Precision	B	76

Sample 2

	Type (A or B)	Final uncertainty budget (%)
Preparation of sample blends (weighings)	B	3
Calibration model	B	4
Preparation of calibration blends (weighings)	B	10
Precision	B	83

PTB

Determination of the mass fraction of urea in Human Serum CCQM K109, Level I

Model Equation:
$w_{sample}=w_{sample}exp*P_{urea}*K_W*Sys$

List of Quantities:

Quantity	Unit	Definition
w_{sample}	mg/kg	Mass fraction of urea in serum
$w_{sample}exp$	mg/kg	Mass fraction of urea in serum per subsample, mean of 6 single observations
P_{urea}		Purity of the reference material
K_W		Uncertainty of weighing
Sys		Estimated factor for unidentified systematic error

$w_{sample}exp$:
Type A
Method of observation: Direct
Number of observation: 6

No.	Observation
1	1487.6 mg/kg
2	1485.5 mg/kg
3	1488.8 mg/kg
4	1485.4 mg/kg
5	1487.6 mg/kg
6	1485.0 mg/kg

Arithmetic Mean: 1486.650 mg/kg
Standard Deviation: 1.6 mg/kg
Standard Uncertainty: 0.633 mg/kg
Degrees of Freedom: 5

The observations (w) are the determined mass fractions of urea in serum per subsample (in mg/kg) - 2 vials, 3 subsamples per vial used.

P_{urea}:
Type B rectangular distribution
Value: 1
Halfwidth of Limits: 0.001

Uncertainty purity of the reference compound urea in used NIST SRM 912a, according to certificate +/- 0,1%
The purity (99,9%) was already calculated in the excel sheet for the determination of w. Therefore the value was set here to 1.

K_W:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.00017
Coverage Factor: 2

Uncertainty of the microbalance MC 5 (Sartorius)
calibration certificate dated 12.11.2015
U = 0,0008 mg + 1,20 * 10 E- 0 5 * m (w);
= 0,017% (5 mg)

Sys:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.01
Coverage Factor: 2

Unknown factor for systematic unidentified discrepancies including sample preparation and GC-MS interferences (estimated value = +/- 1,0 %).

Uncertainty Budgets:
w_{sample}: Mass fraction of urea in serum

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
w_{sample}exp	1486.650 mg/kg	0.633 mg/kg	normal	1.0	0.63 mg/kg	0.7 %
P_{urea}	1.000000	$577 \cdot 10^{-6}$	rectangular	1500	0.86 mg/kg	1.3 %
K_W	1.0000000	$85.0 \cdot 10^{-6}$	normal	1500	0.13 mg/kg	0.0 %
Sys	1.00000	$5.00 \cdot 10^{-3}$	normal	1500	7.4 mg/kg	98.0 %
w_{sample}	1486.65 mg/kg	7.51 mg/kg				

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor	Coverage
w_{sample}	1487 mg/kg	15 mg/kg	2.00	95% (t-table 95.45%)

Determination of the mass fraction of urea in Human Serum CCQM K109, Level II

Model Equation:
$w_{sample}=w_{sample}exp*P_{urea}*K_W*Sys$

List of Quantities:

Quantity	Unit	Definition
w_{sample}	mg/kg	Mass fraction of urea in serum
$w_{sample}exp$	mg/kg	Mass fraction of urea in serum per subsample, mean of 6 single observations
P_{urea}		Purity of the reference material
K_W		Uncertainty of weighing
Sys		Estimated factor for unidentified systematic error

$w_{sample}exp$:
Type A
Method of observation: Direct
Number of observation: 6

No.	Observation
1	333.2 mg/kg
2	334.8 mg/kg
3	334.8 mg/kg
4	333.5 mg/kg
5	334.1 mg/kg
6	334.7 mg/kg

Arithmetic Mean: 334.183 mg/kg
Standard Deviation: 0.70 mg/kg
Standard Uncertainty: 0.287 mg/kg
Degrees of Freedom: 5

The observations (w) are the determined mass fractions of urea in serum per subsample (in mg/kg) - 2 vials, 3 subsamples per vial used.

P_{urea}:
Type B rectangular distribution
Value: 1
Halfwidth of Limits: 0.001

Uncertainty purity of the reference compound urea in used NIST SRM 912a, according to certificate +/- 0,1%
The purity (99,9%) was already calculated in the excel sheet for the determination of w. Therefore the value was set here to 1 .
K_W:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.00017
Coverage Factor: 2

Uncertainty of the microbalance MC 5 (Sartorius)

calibration certificate dated 12.11.2015
U = 0,0008 mg + 1,20 * 10 E- 0 5 * m (w);
= 0,017% (5 mg)

Sys:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.01
Coverage Factor: 2

Unknown factor for systematic unidentified discrepancies including sample preparation and GC-MS interferences (estimated value = +/- 1,0 %).

Uncertainty Budgets:
w_{sample}: Mass fraction of urea in serum

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
w_{sample}exp	334.183 mg/kg	0.287 mg/kg	normal	1.0	0.29 mg/kg	2.8 %
P_{urea}	1.000000	$577 \cdot 10^{-6}$	rectangular	330	0.19 mg/kg	1.3 %
K_W	1.0000000	$85.0 \cdot 10^{-6}$	normal	330	0.028 mg/kg	0.0 %
Sys	1.00000	$5.00 \cdot 10^{-3}$	normal	330	1.7 mg/kg	95.9 %
w_{sample}	334.18 mg/kg	1.71 mg/kg				

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor	Coverage
w_{sample}	334.2 mg/kg	3.4 mg/kg	2.00	95% (t-table 95.45%)

Determination of the mass fraction of uric acid in Human Serum CCQM K109, Level I

Key comparison CCQM-K109
Model Equation:
$w_{sample}=w_{sample}exp*P_{uricacid}*K_W*Sys$

List of Quantities:

Quantity	Unit	Definition
w_{sample}	mg/kg	Mass fraction of uric acid in serum
w_{sample}exp	mg/kg	Mass fraction of uric acid in serum per subsample, mean of 6 single observations
$P_{uricacid}$		Purity of the reference material
K_W		Uncertainty of weighing
Sys		Estimated factor for unidentified systematic error

w_{sample}exp:
Type A
Method of observation: Direct
Number of observation: 6

No.	Observation
1	134.39 mg/kg
2	134.12 mg/kg
3	134.02 mg/kg
4	134.43 mg/kg
5	134.40 mg/kg
6	135.75 mg/kg

Arithmetic Mean: 134.518 mg/kg
Standard Deviation: 0.63 mg/kg
Standard Uncertainty: 0.256 mg/kg
Degrees of Freedom: 5

The observations (w) are the determined mass fractions of uric acid in serum per subsample (in mg/kg) - 2 vials, 3 subsamples per vial used.

$P_{uricacid}$:
Type B rectangular distribution
Value: 1
Halfwidth of Limits: 0.001

Uncertainty purity of the reference compound uric acid in used NIST SRM 913a, according to certificate +/- 0,1%
The purity (99,6%) was already calculated in the excel sheet for the determination of w. Therefore the value was set here to 1 .

K_W:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.00017
Coverage Factor: 2

Uncertainty of the microbalance MC 5 (Sartorius)
calibration certificate dated 12.11.2015

U = 0,0008 mg + 1,20 * 10 E- 0 5 * m (w);
= 0,017% (5 mg)

Sys:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.01
Coverage Factor: 2

Unknown factor for systematic unidentified discrepancies including sample preparation and GC-MS interferences (estimated value = +/- 1,0 %).

Uncertainty Budgets:
w_{sample}: Mass fraction of uric acid in serum

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
w_{sample}exp	134.518 mg/kg	0.256 mg/kg	normal	1.0	0.26 mg/kg	12.5 %
$P_{uricacid}$	1.000000	$577 \cdot 10^{-6}$	rectangular	130	0.078 mg/kg	1.2 %
K_W	1.0000000	$85.0 \cdot 10^{-6}$	normal	130	0.011 mg/kg	0.0 %
Sys	1.00000	$5.00 \cdot 10^{-3}$	normal	130	0.67 mg/kg	86.3 %
w_{sample}	134.518 mg/kg	0.724 mg/kg				

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor	Coverage
w_{sample}	134.5 mg/kg	1.5 mg/kg	2.03	95% (t-table 95.45%)

Determination of the mass fraction of uric acid in Human Serum CCQM K109, Level II

Model Equation:

$w_{sample}=w_{sample}exp*P_{uricacid}*K_W*Sys$

List of Quantities:

Quantity	Unit	Definition
w_{sample}	mg/kg	Mass fraction of uric acid in serum
w_{sample}exp	mg/kg	Mass fraction of uric acid in serum per subsample, mean of 6 single observations
$P_{uricacid}$		Purity of the reference material
K_W		Uncertainty of weighing
Sys		Estimated factor for unidentified systematic error

w_{sample}exp:

Type A
Method of observation: Direct
Number of observation: 6

No.	Observation
1	39.31 mg/kg
2	39.35 mg/kg
3	39.43 mg/kg
4	39.37 mg/kg
5	39.57 mg/kg
6	39.21 mg/kg

Arithmetic Mean: 39.3733 mg/kg
Standard Deviation: 0.12 mg/kg
Standard Uncertainty: 0.0494 mg/kg
Degrees of Freedom: 5

The observations (w) are the determined mass fractions of uric acid in serum per subsample (in mg/kg) - 2 vials, 3 subsamples per vial used.

$P_{uricacid}$:

Type B rectangular distribution
Value: 1
Halfwidth of Limits: 0.001

Uncertainty purity of the reference compound uric acid in used NIST SRM 913a, according to certificate +/- 0,1%
The purity (99,6%) was already calculated in the excel sheet for the determination of w. Therefore the value was set here to 1 .

K_W:

Type B normal distribution
Value: 1
Expanded Uncertainty: 0.00017
Coverage Factor: 2

Uncertainty of the microbalance MC 5 (Sartorius)
calibration certificate dated 12.11.2015

U = 0,0008 mg + 1,20 * 10 E- 0 5 * m (w);
= 0,017% (5 mg)

Sys:
Type B normal distribution
Value: 1
Expanded Uncertainty: 0.01
Coverage Factor: 2

Unknown factor for systematic unidentified discrepancies including sample preparation and GC-MS interferences (estimated value = +/- 1,0 %).

