CCQM-K142
Comparison of CRMs and Value-Assigned Quality Controls: Urea and Uric Acid in Human Serum or Plasma

Track A Model 2 Key Comparison

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ABSTRACT

The 2017 CCQM-K142 “Comparison of CRMs and Value-Assigned Quality Controls: Urea and Uric Acid in Human Serum or Plasma” is the third Key Comparison directly testing the chemical measurement services provided to customers by National Metrology Institutes (NMIs) and Designated Institutes (DIs) through certified reference materials (CRMs). CRMs certified for urea and/or uric acid content in human serum or plasma were compared using measurements made on these materials under repeatability conditions. Four NMIs/DIs submitted 10 CRMs certified for urea; five NMIs/DIs submitted 12 CRMs certified for uric acid. These materials represent most of the higher-order reference materials then available for these clinically important measurands.

Uncertainty-weighted generalised distance regression was used to establish the Key Comparison Reference Function (KCRF) relating the CRM certified values to the repeatability measurements. The urea results for all 10 CRMs were deemed equivalent at the 95 % level of confidence and were used to define the KCRF for urea. The uric acid result for one of the 12 CRMs was deemed non-equivalent: the submitting NMI reevaluated their result and withdrew that material from use in defining the KCRF for uric acid. The remaining 11 CRMs were used to define the KCRF for uric acid.

Monte Carlo methods were used to estimate 95 % level-of-confidence coverage intervals for the relative degrees of equivalence of materials, $\%d \pm U_{95}(\%d)$, and of the participating NMIs/DIs, $\%D \pm U_{95}(\%D)$. For the urea materials, the $\%D \pm U_{95}(\%D)$ intervals were within (-3 to 5) % of the consensus results. For the uric acid materials from four of the five NMIs/DIs, the $\%D \pm U_{95}(\%D)$ intervals were within (-4 to 5) % of the consensus results. These results demonstrate that with the exception of one material, the participating institutions can value-assign CRMs for urea and/or uric acid in human serum and plasma.

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ACRONYMS

ANOVA	analysis of variance
CCQM	Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology
CENAM	Centro Nacional de Metrologia, NMI: Mexico
CID	Collision-induced dissociation
CIPM MRA	Comité International des Poids et Measures Mutual Recognition Arrangement
CMC	Calibration and Measurement Capability
CRM	certified reference material
CV	coefficient of variation, expressed in %: CV = 100·s/$\bar{x}$
DI	designated institute
DoE	degrees of equivalence
GC-IDMS	gas chromatography isotope dilution mass spectrometry
GDR	generalised distance regression
HSA	Health Sciences Authority, DI: Singapore
HR	high resolutions
ID	isotope dilution
JCTLM	Joint Committee for Traceability in Laboratory Medicine
KC	Key Comparison
KCRF	Key Comparison Reference Function
KCRV	Key Comparison Reference Value
KRISS	Korea Research Institute of Standards and Science, NMI: Republic of Korea
LAI	leave all in
LC	liquid chromatography
LC-IDMS/MS	liquid chromatography isotope dilution tandem mass spectrometry
LOO	leave one out
MRM	multiple reaction monitoring
MS	mass spectrometry
m/z	mass divide by change
NIM	National Institute of Metrology, NMI: China
NIST	National Institute of Standards and Technology, NMI: USA
NMI	national metrology institute
OAWG	Organic Analysis Working Group
PBMC	parametric bootstrap Monte Carlo
PI	participating institution
pKow	logarithm of the octanol-water partition coefficient
PT	proficiency test

SYMBOLS

~	signifies “distributed as”
ˆ	signifies an estimated quantity when above a symbol
α	intercept
β	slope
ε_i	residual difference for a given material
E	residual random error
γ_j	between-unit differences for a given material
δ_{jk}	within-unit differences of the k^{th} aliquot of the j^{th} unit of a given material
μ	mean
$\sigma_{a\%}$	between-aliquot imprecision/within-material heterogeneity
$\sigma_{c\%}$	between-campaign imprecision/between-unit material heterogeneity
$\sigma_{r\%}$	repeatability imprecision
ν	degrees of freedom
d_i	degree of equivalence for the i^{th} material
D	degree of equivalence for one NMI/DI in a KC
$\%d_i$	degree of equivalence for the i^{th} material as a percentage of the value
$\%D$	degree of equivalence for one NMI/DI in a KC of the value
i	index over materials
j	index over campaigns and/or units
k	index over replicates or aliquots
l	index over replicates
N(.,.)	Normal distribution
n_a	number of aliquots of each unit
n_c	number of measurement campaigns
n_m	number of materials
n_r	number of replicates of each aliquot or unit
n_u	number of units of each material
R	generic representation of “instrument response”
R_{jkl}	instrument response for the l^{th} replicate of the k^{th} aliquot of the j^{th} unit of a given material
SIGN	signum or “sign” (±1) of a value
$t_{0.05,\nu}$	two-tailed Student’s t critical value for 95 % confidence with ν degrees of freedom
u	standard uncertainty
u_∞	“large sample” standard uncertainty
U_{95}	half-width of the 95 % level of confidence expanded uncertainty
V	generic representation of “assigned value”
V_i	assigned value for the i^{th} material

1.0 INTRODUCTION

1.1 Historical Background

The CCQM-K142 “Track B” comparison of value-assigned materials was intended to complement the “Track A” comparison of measurement capability, CCQM-K109/P148 Determination of Mass Fraction of Urea and Uric Acid in Human Serum. All national metrology institutes (NMIs) and designated institutes (DIs) that currently deliver measurement services for urea and/or uric acid in human serum or plasma through one or more value-assigned certified reference materials (CRMs), PT materials, or accuracy quality controls were invited to participate in the CCQM-K142 comparison. The Track B comparison was available for institutes with Calibration and Measurement Claims (CMCs) in the CIPM MRA Key Comparison Database for urea and/or uric acid in human serum or plasma materials, where the delivery mechanism is value-assigned materials.

As with the previous OAWG Track B comparisons, CCQM-K79 and CCQM-K80, participation in CCQM-K142 was accomplished by providing the study’s Measurement Laboratory with materials that the participating institute value-assigned, kept in storage, and shipped to customers. All comparison measurements were made at the Measurement Laboratory under repeatability conditions.

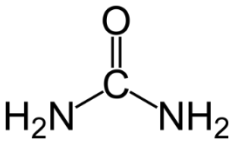
At the April 2018 CCQM meeting the nomenclature for key comparisons sitting under CCQM was revised. The new name for a Track B KC of this type became a “Model 2” comparison, meaning that participants provided samples to the coordinating laboratory. This Model 2 naming convention can be applied to a Track A, C or D comparison. In this case this is considered a Track A model 2 comparison due to its close linkage with the CCQM-K109 Track A KC for similar measurands.

The Health Sciences Authority (HSA) volunteered to provide the repeatability-condition measurements for CCQM-K142 and to jointly coordinate the study with the National Institute for Standards and Technology (NIST). HSA evaluated all study materials by employing the isotope dilution mass spectrometric method, using the liquid chromatography coupled with tandem mass spectrometry (LC-IDMS/MS) measurement system.

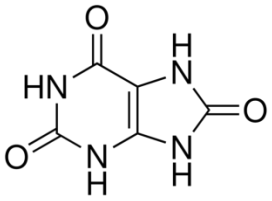
1.2 Measurands

Figure 1 displays the molecular structure, mass, and octanol/water partition coefficient for the two measurands.

Figure 1: Measurands



Urea
CAS Number: 57-13-6
MW: 60.06 g/mol, pK_{OW}: 2.1[1]



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

Urea serves an important role in the metabolism of nitrogen-containing compounds 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.

1.3 Comparison Design Background

Considerations for the design of the comparison were closely referenced to CCQM-K79 and CCQM-K80. Basically, the Measurement Laboratory considered the number of candidate materials and their analyte levels for each potential PI. A limit to the number of candidate materials that each institute could nominate was then established based on the analytical capabilities and available resources of the Measurement Laboratory to conduct measurements under repeatability conditions.

A target date for supplying those materials to the Measurement Laboratory was set and the materials were stored under the conditions specified in their Certificates until measurements were made. The measurements were made under repeatability conditions. The measurement result and the uncertainty for each material were determined.

A consensus model that related to the assigned and measured values, using a technique that considers the uncertainties on both the assigned and measured values, was adopted. For both measurands, the difference between the assigned and measured value for each material and the value predicted from the consensus model was estimated, considering the uncertainties on the definition of the model, as

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

well as those on the observed values. The differences were then converted into degrees of equivalence.

2.0 STEP 1: DESIGN OF THE STUDY

2.1 Timeline

Table 1: Timeline

Date	Action
27 January 2016	Call for Participation
12 February 2016	Deadline for registration
May to August 2016	Measurement campaigns
October 2016	Presentation of preliminary results
April 2017	Submission of Draft A Report
March 2018	Submission of Draft B Report
July 2018	Submission of Final Report

2.2 Participating Institutes (PIs)

Table 2: Participating Institutes

Acronym	Participating Institute	Country	Remarks
CENAM	Centro Nacional de Metrología	México	
HSA	Health Sciences Authority	Singapore	
KRISS	Korea Research Institute of Standards and Science	Korea	
NIM	National Institute of Metrology	China	Uric acid only
NIST	National Institute of Standards and Technology	USA	

2.3 Materials

Only serum and plasma materials with valid certified values and uncertainties were eligible for inclusion in CCQM-K142. Likewise, only materials either directly certified in units of mass fraction or that could be converted into units of mass fraction using the density of the materials were eligible.

To limit the number of materials to a quantity that could be measured under repeatability conditions, the participating institutes (PIs) were asked to provide no more than three materials for each measurand. To ensure that the required repeatability measurements could be made on at least two units of each material, PIs were requested to provide four units of each material. NIM provided three units of their uric acid materials. All other PIs provided at least four units of each material for each analyte.

Tables 3 and 4 summarise the certification information as provided by the participating institutes for the 10 urea materials and the 12 uric acid materials submitted for inclusion in CCQM-K142. In addition to identifying the certifying institute, the certified value "V," the uncertainty on the certified value "$U_{95}(V)$" at a 95 % level of confidence, and the units of certification, these tables list the auxiliary information deemed useful for evaluating the materials' suitability for inclusion in the comparison and for the measurement design. Most of this information was available in the certification documents supplied by the participating institutes in response to the solicitation. When required information was not supplied in submitted documents, it was solicited via email. The repeatability measurements were not begun until all required information was compiled and the accuracy of the compilation confirmed by the participating institutes.

Tables 3 and 4 also list the basic analytical technique used within each institute for certification and the condition of the samples upon arrival at HSA. This information was recorded as a potential aid to the interpretation of results. Two of the six bottles CENAM material for uric acid were thawed upon arrival at HSA. CENAM confirmed the thawed materials could be used for uric acid. All other materials arrived frozen in dry ice in completely intact packaging. Transportation was not an issue for the HSA materials.

All materials submitted by all PIs were CRMs.

Table 3: Urea CRMs

		Certified Values			Auxiliary Information[a]						
PI	**CRM**	***V***	U_{95} (***V***)	**Units**	**Matrix**	**mL**	**°C**	**Year**	**Expires**	**Condition[b]**	**Method[c]**
CENAM	DMR-263a	27.07	0.69	mg/dL	Frozen	1	-80	Nov-04	Mar-13[d]	Frozen	ID-GC/MS
CENAM	DMR-263b	33.2	1.3	mg/dL	Frozen	1	-20 to 80	Oct-14	Oct-19	Frozen	ID-GC/MS
CENAM	DMR-263c	89.6	3.2	mg/dL	Lyoph	3 g per 3 mL H_2O	0 to 8	May-16	May-21	Frozen	ID-GC/MS
KRISS	111-01-01A	156.9	3.4	mg/kg	Frozen	3	-70	1-Apr-16	30-Mar-20	Frozen	ID-LC/MSMS
KRISS	111-01-02A	1129	25	mg/kg	Frozen	3	-70	1-Apr-16	30-Mar-20	Frozen	ID-LC/MSMS
NIST	SRM 909c	25.95	0.53	mg/dL	Frozen	2	-60	14-Dec-10	15-Oct-25	Frozen	ID-GC/MS
NIST	SRM 1950	23.45	0.49	mg/dL	Plasma	1	-60	14-Jul-11	30-Sep-23	Frozen	ID-GC/MS
HSA	HRM-3002B-01	5.415	0.076	mmol/L	Frozen	1	-60	29-Jan-16	29-Jan-20	Frozen	ID-LC/MS
HSA	HRM-3002A-02	7.65	0.13	mmol/L	Frozen	1	-60	12-Apr-13	12-Oct-19	Frozen	ID-LC/MS
HSA	HRM-3002A-03	13.33	0.25	mmol/L	Frozen	1	-60	12-Apr-13	12-Oct-19	Frozen	ID-LC/MS

a **Matrix** is the form of the material, either liquid frozen serum (Frozen), lyophilised serum (Lyoph) or liquid frozen plasma (Plasma); **mL** is the volume of material per unit, **°C** is the specified storage temperature, **Year** indicates the material was originally certified, and **Expires** indicates the expiration date of the material.

b **Condition** refers to the condition at which the material arrived at HSA laboratory. HRM-3002B-01, HRM-3002A-02 and HRM-3002A-03 were taken from storage.

c **Method** refers to the certification method used by the certifying institute to value assign the material: GC = gas chromatography, ID = isotope dilution, LC = liquid chromatography, MS = mass spectrometry, HR = high resolution.

d There was no new CoA for this CRM. However, at the point of the comparison DMR-263a continued to be maintained by CENAM for their long-term stability studies.

Table 4: Uric Acid CRMs

		Certified Values			Auxiliary Information[a]						
PI	**CRM**	***V***	U_{95} (***V***)	**Units**	**Matrix**	**mL**	**°C**	**Year**	**Expires**	**Condition[b]**	**Method[c]**
CENAM	DMR-263a	5.21	0.46	mg/dL	Frozen	1	-80	Nov-04	Mar-13[d]	Frozen/Thawed	LC/MS
CENAM	DMR-263b	5.64	0.41	mg/dL	Frozen	1	-20 to 80	Oct-14	Oct-19	Frozen/Thawed	LC/MS
CENAM	DMR-263c	5.39	0.31	mg/dL	Lyoph	3 g per 3 mL H_2O	0 to 8	May-16	May-21	Frozen/Chilled	LC/MS
KRISS	111-01-01A	38.05	0.82	mg/kg	Frozen	3	-70	1-Apr-16	30-Mar-20	Frozen	ID-LC/MSMS
KRISS	111-01-02A	116.6	4.2	mg/kg	Frozen	3	-70	1-Apr-16	30-Mar-20	Frozen	ID-LC/MSMS
NIM	GBW09157	55.9	1.1	µg/g	Frozen	1	-70	1-Aug-08	30-Aug-17	Frozen	ID-LC/MS
NIM	GBW09169	72.2	1.9	µg/g	Frozen	1	-70	1-Aug-08	30-Aug-17	Frozen	ID-LC/MS
NIST	SRM 909c	4.68	0.1	mg/dL	Frozen	2	-60	14-Dec-10	15-Oct-25	Frozen	ID-GC/MS
NIST	SRM 1950	4.274	0.089	mg/dL	Plasma	1	-60	14-Jul-11	30-Sep-23	Frozen	ID-GC/MS
HSA	HRM-3002B-01	0.2925	0.0068	mmol/L	Frozen	1	-60	29-Jan-16	29-Jan-20	Frozen	ID-LC/MS
HSA	HRM-3002A-02	0.599	0.02	mmol/L	Frozen	1	-60	12-Apr-13	12-Oct-19	Frozen	ID-LC/MS
HSA	HRM-3002A-03	0.762	0.02	mmol/L	Frozen	1	-60	12-Apr-13	12-Oct-19	Frozen	ID-LC/MS

a **Matrix** is the form of the material, either liquid frozen serum (Frozen), lyophilised serum (Lyoph) or liquid frozen plasma (Plasma); **mL** is the volume of material per unit, **°C** is the specified storage temperature, **Year** indicates the material was originally certified, and **Expires** indicates the expiration date of the material.

b **Condition** refers to the condition at which the material arrived at HSA laboratory. HRM-3002B-01, HRM-3002A-02 and HRM-3002A-03 were taken from storage.

c **Method** refers to the certification method used by the certifying institute to value assign the material: GC = gas chromatography, ID = isotope dilution, LC = liquid chromatography, MS = mass spectrometry, HR = high resolution.