Uncertainty Budgets:
w_{sample}: Mass fraction of uric acid in serum

Quantity	Value	Standard Uncertainty	Distribution	Sensitivity Coefficient	Uncertainty Contribution	Index
w_{sample}exp	39.3733 mg/kg	0.0494 mg/kg	normal	1.0	0.049 mg/kg	5.8 %
$P_{uricacid}$	1.000000	$577 \cdot 10^{-6}$	rectangular	39	0.023 mg/kg	1.2 %
K_W	1.0000000	$85.0 \cdot 10^{-6}$	normal	39	$3.3 \cdot 10^{-3}$ mg/kg	0.0 %
Sys	1.00000	$5.00 \cdot 10^{-3}$	normal	39	0.20 mg/kg	92.9 %
w_{sample}	39.373 mg/kg	0.204 mg/kg				

Results:

Quantity	Value	Expanded Uncertainty	Coverage factor	Coverage
w_{sample}	39.37 mg/kg	0.41 mg/kg	2.00	95% (t-table 95.45%)

NMIJ

Urea sample 1

Factor of uncertainty	Value *xi*		Standard uncertainty *u(xi)*		*ui(Csample)* =|*Ci*|*u(xi)*	Degree of freedom
Concentration of analyte	1475.06	mg/kg		mg/kg		
Vial (n=2)			1.96	mg/kg	1.96	1
Sample preparation (n=3)			0.76	mg/kg	0.76	4
Analysis (n=3)			0.95	mg/kg	0.95	12
Weight of sample	103.40	mg	0.05	mg	0.71	large
Weight of I.S. for sample	99.70	mg	0.05	mg	0.74	large
Concentrations of normalized std1 solution	1303.56	mg/kg	2.30	mg/kg	0.76	
Concentrations of normalized std2 solution	1602.76	mg/kg	2.78	mg/kg	1.76	
Peak area ratio of the analyte and I.S. by the measurement of the sample	1.22		0.0029		3.14	
Peak area ratio of the analyte and I.S. by the measurement of std1	1.04		0.0012		0.45	
Peak area ratio of the analyte and I.S. by the measurement of std2	1.31		0.0020		1.40	
	Mass Fraction (mg/kg)		Expanded Uncertainty (k=2)	*%*	Combined Standard Uncertainty (mg/kg)	
	1475.06		9.40	0.64	4.70	

Urea sample 2

Factor of uncertainty	Value *xi*		Standard uncertainty *u(xi)*		*ui(Csample)* =|*Ci*|*u(xi)*	Degree of freedom
Concentration of analyte	333.70	mg/kg		mg/kg		
Vial (n=2)			0.00	mg/kg	0.00	1
Sample preparation (n=3)			0.33	mg/kg	0.33	4
Analysis (n=3)			0.36	mg/kg	0.36	12
Weight of sample	103.69	mg	0.05	mg	0.16	large
Weight of I.S. for sample	100.35	mg	0.05	mg	0.17	large
Concentrations of normalized std1 solution	307.61	mg/kg	0.38	mg/kg	0.16	
Concentrations of normalized std2 solution	371.97	mg/kg	0.45	mg/kg	0.25	
Peak area ratio of the analyte and I.S. by the measurement of the sample	1.20		0.0062		1.66	
Peak area ratio of the analyte and I.S. by the measurement of std1	1.06		0.0017		0.20	
Peak area ratio of the analyte and I.S. by the measurement of std2	1.30		0.0028		0.42	
	Mass Fraction (mg/kg)		Expanded Uncertainty (k=2)	*%*	Combined Standard Uncertainty (mg/kg)	
	333.70		3.658	1.10	1.829	

Uric acid sample 1

Factor of uncertainty	Value *xi*		Standard uncertainty *u(xi)*		*ui(Csample)* =|*Ci*|*u(xi)*	Degree of freedom
Concentration of analyte	138.34	mg/kg		mg/kg		
Vial (n=2)			0.46	mg/kg	0.46	1
Sample preparation (n=3)			0.07	mg/kg	0.07	4
Analysis (n=6)			0.22	mg/kg	0.22	30
Weight of sample	103.28	mg	0.05	mg	0.07	large
Weight of I.S. for sample	98.96	mg	0.05	mg	0.07	large
Concentrations of normalized std1 solution	104.42	mg/kg	0.23	mg/kg	0.05	
Concentrations of normalized std2 solution	157.46	mg/kg	0.33	mg/kg	0.24	
Peak area ratio of the analyte and I.S. by the measurement of the sample	1.14		0.0052		0.62	
Peak area ratio of the analyte and I.S. by the measurement of std1	0.82		0.0026		0.08	
Peak area ratio of the analyte and I.S. by the measurement of std2	1.25		0.0039		0.35	
	Mass Fraction (mg/kg)		Expanded Uncertainty (k=2)	*%*	Combined Standard Uncertainty (mg/kg)	
	138.34		1.846	1.33	0.923	

Uric acid sample 2

Factor of uncertainty	Value *xi*		Standard uncertainty *u(xi)*		*ui(Csample)* =|*Ci*|*u(xi)*	Degree of freedom
Concentration of analyte	39.37	mg/kg				
Vial (n=2)			0.00	mg/kg	0.00	1
Sample preparation (n=3)			0.04	mg/kg	0.04	4
Analysis (n=6)			0.06	mg/kg	0.06	30
Weight of sample	103.12	mg	0.05	mg	0.02	large
Weight of I.S. for sample	99.80	mg	0.05	mg	0.02	large
Concentrations of normalized std1 solution	32.04	mg/kg	0.07	mg/kg	0.03	
Concentrations of normalized std2 solution	37.99	mg/kg	0.10	mg/kg	0.05	
Peak area ratio of the analyte and I.S. by the measurement of the sample	1.08		0.0085		0.12	
Peak area ratio of the analyte and I.S. by the measurement of std1	0.85		0.0033		0.02	
Peak area ratio of the analyte and I.S. by the measurement of std2	1.26		0.0061		0.05	
	Mass Fraction (mg/kg)		Expanded Uncertainty (k=2)	*%*	Combined Standard Uncertainty (mg/kg)	
	39.37		0.331	0.84	0.166	

KRISS

Urea

			STY-0049-001	STY-0049-002
Mean (mg/kg)			1604.2	336.9
Source of uncertainty	Systematic Unc.	Calibration standard mixture (Preparation of standard solution and calibration blends, Purity of standard compound)	11.2	2.3
	Random Unc.	Measurement uncertainty (Measurement of isotope ratio of sample and standard solution, Weighing of sample and isotipe solution added to the sample for analysis)	6.4	0.8
Combined standard unc.			13.0	2.5
veff			9	8
k(95%)			2.26	2.31
Uexp (mg/kg)			29.3	5.7
Uexp (rel%)			1.8%	1.7%

Uric acid

STY-0049-001

Uric acid

Parameter	Input	Unit	Standard uncertainty	Degree of freedom	Type of uncertainty	Uncertainty contribution (%)
$M_{is\text{-}sol,spiked}$	0.1897	g	0.0001	∞	Type B	0.04%
AR_{sample}	0.9373		0.0175	5	Type A	0.77%
W_s	0.0715	g	0.0001	∞	Type B	0.12%
AR_{std}	0.9476		0.0208	2	Type A	0.89%
			Average (ng/g)			146.374
			Combined uncertainty (%)			1.05%
			Effective degree of freedom			3
			k (95% level of confidence)			3.18
			Expanded uncertainty			3.356%

STY-0049-002

Uric acid

Parameter	Input	Unit	Standard uncertainty	Degree of freedom	Type of uncertainty	Uncertainty contribution (%)
$M_{is\text{-}sol,spiked}$	0.0828	g	0.0001	∞	Type B	0.10%
AR_{sample}	0.9676		0.0125	5	Type A	0.54%
W_s	0.1089	g	0.0001	∞	Type B	0.08%
AR_{std}	0.9476		0.0208	2	Type A	0.89%
			Average (ng/g)			42.335
			Combined uncertainty (%)			1.24%
			Effective degree of freedom			5
			k (95% level of confidence)			2.57
			Expanded uncertainty			3.189%

CENAM

Urea, Budget of the uncertainty:

Source	**Value**	**units**	**uncertainty**	**standard uncertainty**
Mass of measurand for calibration solution	0.29447	g	Experimental, repeatability and calibration	0.000080
Mass of labeled compound for calibration solution	0.29204	g	Experimental, repeatability and calibration	0.000080
Sample mass	0.30408	g	Experimental, repeatability and calibration	0.000050
Mass of labeled solution for sample	0.30339	g	Experimental, repeatability and calibration	0.000044
Area ratio for calibration solution	1.0203		Experimental, repeatability	0.0011
Area ratio for sample	1.0126		Experimental, repeatability	0.0073
Mass fraction of measurand in calibration solution	0.3388	mg/g	Experimental, weight repeatability and purity	0.0014

The expanded uncertainty was obtained by multiplying the combined standards uncertainty by k =2 for a 95 % confident level

Source	**Value**	**units**	**uncertainty**	**standard uncertainty**
Matematic model	0.33843	mg/g	Experimental, repeatibility and calibration	0.00284
Reproducibility	----	mg/g	Experimental, reproducibility	0.00145
Instrument performance	----	mg/g	Experimental, repeatibility	0.00219
			combined standards uncertainty	0.3866
			U k: 2	0.77

Uric acid, Budget of the uncertainty

Source	**Value**	**units**	**uncertainty**	**standard uncertainty**
Mass of measurand for calibration solution	0.24987	g	Experimental, repeatability and calibration	0.00006
Mass of labeled compound for calibration solution	0.25180	g	Experimental, repeatability and calibration	0.00006
Sample mass	0.20088	g	Experimental, repeatability and calibration	0.0000075
Mass of labeled solution for sample	0.19710	g	Experimental, repeatability and calibration	0.0000073
Area ratio for calibration solution	0.99253		Experimental, repeatability	0.0383
Area ratio for sample	1.00493		Experimental, repeatability	0.002050
Mass fraction of measurand in calibration solution	0.1504	mg/g	Experimental, weight repeatability and purity	0.0058

The expanded uncertainty was obtained by multiplying the combined standards uncertainty by k =2 for a 95 % confident level

Source	**Value**	**units**	**uncertainty**	**standard uncertainty**
Matematic model	0.1504	mg/g	Experimental, repeatability and calibration	0.0058
Reproducibility	----	mg/g	Experimental, reproducibility	0.0010
Instrument performance	----	mg/g	Experimental, repeatability	0.00049
			combined standards uncertainty	0.00595
			U k: 2	0.0119

VNIIM

UREA

Source of uncertainty		u, % Level 1		u, % Level 2
mass of sample (m)		**0,057**		**0,057**
response factor (F)		**1,98**		**1,02**
preparation of calibration solution	*0,82*		*0,82*	
purity of reference standard	*0,1*		*0,1*	
RSD of F determination	*1,80*		*0,60*	
mass of internal standard added to sample before extraction (m_{IS})		**0,58**		**0,61**
preparation of IS solution	*0,58*		*0,58*	
mass of IS solution added to sample	*0,04*		*0,19*	
RSD of results, %		**1,4**		**0,87**
comb.std uncertainty		**2,49**		**1,47**
expanded uncertainty (k=2)		**5,0**		**3,0**

URIC ACID

Source of uncertainty		u, % Level 1		u, % Level 2
mass of sample (m)		**0,057**		**0,057**
response factor (F)		**1,71**		**1,54**
preparation of calibration solution	*0,82*		*0,82*	
purity of reference standard	*0,1*		*0,1*	
RSD of F determination	*1,5*		*1,3*	
mass of internal standard added to sample before extraction (m_{IS})		**0,58**		**0,61**
preparation of IS solution	*0,58*		*0,58*	
mass of IS solution added to sample	*0,08*		*0,19*	
RSD of results, %		**2,0**		**1,98**
comb.std uncertainty		**2,7**		**2,6**
expanded uncertainty (k=2)		**5,4**		**5,2**

HSA

As C_Y does not contribute to the measurement uncertainty of C_X, 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:

$$C_X = R_M' \times \frac{M_Y C_Z}{M_X} \tag{2}$$

where
R_M = isotope mass ratio in sample blend
C_Z = concentration of urea or uric acid 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 urea or uric acid in human serum sample. A coverage factor *k* with a value of 2 was used to expand the combined standard uncertainty at a 95 % confidence interval. Possible sources of biases [method precision (F_P) and choice of using different ion pairs (F_I)] were accounted for in the final uncertainty budget with the use of the measurement equation:

$$C_X = F_P \times F_I \times R_M' \times \frac{M_Y C_Z}{M_X} \tag{3}$$

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

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

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

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 LC-MS/MS and GC-MS results for each sample was used as the standard uncertainty of method precision.
(3) F_I: The standard deviation of mean 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) C_Z: The certified purity and uncertainty of NIST SRM 912a (for urea) and SRM 913a (for uric acid) in combination with the uncertainty of weighing for preparation of the calibration standard solutions.
(5) 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:

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

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_{Mci} = 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:

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

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

The full uncertainty budget is given in the Tables 1 – 5 below:

Table 1 Uncertainty budget of urea in Serum I.

	x	u_{xi}	u_{xi}/x	c_i	$c_i^2 \,.\, u_{xi}^2$	Contribution
M_X (g)	0.0973	0.000085	0.0872 %	15174	1.658	1.12%
M_Y (g)	0.0720	0.000085	0.1179 %	20522	3.032	2.05%
C_Z (mg/kg)	1927.9	5.4809	0.2843 %	0.77	17.636	11.90%
R_M'	1.0236	0.007217	0.7051 %	1443.1	108.48	73.20%
F_P (mg/kg)	1477.2	4.1247	0.2792 %	1.00	17.013	11.48%
F_I (mg/kg)	1477.2	0.6066	0.0411 %	1.00	0.368	0.25%

Table 2 Uncertainty budget of uric acid in Serum I.

	x	u_{xi}	u_{xi}/x	c_i	$c_i^2 \cdot u_{xi}^2$	Contribution
M_X (g)	0.0989	0.000085	0.0858 %	1383.1	0.01377	0.69%
M_Y (g)	0.0668	0.000127	0.1905 %	2047.1	0.06789	3.42%
C_Z (mg/kg)	201.77	0.9949	0.4931 %	0.68	0.4550	22.91%
R_M'	0.9831	0.007023	0.7144 %	139.15	0.9550	48.09%
F_P (mg/kg)	136.80	0.2905	0.2124 %	1.00	0.08441	4.25%
F_I (mg/kg)	136.80	0.6401	0.4679 %	1.00	0.4097	20.63%

Table 3 Uncertainty budget of urea in Serum II.

	x	u_{xi}	u_{xi}/x	c_i	$c_i^2 \cdot u_{xi}^2$	Contribution
M_X (g)	0.0969	0.000085	0.0876 %	3441.2	0.08526	1.15%
M_Y (g)	0.0823	0.000085	0.1032 %	4052.6	0.1182	1.60%
C_Z (mg/kg)	1927.9	5.4809	0.2843 %	0.17	0.8982	12.12%
R_M'	0.2033	0.001422	0.6997 %	1640.1	5.4402	73.40%
F_P (mg/kg)	333.37	0.9284	0.2785 %	1.00	0.8620	11.63%
F_I (mg/kg)	333.37	0.08723	0.0262 %	1.00	0.00761	0.10%

Table 4 Uncertainty budget of uric acid in Serum II.

	x	u_{xi}	u_{xi}/x	c_i	$c_i^2 \cdot u_{xi}^2$	Contribution
M_X (g)	0.1483	0.000127	0.0858 %	264.92	0.00114	0.69%
M_Y (g)	0.0729	0.000127	0.1745 %	538.73	0.00470	2.84%
C_Z (mg/kg)	201.77	0.9949	0.4931 %	0.19	0.03754	22.65%
R_M'	0.3370	0.00246	0.7304 %	116.61	0.08236	49.70%
F_P (mg/kg)	39.294	0.08210	0.2089 %	1.00	0.00674	4.07%
F_I (mg/kg)	39.294	0.1824	0.4641 %	1.00	0.03325	20.06%

NIMT

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

$u(w_{z,c})$ 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 precision 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 precipitation

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.

UREA STY-0049-001 (GREEN CAP)

Cx = 1456 mg/kg
u(x) = 16 mg/kg
u(x)/x = 1.08%
Veff (total) = 18.189
k = 2.10 (@ 95% confidence level)
U(x) = 33
% U(x) = 2.27 %

Measurement equation factors	Values x	Uncertainties	
		u(x)	u(x)/(x)
Method Precision	1.0000	0.00791	0.791%
$\mathbf{m_{zc}}$	0.13626	0.000046	0.0335%
$\mathbf{m_y}$	0.14002	0.000046	0.0326%
$\mathbf{m_{yc}}$	0.14149	0.000046	0.0323%
$\mathbf{m_x}$	0.09806	0.000046	0.0466%
$\mathbf{w_z}$	998.0268	3.914423	0.3922%
R'b	0.7959	0.002489	0.3127%
R'bc	0.7434	0.001369	0.1842%
Extraction effects	1.000	0.0050	0.500%

URIC ACID STY-0049-001 (GREEN CAP)

Cx = 134.6 mg/kg
u(x) = 1.5 mg/kg
u(x)/x = 1.07 %
Veff (total) = 13.998
k = 2.16 (@ 95% confidence level)
U(x) = 3.2
% U(x) = 2.31 %

Measurement equation factors	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Method Precision	1.0000	0.00471	0.471%
m_{zc}	0.03940	0.000046	0.1159%
m_y	0.04147	0.000046	0.1101%
m_{yc}	0.04147	0.000046	0.1101%
m_x	0.09926	0.000046	0.0460%
w_z	99.8547	0.483411	0.4841%
R'b	0.9406	0.004655	0.4949%
R'bc	0.9364	0.003690	0.3940%
Extraction effects	1.000	0.0050	0.500%

UREA STY-0049-002 (RED CAP)

Cx = 329.0 mg/kg
u(x) = 6.1 mg/kg
u(x)/x = 1.85%
Veff (total) = 15.027
k = 2.13 (@ 95% confidence level)
U(x) = 13
% U(x) = 3.95%

Measurement equation factors	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Method Precision	1.0000	0.01252	1.252%
m_{zc}	0.03208	0.000046	0.1424%
m_y	0.03485	0.000046	0.1311%
m_{yc}	0.03442	0.000046	0.1327%
m_x	0.09818	0.000046	0.0465%
w_z	1000.0173	6.558208	0.6558%
R'b	0.9922	0.007584	0.7644%
R'bc	0.7394	0.005449	0.7370%
Extraction effects	1.000	0.0050	0.500%

URIC ACID STY-0049-002 (RED CAP)

Cx = 38.66 mg/k

u(x) = 0.52 mg/kg

u(x)/x = 1.35%

Veff (total) = 31.234

k = 2.04 (@ 95% confidence level)

U(x) = 1.07

% U(x) = 2.74 %

Measurement equation factors	Values	Uncertainties	
	x	u(x)	u(x)/(x)
Method Precision	1.0000	0.00964	0.964%
$\mathbf{m_{zc}}$	0.03940	0.000046	0.1159%
$\mathbf{m_{y}}$	0.04147	0.000046	0.1101%
$\mathbf{m_{yc}}$	0.04147	0.000046	0.1101%
$\mathbf{m_{x}}$	0.09926	0.000046	0.0460%
$\mathbf{w_{z}}$	99.8547	0.528244	0.5290%
R'b	0.8843	0.003925	0.4439%
R'bc	1.0009	0.003384	0.3381%
Extraction effects	1.000	0.0050	0.500%

UME

Uncertainty budget of Urea				
		Value	u(x)	u(x)/x
Weighing of sample (mg)		100	0.0001	1.45E-06
Weighing of IS (mg)		100	0.0002	1.71E-06
Native stock solution (mg/kg)		10000	0.0050	5.01E-07
Labelled stock solution (mg/kg)		2500	0.0367	1.47E-05
Intermediate precision		100	0.6853	6.85E-03
Recovery		253	0.0003	1.11E-06
Repeatability		1	0.0008	7.75E-04
Calibration graph		20	0.1416	7.08E-03
				9.88E-03
Result (mg/kg)	356.720			
Combined uncertainty		3.526		
Expanded uncertainty		7.052		
% Relative uncertainty		1.977		
% Relative standard uncertainty		0.988		

Uncertainty budget of Uric acid				
		Value	u(x)	u(x)/x
Weighing of sample (mg)		100	1.71E-04	1.71E-06
Weighing of IS (mg)		100	1.45E-04	1.45E-06
Native stock solution (mg/kg)		2500	4.01E-03	1.60E-06
Labelled stock solution (mg/kg)		200	2.94E-02	1.47E-04
Intermediate precision		100	3.78E-01	3.78E-03
Recovery		46	1.50E-03	3.26E-05
Repeatability		1	8.16E-03	8.16E-03
Calibration graph		1	0.0142	1.42E-02
				1.68E-02
Result (mg/kg)	39.940			
Combined uncertainty		0.566		
Expanded uncertainty		1.132		
% Relative uncertainty		2.835		
% Relative standard uncertainty		1.418		

LGC

Calculation of Sample Blend Uncertainty

The standard and combined uncertainties were calculated in accordance with Eurachem guidelines. The uncertainty calculated for a sample blend was determined by combining the relative standard uncertainties as described in equation 1.

Equation 1:

$$u(w'_x) = w'_x \sqrt{\left(\frac{u(w_z)}{w_z}\right)^2 + \left(\frac{u(m_x)}{m_x}\right)^2 + \left(\frac{u(m_z)}{m_z}\right)^2 + \left(\frac{u(m_y)}{m_y}\right)^2 + \left(\frac{u(m_{yc})}{m_{yc}}\right)^2 + \left(\frac{u(R'_B / R'_{BC})}{R'_B / R'_{BC}}\right)^2}$$

Where:

u = Standard uncertainty

Calculation of Total Uncertainty for the Two Bottles

The combined uncertainty was calculated by combining the average blend standard uncertainty (of the 6 aliquots) with the blend to blend variation as described in equation 2.