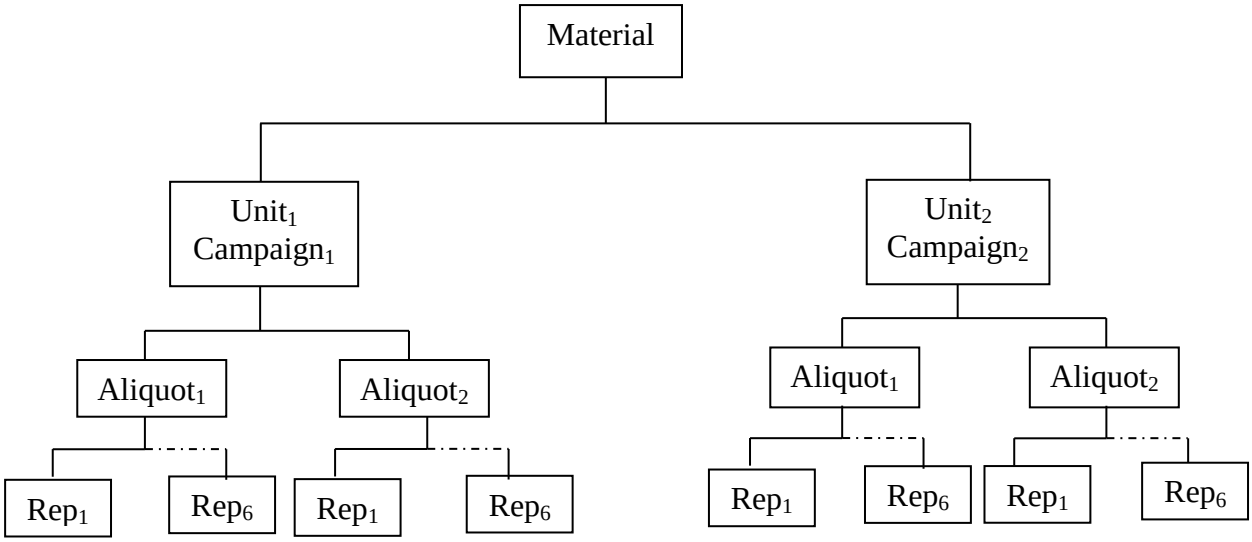
d There was no new CoA for this CRM. However, at the point of the comparison DMR-263a continued to be maintained by CENAM for their long-term stability studies.

3.0 STEP 2: MEASUREMENTS

3.1 Measurement Design

Participating institutes provided HSA with at least three units of each of their submitted materials, two to be analysed and at least one other for use in case of technical failure or to facilitate investigation of disputed results. Given the number of materials and the time required for each analysis, the measurements were made in two measurement campaigns (runs) conducted by two different Analysts. In both campaigns, six measurements were made on two independently prepared aliquots from one randomly selected unit of each material. Figure 2 summarises this three-level nested design.

Figure 2: Repeatability Measurement Design



Measurements on the comparison materials were performed following a randomised block design with blocking on aliquot and replicate. Quality control materials were interspersed at regular intervals in the measurements. All measurements within each campaign were made under repeatability conditions. No intentional changes were made to the equipment, reagents, or quality control materials between campaigns. The measurements of both urea and uric acid were conducted by two Analysts. One Analyst made the Campaign 1 measurements and the other Campaign 2 measurements.

The above design confounds between-unit and between-campaign sources of measurement imprecision. Hence, the measurements made for this study cannot be used to estimate between-unit inhomogeneity for any of the study materials.

3.2 Analytical Method

All materials were analysed under repeatability conditions by HSA using LC-IDMS/MS. The Experimental details are provided in Appendix A. Quantification was based on the relative peak areas for urea (m/z 61→44) and $^{13}C,^{15}N_2$-urea (m/z 64→46), and for uric acid (m/z 167→126) and 1,3-$^{15}N_2$-uric acid (m/z 169→125). Tables 5 and 6 list all the measurement results for the CCQM-K142 materials for urea and uric acid, respectively.

3.2.1 Measurement Quality Assurance

In addition to the measurements made on the CCQM-K142 materials, a control solution was analysed at regularly spaced intervals within each campaign.

Table 5: Urea Measurement

$Unit_1$ ($Campaign_1$)

		$Aliquot_1$						$Aliquot_2$					
PI	CRM	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6
CENAM	DMR-263a	273.112	263.107	268.609	266.129	272.672	269.231	266.934	264.413	267.007	266.210	267.269	265.209
CENAM	DMR-263b	317.433	310.763	322.585	315.165	318.392	315.810	313.448	311.819	316.269	320.282	319.142	315.324
CENAM	DMR-263c	853.538	835.607	837.379	837.735	841.379	844.596	852.319	828.052	838.884	848.150	844.413	853.802
KRISS	111-01-01A	156.218	157.198	157.265	158.725	157.638	157.266	156.969	155.904	155.695	155.416	154.969	157.844
KRISS	111-01-02A	1116.959	1145.762	1105.628	1125.694	1117.021	1142.785	1121.070	1139.803	1130.295	1133.167	1125.823	1145.700
NIST	SRM 909c	254.761	246.263	254.750	252.290	256.222	254.228	249.229	249.351	248.648	250.667	250.587	255.495
NIST	SRM 1950	221.166	220.712	222.034	228.447	224.924	226.948	222.182	219.179	219.906	221.388	228.308	225.131
HSA	HRM-3002B-01	321.630	313.537	321.305	317.498	325.018	322.381	316.214	314.520	323.620	315.376	320.533	322.610
HSA	HRM-3002A-02	445.909	446.649	452.884	442.676	456.118	452.191	440.737	440.321	448.216	443.655	455.929	447.598
HSA	HRM-3002A-03	789.039	783.710	782.099	777.961	788.245	790.696	799.141	769.611	770.279	769.846	789.800	773.318

$Unit_2$ ($Campaign_2$)

		$Aliquot_1$						$Aliquot_2$					
PI	CRM	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6
CENAM	DMR-263a	265.118	264.396	264.312	266.797	264.624	262.995	261.851	261.633	263.727	262.273	264.589	265.936
CENAM	DMR-263b	312.546	314.012	318.249	316.691	315.413	318.805	309.991	316.344	316.116	314.799	315.471	314.161
CENAM	DMR-263c	851.240	847.722	859.783	866.089	880.383	875.292	846.342	858.454	853.467	875.550	870.518	881.092
KRISS	111-01-01A	155.162	156.766	158.144	157.288	159.873	156.669	156.667	154.482	157.023	157.477	155.457	156.119
KRISS	111-01-02A	1130.389	1124.357	1133.044	1131.544	1130.141	1129.057	1122.244	1129.328	1122.145	1123.270	1130.418	1129.916
NIST	SRM 909c	250.279	251.042	254.822	253.763	253.840	251.925	246.609	252.666	253.361	252.545	253.243	254.449
NIST	SRM 1950	221.735	223.773	221.094	226.865	225.173	222.644	219.630	224.546	223.280	228.115	222.681	223.927
HSA	HRM-3002B-01	315.655	320.493	318.125	319.918	319.507	322.106	314.951	316.344	319.388	318.420	322.903	313.290
HSA	HRM-3002A-02	446.284	449.097	444.905	445.927	446.555	448.734	441.460	450.857	449.204	447.694	450.999	448.297
HSA	HRM-3002A-03	760.811	777.947	780.893	784.772	792.357	784.946	791.900	779.190	777.054	782.862	792.583	784.600

Table 6: Uric Acid Measurements

$Unit_1$ ($Campaign_1$)

		$Aliquot_1$						$Aliquot_2$					
PI	CRM	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6
CENAM	DMR-263a	53.221	53.849	54.687	52.471	54.479	52.812	52.618	53.781	53.314	53.899	52.776	52.262
CENAM	DMR-263b	50.285	50.925	50.735	48.261	50.829	50.387	49.975	49.743	49.214	49.738	49.933	50.031
CENAM	DMR-263c	53.924	51.502	54.107	53.997	53.520	55.247	53.716	53.353	53.989	52.973	54.663	54.303
KRISS	111-01-01A	37.815	37.249	35.924	37.629	37.629	38.234	37.273	36.829	37.647	36.878	37.582	37.634
KRISS	111-01-02A	114.457	111.671	114.113	114.431	114.123	109.717	114.219	113.634	113.228	114.234	115.508	114.278
NIM	GBW09157	57.545	55.487	57.292	56.094	55.299	56.084	56.532	55.812	56.119	55.391	55.555	56.195
NIM	GBW09169	73.472	74.088	70.497	72.348	71.465	72.709	72.013	71.224	70.720	73.693	72.090	74.158
NIST	SRM 909c	46.007	45.589	46.422	46.874	45.831	45.217	44.089	46.156	45.537	46.166	46.342	46.777
NIST	SRM 1950	42.076	41.642	42.021	43.036	41.504	43.596	40.861	42.403	43.793	41.518	42.198	43.013
HSA	HRM-3002B-01	49.997	48.209	49.777	48.737	48.046	48.728	48.826	51.023	49.193	47.918	48.761	48.213
HSA	HRM-3002A-02	100.777	101.621	96.695	99.235	98.023	99.730	100.098	103.531	99.424	101.493	97.843	98.817
HSA	HRM-3002A-03	126.397	124.503	120.073	125.775	125.775	127.795	128.768	126.867	129.412	124.985	125.751	126.046

Table 6: Uric Acid Measurements (Continued)

$Unit_2$ ($Campaign_2$)

		$Aliquot_1$						$Aliquot_2$					
PI	CRM	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6	Rep_1	Rep_2	Rep_3	Rep_4	Rep_5	Rep_6
CENAM	DMR-263a	51.715	52.067	51.942	52.117	53.439	52.597	53.097	52.816	52.942	52.470	52.389	52.885
CENAM	DMR-263b	49.124	49.567	48.932	49.268	50.662	49.073	48.574	48.590	48.435	47.972	47.446	50.155
CENAM	DMR-263c	53.103	52.970	54.181	53.301	54.332	53.914	54.897	52.513	54.917	53.191	53.355	53.328
KRISS	111-01-01A	36.534	36.679	36.881	37.303	36.567	37.338	37.568	36.901	37.236	36.931	35.699	36.941
KRISS	111-01-02A	114.640	112.476	116.623	113.220	112.125	114.053	114.985	112.770	113.541	111.687	112.764	111.965
NIM	GBW09157	54.878	55.462	55.268	54.376	55.274	55.798	54.985	55.623	55.098	55.990	55.393	55.794
NIM	GBW09169	72.194	73.169	72.194	70.797	71.879	72.518	72.677	72.713	72.070	72.826	70.599	70.546
NIST	SRM 909c	44.812	45.920	45.895	45.362	45.359	46.159	45.154	44.964	44.372	44.360	45.576	45.000
NIST	SRM 1950	41.751	41.578	41.870	41.572	42.236	40.083	41.776	42.062	41.350	42.163	41.848	42.254
HSA	HRM-3002B-01	48.469	48.777	48.723	48.303	48.644	47.976	48.018	47.218	47.226	48.670	48.243	47.369
HSA	HRM-3002A-02	98.046	100.783	99.651	98.344	98.693	97.546	96.912	99.383	96.747	102.117	98.690	100.001
HSA	HRM-3002A-03	125.377	124.542	124.112	123.267	122.339	125.597	123.560	123.946	125.443	126.721	123.470	125.304

3.3 Frequentist Estimation of Value and Uncertainty

The three-level nested measurement design for the CCQM-K142 materials addresses instrumental, sample preparation and between-campaign sources of measurement variability by making six measurements on two independent aliquots of two different units of each material.

The least complex model for describing measurements made using this design is:

$$R_{jkl} \sim \mathrm{N}\left(\mu + \gamma_j + \delta_{jk}, \sigma_{\mathrm{r}}^2\right)$$

where "~" indicates "is distributed as", N(p,q^2) defines a normal distribution with mean p and standard deviation q, j indexes the units, k indexes the aliquots, l indexes the replicates per aliquot, μ is the population mean, γ_j are between-campaign differences, δ_{jk} are between-aliquot differences, and σ_{r} is the limiting LC-IDMS/MS imprecision for the material. The γ_j and δ_{jk} are assumed to be

$$\gamma_j \sim \mathrm{N}\left(0, \sigma_{\mathrm{c}}^2\right) \text{ and } \delta_{jk} \sim \mathrm{N}\left(0, \sigma_{\mathrm{a}}^2\right)$$

where σ_{c} reflects the true between-campaign and/or between-unit variability and σ_{a} reflects the true between-aliquot and/or within-unit variability.

3.3.1 Value

The repeatability measurement for each material, R, can be estimated as the mean of the individual measurements:

$$R = \sum_{i=1}^{N_c} \sum_{j=1}^{N_a} \sum_{k=1}^{N_r} R_{ijk} \,/\left(N_c \times N_a \times N_r\right)$$

where N_c is the number of measurement campaigns, N_{a} is the number of aliquots taken from each campaign, and N_{r} is the number of replicates of each aliquot. For all urea and uric acid materials in CCQM-K142: N_{c} =2, N_{a} =2, and N_r = 6.

3.3.2 Measurement Standard Uncertainty

The usual estimate of the standard uncertainty of this mean is:

$$u\left(R\right) = \sqrt{\frac{N_a \times N_r \times \sigma_c^2 + N_r \times \sigma_a^2 + \sigma_r^2}{N_c \times N_a \times N_r}} \quad .$$

These standard deviations must be estimated from the data, most practically calculated with linear mixed model statistical analysis systems [1]. Tables 7 and 8 list the estimated standard deviation estimates for urea and uric acid, respectively, expressed as percent relative values:

$$_{\%}\sigma = 100 \times \sigma / R \,.$$

Tables 7 and 8 also list the relative standard uncertainties of the certified values expressed as percent:

$$_{\%}u(V) = 100 \times u_{\infty}(V)/V$$

where $u_\infty(V)$ is the "large sample" standard uncertainty and is equal to one-half of the certified $U_{95}(V)$

$$u_\infty(V) = U_{95}(V)/2 .$$

Note that σ_r estimates just the instrumental precision, independent of within- and between-unit sample preparation and/or heterogeneity issues. The pooled relative instrumental precision, $_{\%}\sigma_r$, is 1.05 % for the urea measurements and 1.54 % for the uric acid measurements. The $_{\%}\sigma_a$ and $_{\%}\sigma_c$ estimates are not easily interpreted since σ_a combines all aliquot preparation-related differences with within-unit heterogeneity and σ_c combines all Analyst- or time-related differences in the method with between-unit heterogeneity.

3.3.3 Large-Sample Standard Uncertainties

Ideally the $u(R)$ should be representative of the material rather than just the specific units of the material used in the study. As discussed in [1], one approach to accomplishing this is to first expand the estimated standard uncertainty by the appropriate two-tailed Student's t 95 % level of confidence factor

$$U_{95}(R) = t_{0.05,\nu} \times u(R)$$

and then divide the expanded uncertainty by the conventional metrological large-sample coverage factor of 2, giving a "large sample" standard uncertainty:

$$u_\infty(R) = U_{95}(R)/2$$

where ν is the number of degrees of freedom associated with $u(R)$.

Unfortunately, determining ν is problematic. The usual interpretation of the analysis of variance (ANOVA) results presented in Tables 7 and 8 provides $\nu = N_r \times N_a \times N_c - 1 = 23$ when both σ_a and σ_c are statistically insignificant (here, when $_{\%}\sigma_a$ and $_{\%}\sigma_c$ are zero), $\nu = N_a \times N_c - 1 = 3$ when just σ_c is insignificant, and $\nu = N_c - 1 = 1$ when σ_c is significant (here, when $_{\%}\sigma_c$ is greater than zero). Under this interpretation, $t_{0.05,\nu}$ / 2 for the different materials is ≈ 1.03 when ν is 23, ≈ 1.59 when ν is 3, and ≈ 6.35 when ν is 1.

This interpretation only considers the evidence of the measurements and does not include information about the materials inherent in the uncertainty assigned to the certified values, $u(V)$. For all the urea materials and for all the uric acid materials, the estimated $_{\%}u(R)$ is less than the certified $_{\%}u(V)$ suggesting that any within- and between-unit heterogeneity sources of variability were recognised and accounted for during certification. Expanding the $u(R)$ to be greater than $u_\infty(V)$ for these materials yields $u_\infty(R)$ that are unreasonably large.

For the frequentist analysis discussed in Section 4, based on this insight we assert that the "real" ν for all the urea and uric acid materials is "large" and therefore:

$$u_{\infty}(R) \cong u(R)$$.

Table 7: Measurement Summary for Urea Materials*

PI	CRM	R	$_{\%}\sigma_r$	$_{\%}\sigma_a$	$_{\%}\sigma_c$	$_{\%}u(R)$	$_{\%}u(V)$
CENAM	DMR-263a	265.76	0.85	0.44	0.83	0.65	1.27
CENAM	DMR-263b	315.79	0.97	0	0.18	0.24	1.96
CENAM	DMR-263c	853.41	1.30	0	1.72	1.25	1.79
HSA	HRM-3002B-01	318.97	1.09	0	0	0.22	0.70
HSA	HRM-3002A-02	447.62	0.97	0	0	0.20	0.85
HSA	HRM-3002A-03	782.24	1.18	0.17	0	0.25	0.94
KRISS	111-01-01A	156.76	0.75	0.44	0	0.27	1.08
KRISS	111-01-02A	1128.57	0.84	0.06	0	0.17	1.11
NIST	SRM-1950	223.49	1.32	0	0	0.27	1.04
NIST	SRM-909c	252.13	1.09	0.21	0	0.24	1.02

Table 8: Measurement Summary for Uric Acid Materials*

PI	CRM	R	$_{\%}\sigma_r$	$_{\%}\sigma_a$	$_{\%}\sigma_c$	$_{\%}u(R)$	$_{\%}u(V)$
CENAM	DMR-263a	52.94	1.24	0.36	0.98	0.76	4.42
CENAM	DMR-263b	49.49	1.54	0.82	1.26	1.03	3.64
CENAM	DMR-263c	53.72	1.67	0	0.11	0.35	2.88
HSA	HRM-3002B-01	48.54	1.57	0.32	1.08	0.84	1.16
HSA	HRM-3002A-02	99.34	1.81	0	0.53	0.53	1.67
HSA	HRM-3002A-03	125.24	1.46	0.52	0.66	0.61	1.31
KRISS	111-01-01A	37.12	1.54	0	0.91	0.71	1.08
KRISS	111-01-02A	113.52	1.29	0.34	0	0.31	1.80
NIM	GBW09157	55.72	1.08	0	0.95	0.71	0.98
NIM	GBW09169	72.19	1.59	0	0.33	0.40	1.32
NIST	SRM-1950	42.01	1.86	0	0.94	0.77	1.05
NIST	SRM-909c	45.58	1.44	0.49	0.89	0.74	1.07

* Table Legend

R Mean of repeatability measurements, arbitrary units

$_{\%}\sigma_r$ Relative within-replicate precision, expressed as percent of R

$_{\%}\sigma_a$ Relative between-aliquot precision, expressed as percent of R

$_{\%}\sigma_c$ Relative between-campaign precision, expressed as percent of R

$_{\%}u(R)$ Relative standard uncertainty of measurements, expressed as percent of R

$_{\%}u(V)$ Relative standard uncertainty of certification, expressed as percent of V

3.3.4 Data Used in the RegViz Frequentist Analyses

Tables 9 and 10 summarise the certified values and measured values for the study materials used in the frequentist analysis of urea and uric acid, respectively. In

these Tables, the materials are sorted in order of increasing certified value, *V*. Each material is assigned a one-character identifying code to simplify graphical presentation.

Table 9: Data Used in the Analysis of Urea Materials

		mg/kg		Arbitrary Units		
Code	CRM	*V*	$u_\infty(V)$	*R*	$u_\infty(R)$	Study
A	111-01-01A	156.90	1.70	156.76	0.42	K142
B	SRM 1950	229.71	2.40	223.49	0.60	K142
C	SRM 909c	253.39	2.59	252.13	0.62	K142
D	DMR-263a	264.80	3.37	265.76	1.74	K142
E	HRM-3002B-01	319.60	2.24	318.97	0.71	K142
F	DMR-263b	323.78	6.34	315.79	0.75	K142
G	HRM-3002A-02	449.00	3.82	447.62	0.89	K142
H	HRM-3002A-03	781.68	7.33	782.24	1.99	K142
I	DMR-263c	871.09	15.56	853.41	10.64	K142
J	111-01-02A	1129.00	12.50	1128.57	1.96	K142

Table 10: Data Used in the Analysis of Uric Acid Materials

		mg/kg		Arbitrary Units		
Code	CRM	*V*	$u_\infty(V)$	*R*	$u_\infty(R)$	Study
A	111-01-01A	38.05	0.41	37.12	0.27	K142
B	SRM 1950	41.87	0.44	42.01	0.32	K142
C	SRM 909c	45.70	0.49	45.58	0.34	K142
D	HRM-3002B-01	48.32	0.56	48.54	0.41	K142
E	DMR-263a	50.96	2.25	52.94	0.40	K142
F	DMR-263c	52.40	1.51	53.72	0.19	K142
G[a]	*DMR-263b*	*55.00*	*2.00*	*49.49*	*0.51*	*K142*
H	GBW09157	55.90	0.55	55.72	0.40	K142
I	GBW09169	72.20	0.95	72.19	0.29	K142
J	HRM-3002A-02	98.41	1.64	99.34	0.52	K142
K	111-01-02A	116.60	2.10	113.52	0.36	K142
L	HRM-3002A-03	125.07	1.64	125.24	0.77	K142

a Withdrawn by CENAM from use in the Key Comparison Reference Function (KCRF)

3.4 Bayesian Estimation

Bayesian analysis is based on a somewhat different definition of probability than the usual frequentist interpretation underpinning classical statistical inference. Under the Bayesian paradigm, parameters such as the measurand value and variance components have probability distributions that quantify our knowledge about them. The estimation process starts with quantification of prior knowledge about the parameters followed by specification of the statistical model that relates the parameters to the data.

The components of the model specified in Section 3.3 are combined via Bayes Theorem to obtain posterior distributions for the parameters. These distributions update our knowledge about the parameters based on the evidence provided by the data. This analysis can produce a probability distribution for each μ (the true value of analyte quantity estimated by the measurement mean, R) which encompasses all information and variability present in the data but is confined by bounds based on prior knowledge. The process yields a probability interval which is interpretable as an uncertainty interval. Markov Chain Monte Carlo (MCMC) empirical Bayesian methods enable computation of coverage intervals. The OpenBUGS software system [http://www.openbugs.net/w/FrontPage] that implements this analysis is freely available and (relatively) easy to use.

Ideally, Bayesian analysis can proceed using very conservative, minimally-informative priors (e.g., very broad Gaussian distributions) and let the data mostly determine the posterior distribution of the measurand. Unfortunately, somewhat informative priors are required with small degrees of freedom. However, when these priors are carefully defined the analysis can validly produce probability distributions for the μ which encompass the available information on the materials and all the variability present in the data.

3.4.1 Differences Between the Frequentist and Bayesian Implementations

Based again on the insight that the "real" v for the urea and uric acid materials is "large", the Bayesian OpenBUGS codes developed for this study assign an informative prior to each material's between-unit/campaign standard deviation, σ_c. For all materials where $u_\infty(V)$ is as large or larger than the ANOVA estimate for $u_\infty(R)$, the prior is $u_\infty(V)$.

The frequentist ANOVA analysis estimates a different σ_r, for every material. However, the relative estimates, ${}_{\%}\sigma_r = 100\times\sigma_r/R$, are approximately constant for both measurands. Based on this observation, for each measurand the OpenBUGS codes estimate a common ${}_{\%}\sigma_r$ for all materials.

3.4.2 Data Used in the Bayesian Analyses

The Bayesian OpenBUGS codes developed for this study use the V and $u_\infty(V)$ values listed in Tables 9 and 10 and the "raw" measurement results listed in Tables 5 and 6. The complete OpenBUGS code and data for both the urea and uric acid materials are listed in Appendix B.

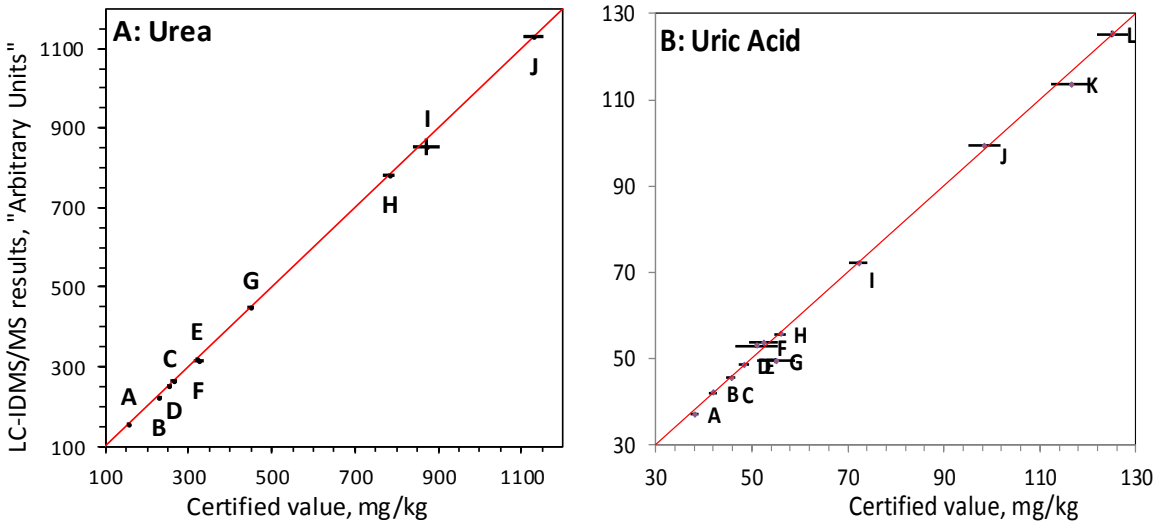
4.0 STEP 3: DEFINE A CONSENSUS MODEL

4.1 The Key Comparison Reference Function (KCRF)

In analogy to the "Key Comparison Reference Value (KCRV)" used with single-material comparisons, whatever model is used to characterise the relationship between the certified values, $V \pm u_\infty(V)$, and the measured summary values, $R \pm u_\infty(R)$, we term the "Key Comparison Reference Function (KCRF)" for the comparison.

Since a definitive method was used for the measurements, a linear relationship is expected between the certified and measured values. Figure 3 provides an overview of the relationship between the certified and measurement values of urea and uric acid. The closeness of the values to the lines confirms that the relationship for both measurands, and thus its KCRF, is linear.

Figure 3: Certified Vs Measured Values



Each cross denotes the $\{V \pm 2\times u_\infty(V), R \pm 2\times u_\infty(R)\}$ for one material. The red line represents exact equality between the certified and measured values: $R = V$. The crosses are labelled in order of increasing V. The materials in panel A, the urea arm of the study, are labeled from A to J. The materials in panel B, the uric acid arm, are labeled from A to L. Refer Tables 9 and 10 for the association between the code and the materials.

4.1.1 Linear Models

A linear relationship can be modelled as:

$$R = \alpha + \beta V + E \qquad [1]$$

where α is the intercept, β is the slope, and E is the residual random error. Alternatively, if α is asserted to be zero, then the relationship can be modelled as:

$$R = \beta V + E \ . \qquad [2]$$

The number of degrees of freedom for the model is the number of materials used to parameterise to model, N_m, minus the number of adjustable parameters in the model. Two such parameters are needed for Equation 1, α and β; only one, β, is needed for Equation 2. In consequence, if α is truly zero then the uncertainty in the estimate of β should be smaller using Equation 2 rather than Equation 1. However, should α be erroneously asserted to be zero then use of Equation 2 will result in a biased model.

4.1.2 Generalised Distance Regression (GDR)

Ordinary least squares regression is not an appropriate approach to estimating the parameters of Equations 1 or 2 since both the certified and measurement results have known and non-negligible uncertainty [1]. However, generalised distance regression (GDR) provides appropriate parameters by iteratively minimising the total uncertainty-scaled residual distances:

$$E = \sum_{i=1}^{N_m} \varepsilon_i^2; \qquad \varepsilon_i^2 = \left(\frac{V_i - \widehat{V}_i}{u_\infty(V_i)}\right)^2 + \left(\frac{R_i - \widehat{R}_i}{u_\infty(R_i)}\right)^2; \qquad \widehat{R}_i = \hat{\alpha} + \hat{\beta}\widehat{V}_i$$

where i indexes the materials, N_m is the number of materials, and $\widehat{V}_i$, $\widehat{R}_i$, $\hat{\alpha}$, and $\hat{\beta}$ are predicted estimates for the parameters. Note that the residual uncertainty-weighted distance for a given material, ε_i is symmetric in V_i and R_i.

There are several available Frequentist implementations of GDR [1]. In this report, these results were obtained using the RegViz system developed by NIST.

4.1.3 Parametric Bootstrap Monte Carlo Uncertainty Evaluation

The RegViz system incorporates a parametric bootstrap Monte Carlo (PBMC) technique that facilitates the estimation of the variability for all quantities estimated with GDR. With PBMC, the entire set of V_i and R_i values used in the GDR analysis are repeatedly replaced with corresponding "pseudo-values" randomly drawn from each of the $\mathrm{N}\left(V_i, u_\infty^2(V_i)\right)$ and $\mathrm{N}\left(R_i, u_\infty^2(R_i)\right)$ normal kernels. The parameters and associated quantities are stored and, once a suitably large number have been generated, approximate 95 % expanded uncertainty intervals are estimated from the percentiles of the empirical distributions. Since only the central 95 % of the distributions are of interest, relatively few pseudo-sets are required for stable estimates.

4.1.4 Bayesian GDR

The OpenBUGS Bayesian codes developed for this project treat both the *V* and *R* values as distributions rather than fixed values. As such, they inherently produce result distributions that can be summarised as GDR parameter and parameter uncertainty estimates.

4.2 Graphical Analyses Using the RegViz GDR System

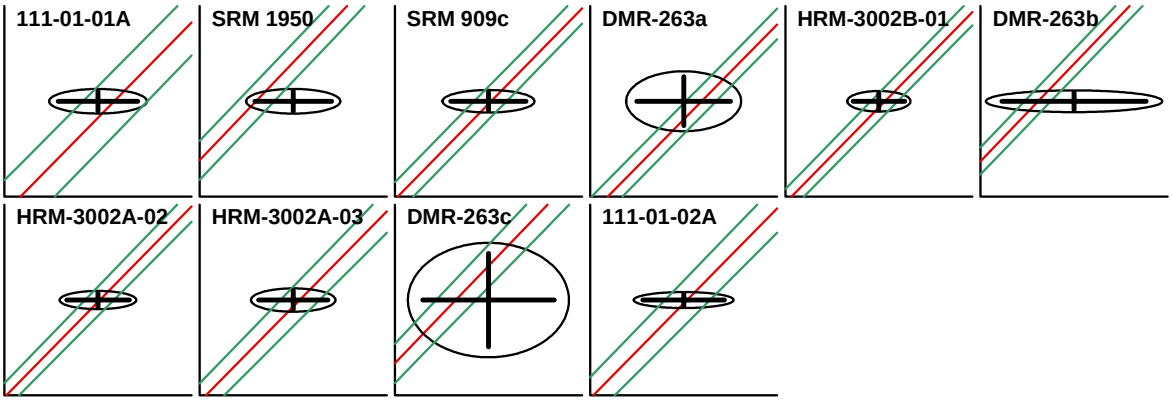
4.2.1 Overview

Figure 4 displays summary GDR results for the urea materials; Figure 5 displays summary results for the uric acid materials. In both Figures, panel A displays the results based on the $R = \alpha + \beta V$ model and panel B displays the results for $R = \beta V$. The graphical resolution required for simultaneously displaying all materials in single scatterplot is insufficient for adequately visualising the analyses. Therefore, Figures 4 and 5 display each material in a series of high-resolution individual "thumbnail" scatterplots.

Note: Following discussion of the Draft A report in April 2017, CENAM withdrew the value for DMR-263b from use in parameterising the uric acid KCRF. The following uric acid analyses do not use the DMR-263b to parameterise the models.

Figure 4: GDR Results for Urea Materials

A: $R = \alpha+\beta V$



B: $R = \beta V$

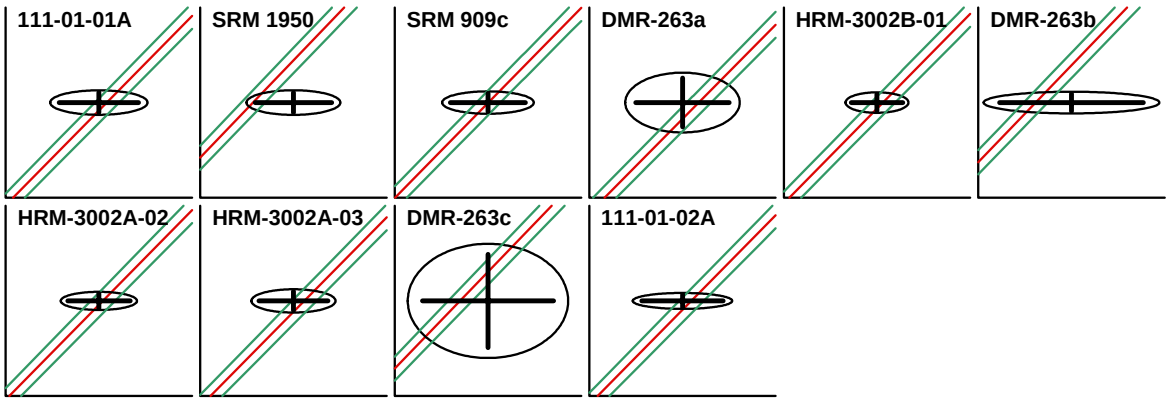
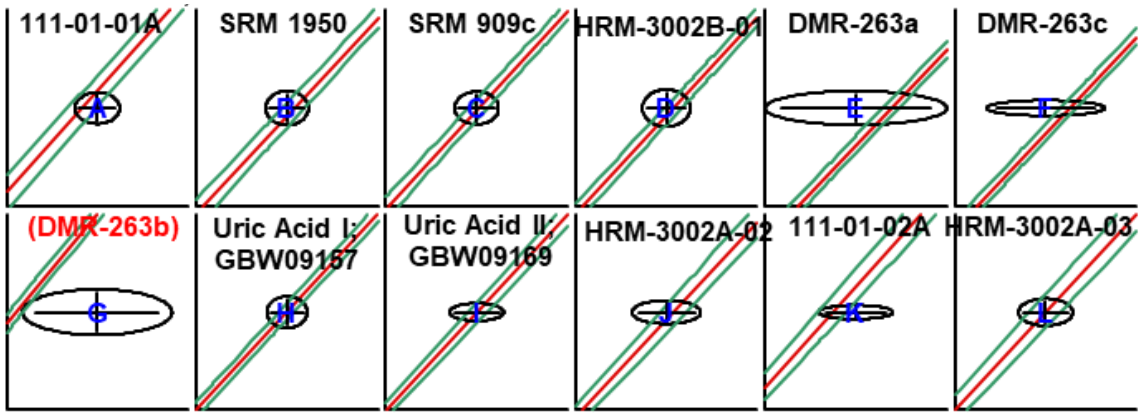
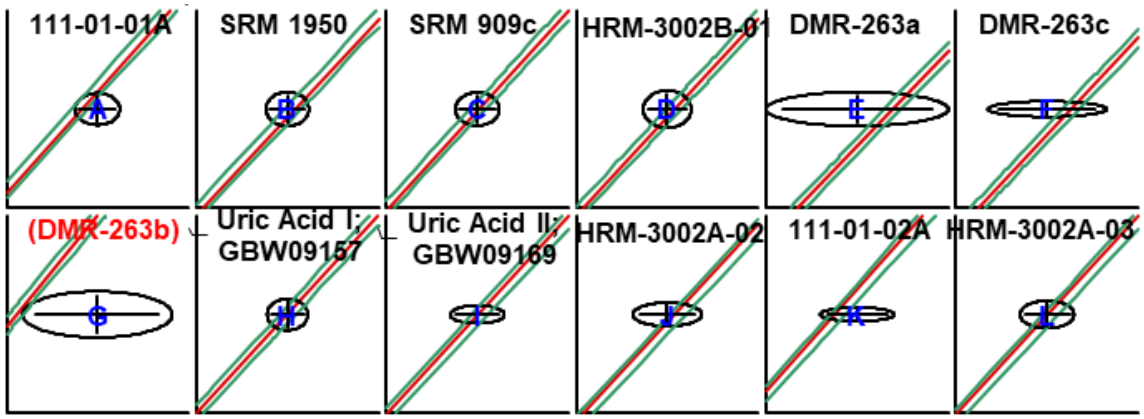