Equation 2 – Combined Uncertainty Equation

$$u(w_X) = \sqrt{u(\overline{w'_X})^2 + b_{\text{var}}^2}$$

Where:

bvar = Blend to blend variation = standard deviation of the 6 aliquots mass fraction

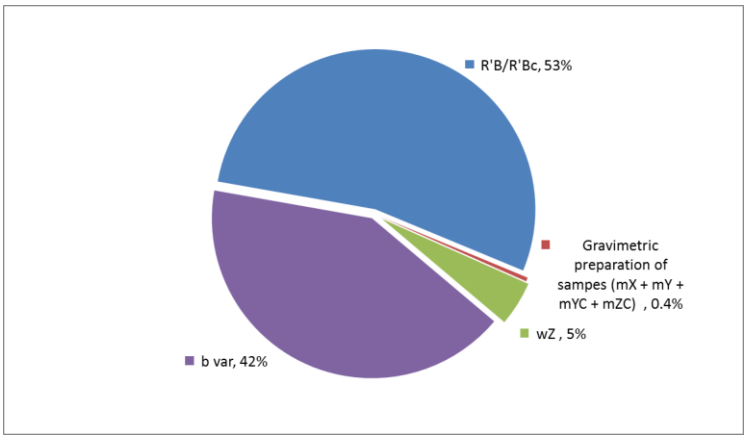

Figure 1 – Average Sample Blend Uncertainty Budget for 342.8 mg/kg Sample:

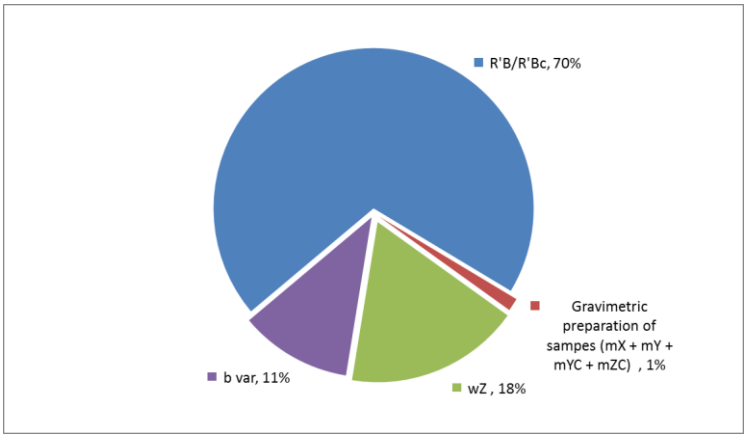

Figure 2 – Average Sample Blend Uncertainty Budget for 1553 mg/kg Sample:

The uncertainty budgets illustrated are average ones. The proportion of the measured ratios will vary with the precision of the measurements.

The major contributions to the uncertainty budget are:

R'_B/R'_{Bc} = the precision of the measured isotope amount ratio

b_{var} = the standard deviation mean of the mass factions (blend to blend precision)

w_z = the mass fraction of the analyte in the primary standard (the purity uncertainty being the major component and the gravimetric preparation only a minor component).

Minor contributions to the uncertainty budget are:

m_x, m_z, m_y and m_{yc} = the uncertainty associated with gravimetric preparation of the blends

NIST

Urea

Results with Type A uncertainty

	Set 1	Set 2
	mg/kg	mg/kg
Level 1		
Value Y_{1j}	1499.7	1511.8
Type A uncertainty	11.85	8.04
	mg/kg	mg/kg
Level 2		
Value Y_{2j}	329.23	334.4
Type A uncertainty	3.71	2.60

Complete Uncertainty Budget

Component	Type	Level 1	Level 2
		uncertainty in %	uncertainty in %
Repeatability and calibration	A	0.48	0.68
Reference compound	B	0.05	0.05
Isotope effects	B	0.1	0.1
Weighing	B	0.1	0.1
Undetected bias	B	1	1
Total relative standard uncertainty if errors are additive	B	1.22	1.32

Summary of Results:

For Level 1: value of urea is 1505.8, standard uncertainty = 18.4, expanded uncertainty (U)= 36.8 mg/kg, coverage factor (k=2) for 95% confidence interval.
For Level 2: value of urea is 331.8, standard uncertainty = 4.4, expanded uncertainty (U) = 8.8 mg/kg, coverage factor (k=2) for 95% confidence interval.

Uric acid

Results with Type A uncertainty

	mg/kg
Level 1	
Value Y_1	135.83
Type A uncertainty	1.01
	mg/kg
Level 2	
Value Y_2	39.08
Type A uncertainty	0.30

Complete Uncertainty Budget

Component	Type	Level 1	Level 2
		uncertainty in %	uncertainty in %
Repeatability and calibration	A	0.74	0.76
Reference compound	B	0.05	0.05
Isotope effects	B	0.1	0.1
Weighing	B	0.1	0.1
Undetected bias	B	1	1
Total relative standard uncertainty if errors are additive	B	1.25	1.26

Summary of Results:

For Level 1: value of uric acid is 135.8 mg/kg, standard uncertainty = 1.7 mg/kg, expanded uncertainty (U) = 3.39 mg/kg , coverage factor (k=2) for 95% confidence interval.
For Level 2: value of uric acid is 39.1 mg/kg, standard uncertainty = 0.5 mg/kg, expanded uncertainty (U) = 1.0 mg/kg, coverage factor (k=2) for 95% confidence interval.

20. APPENDIX C: WEIGHTED MEDIAN ANALYSIS

Blaza Toman

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The Laplace random effects model [1] is similar to the Gaussian random effects model [2], the only difference being that the laboratory effects are assumed to come from a double exponential (Laplacian) distribution rather than the normal (Gaussian). The result is that the KCRV is a type of weighted median. The Laplace probability distribution has “fatter” tails and so is more robust to outliers. The following results were obtained using Markov Chain Monte Carlo (MCMC) methods programmed in OpenBUGS [3].

Of the results that could be considered possible outliers only UME did not withdraw their results from the KCRV calculations, so the data was tested with and without their results to assess the impact of this.

Uric acid (serum I)

With UME included in the weighted median calculation, the KCRV is 136.50 mg/kg with standard uncertainty of 0.98 mg/kg, giving a relative standard uncertainty of 0.72 %. Excluding UME, the KCRV is 136.00 mg/kg with standard uncertainty of 0.59 mg/kg, giving a relative standard uncertainty of 0.43 %

Table C-1. Uric acid (serum I) DOE and expanded uncertainty

UME Included				UME Excluded			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$	NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-0.12	4.45	-0.03	NMIA	0.40	4.16	0.10
INMETRO*	15.48	20.26	0.76	INMETRO*	16.14	20.16	0.80
NIM	-0.03	3.56	-0.01	NIM	0.47	3.23	0.15
LNE	-0.10	3.44	-0.03	LNE	0.41	3.07	0.13
PTB	-2.02	2.41	-0.84	PTB	-1.53	1.84	-0.83
GLHK	-0.34	5.39	-0.06	GLHK	0.21	5.19	0.04
NMIJ	1.78	2.66	0.67	NMIJ	2.29	2.13	1.07
KRISS*	9.89	3.61	2.74	KRISS*	10.35	3.25	3.19
CENAM*	13.90	12.01	1.16	CENAM*	14.27	11.97	1.19
VNIIM*	-12.74	6.98	-1.82	VNIIM*	-12.19	6.76	-1.80
HSA	0.28	3.42	0.08	HSA	0.76	3.06	0.25
NIMT	-1.93	3.61	-0.53	NIMT	-1.43	3.21	-0.44
UME	12.04	4.61	2.61	UME*	12.49	4.35	2.87
NIST	-0.72	3.89	-0.19	NIST	-0.21	3.63	-0.06

**Excluded from KCRV calculation*

Uric acid (serum II)

With UME included in the weighted median calculation, the KCRV is 39.39 mg/kg with standard uncertainty of 0.11 mg/kg, giving a relative standard uncertainty of 0.28 %. Excluding UME, the KCRV is 39.32 mg/kg with standard uncertainty of 0.11 mg/kg, giving a relative standard uncertainty of 0.28 %

Table C-2. Uric acid (serum II) DOE and expanded uncertainty

UME Included			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-0.18	1.21	-0.15
INMETRO*	3.67	6.06	0.61
NIM	0.28	1.31	0.21
LNE	0.20	1.25	0.16
PTB	-0.02	0.46	-0.04
GLHK	-0.59	1.62	-0.36
NMIJ	-0.02	0.40	-0.05
KRISS*	2.92	1.03	2.84
CENAM	5.88	1.37	4.30
VNIIM	-0.39	2.01	-0.19
HSA	-0.10	0.85	-0.12
NIMT	-0.73	1.06	-0.69
UME	0.55	1.16	0.48
NIST	-0.28	1.02	-0.28

UME Excluded			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-0.12	1.22	-0.10
INMETRO*	3.68	6.04	0.61
NIM	0.35	1.32	0.26
LNE	0.29	1.24	0.23
PTB	0.05	0.46	0.11
GLHK	-0.53	1.61	-0.33
NMIJ	0.05	0.41	0.12
KRISS*	2.98	1.02	2.92
CENAM	5.93	1.37	4.32
VNIIM	-0.31	2.01	-0.16
HSA	-0.03	0.84	-0.04
NIMT	-0.65	1.05	-0.62
UME*	0.62	1.16	0.53
NIST	-0.22	1.01	-0.22

**Excluded from KCRV calculation*

Urea (serum I)

With UME included in the weighted median calculation, the KCRV is 1486.0 mg/kg with standard uncertainty of 9.03 mg/kg, giving a relative standard uncertainty of 0.61 %. Excluding UME, the KCRV is 1483.0 mg/kg with standard uncertainty of 6.52 mg/kg, giving a relative standard uncertainty of 0.44 %.

Table C-3. Urea (serum I) DOE and expanded uncertainty

UME Included			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-16.85	26.76	-0.63
INMETRO*	-88.71	260.40	-0.34
NIM	-4.81	36.56	-0.13
GLHK	27.79	47.40	0.59
LNE	-13.07	33.08	-0.40
PTB	0.91	23.58	0.04
NMIJ	-10.97	20.74	-0.53
KRISS*	118.20	31.88	3.71
CENAM	-0.04	26.86	0.00
VNIIM	18.28	78.88	0.23
HSA	-9.02	30.28	-0.30
NIMT	-29.75	37.20	-0.80
UME	128.40	36.74	3.49
LGC	66.97	26.98	2.48
NIST	20.13	41.10	0.49

UME Excluded			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-13.76	24.20	-0.57
*INMETRO**	-83.14	263.80	-0.32
NIM	-2.07	34.12	-0.06
GLHK	31.27	45.70	0.68
LNE	-10.04	30.86	-0.33
PTB	3.83	20.14	0.19
NMIJ	-8.00	16.54	-0.48
*KRISS**	121.40	29.20	4.16
CENAM	2.93	23.48	0.12
VNIIM	21.14	76.28	0.28
HSA	-5.84	28.22	-0.21
NIMT	-26.89	34.66	-0.78
*UME**	131.50	34.02	3.87
LGC	70.21	23.92	2.94
NIST	22.64	38.98	0.58

**Excluded from KCRV calculation*

Urea (serum II)

With UME included in the weighted median calculation, the KCRV is 334.7 mg/kg with standard uncertainty of 1.81 mg/kg, giving a relative standard uncertainty of 0.54 %. Excluding UME, the KCRV is 334.1 mg/kg with standard uncertainty of 1.29 mg/kg, giving a relative standard uncertainty of 0.39 %.