Figure 5: GDR Results for Uric Acid Materials

A: $R = \alpha+\beta V$



B: $R = \beta V$



Each thumbnail plots the certified value of a given material along the horizontal axis and the results of the repeatability measurements along the vertical. Each thumbnail is centered at $\{V_i, R_i\}$. The crosses represent $\{V_i \pm 2 \times u_\infty(V_i), R_i \pm 2 \times u_\infty(R_i)\}$. The thumbnails are arranged in order of increasing Vi. All thumbnails for a given measurand have the same relative scale. The thumbnails labeled in red font in Figure 5 denote the materials that are not used in parameterising the models. The red lines represent the candidate KCRF. The green lines are approximate 95 % level of confidence intervals on the candidate KCRF, U_{95}(KCRF). As expected, the KCRF ± U_{95}(KCRF) intervals are somewhat narrower for the R = βV models.

The ellipses bound all points that are within the 95 % confidence region around the $\{V_i, R_i\}$. The square of the GDR uncertainty-weighted residuals, ε_i^2 , are expected to be distributed as χ^2 with two degrees of freedom. Therefore, ε_i less than the value for the 95th percentile expected from this distribution, $\sqrt{5.99} \approx 2.45$, indicate that the uncertainties adequately cover the difference between the estimated and observed values at the 95 % level of confidence. Ellipses that overlap the candidate KCRF line suggest that the observed values are consistent with the KCRF. Ellipses that do not substantially overlap the KCRF ±U_{95}(KCRF) interval suggest that the observed values are not consistent with the KCRF. By this criterion, only the DMR-263b uric acid measurand appears inconsistent.

4.2.2 Identifying Influential Materials

The GDR solution can be strongly influenced by materials having small $u_\infty(V_i)$ and/or $u_\infty(R_i)$. The magnitude of this influence depends not only on the magnitudes of the uncertainties but also on where the $\{V_i, R_i\}$ pair is located relative to the other materials.

Leave-One-Out (LOO) validation is an efficient approach to establishing which, if any, materials are sufficiently influential to distort the consensus estimation of the candidate KCRF. A LOO analysis proceeds by excluding each material in turn from its own evaluation. For the urea materials, this involves 11 GDR analyses: one solution with all 10 materials included in the analysis and 10 solutions each with one material excluded. For the uric acid materials, this involves 12 GDR analyses: one solution with the 12 eligible materials included in the analysis and 11 solutions each with one material excluded.

Figures 6 and 7 compare the "exact" ε_i, calculated using all urea and uric acid materials ("Leave-All-In" or "LAI" analysis), with their LOO-estimated analogues. In both Figures 6 and 7, the A panels display results for the $R = \alpha+\beta V$ model and the B panels display results for the $R = \beta V$ model.

Of the urea materials, only "B" (SRM 1950) is strongly influential in both models. By this criterion, "B" is nearly inconsistent with the consensus GDR solutions. Of the uric acid materials, only "A" (111-01-01A) and "K" (111-01-02A) are moderately influential in both models. By this criterion, "A" and "K" are marginally consistent with the consensus GDR solutions.

Figure 6: Strongly Influential Urea Materials

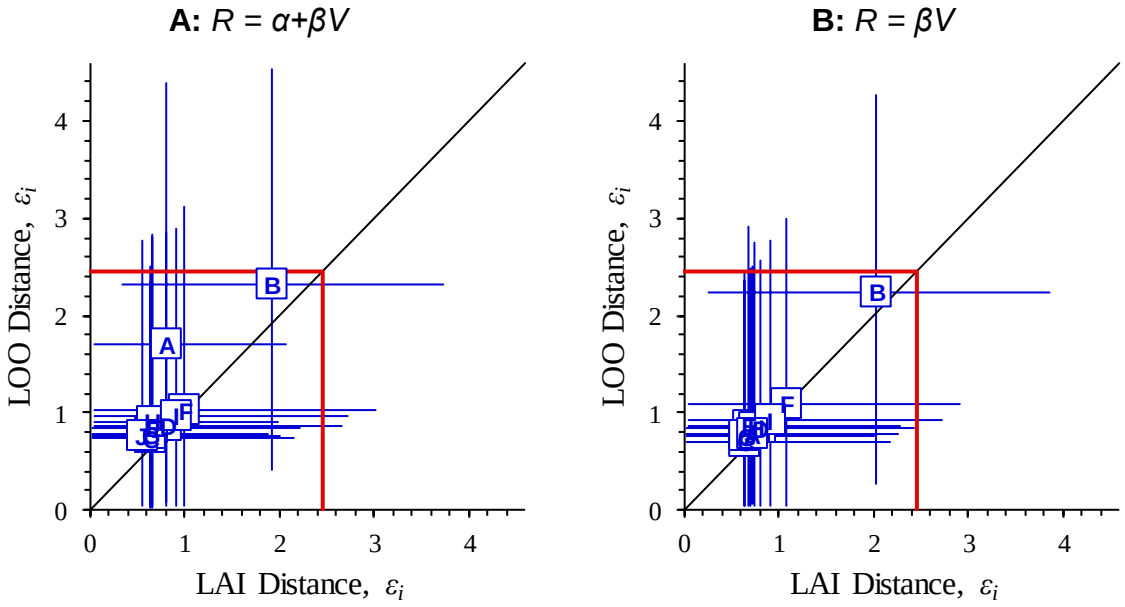
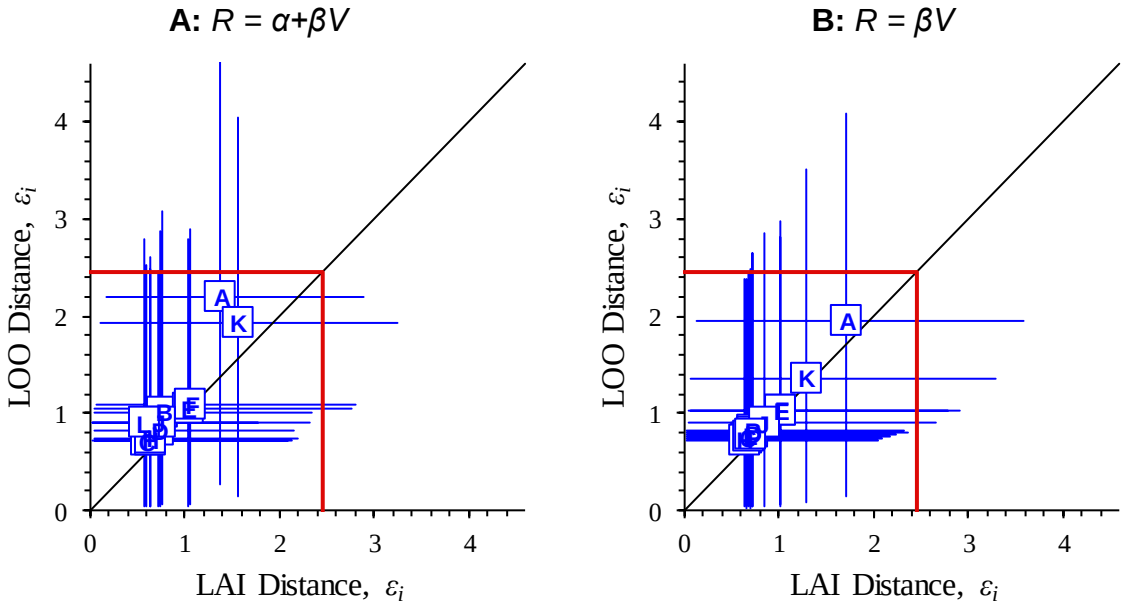


Figure 7: Strongly Influential Uric Acid Materials



The open squares represent estimates for individual materials; the crosses represent the PBMC-estimated 95 % level of confidence intervals on the estimates. Results inside the red lines indicate materials that are consistent with the consensus GDR solution. The diagonal line represents equality between the two estimates. Results far from the diagonal line indicate materials that strongly influence the consensus solution.

4.2.4 Identifying Consequential Materials

Figures 8 and 9 display the negative and positive consequences for ε_i estimated from 1000 PBMC iterations for urea and uric acid materials. In both Figures 8 and 9, the A panels display results for the $R = \alpha+\beta V$ model and the B panels display results for the $R = \beta V$ model.

Figure 8: Strongly Consequential Urea Materials

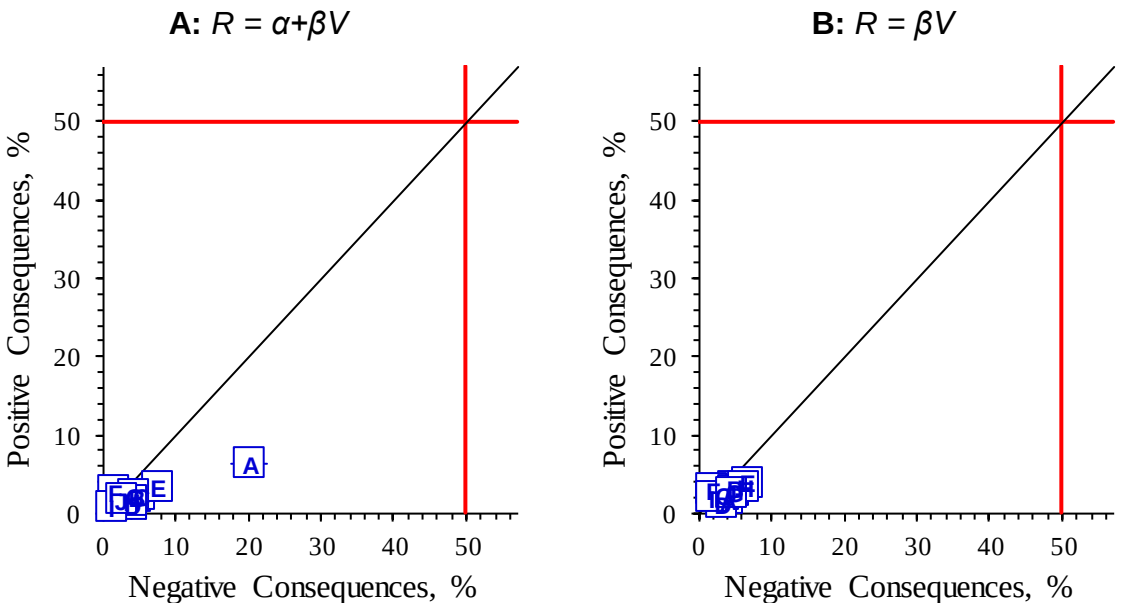
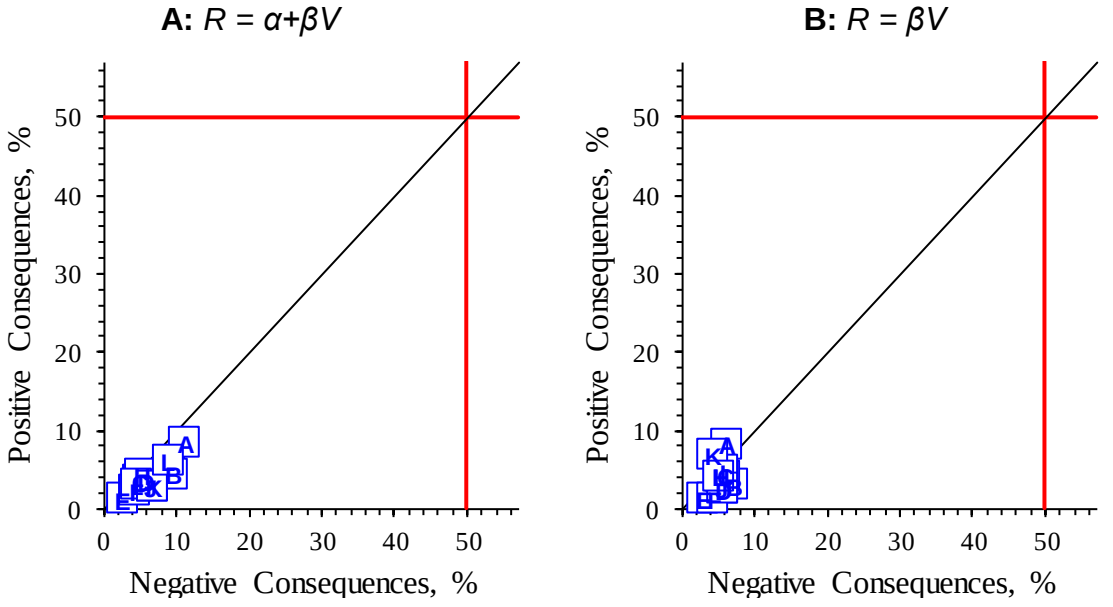


Figure 9: Strongly Consequential Uric Acid Materials



The open squares represent estimates for individual materials; the crosses represent the PBMC-estimated 95 % level of confidence intervals on the estimates. The red lines enclose materials whose presence in the GDR model do not have strong negative or positive consequence for other materials.

None of the urea materials are strongly consequential. Material “A” (111-01-01A), which has the smallest V_i, does have more consequence than the other materials in the $R = \alpha+\beta V$ model. This indicates that A, having “leverage” on the regression line when the intercept is not forced to zero, is not perfectly aligned with the consensus relationship. On average, the presence of A in the $R = \alpha+\beta V$ GDR increases the ε_i of the other materials in about 20 % of the PBMC analyses.

None of the uric acid materials are strongly consequential.

4.3 Parameter Values for the Candidate KCRFs

In addition to identifying materials that could distort the consensus GDR solution, LOO-PBMC enables a more robust estimate of the variability of the GDR parameters. The LOO estimates are influenced by biases (systematic differences in the GDR solutions with-and-without each material in the models) that are not present with LAI models. Thus, the LOO-PBMC parameter uncertainties are constrained to be somewhat larger than those determined with LAI-PBMC analysis.

Table 11 lists the consensus solution parameters for urea and uric acid materials based on the $R = \alpha + \beta V$ and $R = \beta V$ models as estimated using the frequentist RegViz and the Bayesian OpenBUGs systems. The slightly larger LOO-based asymptotic standard uncertainty estimates obtained from the RegViz system provide more conservative coverage than do the LAI. The OpenBUGs implementations that were developed for this study do not provide LOO estimates.

Table 11: Model Parameter Estimates

				$u(\hat{\alpha})^a$			$u(\hat{\beta})^b$	
Measurand	Model	Method	$\hat{\alpha}^a$	LAI[c]	LOO[d]	$\hat{\beta}^b$	LAI[c]	LOO[d]
Urea	$R = \alpha+\beta V$	RegViz	-1.3	2.3	2.6	0.9989	0.0078	0.0086
		BUGs	-1.6	2.3		0.9999	0.0079	
	$R = \beta V$	RegViz				0.9948	0.0034	0.0037
		BUGs				0.9952	0.0036	
Uric Acid	$R = \alpha+\beta V$	RegViz	-0.55	0.76	0.84	1.006	0.015	0.015
		BUGs	-0.33	0.76		1.003	0.015	
	$R = \beta V$	RegViz				0.9969	0.0046	0.0048
		BUGs				0.9971	0.0045	

a Intercept and its uncertainty estimates are expressed in arbitrary units

b Slope and its uncertainty estimates are expressed in arbitrary units per mg/kg

c Standard deviation of Leave-All-In PBMC parameter estimates where all eligible materials were included in model

d Standard deviation of Leave-One-Out PBMC parameter estimates where one eligible material is in turn left out of the model in each set

Using the RegViz parameter estimates and the LOO estimates of parameter uncertainty, the candidate KCRFs for

- the urea materials are
  - $\hat{R} = (-1.3 \pm 2.6) + (0.9989 \pm 0.0086)\hat{V}$
  - $\hat{R} = (0.9948 \pm 0.0037)\hat{V}$

- And for the uric acid materials
  - $\hat{R} = (-0.55 \pm 0.84) + (1.006 \pm 0.015)\hat{V}$
  - $\hat{R} = (0.9969 \pm 0.0048)\hat{V}$

4.4 GDR Predicted Values

Tables 12 and 13 list the frequentist RegViz estimates for the assigned values and repeatability measurements along with their LOO-estimated asymptotic standard uncertainties for urea and uric acid materials.

The OpenBUGS Bayesian estimates for the assigned values and repeatability measurements are qualitatively like the values in these Tables. They are not separately listed in this final report. See Figures 10 to 13 for graphical comparisons of the RegViz and OpenBUGS estimates.

Table 12: GDR Predicted Values for Urea Materials

		$R = \alpha + \beta V$ model				$R = \beta V$ model			
		mg/kg		Arbitrary Units		mg/kg		Arbitrary Units	
Code	CRM	$\hat{V}_i$	$u(\hat{V}_i)$	$\hat{R}_i$	$u(\hat{R}_i)$	$\hat{V}_i$	$u(\hat{V}_i)$	$\hat{R}_i$	$u(\hat{R}_i)$
A	111-01-01A	159.6	1.9	156.59	0.41	157.60	0.68	156.72	0.40
B	SRM 1950	224.4	1.2	223.82	0.59	224.44	0.93	223.82	0.56
C	SRM 909c	253.8	1.2	252.11	0.60	253.44	1.04	252.13	0.59
D	DMR-263a	267.0	1.8	265.17	1.57	266.75	1.71	265.24	1.57
E	HRM-3002B-01	320.8	1.3	318.85	0.68	320.79	1.36	318.85	0.69
F	DMR-263b	317.4	1.3	315.88	0.73	317.33	1.35	315.88	0.73
G	HRM-3002A-02	449.5	2.0	447.59	0.89	450.05	1.76	447.56	0.87
H	HRM-3002A-03	785.3	4.9	781.97	1.89	786.59	3.31	781.88	1.99
I	DMR-263c	859.9	9.4	858.65	8.94	861.86	8.78	857.75	8.58
J	111-01-02A	1131.9	8.0	1128.50	1.95	1134.84	4.42	1128.43	1.93

Table 13: GDR Predicted Values for Uric Acid Materials

		$Y = \alpha + \beta X$ model				$Y = \beta X$ model			
		mg/kg		Arbitrary Units		mg/kg		Arbitrary Units	
Code	CRM	$\hat{V}_i$	$u(\hat{V}_i)$	$\hat{R}_i$	$u(\hat{R}_i)$	$\hat{V}_i$	$u(\hat{V}_i)$	$\hat{R}_i$	$u(\hat{R}_i)$
A	111-01-01A	37.28	0.33	37.46	0.25	37.39	0.26	37.41	0.23
B	SRM 1950	42.17	0.32	41.85	0.27	42.06	0.28	41.91	0.26
C	SRM 909c	45.76	0.32	45.55	0.30	45.70	0.31	45.58	0.29
D	HRM-3002B-01	48.62	0.36	48.38	0.34	48.58	0.38	48.40	0.34
E	DMR-263a	53.06	0.46	52.87	0.39	53.04	0.48	52.87	0.41
F	DMR-263c	53.90	0.31	53.70	0.19	53.88	0.31	53.70	0.18
G	DMR-263b	49.99	0.55	49.81	0.50	49.96	0.54	49.82	0.51
H	GBW09157	55.88	0.36	55.73	0.34	55.88	0.37	55.73	0.33
I	GBW09169	72.27	0.49	72.18	0.28	72.39	0.43	72.17	0.28
J	HRM-3002A-02	99.40	0.92	99.24	0.51	99.58	0.65	99.22	0.48
K	111-01-02A	112.76	1.10	113.63	0.37	113.74	0.64	113.60	0.36
L	HRM-3002A-03	125.09	1.37	125.24	0.74	125.54	0.83	125.14	0.70

5.0 STEP 4: DEGREES OF EQUIVALENCE

5.1 Degrees of Equivalence for Materials

An appropriate definition for the degrees of equivalence for materials in the present comparison is the percent relative signed orthogonal distance [1]:

$$\%d_i = 100 \times SIGN\left(V_i - \hat{V}\right) \times \frac{\sqrt{\left(V_i - \hat{V}\right)^2 + \left(\left(R_i - \hat{R}_i\right)/\hat{\beta}\right)^2}}{\left(V_i + \left(R_i - \hat{\alpha}\right)/\hat{\beta}\right)/2}$$

where the measurement-related terms are transformed to have the same scale as the assigned values. The function SIGN returns the sign (±1) of its argument and defines whether the observed $\{V_i, R_i\}$ pair is "above" or "below" the candidate KCRF.

5.1.1 Degree of Equivalence Uncertainty for Individual Materials

The $d_i \pm U_{95}(\%d_i)$ can be estimated from the empirical distribution of the $\%d_i$values calculated for each set of PBMC pseudo-values, using the LOO analysis to make the uncertainty estimates robust to each material's "self-referential" influence. The $U_{95}(\%d_i)$ for each material can be estimated from the distribution of the $\%d_i$ calculated when its own values are not used in the GDR solution. While requiring many more calculations, these LOO-PBMC estimates are free of correlation between each material's observed values and the candidate KCRF.

5.1.2 Graphical Representation of Degrees of Equivalence for Materials

Figure 10 displays RegViz (panels A and B) and OpenBUGS (panels C and D) estimates of $\%d_i \pm U_{95}(\%d_i)$ estimates for the urea materials in dot-and-bar format. Figure 11 displays the analogous estimates for the uric acid materials. The red line denotes zero bias relative to the KCRF; the $\%d_i$ for materials with bars that cross this line are consistent with the consensus model with about a 95 % level of confidence. The horizontal axis in these figures displays the V_i of each material. The green circles in Figure 11 denote the CENAM DMR-263b that was not used in defining the model parameters.

Figure 10: DoE for Urea Materials

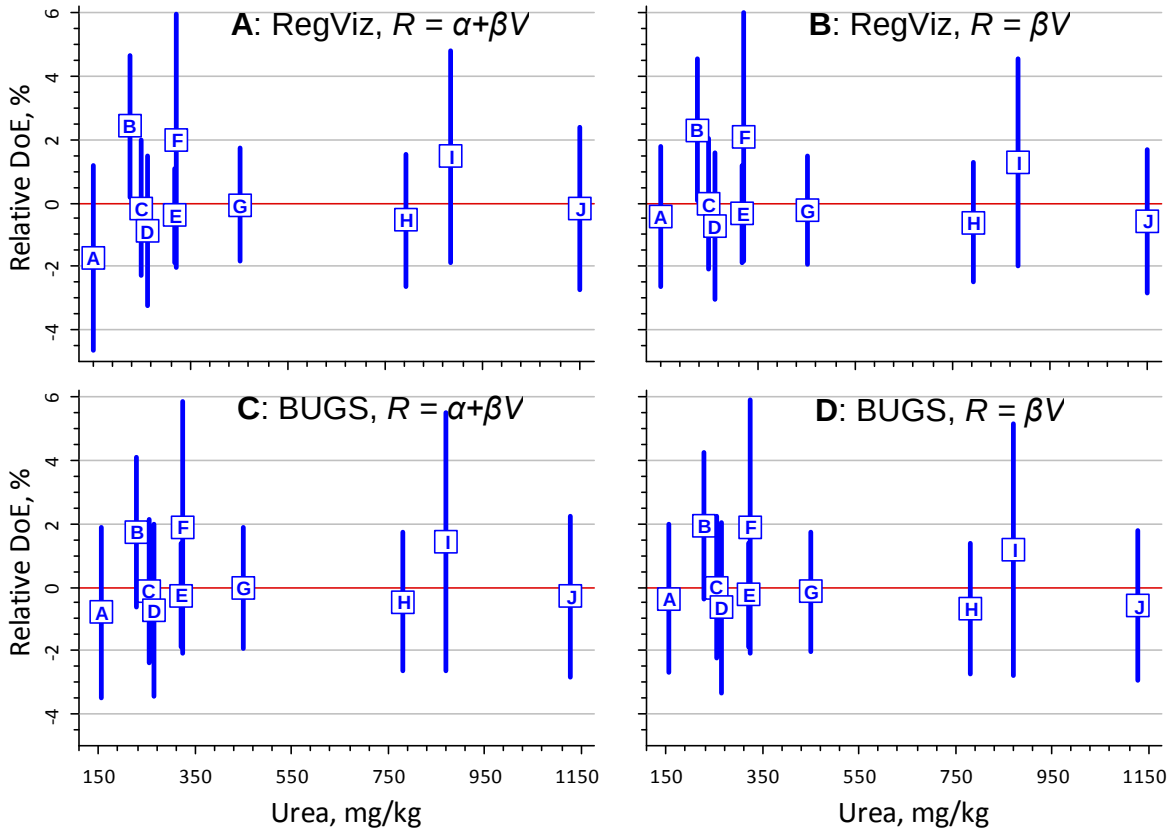
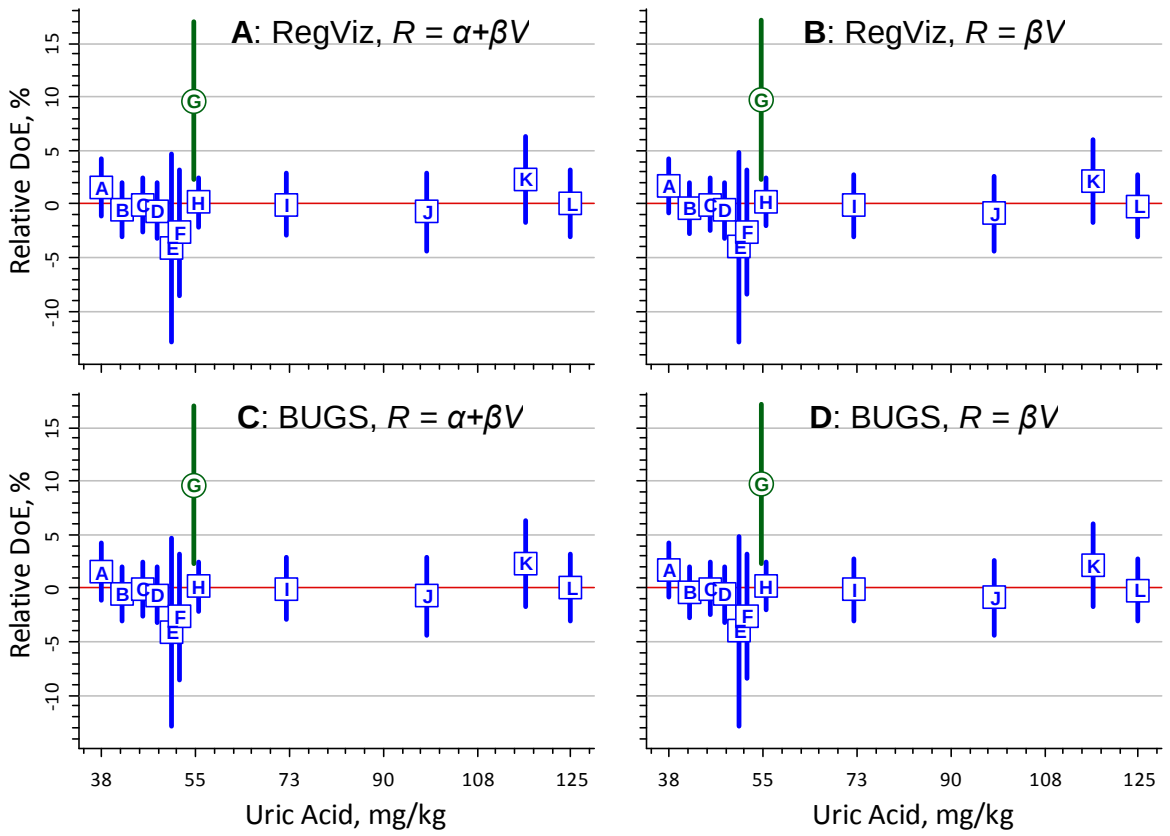


Figure 11: DoE for Uric Acid Materials



5.2 Degrees of Equivalence for Participating Institutes

All the PIs in CCQM-K142 are represented by more than one material. The results for all the materials from each PI contributing more than one material can therefore be combined in some way to provide the desired goal of the comparison: the expected degrees of equivalence of the PIs, $\%D$.

For the RegViz estimates, the $\%D$ for each PI are estimated from the PBMC pseudo-values as the mean and standard deviation of the pseudo-values for all the materials contributed by that PI combined and treated as a single distribution [1]. For the OpenBUGS estimates, the $\%D$ are estimated from the median and empirical 95 % confidence interval of the probability density function produced by combining the $N(\%d_i,(U_{95}(\%d_i)/2)^2)$ kernels of each material. This method is described as the "Mixture Model Median" in [2] and the "Linear Pool" in [3,4].

5.2.1 Graphical Representation of Degrees of Equivalence for PIs

Figure 12 displays the RegViz (panels A and B) and OpenBUGS (panels C and D) estimates of $\%D \pm U_{95}(\%D)$ and $\%d_i \pm U_{95}(\%d_i)$ in dot-and-bar format for urea. Figure 13 displays the analogous estimates for uric acid. The thick black bars and black solid dots represent the $\%D$ and thin blue bars and blue open dots the $\%d_i$. The green circle in Figure 13 denotes the CENAM DMR-263b that was not used in defining the model parameters. The PIs are arranged in alphabetical order.

Figure 12: DoE for PIs That Submitted Urea Materials

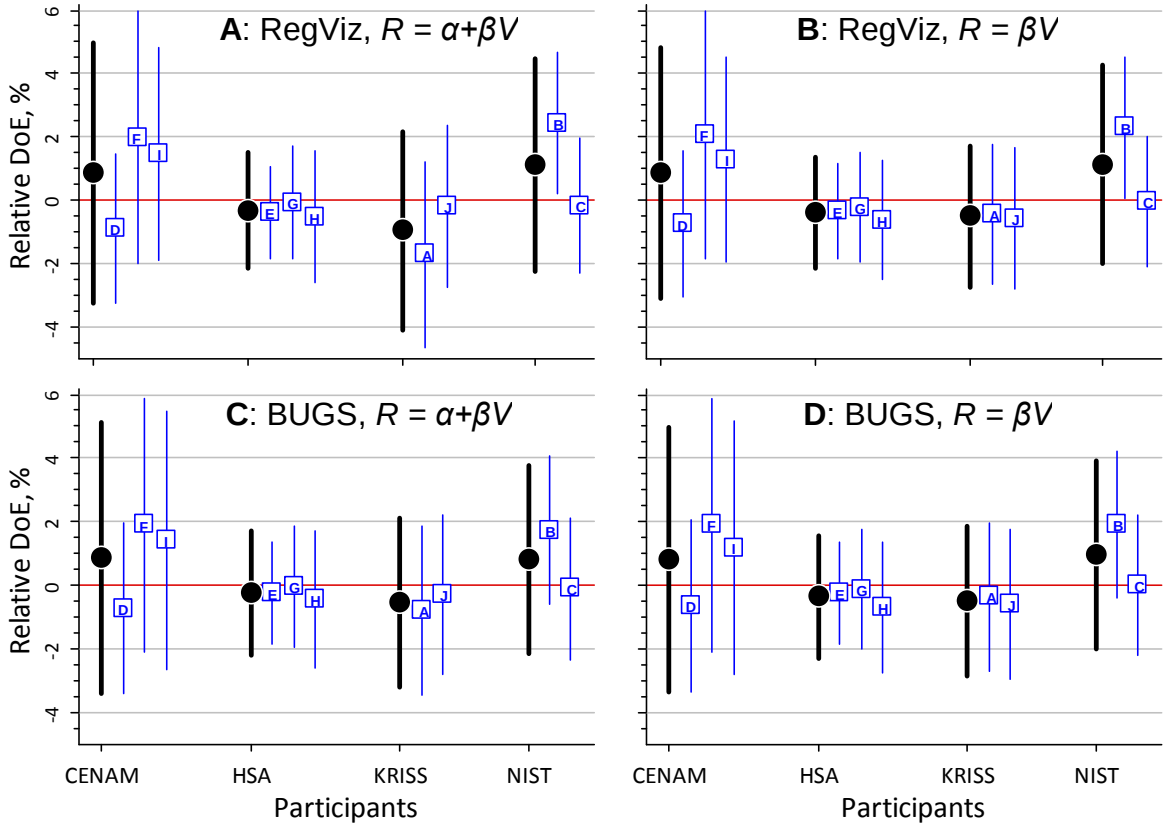
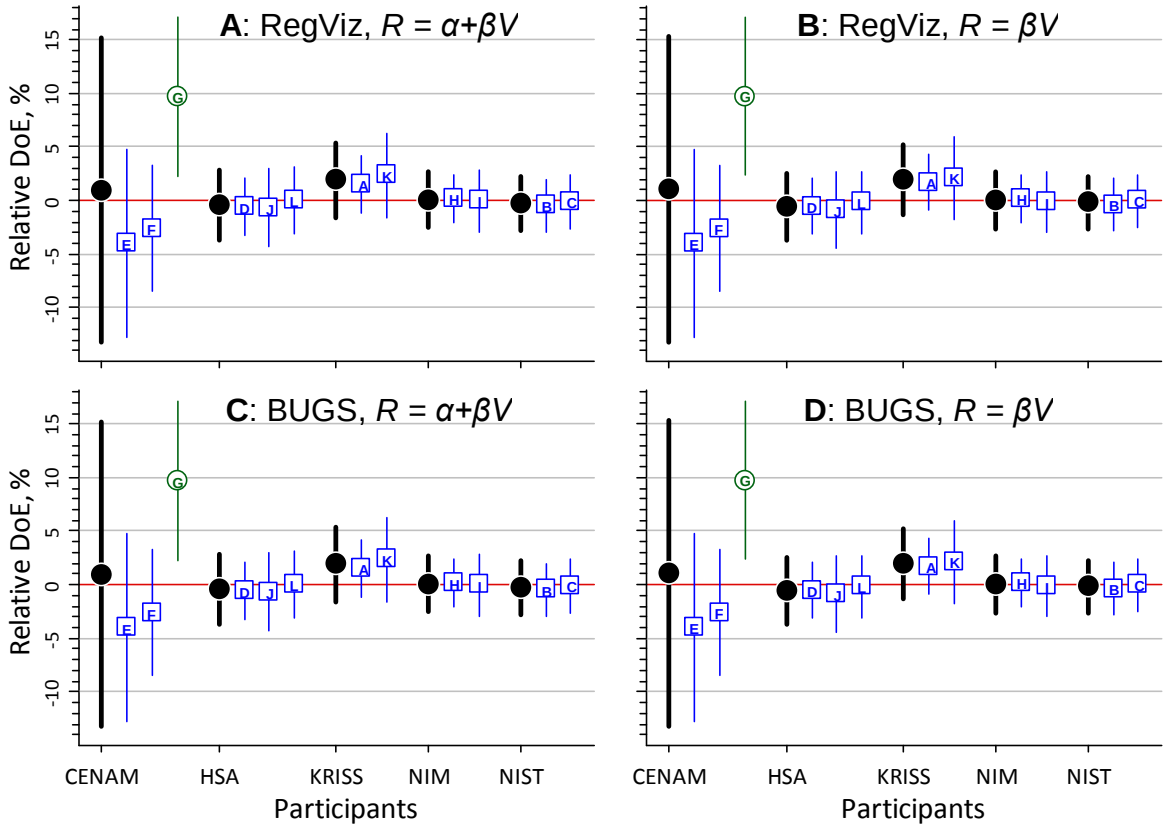