Table C-4. Urea (serum II) DOE and expanded uncertainty

UME Included			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-6.26	6.33	-0.99
INMETRO*	-33.06	55.62	-0.59
NIM	0.15	8.70	0.02
GLHK	-4.14	10.24	-0.40
LNE	2.24	16.34	0.14
PTB	-0.45	4.95	-0.09
NMIJ	-1.00	5.25	-0.19
KRISS*	2.21	6.13	0.36
CENAM	3.80	8.55	0.44
VNIIM	1.35	10.87	0.12
HSA	-1.30	6.52	-0.20
NIMT	-5.61	12.89	-0.44
UME	22.09	7.91	2.79
LGC	8.09	6.18	1.31
NIST	-2.82	9.58	-0.29

UME Excluded			
NMI	D_i	$U(D_i)$	$D_i/U(D_i)$
NMIA	-5.67	5.85	-0.97
INMETRO*	-31.94	56.38	-0.57
NIM	0.65	8.26	0.08
GLHK	-3.47	9.92	-0.35
LNE	2.80	16.24	0.17
PTB	0.10	4.31	0.02
NMIJ	-0.45	4.63	-0.10
KRISS*	2.81	5.64	0.50
CENAM	4.34	8.09	0.54
VNIIM	1.90	10.40	0.18
HSA	-0.69	6.12	-0.11
NIMT	-5.11	12.57	-0.41
UME*	22.68	7.45	3.05
LGC	8.72	5.62	1.55
NIST	-2.37	9.20	-0.26

**Excluded from KCRV calculation*

References

[1] Rukhin A, Possolo A, (2011) *Computational Statistics and Data Analysis* 55, 1815 – 1825.

[2] Toman B, Possolo A (2009) *Accred Qual Assur* 14:553-563.

[3] Lunn DJ, Spiegelhalter D, Thomas A, Best N (2009) *Statistics in Medicine* 28:3049–3082.

Example OpenBUGS code

Double exponential (Laplace) random effects model with mu as the measurand.
Data is from CCQM-K109 uric acid (Serum I).

The posterior mean of mu is 136.5 with posterior standard deviation of 0.96. Compare this with median = 136.4 with standard uncertainty of 0.29.

The sigbeta parameter estimates the “dark uncertainty” in standard deviation units. When the posterior distribution of this parameter is appreciably asymmetric, the median of the distribution may provide a more characteristic estimate than the mean. Here, the (1.508 ± 0.300) mg/kg estimate provided by the mean and standard deviation appears to adequately represent the distribution.

The DoEi are degrees of equivalence for labs that are included in KCRV. The DoEo are the degrees of equivalence for the labs excluded from KCRV.

 # Model

```
ModelBegin{
mu~dnorm(0,1.0E-6); beta~dgamma(1.0E-5,1.0E-5); sigbeta<-sqrt(1/beta)
#
# Participants included in the KCRV
for(k in 1:Ni){delta[k]~ddexp(mu,beta); prec[k]<-1/pow(ui[k],2);
  xi[k]~dnorm(delta[k],prec[k]); pred[k]~dnorm(mu, prec[k]);
  DoEi[k]<-xi[k]-pred[k]}
#
# Participants excluded from the KCRV
for(k in 1:No){preco[k]<-1/pow(uo[k],2); predo[k]~dnorm(mu, preco[k]); DoEo[k]<-xo[k]-predo[k]}
}ModelEnd
```

 # Inits

```
list(beta=1)
```

 # Data

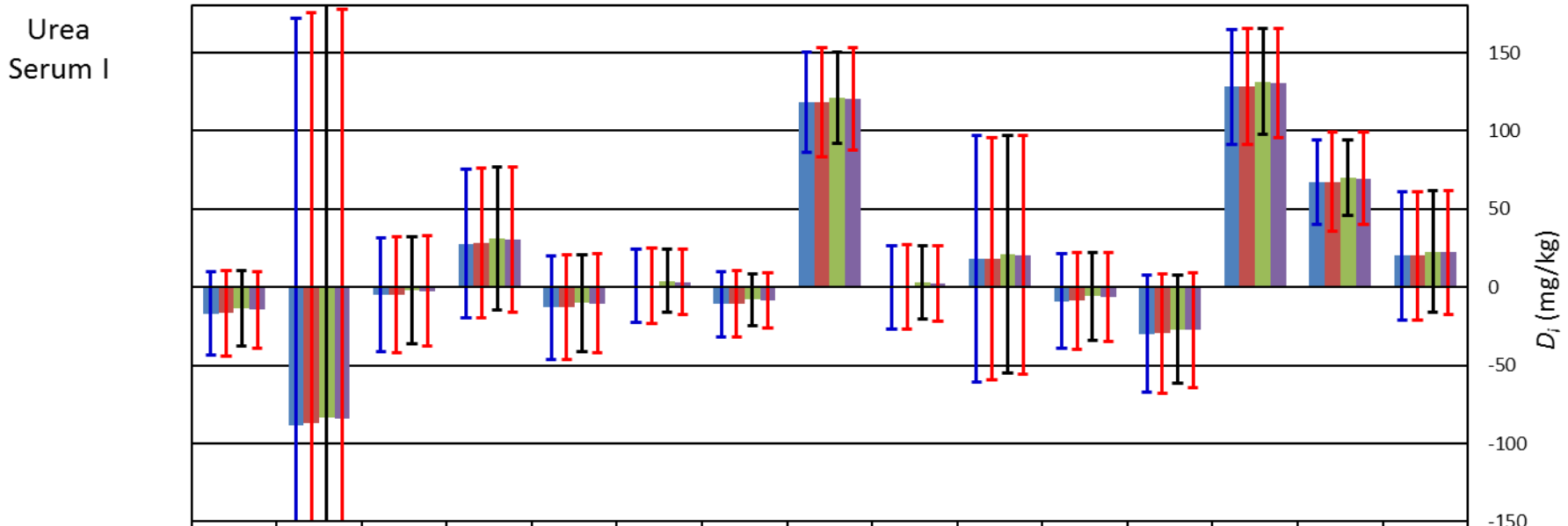
```
list(Ni=10, No=4)

xi[]    ui[]
136.4   2       #NMIA
136.5   1.5     #NIM
136.4   1.4     #LNE
134.52  0.72    #PTB
136.2   2.5     #GLHK
138.3   0.9     #NMIJ
136.8   1.41    #HAS
134.6   1.5     #NIMT
148.53  2.105   #UME
135.8   1.7     #NIST
END

xo[]    uo[]
152     10      #INMETRO
146.4   1.5     #KRISS
150.42  5.95    #CENAM
123.8   3.34    #VNIIM
END
```

Example OpenBUGS output

	mean	sd	MC_error	val2.5pc	median	val97.5pc	start	sample
DoEi[1]	-0.1192	2.206	0.009516	-4.473	-0.1098	4.196	20001	50000
DoEi[2]	-0.03022	1.771	0.008393	-3.509	-0.02797	3.423	20001	50000
DoEi[3]	-0.1285	1.686	0.007474	-3.441	-0.121	3.191	20001	50000
DoEi[4]	-2.011	1.195	0.005769	-4.377	-2.002	0.3056	20001	50000
DoEi[5]	-0.3335	2.665	0.01146	-5.525	-0.3252	4.913	20001	50000
DoEi[6]	1.772	1.307	0.006081	-0.8245	1.786	4.327	20001	50000
DoEi[7]	0.2803	1.692	0.008057	-3.098	0.2918	3.595	20001	50000
DoEi[8]	-1.93	1.783	0.008895	-5.467	-1.929	1.569	20001	50000
DoEi[9]	11.99	2.301	0.01057	7.521	11.99	16.5	20001	50000
DoEi[10]	-0.7257	1.947	0.009103	-4.562	-0.741	3.078	20001	50000
DoEo[1]	15.5	10.08	0.04702	-4.375	15.52	35.1	20001	50000
DoEo[2]	9.869	1.773	0.008449	6.387	9.867	13.34	20001	50000
DoEo[3]	13.84	6.019	0.02854	2.005	13.83	25.67	20001	50000
DoEo[4]	-12.73	3.463	0.01393	-19.52	-12.72	-5.929	20001	50000
mu	136.5	0.9499	0.004784	134.7	136.5	138.4	20001	50000
sigbeta	1.508	0.3001	0.001401	1.02	1.473	2.202	20001	50000

Column 1 The model parameters of interest, as named in the model code.
"mean" and "sd" The mean and standard deviation of the posterior distribution of the parameter.
"MC error" The estimated uncertainty in the posterior mean due to finite number of recorded draws.
"val2.5pc" The 2.5th percentile of the posterior distribution.
"median" The 50th percentile of the posterior distribution.
"val2.5pc" The 97.5th percentile of the posterior distribution.
"start" The number of MCMC iterations used in the burn-in phase of the analysis. These results used a burn-in of 20000 iterations with 10-fold thinning.
"sample" The number of MCMC iterations used to estimate the parameters. These results are based on 50000 iterations with 20-fold thinning. The entire analysis, including burn-in, required less than 20 seconds on a 2013 laptop PC.