Figure 13: DoE for PIs That Submitted Uric Acid Materials



5.3 Tabular Presentation of Degrees of Equivalence

Tables 14 to 17 list the RegViz-and BUGS estimates of the degrees of equivalence for the urea materials and for their submitting PIs using the $R = \alpha + \beta V$ and $R = \beta V$ candidate KCRFs. Tables 18 to 21 list the RegViz-and BUGS estimates for the uric acid materials and for their submitting PIs using the $R = \alpha + \beta V$ and $R = \beta V$ candidate KCRFs.

5.4 Choice of Model for Degrees of Equivalence

The $\hat{\alpha} \pm u(\hat{\alpha})$ for both the urea and uric acid include $\hat{\alpha} = 0$, suggesting that the intercept parameter for both sets of materials is effectively zero. In addition to smaller uncertainties for the slope parameters, the $R = \beta V$ models on average provide slightly better DoEs than do the $R = \alpha + \beta V$ models.

The presence of the low urea and uric acid concentration 111-01-01A ("A") material in the regression negatively impacts the $\%d$ of the other materials using the $R = \alpha + \beta V$ models. The impact disappears in the $R = \beta V$ models for urea and is reduced for uric acid.

While not fully in agreement with the candidate KCRF models, the presence of the urea material SRM 1950 ("B") does not significantly impact the $\%d$ of the other materials in either model.

The RegViz and BUGS systems provide essentially equivalent KCRFs, $d_i \pm U_{95}(\%d_i)$, and $\%D \pm U_{95}(\%D)$ values for both the $R = \alpha + \beta V$ and $R = \beta V$ models. While relatively unfamiliar within the chemical metrology community, the Bayesian approach implemented by the BUGS models is statistically sound, explicitly identifies its assumptions, facilitates exploring those assumptions, is computationally efficient, and can performed using freely accessible and well-supported software. The more familiar frequentist approach implemented by the RegViz system supports specialised data visualization tools but is computationally inefficient and while freely available is implemented in an old spreadsheet macro language that is supported by one programmer at NIST. The use of the Bayesian approach is thus recommended for estimating parameters of interest in this and future Track B or "Model 2" studies.

Table 14: RegViz Urea DoEs Using the R = α+βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	0.8	2.1	4.3	DMR-263a	D	-0.9	1.2	2.3	264.8	2.5
				DMR-263b	F	2.0	2.0	4.0	323.8	3.9
				DMR-263c	I	1.5	1.7	3.3	871.1	3.6
HSA	-0.3	0.9	1.9	HRM-3002B-01	E	-0.4	0.7	1.5	319.6	1.4
				HRM-3002A-02	G	-0.1	0.9	1.8	449.0	1.7
				HRM-3002A-03	H	-0.5	1.0	2.1	781.7	1.9
KRISS	-1.0	1.6	3.2	111-01-01A	A	-1.7	1.5	2.9	156.9	2.2
				111-01-02A	J	-0.2	1.3	2.6	1129	2.2
NIST	1.1	1.7	3.2	SRM 1950	B	2.4	1.1	2.2	229.7	2.1
				SRM 909c	C	-0.2	1.1	2.1	253.4	2.0

Table 15: Bugs Urea DoEs Using the R = α+βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	0.9	2.2	4.5	DMR-263a	D	-0.7	1.3	2.7	264.8	2.5
				DMR-263b	F	1.9	2.0	4.0	323.8	3.9
				DMR-263c	I	1.4	2.0	4.0	871.1	3.6
HSA	-0.2	1.0	2.0	HRM-3002B-01	E	-0.3	0.8	1.6	319.6	1.4
				HRM-3002A-02	G	0.0	1.0	1.9	449.0	1.7
				HRM-3002A-03	H	-0.5	1.1	2.2	781.7	1.9
KRISS	-0.5	1.4	2.7	111-01-01A	A	-0.8	1.3	2.7	156.9	2.2
				111-01-02A	J	-0.3	1.3	2.5	1129	2.2
NIST	0.8	1.5	2.9	SRM 1950	B	1.7	1.2	2.3	229.7	2.1
				SRM 909c	C	-0.1	1.1	2.2	253.4	2.0

[a] Percent relative expanded uncertainty, $100 \times U_{95}(V_i)/V_i$

Table 16: RegViz Urea DoEs Using the R = βV Candidate KCRF

PIs				Materials							
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	0.9	2.0	4.4	DMR-263a	D	-0.7	1.2	2.3	264.8	2.5
				DMR-263b	F	2.1	2.0	3.9	323.8	3.9
				DMR-263c	I	1.3	1.6	3.2	871.1	3.6
HSA	-0.4	0.9	1.8	HRM-3002B-01	E	-0.3	0.8	1.5	319.6	1.4
				HRM-3002A-02	G	-0.2	0.9	1.7	449.0	1.7
				HRM-3002A-03	H	-0.6	0.9	1.9	781.7	1.9
KRISS	-0.5	1.1	2.2	111-01-01A	A	-0.4	1.1	2.2	156.9	2.2
				111-01-02A	J	-0.6	1.1	2.2	1129	2.2
NIST	1.1	1.6	3.0	SRM 1950	B	2.3	1.1	2.2	229.7	2.1
				SRM 909c	C	0.0	1.0	2.1	253.4	2.0

Table 17: BUGS Urea DoEs Using the R = βV Candidate KCRF

PIs				Materials							
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	0.8	2.1	4.4	DMR-263a	D	-0.7	1.3	2.7	264.8	2.5
				DMR-263b	F	1.9	2.0	4.0	323.8	3.9
				DMR-263c	I	1.4	2.0	4.0	871.1	3.6
HSA	-0.4	1.0	2.0	HRM-3002B-01	E	-0.3	0.8	1.6	319.6	1.4
				HRM-3002A-02	G	0.0	1.0	1.9	449.0	1.7
				HRM-3002A-03	H	-0.5	1.1	2.2	781.7	1.9
KRISS	-0.5	1.2	2.4	111-01-01A	A	-0.8	1.3	2.7	156.9	2.2
				111-01-02A	J	-0.3	1.3	2.5	1129	2.2
NIST	1.0	1.5	2.9	SRM 1950	B	1.7	1.2	2.3	229.7	2.1
				SRM 909c	C	-0.1	1.1	2.2	253.4	2.0

[a] Percent relative expanded uncertainty, $100 \times U_{95}(V_i)/V_i$

Table 18: RegViz Uric Acid DoEs Using the R = α+βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	1.2	7.4	14.7	DMR-263a	E	-3.9	4.1	8.2	50.96	8.8
				DMR-263c	F	-2.8	2.7	5.5	55.00	5.8
				DMR-263b[b]	G	10.1	3.9	7.9	52.40	7.3
HSA	-0.6	1.5	3.1	HRM-3002B-01	D	-0.6	1.1	2.1	48.32	2.3
				HRM-3002A-02	J	-1.1	1.7	3.4	98.41	3.3
				HRM-3002A-03	L	0.0	1.5	3.0	125.07	2.6
KRISS	2.9	1.8	4.0	111-01-01A	A	2.3	1.2	2.4	38.05	2.2
				111-01-02A	K	3.5	2.0	4.0	116.60	3.6
NIM	0.0	1.2	2.5	GBW09157	H	0.1	1.0	2.0	55.90	2.0
				GBW09169	I	-0.2	1.4	2.8	72.20	2.6
NIST	-0.5	1.1	2.2	SRM 1950	B	-0.8	1.0	2.1	41.87	2.1
				SRM 909c	C	-0.2	1.1	2.1	45.70	2.1

Table 19: BUGS Uric Acid DoEs Using the R = α+βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	1.0	7.2	14.1	DMR-263a	E	-4.0	4.4	8.8	50.96	8.8
				DMR-263c	F	-2.6	2.9	5.9	55.00	5.8
				DMR-263b[b]	G	9.6	3.7	7.4	52.40	7.3
HSA	-0.4	1.6	3.3	HRM-3002B-01	D	-0.6	1.3	2.7	48.32	2.3
				HRM-3002A-02	J	-0.7	1.8	3.7	98.41	3.3
				HRM-3002A-03	L	0.0	1.6	3.1	125.07	2.6
KRISS	1.9	1.8	3.8	111-01-01A	A	1.5	1.3	2.7	38.05	2.2
				111-01-02A	K	2.3	2.0	4.0	116.60	3.6
NIM	0.1	1.3	2.7	GBW09157	H	0.2	1.1	2.3	55.90	2.0
				GBW09169	I	0.0	1.5	2.9	72.20	2.6
NIST	-0.3	1.3	2.5	SRM 1950	B	-0.5	1.2	2.5	41.87	2.1
				SRM 909c	C	-0.1	1.3	2.5	45.70	2.1

*a*Percent relative expanded uncertainty, $100 \times U_{95}(V_i)/V_i$

b Material's certification withdrawn; not used to parameterize the KCRF

Table 20: RegViz Uric Acid DoEs Using the R = βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	1.1	7.3	14.6	DMR-263a	E	-3.9	4.0	8.0	50.96	8.8
				DMR-263c	F	-2.7	2.9	5.7	55.00	5.8
				DMR-263b[b]	G	10.0	4.0	8.0	52.40	7.3
HSA	-0.7	1.4	2.9	HRM-3002B-01	D	-0.6	1.1	2.2	48.32	2.3
				HRM-3002A-02	J	-1.2	1.6	3.2	98.41	3.3
				HRM-3002A-03	L	-0.4	1.3	2.6	125.07	2.6
KRISS	2.2	1.6	3.6	111-01-01A	A	2.0	1.1	2.1	38.05	2.2
				111-01-02A	K	2.5	1.9	3.8	116.60	3.6
NIM	-0.1	1.2	2.4	GBW09157	H	0.0	1.0	1.9	55.90	2.0
				GBW09169	I	-0.3	1.3	2.7	72.20	2.6
NIST	-0.3	1.1	2.2	SRM 1950	B	-0.5	1.0	2.1	41.87	2.1
				SRM 909c	C	0.0	1.0	2.1	45.70	2.1

Table 21: BUGS Uric Acid DoEs Using the R = βV Candidate KCRF

PIs				Materials						
	%*D*, percent					$\%d_i$, percent			V_i	
PI	Value	*u*	U_{95}	Material	Code	Value	*u*	U_{95}	mg/kg	$\%U_{95}$[a]
CENAM	1.1	7.3	14.1	DMR-263a	E	-4.0	4.4	8.8	50.96	8.8
				DMR-263c	F	-2.6	2.9	5.8	55.00	5.8
				DMR-263b[b]	G	9.7	3.7	7.4	52.40	7.3
HSA	-0.5	1.6	3.2	HRM-3002B-01	D	-0.6	1.3	2.6	48.32	2.3
				HRM-3002A-02	J	-0.9	1.8	3.5	98.41	3.3
				HRM-3002A-03	L	-0.2	1.5	2.9	125.07	2.6
KRISS	1.9	1.7	3.5	111-01-01A	A	1.7	1.3	2.5	38.05	2.2
				111-01-02A	K	2.1	1.9	3.8	116.60	3.6
NIM	0.0	1.3	2.7	GBW09157	H	0.2	1.1	2.3	55.90	2.0
				GBW09169	I	-0.1	1.4	2.9	72.20	2.6
NIST	-0.2	1.3	2.5	SRM 1950	B	-0.4	1.2	2.4	41.87	2.1
				SRM 909c	C	0.0	1.2	2.5	45.70	2.1

a Percent relative expanded uncertainty, $100 \times U_{95}(V_i)/V_i$

b Material's certification withdrawn; not used to parameterize the KCRF

6.0 REFERENCES

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APPENDIX A: SOURCES OF INFORMATION

A.1 Reagents and Materials

Urea CRM (SRM 912a from NIST) with a purity of (99.9 ± 0.1) %, and uric acid CRM (SRM 913a from NIST) with a purity of (99.6 ± 0.1) % were used as the calibration standards. Isotope-labelled internal standards, $^{13}C,^{15}N_2$-urea (purity ≥ 98 %) and 1,3-$^{15}N_2$-Uric acid (Purity ≥ 98 %), were commercially obtained. All solutions and LC mobile phase were prepared using ultrapure water (resistivity=18.2 MΩ cm) from Mili-Q Integral System. HPLC-grade acetonitrile, LCMS-grade formic acid and high-purity (>99 %) ammonium formate were used to prepare the mobile phases.

A.2 Calibration and Internal Standard Solutions

Stock solutions of urea and $^{13}C,^{15}N_2$-urea were prepared gravimetrically in ethanol/water (v/v=10/90) using a balance with a readability of 0.01 mg and stored at -20 ºC when not in use. A 40-mL amber vial was used to prepare the solutions. Approximately 30 mg of urea or $^{13}C,^{15}N_2$-urea was weighed into the amber vial and about 16 mL of ethanol/water (v/v=10/90) was added to the vial. The final mass fraction was determined. This gave solutions of urea and $^{13}C,^{15}N_2$-urea with mass fractions of about 2000 µg/g. These solutions were combined to yield four calibration blends with isotope mass ratio (urea: $^{13}C,^{15}N_2$-urea) being close to 0.8, 0.9, 1.1, and 1.2, respectively. The calibration blends were diluted to about 2000 ng/g with acetonitrile for LC-MS/MS measurement. A working solution of $^{13}C,^{15}N_2$-urea internal standard for spiking in serum/plasma (see Section A.4) was prepared by diluting the stock solution of $^{13}C,^{15}N_2$-urea to about 100 µg/g.

Stock solutions of uric acid and 1,3-$^{15}N_2$-uric acid were prepared gravimetrically in 2 mmol/L aqueous ammonia using a balance with a readability of 0.01 mg and stored at -20 ºC when not in use. A 250 mL plastic bottle was used to prepare the solutions. Approximately 25 mg of uric acid or $^{13}C,^{15}N_2$-uric acid was weighed into the plastic bottle and about 125 mL of aqueous ammonia was added to the bottle and the final mass fraction was determined. This gave solutions of uric acid and 1,3-$^{15}N_2$-uric acid with mass fractions of about 200 µg/g. These solutions were combined to yield four calibration blends with isotope mass ratio (uric acid: 1,3-$^{15}N_2$-uric acid) being close to 0.85, 0.95, 1.05, and 1.15, respectively. The calibration blends were diluted to about 2000 ng/g with 2 mmol/L aqueous ammonia for LC-MS/MS measurement. A working solution of 1,3-$^{15}N_2$-uric acid internal standard for spiking in serum/plasma (see Section A.4) was prepared by diluting the stock solution of 1,3-$^{15}N_2$-uric acid to about 100 µg/g.

A.3 Reconstitution of Lyophilised Material

The lyophilised material from CENAM (DMR-263c) was gravimetrically reconstituted following the instruction in the Certificate of Analysis of DMR-263c. The actual mass of water added for reconstitution was recorded, and was used to correct the measurement results.

A.4 Sample Preparation

Frozen serum/plasma materials to be analysed were removed from -70 ºC storage and allowed to equilibrate to room temperature. Lyophilised material (DMR-263c) was freshly reconstituted before measurement. The sampling size was 0.1 mL for urea measurement and 0.2 mL for uric acid measurement. Based on the certified values of each material, each sample blend was prepared gravimetrically by spiking appropriate amount of isotope-labelled internal standard solution into the material to control the isotope mass ratio to be within the acceptable range of 0.95 – 1.05, with an optimal value of 1.0. Four-point calibration curve was used for both urea and uric acid measurements (see Section A.2 for the isotope mass ratio of the calibration blends).

For urea measurement, the prepared sample blends were kept at ambient temperature (18 to 25) ^{o}C for at least 1 h for equilibration. Acetonitrile (three-fold of aqueous volume) was then added to each sample blend for protein precipitation. The mixtures were vortexed vigorously and centrifuged for 5 min at 419 rad/s (4000 rpm). The supernatant of each mixture was filtered through 0.22 µm syringe filter, and diluted to approximately 2000 ng/g with acetonitrile for LC-MS/MS measurement.

For uric acid measurement, the prepared sample blends were kept at ambient temperature (18 to 25) ^{o}C for at least 2 h for equilibration. Appropriate amount of water was added so that the total volume of the aqueous phase (isotope-labelled internal standard solution and the top-up water) was the same as that of the material (0.2 mL of serum/plasma material). Acetonitrile (one-fold of aqueous volume) was then added to each sample blend for protein precipitation. The mixtures were vortexed vigorously and centrifuged for 5 min at 419 rad/s (4000 rpm). The supernatant of each mixture was evaporated to dryness under N_2 at 30 ^{o}C, and reconstituted with appropriate amount of 2 mmol/L aqueous ammonia so that the concentration was about 2000 ng/g before LC-MS/MS measurement.

A.5 Quality Control

Two serum CRMs from HSA (HRM-3003A-01 and HRM-3002B-02) that were not included as study materials in CCQM-K142 were used as the quality control materials. The quality control materials were measured together with the study

materials in both campaigns, and the obtained values for both urea and uric acid were found to be well within the uncertainty ranges of the certified values.

A.6 Instrumentation

A Shimadzu 8040 mass spectrometer coupled with a Prominence UFLC LC20AD system was used to analyse all materials.

The column used for urea measurement was an Agilent RX-SIL column, 150 mm × 2.1 mm, 5 µm. The LC parameters were: mobile phase A, 0.1 % formic acid in water; mobile phase B, 0.1 % formic acid in acetonitrile; flow rate, 0.5 mL/min; gradient, isocratic (95 % mobile phase B); column temperature, ambient temperature (18 to 25) ^{o}C; injection volume, 10 µL. The MS detection parameters were: positive-mode electrospray ionisation; CID gas, 230 kPa; conversion dynode, - 6.00 kV; interface volt, 4.50 kV; DUIS corona needle, 4.50 kV; interface temperature, 350 ^{o}C; DL temperature, 300 ^{o}C; nebulising gas, 3.00 L/min; heat block, 500 ^{o}C; dying gas, 15.00 L/min. Multiple Reaction Monitoring (MRM) was used to detect urea at m/z 61→44 and ^{13}C,$^{15}N_2$-urea at m/z 64→46.

The column used for uric acid measurement was an Agilent Zorbax SB-Aq, 100 mm × 2.1 mm × 3.5 µm. The LC parameters were: mobile phase A, 5 mmol/L ammonium formate in water with 0.05 % formic acid; mobile phase B, acetonitrile; flow rate, 0.3 mL/min; gradient, isocratic (2 % mobile phase B); column temperature, ambient temperature (18 ^{o}C to 25 ^{o}C); injection volume, 10 µL. The MS detection parameters were: negative-mode electrospray ionisation; CID gas, 230 kPa; conversion dynode, 6.00 kV; interface volt, - 3.50 kV; DUIS corona needle, - 3.50 kV; interface temperature, 350 ^{o}C; DL temperature, 250 ^{o}C; nebulising gas, 3.00 L/min; heat block, 400 ^{o}C; dying gas, 15.00 L/min. Multiple Reaction Monitoring (MRM) was used to detect uric acid at m/z 167→124 and 1,3-$^{15}N_2$-uric acid at m/z 169→125.

APPENDIX B: OpenBUGS Analysis Code

B.1 Urea Materials

```
# Scalars
# a...... intercept
# b...... slope
# n0..... number of materials (here, 10)
# n1..... number of units per material (here, 2)
# n2..... number of aliquots per unit materials (here, 2)
# n3..... number of repeats per aliquot (here, 6)
# pmthd.. instrumental 1/(relative variance)
# smthd.. instrumental relative SD
#
# Vectors
# doe[n0]..... degree of equivalance
# prept[n0]... instrumental 1/variance
# pVhat[n0]... 1/(uVda2 * uVda2)
# pVtru[n0]... 1/(uVda1 * uVda1)
# uVda1[n0].. certified uncertainties
# uVda2[n0].. same as uVda1
# Vda1[n0]... Certified values
# Vda2[n0]... identical to Vdat1
# Rhat[n0].... predicted R values
# srept[n0]... instrumental SDs
#
# Matrices
# dlta[n0,n1,n2].... unit-related bias
# gmma[n0,n1]....... aliquot-related bias
# pdlta[n0,n1,n2]... unit-related 1/variance
# pgmma[n0,n1]...... aliquot-related 1/variance
# Rdat[0,n1,n2,n3].. individual R measurments

Models... R=a+bV and R=bV{
# Regression parameters: you must de-comment one of the two "a" definitions
#a~dnorm(0,1.0E-5)  #Remove the initial "#" for R=a+bV
#a<-0               #Remove the initial "#" for R=bV
b~dnorm(1,1.0E-5)
#
# Instrumental variability-related parameter & distributions
pmthd~dgamma(1.0E-5,1.0E-5);smthd<-100/sqrt(pmthd)
for(i in 1:n0){prept[i]<-pmthd/pow(Vda1[i],2);srept[i]<-1/sqrt(prept[i])}
#
# Certified value-related distributions
for(i in 1:n0){Vtru[i]~dnorm(0,1.0E-5);
             pVtru[i]<-1/pow(uVda1[i],2);Vda1[i]~dnorm(Vtru[i],pVtru[i])}
for(i in 1:n0){Vhat[i]~dnorm(0,1.0E-5);
             pVhat[i]<-1/pow(uVda2[i],2);Vda2[i]~dnorm(Vhat[i],pVhat[i])}
#
# Regression-related predictions
for(i in 1:n0){Rhat[i]<-a+b*Vtru[i]}
#
# Measurement/ANOVA-related distributions
for(i in 1:n0){for(j in 1:n1)
             {pgmma[i,j]~dgamma(1.0E-5,1.0E-5);gmma[i,j]~dnorm(Rhat[i],prept[i])}}
for(i in 1:n0){for(j in 1:n1){for(k in 1:n2)
             {pdlta[i,j,k]~dgamma(1.0E-3,1.0E-3);
             dlta[i,j,k]~dnorm(gmma[i,j],pgmma[i,j])}}}
for(i in 1:n0){for(j in 1:n1){for(k in 1:n2)
             {for(l in 1:n3){Rdat[i,j,k,l]~dnorm(dlta[i,j,k],pdlta[i,j,k])}}}}
#
# doe estimation
for(i in 1:n0){doe[i]<-200*(Vhat[i]-Vtru[i])/(Vtru[i]+Vhat[i])}
}
```

```
#
# CRMs
Vda1[]        uVda1[]       Vda2[] uVda2[]             #PI          CRM
264.8         3.37          264.8         3.37         #CENAM       DMR-263a
323.78        6.34          323.78        6.34         #CENAM       DMR-263b
871.09        15.56         871.09        15.56        #CENAM       DMR-263c
156.9         1.7           156.9         1.7          #KRISS       111-01-01A
1129          12.5          1129          12.5         #KRISS       111-01-02A
253.39        2.59          253.39        2.59         #NIST        SRM-1950
229.71        2.4           229.71        2.4          #NIST        SRM-909c
319.6         2.24          319.6         2.24         #HSA         HRM-3002A-03
449           3.82          449           3.82         #HSA         HRM-3002B-01
781.68        7.33          781.68        7.33         #HSA         HRM-3002A-02
END

#
# Measurements
list(n0=10,n1=2, n2=2, n3=6,
Rdat=structure(.Data=c(
273.112,263.107,268.609,266.129,272.672,269.231,266.934,264.413,267.007,266.210,267.269,265.209,
265.118,264.396,264.312,266.797,264.624,262.995,261.851,261.633,263.727,262.273,264.589,265.936,
317.433,310.763,322.585,315.165,318.392,315.810,313.448,311.819,316.269,320.282,319.142,315.324,
312.546,314.012,318.249,316.691,315.413,318.805,309.991,316.344,316.116,314.799,315.471,314.161,
853.538,835.607,837.379,837.735,841.379,844.596,852.319,828.052,838.884,848.150,844.413,853.802,
851.240,847.722,859.783,866.089,880.383,875.292,846.342,858.454,853.467,875.550,870.518,881.092,
156.218,157.198,157.265,158.725,157.638,157.266,156.969,155.904,155.695,155.416,154.969,157.844,
155.162,156.766,158.144,157.288,159.873,156.669,156.667,154.482,157.023,157.477,155.457,156.119,
1116.959,1145.762,1105.628,1125.694,1117.021,1142.785,1121.070,1139.803,1130.295,1133.167,1125.823,1145.700,
1130.389,1124.357,1133.044,1131.544,1130.141,1129.057,1122.244,1129.328,1122.145,1123.270,1130.418,1129.916,
254.761,246.263,254.750,252.290,256.222,254.228,249.229,249.351,248.648,250.667,250.587,255.495,
250.279,251.042,254.822,253.763,253.840,251.925,246.609,252.666,253.361,252.545,253.243,254.449,
221.166,220.712,222.034,228.447,224.924,226.948,222.182,219.179,219.906,221.388,228.308,225.131,
221.735,223.773,221.094,226.865,225.173,222.644,219.630,224.546,223.280,228.115,222.681,223.927,
321.630,313.537,321.305,317.498,325.018,322.381,316.214,314.520,323.620,315.376,320.533,322.610,
315.655,320.493,318.125,319.918,319.507,322.106,314.951,316.344,319.388,318.420,322.903,313.290,
445.909,446.649,452.884,442.676,456.118,452.191,440.737,440.321,448.216,443.655,455.929,447.598,
446.284,449.097,444.905,445.927,446.555,448.734,441.460,450.857,449.204,447.694,450.999,448.297,
789.039,783.710,782.099,777.961,788.245,790.696,799.141,769.611,770.279,769.846,789.800,773.318,
760.811,777.947,780.893,784.772,792.357,784.946,791.900,779.190,777.054,782.862,792.583,784.600
),.Dim=c(10,2,2,6)))

#
# Inits
list(pmthd=1,
pgmma=structure(.Data=c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1),.Dim=c(10,2)),
pdlta=structure(.Data=c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1
,1,1,1,1,1,1,1,1,1,1),.Dim=c(10,2,2)))
```

B.2 Uric Acid Materials

```
# Scalars
# a..... intercept parameterized on 11 CCQM-K142 materials
# a.cut.. intercept applied to DMR-263b
# b..... slope parameterized on 11 eligible CCQM-K142 materials
# b.cut.. slope applied to DMR-263b
# n0..... number of materials (here, 15)
# n1..... number of units per material (here, 2)
# n2..... number of aliquots per unit materials (here, 2)
# n3..... number of repeats per aliquot (here, 6)
# pmthd.. instrumental 1/(relative variance)
# smthd.. instrumental relative SD
#
# Vectors
# doe[n0]..... degree of equivalance
# prept[n0]... instrumental 1/variance
# pVhat[n0]... 1/(uVda2 * uVda2)
# pVtru[n0]... 1/(uVda1 * uVda1)
# uVda1[n0]... certified uncertainties
# uVda2[n0]... same as uVda1
# Vda1[n0].... Certified values
# Vda2[n0].... identical to Vdat1
# Rhat[n0].... predicted R values
# srept[n0]... instrumental SDs
#
# Matrices
# dlta[n0,n1,n2].... unit-related bias
# gmma[n0,n1]....... aliquot-related bias
# pdlta[n0,n1,n2]... unit-related 1/variance
# pgmma[n0,n1]...... aliquot-related 1/variance
# Rdat[0,n1,n2,n3].. individual R measurments

Models... R=a+bV and R=bV{
# Regression parameters: you must de-comment one of the two "a" definitions
#a~dnorm(0,1.0E-5)  #Remove the initial "#" for R=a+bV
#a<-0               #Remove the initial "#" for R=bV
b~dnorm(1,1.0E-5)
#
# instrumental variability-related parameter & distributions
pmthd~dgamma(1.0E-5,1.0E-5);smthd<-100/sqrt(pmthd)
for(i in 1:n0){prept[i]<-pmthd/pow(Vda1[i],2);srept[i]<-1/sqrt(prept[i])}
#
# Certified value-related distributions
for(i in 1:n0){Vtru[i]~dnorm(0,1.0E-5);
              pVtru[i]<-1/(uVda1[i]*uVda1[i]);Vda1[i]~dnorm(Vtru[i],pVtru[i])}
for(i in 1:n0){Vhat[i]~dnorm(0,1.0E-5);
              pVhat[i]<-1/(uVda2[i]*uVda2[i]);Vda2[i]~dnorm(Vhat[i],pVhat[i])}
#
# Regression-related predictions
# The "cut" function is used limit updating a & b to just the eligible materials.
for(i in 1:11){Rhat[i]<-a+b*Vtru[i]}
a.cut<-cut(a);b.cut<-cut(b)
for(i in 12:n0){Rhat[i]<-a.cut+b.cut*Vtru[i]}
#
# Measurement/ANOVA-related distributions
for(i in 1:n0){for(j in 1:n1)
              {pgmma[i,j]~dgamma(1.0E-5,1.0E-5);gmma[i,j]~dnorm(Rhat[i],prept[i])}}
for(i in 1:n0){for(j in 1:n1){for(k in 1:n2)
              {pdlta[i,j,k]~dgamma(1.0E-3,1.0E-3);
              dlta[i,j,k]~dnorm(gmma[i,j],pgmma[i,j])}}}
for(i in 1:n0){for(j in 1:n1){for(k in 1:n2)
              {for(l in 1:n3){Rdat[i,j,k,l]~dnorm(dlta[i,j,k],pdlta[i,j,k])}}}}
#
# doe estimation
for(i in 1:n0){doe[i]<-200*(Vhat[i]-Vtru[i])/(Vtru[i]+Vhat[i])}
}
```

```
#
# CRMs
Vda1[]        uVda1[]       Vda2[]        uVda2[]       #PI           CRM
50.96         2.25          50.96         2.25          #CENAM        DMR-263a
52.40         1.51          52.40         1.51          #CENAM        DMR-263c
38.05         0.41          38.05         0.41          #KRISS        111-01-01A
116.6         2.1           116.6         2.1           #KRISS        111-01-02A
55.90         0.55          55.90         0.55          #NIM          GBW09n07
72.20         0.95          72.20         0.95          #NIM          GBW09169
45.70         0.49          45.70         0.49          #NIST         SRM-909c
41.87         0.44          41.87         0.44          #NIST         SRM-1950
48.32         0.561         48.32         0.561         #HSA          HRM-3002B-01
98.41         1.64          98.41         1.64          #HSA          HRM-3002A-02
125.07        1.64          125.07        1.64          #HSA          HRM-3002A-03
55.00         2.00          55.00         2.00          #CENAM        DMR-263b
END

#
# Measurements
list(n0=12,n1=2, n2=2, n3=6,
Rdat=structure(.Data=c(
53.221,53.849,54.687,52.471,54.479,52.812,52.618,53.781,53.314,53.899,52.776,52.262,
51.715,52.067,51.942,52.117,53.439,52.597,53.097,52.816,52.942,52.470,52.389,52.885,
53.924,51.502,54.107,53.997,53.520,55.247,53.716,53.353,53.989,52.973,54.663,54.303,
53.103,52.970,54.181,53.301,54.332,53.914,54.897,52.513,54.917,53.191,53.355,53.328,
37.815,37.249,35.924,37.629,37.629,38.234,37.273,36.829,37.647,36.878,37.582,37.634,
36.534,36.679,36.881,37.303,36.567,37.338,37.568,36.901,37.236,36.931,35.699,36.941,
114.457,111.671,114.113,114.431,114.123,109.717,114.219,113.634,113.228,114.234,115.508,114.278,
114.640,112.476,116.623,113.220,112.125,114.053,114.985,112.770,113.541,111.687,112.764,111.965,
57.545,55.487,57.292,56.094,55.299,56.084,56.532,55.812,56.119,55.391,55.555,56.195,
54.878,55.462,55.268,54.376,55.274,55.798,54.985,55.623,55.098,55.990,55.393,55.794,
73.472,74.088,70.497,72.348,71.465,72.709,72.013,71.224,70.720,73.693,72.090,74.158,
72.194,73.169,72.194,70.797,71.879,72.518,72.677,72.713,72.070,72.826,70.599,70.546,
46.007,45.589,46.422,46.874,45.831,45.217,44.089,46.156,45.537,46.166,46.342,46.777,
44.812,45.920,45.895,45.362,45.359,46.159,45.154,44.964,44.372,44.360,45.576,45.000,
42.076,41.642,42.021,43.036,41.504,43.596,40.861,42.403,43.793,41.518,42.198,43.013,
41.751,41.578,41.870,41.572,42.236,40.083,41.776,42.062,41.350,42.163,41.848,42.254,
49.997,48.209,49.777,48.737,48.046,48.728,48.826,51.023,49.193,47.918,48.761,48.213,
48.469,48.777,48.723,48.303,48.644,47.976,48.018,47.218,47.226,48.670,48.243,47.369,
100.777,101.621,96.695,99.235,98.023,99.730,100.098,103.531,99.424,101.493,97.843,98.817,
98.046,100.783,99.651,98.344,98.693,97.546,96.912,99.383,96.747,102.117,98.690,100.001,
126.397,124.503,120.073,125.775,125.775,127.795,128.768,126.867,129.412,124.985,125.751,126.046,
125.377,124.542,124.112,123.267,122.339,125.597,123.560,123.946,125.443,126.721,123.470,125.304,
50.285,50.925,50.735,48.261,50.829,50.387,49.975,49.743,49.214,49.738,49.933,50.031,
49.124,49.567,48.932,49.268,50.662,49.073,48.574,48.590,48.435,47.972,47.446,50.155),
.Dim=c(12,2,2,6)))

#
# Inits
list(pmthd=1,
pgmma=structure(.Data=c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1),.Dim=c(12,
2)),
pdlta=structure(.Data=c(1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1
,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1),.Dim=c(12,2,2)))
```