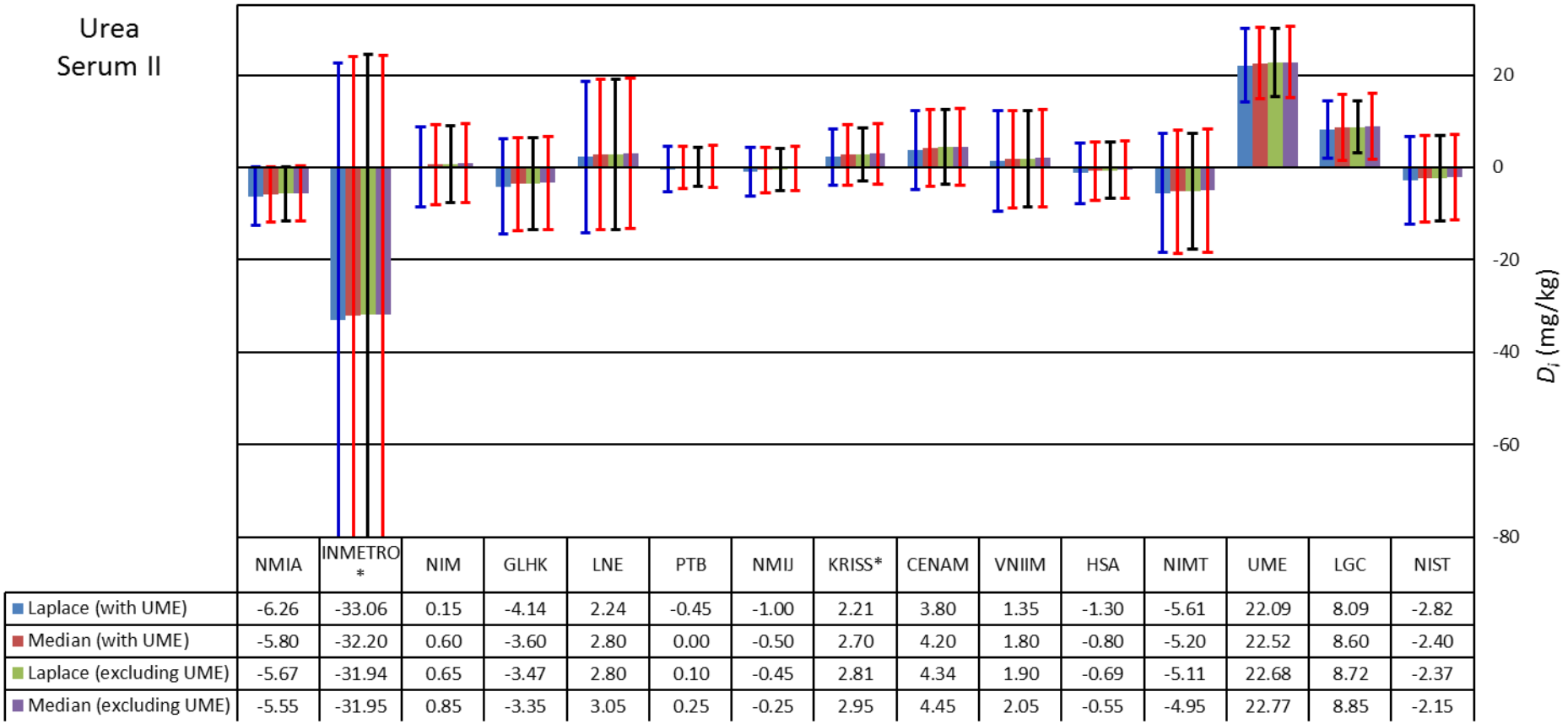
21. APPENDIX D: COMPARISON OF DEGREE OF EQUIVALENCE ESTIMATES



	NMIA	INMETRO*	NIM	GLHK	LNE	PTB	NMIJ	KRISS*	CENAM	VNIIM	HSA	NIMT	UME	LGC	NIST
Laplace (with UME)	-16.85	-88.71	-4.81	27.79	-13.07	0.91	-10.97	118.20	-0.04	18.28	-9.02	-29.75	128.40	66.97	20.13
Median (with UME)	-16.70	-86.70	-4.70	28.30	-12.70	1.00	-10.70	118.50	0.00	18.30	-8.70	-29.70	128.38	67.30	20.10
Laplace (excluding UME)	-13.76	-83.14	-2.07	31.27	-10.04	3.83	-8.00	121.40	2.93	21.14	-5.84	-26.89	131.50	70.21	22.64
Median (excluding UME)	-14.35	-84.35	-2.35	30.65	-10.35	3.35	-8.35	120.85	2.35	20.65	-6.35	-27.35	130.73	69.65	22.45

Figure D-1: Degrees of equivalence, $D_i \pm U(D_i)$, for urea (Serum I)

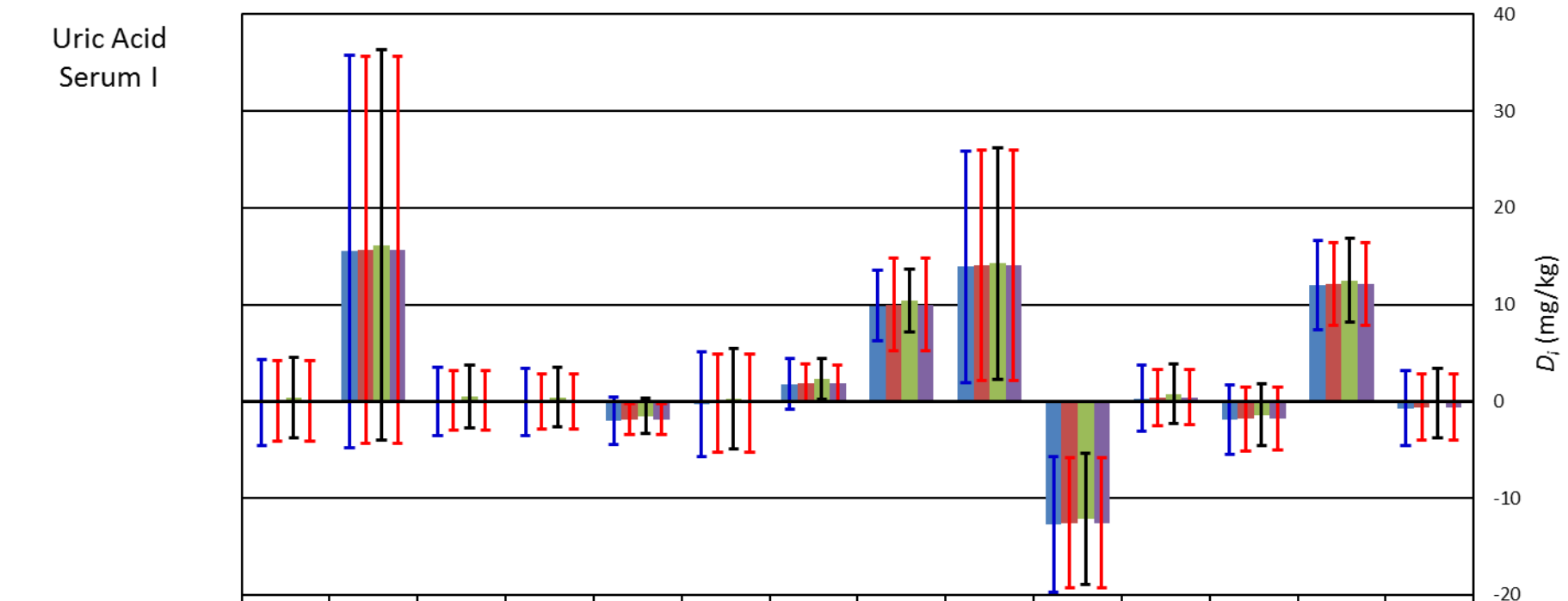
This displays D_i, ± (D_i,i) for the median and weighted median estimators, with and without the UME results. The weighted median is called “Laplace” in the legend.



	NMIA	INMETRO*	NIM	GLHK	LNE	PTB	NMIJ	KRISS*	CENAM	VNIIM	HSA	NIMT	UME	LGC	NIST
Laplace (with UME)	-6.26	-33.06	0.15	-4.14	2.24	-0.45	-1.00	2.21	3.80	1.35	-1.30	-5.61	22.09	8.09	-2.82
Median (with UME)	-5.80	-32.20	0.60	-3.60	2.80	0.00	-0.50	2.70	4.20	1.80	-0.80	-5.20	22.52	8.60	-2.40
Laplace (excluding UME)	-5.67	-31.94	0.65	-3.47	2.80	0.10	-0.45	2.81	4.34	1.90	-0.69	-5.11	22.68	8.72	-2.37
Median (excluding UME)	-5.55	-31.95	0.85	-3.35	3.05	0.25	-0.25	2.95	4.45	2.05	-0.55	-4.95	22.77	8.85	-2.15

Figure D-2: Degrees of equivalence, $D_i \pm U(D_i)$, for urea (Serum II)

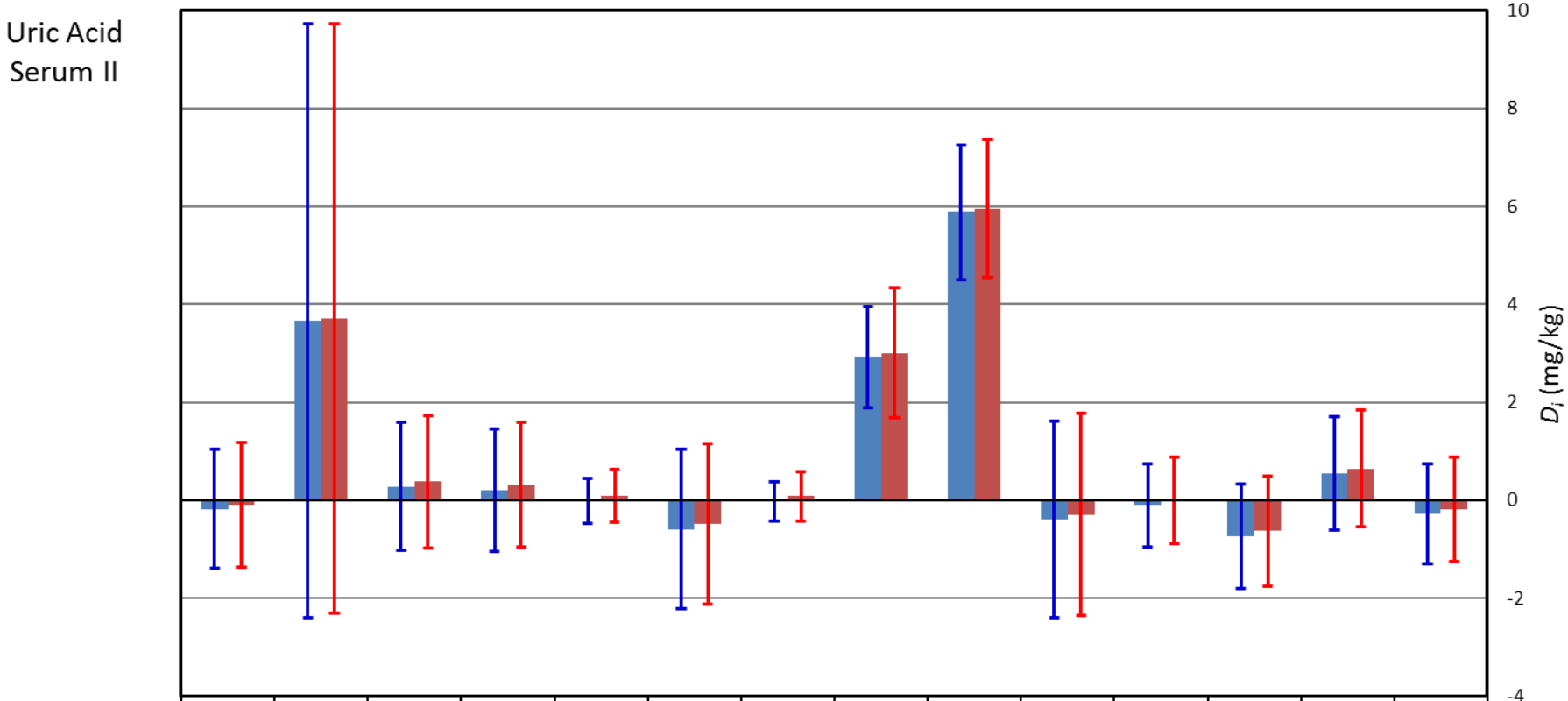
This displays D_i, ± (D_i,i) for the median and weighted median estimators, with and without the UME results. The weighted median is called “Laplace” in the legend.



	NMIA	INMETRO*	NIM	LNE	PTB	GLHK	NMIJ	KRISS*	CENAM*	VNIIM*	HSA	NIMT	UME	NIST
Laplace (with UME)	-0.12	15.48	-0.03	-0.1	-2.02	-0.34	1.78	9.89	13.9	-12.74	0.28	-1.93	12.04	-0.72
Median (with UME)	0.00	15.60	0.10	0.00	-1.88	-0.20	1.90	10.00	14.02	-12.60	0.40	-1.80	12.13	-0.60
Laplace (excluding UME)	0.4	16.14	0.47	0.41	-1.53	0.21	2.29	10.35	14.27	-12.19	0.76	-1.43	12.49	-0.21
Median (excluding UME)	0.00	15.60	0.10	0.00	-1.88	-0.20	1.90	10.00	14.02	-12.60	0.40	-1.80	12.13	-0.60

Figure D-3: Degrees of equivalence, $D_i \pm U(D_i)$, for uric acid (Serum I)

This displays D_i, ± (D_i,i) for the median and weighted median estimators, with and without the UME results. The weighted median is called “Laplace” in the legend.