APPENDIX C: Corrective actions on Uric Acid by CENAM

C.1 Introduction

DMR-263b Frozen Human Serum uric acid was measured at CENAM by an isotope dilution liquid chromatography mass spectrometry (ID-LC-MS) method to assign uric acid value. As follow up of CENAM results of DMR-263b for uric acid in CCQM-K142, and to investigate the CENAM method used for value assignment, the DMR-263b was measured again in CENAM and NIST, using isotope dilution gas chromatography mass spectrometry (ID-GC-MS) methods.

C.2 CENAM Measurements

Materials

Calibrant: SRM 913b Uric Acid, (99.8 ± 0.2) % pure (NIST, Gaithersburg, MD)
CMR CRM 6008-a Uric Acid, (99.6 ± 0.3) % pure (NMIJ, Tsukuba, JP)

Isotope: Uric acid-1,3-$^{15}N_2$ (Cambridge Isotope Laboratories Inc), 98 atom % pure.

Control: SRM 909c Frozen Human Serum (NIST, Gaithersburg, MD)

Samples preparation

Uric acid-$^{15}N_2$ was used, and for derivatization MTBSTFA, imidazole and acetonitrile were used, samples were dried under N_2, then after the internal standard was added and equilibrated overnight. Solid phase extraction was used for cleanup.

Results

The uric acid for CENAM CRM samples by ID-GC-MS are shown in Table-C1. The values obtained for uric acid in the control material SRM 909c (Table C-2) using the ID-GC-MS method modified by CENAM, are in good agreement with the certified value.

Table C-1. Uric acid in DMR-263b Frozen Human Serum samples.

ID sample	# vial	Aliquot	Mass fraction (mg/kg)	Mass Concentration (mg/dL)	Mass Concentration (mg/dL)	Mass Concentration vial mean (mg/dL)	Vial SD (mg/dL)	Vial CV (%)
DMR-263b	180	7	54.5227	5.591	5.5628	5.508	0.064	1.16
			54.1062	5.548				
			54.1213	5.550				
		8	53.3042	5.466	5.4527			
			53.3103	5.466				
			52.9146	5.426				

Table C-2. Uric acid in SRM 909c Frozen Human Serum as a control

ID sample	Aliquot	Mass concentration (mg/dL)	Mass concentration mean (mg/dL)	SD (mg/dL)	CV (%)
SRM 909c	SM-1	4.662	4.6294	0.0315	0.68
		4.658			
		4.650			
	SM-2	4.621			
		4.593			
		4.594			
Certified Value: (4.68 ± 0.10) mg/dL					

C.3 NIST-CENAM Measurements.
Calibrant: SRM 913b Uric Acid, (99.8 ± 0.2) % pure (NIST, Gaithersburg, MD)
Isotope: Uric acid-1,3-$^{15}N_2$ (Cambridge Isotope Laboratories Inc), 98 atom % pure.
Control: SRM 909c Frozen Human Serum (NIST, Gaithersburg, MD)

Sample Preparation.
Calibrants: Stock solutions of SRM 913b and uric acid labeled (1,3-$^{15}N_2$) were accurately weighed, solved with 0.001 mol/L solution of ammonia in boiled distilled water and allowed to stand overnight. Next day, they were sonicated.

Aliquots of two stock solutions were weighed varying the ratio of unlabeled: labeled material. The calibration standards were mixed and allowed to equilibrate overnight. The following day, the calibrants were concentrated to dryness under a stream of nitrogen in a water bath and derivatized with MTBSTFA with 1 % imidazole.

Units of DMR-263b Frozen Human Serum and control SRM 909c were removed from the freezer, allowed to warm to room temperature. Aliquots of serum were weighed in test tubes with the labeled internal standard.

The samples were mixed and allowed to equilibrate overnight. Next day, the samples were processed through the pre-packed Bio-Rad anion exchange cartridges, solvent was evaporated under nitrogen in a water bath. The dried residues were derivatized with MTBSTFA with imidazole and acetonitrile. Samples and standards were heated and centrifuged. Finally, an aliquot of each standard and samples were diluted with acetonitrile and injected into the GC/MS.

Results
The uric acid for CENAM CRM samples by ID-GC-MS are shown in Table C-3. The values obtained for uric acid in the control material SRM 909c (Table C-4) using the ID-GC-MS method at NIST, are in good agreement with the certified value.

Table C-3. Uric acid in DMR-263b Frozen Human Serum samples.

ID sample	# vial	Aliquot	Mass fraction (mg/kg)	Mass Concentration (mg/dL)	Mass Concentration (mg/dL)	Mass Concentration vial mean (mg/dL)	Vial SD (mg/dL)	Vial CV (%)
DMR-263b	109	5	50.7933	5.2083	5.2002	5.2018	0.0022	0.043
			50.6342	5.1920				
		6	50.7053	5.1993	5.2034			
			50.7839	5.2074				
	143	7	50.5413	5.1825	5.1880	5.1949	0.0098	0.188
			50.6310	5.1917				
			50.6121	5.1898				
		8	50.5878	5.1873	5.2018			
			50.9964	5.2292				
			50.6047	5.1890				
Average				**5.1976**	mg/dL	**5.1977**	mg/dL	
SD				**0.0139**	mg/dL	**0.0085**	mg/dL	
CV				**0.2680**	%	**0.1628**	%	

Table C-4. Uric acid in SRM 909c Frozen Human Serum as a control

ID sample	Aliquot	Mass Concentration (mg/dL)	Mass Concentration mean (mg/dL)	SD (mg/dL)	CV (%)
SRM 909c	9	4.6186	4.598	0.028	0.618
		4.6257			
	10	4.5677			
		4.5804			
Certified Value: (4.68 ± 0.10) mg/dl					

C.4 Discussion

Some notable differences between the methods were observed (Table C-5) that may be attributable to the differences between the analytical methodologies.

Table C-5. Main differences observed between methodologies

LC Method	GC Method
Stock solutions	
- Preparation of stock solutions using 5 mmol/L ammonium hydroxide as solvent, starting from ammonium hydroxide 36 % pure	- Preparation of stock solutions using 1 mmol/L ammonium hydroxide as solvent, starting from 5 mmol/L ammonium hydroxide and boiled water. - Solutions allowed to stand overnight
Sample and Calibrants Preparation	
- Weighed samples and calibration standards, addition of uric acid unlabeled	- Weighed samples and calibration standards, addition of uric acid unlabeled - Equilibration overnight
Sample Cleaning	
- Precipitation of proteins adding MeOH and ACN (twice). - Centrifugation - Concentration of sample to dryness under a stream of nitrogen in a water bath at 60 °C - Reconstitution of samples with mobile phase	- Use of BIO RAD SPE cartridge to isolate target analyte (cartridge conditioned using water, MeOH). - Elution of uric acid with 1 mmol/L acetic acid - Concentration of sample to dryness under a stream of nitrogen in a water bath at (45 to 50) °C - Derivatization (12 h) - Centrifugation
Injection	
- Aliquot of reconstituted sample is filtered and injected - Isocratic flow, mobile phase is ammonium acetate 2 mmol/L adjusted to pH 5.5 with formic acid.	- Aliquot of derivatized sample is diluted with ACN and injected - Temperature ramp (200 to 300) °C

Table C-6 summarises the main differences observed in the procedures; they are: 1) concentration of ammonium hydroxide; uric acid can be decomposed to at least four products when it is dissolved with high concentration[2], and this could affect the standard and internal standard reaction in a different manner 2) sample cleaning process; solid extraction phase for GC-MS vs precipitation of proteins for LC-MS, and 3) the sensitivity of the MS instrument, even if the same technique is employed.

Table C-6. Main differences observed between methodologies

NIST GC Method	**CENAM GC Method**
Stock solutions	
- Preparation of stock solutions using 1 mmol/L ammonium hydroxide as solvent, starting from 5 mmol/L ammonium hydroxide and boiled water. - Solutions allowed to stand overnight	- Preparation of stock solutions using 1 mmol/L ammonium hydroxide as solvent, starting from 35 % ammonium hydroxide and boiled water. - Solutions allowed to stand overnight
Sample and Calibrants Preparation	
- Weighed samples and calibration standards, addition of uric acid unlabeled - Equilibration overnight	- Weighed samples and calibration standards, addition of labeled uric acid - Equilibration overnight
Sample Cleaning	
- Use of BIO RAD SPE cartridge to isolate target analyte (cartridge conditioned using water, MeOH). - Elution of uric acid with 1M acetic acid - Concentration of sample to dryness under a stream of nitrogen in a water bath at (45 to 50) °C - Derivatization (12 h)	- Use of Waters Oasis MAX cartridge to isolate target analyte (cartridge conditioned using water, MeOH). - Elution of uric acid with 1 mol/L acetic acid - Concentration of sample to dryness under a stream of nitrogen in a water bath at (45 to 50) °C - Derivatization (18 h) - Centrifugation
Injection	
- Aliquot of derivatized sample is diluted with ACN and injected	- Aliquot of derivatized sample is injected

In consequence CENAM will further review its HPLC MS-MS method for uric acid in serum.

Table C-7 and Figure C-1 compare GC-MS DMR-263b values. Table C-8 and Figure C-2 compare GC-MS SRM 909c values.

Table C-7. Comparison of DMR-263b results

Method	Mass fraction (mg/kg)	*u*(Mass fraction) (mg/kg)	Difference (mg/kg)	Relative Difference (%)
Measured value K142 Reg 1	49.990	2.000	-	-
Measured value K142 Reg 2	50.180	0.960	-	-
Certified value CENAM [a]	55.000	2.000	5.010	10
Measured value GC-MS CENAM-NIST	50.708	0.014	0.718	1
Measured value GC-MS CENAM	53.737	0.064	3.747	7

a CENAM certified value by LC-MS-MS GC-MS

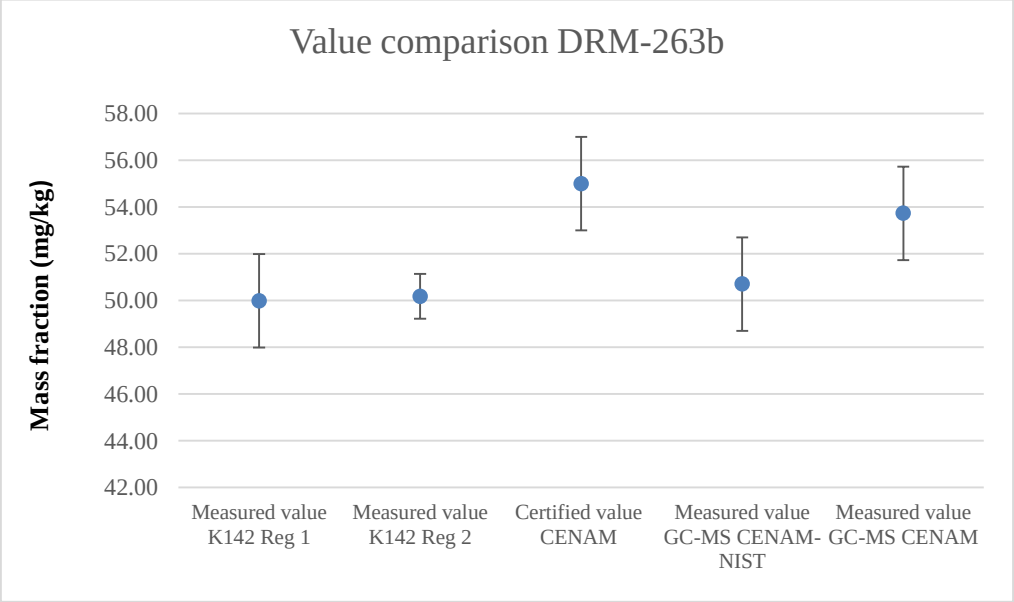

Figure C-1. Comparison of DMR-263b results.
Bars span ± 1 standard uncertainties.

As can be observed, the quantified value at NIST by CENAM staff, using the methodology established there, is in good agreement with the assigned values by the comparison CCQM-K142, and with measured value by GC-MS in CENAM but in less grade, and is not in agreement with the CENAM certified value. In consequence, the CENAM CRM DMR-263b is under review and is not currently available for sale.

Table C-8. Comparison of SRM 909c results

Method	Concentration (mg/dL)	*u*(Concentration) mg/dL	Difference (mg/dL)	Relative Difference (%)
Certified value SRM-909c	4.68	0.05	-	-
Measured value GC-MS CENAM-NIST	4.75	0.01[a]	0.069	1
Measured value GC-MS CENAM	4.63	0.03[a]	-0.051	-1

a Measurement precision, excludes bias components

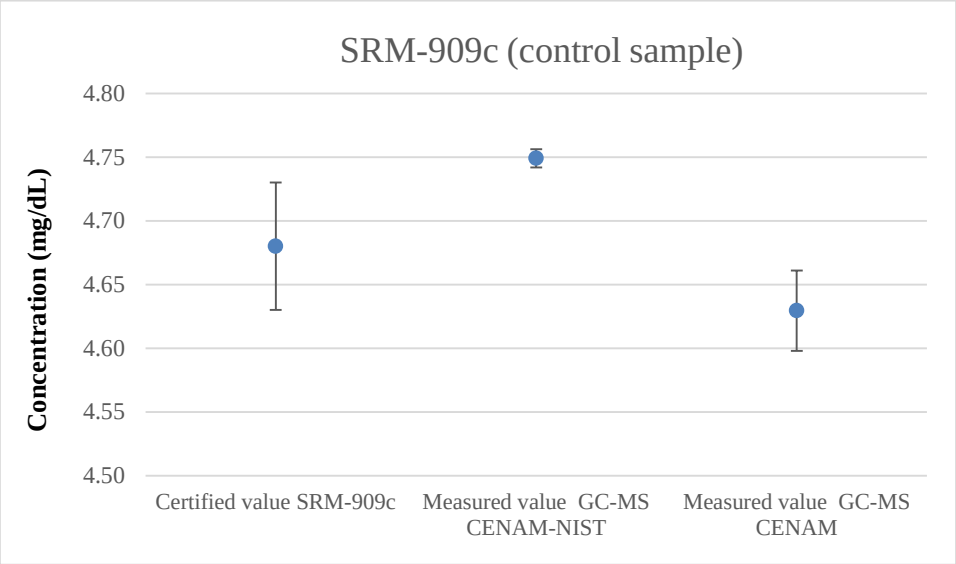

Figure C-2. Comparison of SRM-909c used as control
Bars span ± 1 standard uncertainties.

As Figure C-2 shows, the results for the control sample in both GC-MS methods are near the reference value but in reverse directions. This is just the opposite of the DMR-263b results shown in Figure C-1 for the two GC-MS methods. This behavior could be due to several reasons, such as matrix and method differences and measurement correlations.