	NMIA	INMETRO*	NIM	LNE	PTB	GLHK	NMIJ	KRISS*	CENAM*	VNIIM*	HSA	NIMT	UME	NIST
Laplace random effect	-0.18	3.67	0.28	0.2	-0.02	-0.59	-0.02	2.92	5.88	-0.39	-0.1	-0.73	0.55	-0.28
Median	-0.09	3.71	0.38	0.31	0.08	-0.49	0.08	3.01	5.96	-0.29	0.00	-0.63	0.65	-0.19

Figure D-4: Degrees of equivalence, $D_i \pm U(D_i)$, for uric acid (Serum II)

This displays D_i, ± (D_i,i) for the median and weighted median estimators. The weighted median is called “Laplace” in the legend.

22. APPENDIX E: CORE COMPETENCY TABLES

NMIA (Urea)

CCQM-K109	***NMIA***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Urea: Certified pure material, NMIJ 6006-a
Identity verification of analyte(s) in calibration material.[#]	N/A	N/A
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	N/A
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	N/A
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	- Chromatographic retention time - UPLC-MS/MS: a minimum of 2 MRM transitions monitored - GC-MS/MS: a minimum of 2 MRM transitions monitored - GC-HRMS: a minimum of 2 ions monitored at a resolution of 1500 (5% valley)
Extraction of analyte(s) of interest from matrix	✓	- protein precipitation with ethanol
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	- (1) HILIC HPLC clean-up of the 2-hydroxypyrimidine derivative of urea (2) reversed-phase HPLC clean-up of the camphanate derivative of urea

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	- (1) derivatisation of urea with malonaldehyde bis(dimethylacetal) to its 2-hydroxypyrimidine derivative for analysis by HILIC UPLC-MS/MS (2) derivatisation of urea with malonaldehyde bis(dimethylacetal to its 2-hydroxypyrimidine derivative followed by further derivatisation with MSTFA for analysis by GC-HRMS (3) derivatisation of urea using camphanic chloride for analysis by reversed phase UPLC-MS/MS
Analytical system	✓	- (1) GC-HRMS (EI) (2) reversed-phase UPLC-MS/MS (ESI+) (3) HILIC UPLC-MS/MS (ESI+)
Calibration approach for value-assignment of analyte(s) in matrix	✓	- double IDMS with single-point exact-matching calibration with bracketing
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Comparison of results using independent analysis techniques (LC vs GC, alternative stationary phases differing in selectivity and polarity, reversed-phase vs HILIC, MS/MS vs HRMS): - (1) GC-HRMS (EI) (2) reversed-phase UPLC-MS/MS (ESI+) (3) HILIC UPLC-MS/MS (ESI+)
Other	✓	(1) Stable isotope internal standard selection: Comparison of different internal standards ($^{15}N_2$-urea, ^{13}C,$^{15}N_2$-urea, ^{13}C,$^{15}N_2$,^{18}O-urea) (2) Isotopic equilibration for IDMS: Sample blends equilibrated for ~12 hours overnight (3) Control of uncertainty due to preparation and dilution of standard solutions for highly polar analytes: standards comparison of calibration solutions prepared in different solvents (methanol, ethanol) (4) Monitoring potential bias by analysis of matrix CRMs (HSA HRM-01, HRM-02, HRM-03, NIST SRM909c, SRM1950)

NMIA (Uric acid)

CCQM-K109	***NMIA***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Uric acid: Certified pure material, NIST 913B
Identity verification of analyte(s) in calibration material.[#]	N/A	N/A
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	N/A
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	N/A
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	- Chromatographic retention time - UPLC-MS/MS: a minimum of 2 MRM transitions monitored - GC-MS/MS: a minimum of 2 MRM transitions monitored - GC-HRMS: a minimum of 2 ions monitored at a resolution of 1500 (5% valley)
Extraction of analyte(s) of interest from matrix	✓	- protein precipitation with acetonitrile
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	- reversed-phase HPLC fractionation clean-up of uric acid
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	- derivatisation with BSTFA/TMCS for analysis by GC-MS/MS and GC-HRMS
Analytical system	✓	- (1) GC-MS/MS (EI) (2) GC-HRMS (EI) (3) HILIC UPLC-MS/MS (ESI+, ESI-)
Calibration approach for value-assignment of analyte(s) in matrix	✓	- double IDMS with single-point exact-matching calibration with bracketing

Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Comparison of results using independent analysis techniques (LC vs GC, alternative stationary phases differing in selectivity and polarity, reversed-phase vs HILIC, MS/MS vs HRMS): - (1) GC-MS/MS (EI) (2) GC-HRMS (EI) (3) HILIC UPLC-MS/MS (ESI+, ESI-)
Other	✓	(1) Isotopic equilibration for IDMS: Sample blends equilibrated for ~12 hours overnight (2) Control of uncertainty due to preparation, dilution and stability of standard solutions for highly polar analytes: standards comparison of multiple calibration solutions; stock solutions prepared using different sources of pure materials: NIST 913B and NMIJ 6008-a at multiple time points over a 2 month period; working calibration and internal standard solutions freshly prepared from stock solutions just prior to blend preparation (3) Monitoring potential bias by analysis of matrix CRMs (HSA HRM-01, HRM-02, HRM-03, NIST SRM909c, SRM1950)

INMETRO (Urea & Uric acid)

CCQM-K109	***INMETRO***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly-pure substances: NIST SRM 912a for urea and SRM 913b for uric acid
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Comparison of retention time with CRM, mass spectrum
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation with acetonitrile and separation by centrifugation
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization of urea to produce 2-hydroxypyrimidine
Analytical system	✓	LC-MS/MS (uric acid) and HPLC-DAD(urea)
Calibration approach for value-assignment of analyte(s) in matrix	✓	Standard addition with 5-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

NIM (Urea & Uric acid)

CCQM-K109	***NIM***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure substances of urea and uric acid from NIM were used as GBW09201 and GBW09202
Identity verification of analyte(s) in calibration material.[#]	✓	Urea : HNMR, IR Uric acid: IR
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	Urea: mass balance approach (LC-UV, Karl Fischer titration, DSC, HS-GC) Uric acid: mass balance approach (LC-UV, Karl Fischer titration, mass loss upon drying)
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	LC retention time and SIM mode
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation using acetonitrile (4 mL acetonitrile per gram of serum)
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	Single quadrupole LC-MS: Urea ESI positive, Uric acid ESI negative
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS, Single point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Serum CRMs from NIM (GBW09157, GBW09169) and NIST (SRM 909c) were used as the quality control materials
Other	N/A	

GLHK (Urea)

CCQM-K109	***GLHK***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure Material Urea: NIST 912a
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and mass spec ion ratio
Extraction of analyte(s) of interest from matrix	✓	Liquid/liquid extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein participation, filtration.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a)Quantification mode: IDMS b)Calibration mode
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Urea: Verified by LC-MS/MS measurement.
Other	N/A	

GLHK (Uric Acid)

CCQM-K109	***GLHK***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure Material Uric acid: NIST 913b
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and mass spec ion ratio
Extraction of analyte(s) of interest from matrix	✓	Liquid/liquid extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein participation, filtration.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	Uric acid: LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a)Quantification mode: IDMS b)Calibration mode used: bracketing
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

LNE (Urea & Uric acid)

CCQM-K109	***LNE***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance Urea : SRM NIST 912a Uric acid : SRM NIST 913b
Identity verification of analyte(s) in calibration material.[#]	✓	Mass spectrum, abundance of characteristics ions, comparison with the bibliography
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	Urea : SRM NIST 912a Uric acid : SRM NIST 913b
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, specific ions
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Precipitation of proteins
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Uric acid : N/A Urea : derivatization
Analytical system	✓	Uric acid : LC-MS/MS Urea : GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) 5-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

PTB (Urea)

CCQM-K109	***PTB***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure material from NIST: SRM 912a
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS with single-point calibration
Verification method(s) for value- assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

PTB (Uric acid)

CCQM-K109	***PTB***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Pure material from NIST: SRM 913a
Identity verification of analyte(s) in calibration	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Ion exchange chromatography
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS with single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

NMIJ (Urea)

CCQM-K109	***NMIJ***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NMIJ CRM 6006-a
Identity verification of analyte(s) in calibration material.[#]	✓	IR
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	Acidimetric titration Kjeldahl method
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization (MDBMA/BSTFA)
Analytical system	✓	GC-MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) bracketing calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	NIST SRM 909c
Other	N/A	

NMIJ (Uric acid)

CCQM-K109	***NMIJ***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity $pK_{OW} > 2$ in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NMIJ CRM 6008-a
Identity verification of analyte(s) in calibration material.[#]	✓	MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	Acidimetric titration Kjeldahl method
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) bracketing calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	NIST SRM 909c
Other	N/A	

KRISS (Urea & Uric acid)

CCQM-K109	***KRISS***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	SRM 912a and SRM 913b obtained from NIST were used for the calibration solutions of urea and uric acid, respectively. Each calibration solution was gravimetrially prepared for next ID LC-MRM analysis. In particular, the calibration solution of uric acid was stored at – 20 degree before use.
Identity verification of analyte(s) in calibration material.[#]	✓	Both of urea and uric acid were verified using a LC-ESI-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	X	For uric acid, results for both Serum I & II were high due to the degradation of the standard solutions, which were stored at room temperature.
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The identification of each analyte in the sample was carried out by examining both of retention time in UPLC separation and MS/MS spectra, compared to that of calibration solutions (NIST SRM 912a and 913b)
Extraction of analyte(s) of interest from matrix	✓	Prior to LC-MRM analysis, depletion of serum proteins was carried out by precipitation with acetonitrile for uric acid,
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation were carried out by the treatment of acetonitrile and followed by filtration with 0.2-□m membrane
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	For MRM analyses of each analyte, LC-ESI-MS/MS was used in positive ion mode
Calibration approach for value-assignment of analyte(s) in matrix	✓	ID-MS with exact matching single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample	✓	The method for value-assignment of urea and uric acid was verified using KRISS

(if used)		CRM 111-1-001 and -002
Other	✓	The quality control of KRISS CRMs used for this K109 was previously verified using a NIST SRM 909b
	X	For urea (both Serum I & II), the comparison materials were not treated in accordance with the Study Protocol, i.e. they had been subjected to more than one round of freeze-thaw cycle.

The results for urea (Serum I) and uric acid (Serum I & II) are not consistent with the KCRVs as the 95% confidence intervals for the DoEs do not cross zero.

CENAM (Urea)

CCQM-K109	***CENAM***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance: Urea, CRM-6006a National Metrology Institute of Japan (NMIJ)
Identity verification of analyte(s) in calibration material.[#]	N/A	
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and mass spec ion ratios, ions 168 for urea and ions 171 for $^{13}C,^{15}N_2$-Urea.
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatization: derivatized to O-trimethylsilyl-2-hydroxypyrimidine
Analytical system	✓	GC-MS.
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS single-point calibration.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	SRM 909c was used as control RM.
Other	N/A	

CENAM (Uric acid)

CCQM-K109	*CENAM*	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance: Uric acid, CRM-6008a National Metrology Institute of Japan (NMIJ)
Identity verification of analyte(s) in calibration material.[#]	✓	Ion Mass: 169, Retention time
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time and mass spec ion ratios, ions 169 for uric acid and ions 171 for 1,3-$^{15}N_2$-Uric acid
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Liquid- Liquid, Protein precipitation.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	X	LC-MS. The LC-IDMS results were high due to unspecified reasons. After two rounds of investigation using GC-IDMS, the results agreed well with the majority of the reported results.
Calibration approach for value-assignment of analyte(s) in matrix	✓	IDMS single-point calibration.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	SRM 909c was used as control of Reference Material.
Other	N/A	

The results for uric acid (Serum I & II) are not consistent with the KCRVs as the 95% confidence intervals for the DoEs do not cross zero.

VNIIM (Urea & Uric acid)

CCQM-K109	***VNIIM***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	pure material: urea - SRM 912a from NIST; uric acid - SRM 913b from NIST
Identity verification of analyte(s) in calibration material.[#]	✓	LC/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, mass spec ion ratios
Extraction of analyte(s) of interest from matrix	✓	Protein precipitation
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) single-point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The result for uric acid (Serum I) is not consistent with the KCRV as the 95% confidence interval for the DoE does not cross zero. No specific competency was identified as the reason.

HSA (Urea)

CCQM-K109	***HSA***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly-pure urea CRM (SRM 912a) from NIST was used as the calibration standard.
Identity verification of analyte(s) in calibration material.[#]	✓	LC-MS/MS was used to verify the $[M+H]^+$ ion and the corresponding daughter ions.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	Urea CRM was used as calibrant.
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The analytes in the samples were identified against pure urea CRM (SRM 912a) by comparing their MRM transitions and retention times on the LC-MS/MS.
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation was used for clean-up. The details are as follows: After spiking the isotope labelled internal standard solution, the sample was vortexed, and allowed to equilibrate at ambient temperature for 2 h. Acetonitrile (3 fold of aqueous volume) was then added for protein precipitation. The sample was vortexed vigorously and centrifuged for 5 min at 4000 rpm. The supernatant was filtered through 0.22 µm syringe filter. For LC-MS/MS analysis, the filtrate was diluted to approximately 2000 ng/g with acetonitrile.

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Two-step derivatisation was used for GC-MS measurement. The details are as follows: After centrifugation and filtration through 0.22 µm syringe filter, the filtrate containing about 3 µg of urea was added 30 µL of freshly prepared 1,1,3,3-tetramethoxypropane solution (0.3 mol/L) and 60 µL of hydrochloric acid (6.9 mol/L). The mixture was vortexed and allowed to react for 1 h at room temperature. The solution was then evaporated to dryness under nitrogen at 45 °C. The residue was reconstituted with 50 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide and heated at 60 °C for 1 h to complete the derivatisation. After cooling down to ambient temperature, the sample was ready for GC-MS analysis.
Analytical system	✓	LC-MS/MS and GC-MS were used. The averages of results obtained from LC-MS/MS and GC-MS were reported.
Calibration approach for value-assignment of analyte(s) in matrix	✓	Four-point calibration curve IDMS method was used.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Human serum CRMs for urea from NIST (SRM 909c) and HSA (HRM-3002A, level 3) were used as quality control materials, which were measured in parallel with the comparison samples. The obtained values agreed well within the uncertainties of the certified values of the CRMs.
Other	N/A	

HSA (Uric acid)

CCQM-K109	***HSA***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity $pK_{OW} > 2$ in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly-pure uric acid CRM (SRM 913a) from NIST was used as the calibration standard.
Identity verification of analyte(s) in calibration material.[#]	✓	LC-MS/MS was used to verify the $[M-H]^-$ ion and the corresponding daughter ions.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	Uric acid CRM was used as calibrant.
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The analytes in the samples were identified against pure uric acid CRM (SRM 913a) by comparing their MRM transitions and retention times on the LC-MS/MS.
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE was used for clean-up. The details are as follows: After spiking the isotope labelled internal standards, the sample was vortexed, and allowed to equilibrate at ambient temperature for 2 h. The sample was then vortexed, and SPE was conducted using Waters Oasis MAX cartridge (30 µm, 1 cc, 30 mg). The eluent was dried under nitrogen at 45 ^{o}C, and was then reconstituted with 2 mmol/L ammonia solution (concentration about 500 ng/g) for LC-MS/MS analysis.

Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	For GC-MS analysis. Derivatisation was conducted. The details are as follows: The eluent from SPE was dried under nitrogen at 45 ^{o}C, and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) solution in acetonitrile (50% v/v) was added. The uric acid concentration was kept at approximately 45 µg/g. The solution was then vortexed and heated at 60 ^{o}C for 16 h. After cooling down, After cooling down to ambient temperature, the sample was ready for GC-MS analysis.
Analytical system	✓	LC-MS/MS and GC-MS were used. The averages of results obtained from LC-MS/MS and GC-MS were reported.
Calibration approach for value-assignment of analyte(s) in matrix	✓	Four-point calibration curve IDMS method was used.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	Human serum CRMs for uric acid from NIST (SRM 909c) and HSA (HRM-3002A, level 3) were used as the quality control materials, which were measured in parallel with the comparison samples. The obtained values agreed well within the uncertainties of the certified values of the CRMs.
Other	N/A	

NIMT (Urea & Uric acid)

CCQM-K109	***NIMT***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM912a Urea and NIST SRM913b Uric Acid
Identity verification of analyte(s) in calibration material.[#]	✓	LC-MS/MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).[#]	✓	Gravimetric
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	The analytes in the samples were identified against SRM912a Urea and SRM913b Uric Acid standards by comparing their retention times and m/z of LC-MS/MS
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation using Acetonitrile for uric acid and Ethanol for urea.
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) IDMS b) Exact matching double IDMS using one point calibration
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

UME (Urea)

CCQM-K109	***UME***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance Urea, Sigma Aldrich, 33247
Identity verification of analyte(s) in calibration material.[#]	✓	HPLC-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	The purity assessment was done by TUBITAK UME with mass balance approach by TGA/DSC and LC-MS. Urea, (99.80 ± 0.05) %
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time Parent/ Product Ion
Extraction of analyte(s) of interest from matrix	✓	Protein precipitation with acetonitrile, sample dilution, vortex, centrifuge, filtration
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-HRMS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a)Quantification mode used: IDMS b) Calibration mode used :5-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The results for urea (Serum I & II) are not consistent with the KCRVs as the 95% confidence intervals for the DoEs do not cross zero. No specific competency was identified as the reason.

UME (Uric acid)

CCQM-K109	***UME***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance Uric Acid, Sigma, U2625
Identity verification of analyte(s) in calibration material.[#]	✓	HPLC-MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	The purity assessment was done by TUBITAK UME with mass balance approach by TGA/DSC and LC-MS. Uric Acid, (99.82 ± 0.04) %
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time Parent/ Product Ion
Extraction of analyte(s) of interest from matrix	✓	Protein precipitation with acetonitrile, sample dilution, vortex, centrifuge, filtration
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	N/A	
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-HRMS
Calibration approach for value-assignment of analyte(s) in matrix	✓	a)Quantification mode used: IDMS b) Calibration mode used :5-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	N/A	
Other	N/A	

The result for uric acid (Serum I) is not consistent with the KCRV as the 95% confidence interval for the DoE does not cross zero. No specific competency was identified as the reason.

LGC (Urea)

CCQM-K109	***LGC***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	NIST SRM 912a
Identity verification of analyte(s) in calibration material.#	✓	High Resolution Accurate Mass (HRAM) spectrometry was used, but not intentionally for the purpose of identity verification.
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).#	N/A	
For calibrants which are a calibration solution: Value-assignment method(s).#	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	Retention time, single SRM transition, HRAM of intact urea.
Extraction of analyte(s) of interest from matrix	✓	Protein precipitation via acetonitrile solution extraction
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	Protein precipitation via acetonitrile solution extraction
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	N/A	
Analytical system	✓	LC-MS/MS
Calibration approach for value-assignment of analyte(s) in matrix	✓	Linear calibration –IDMS used initially followed by exact matching –IDMS. EM-IDMS used for results submitted. Each sample blend was analysed five times with an associated calibration blend injected before and after each injection.
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	HRAM – of intact molecule using same LC system as for SRM analysis
Other	N/A	

The results for urea (Serum I & II) are not consistent with the KCRVs as the 95% confidence intervals for the DoEs do not cross zero. No specific competency was identified as the reason.

NIST (Urea)

CCQM-K109	***NIST***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity $pK_{OW} > 2$ in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance (NIST SRM 912a Urea)
Identity verification of analyte(s) in calibration material.[#]	✓	^{1}H- and ^{13}C-NMR, MS, melting point
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	DSC; mass balance including Karl Fischer Titration, UV Spectroscopy
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GC-MS (SIM mode), calibrant vs. sample
Extraction of analyte(s) of interest from matrix	✓	Precipitation followed by SPE
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatisation (1,1,3,3-tetramethoxypropane and N-methyl-N-trimethylsilyltrifluroacetamide)
Analytical system	✓	GC-MS (SIM mode)
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) ID-MS: Urea-O^{18} b) calibration mode used:6-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	NIST SRM 909c used as a control.
Other	N/A	

NIST (Uric acid)

CCQM-K109	***NIST***	***High Polarity Analytes in Biological Matrix: Determination of Urea and Uric Acid in Human Serum***
Scope of Measurement: This comparison enables participating NMIs/DIs to demonstrate their measurement capabilities in the determination of analytes with molecular mass of 50 to 500 g/mol, having the polarity pK_{OW} > 2 in the range of 10 to 2,000 mg/kg in a biological matrix such as human serum, blood and urine.		
Competency	**Tick, cross, or "N/A"**	**Specific Information as Provided by NMI/DI**
Competencies for Value-Assignment of Calibrant		
Calibrant: Did you use a "highly-pure substance" or calibration solution?	✓	Highly pure substance (NIST SRM 913a Uric Acid)
Identity verification of analyte(s) in calibration material.[#]	✓	MS
For calibrants which are a highly-pure substance: Value-Assignment / Purity Assessment method(s).[#]	✓	Mass balance, including mass loss upon drying, ashing, and DSC.
For calibrants which are a calibration solution: Value-assignment method(s).[#]	N/A	
Sample Analysis Competencies		
Identification of analyte(s) in sample	✓	GC-MS (SIM mode), calibrant vs. sample
Extraction of analyte(s) of interest from matrix	N/A	
Cleanup - separation of analyte(s) of interest from other interfering matrix components (if used)	✓	SPE
Transformation - conversion of analyte(s) of interest to detectable/measurable form (if used)	✓	Derivatisation (N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide)
Analytical system	✓	GC-MS (SIM mode; EI)
Calibration approach for value-assignment of analyte(s) in matrix	✓	a) ID-MS: 1,3-$^{15}N_2$-Uric acid b) 5-point calibration curve
Verification method(s) for value-assignment of analyte(s) in sample (if used)	✓	NIST SRM 909c used as a control
Other	N/A	