CCQM-K63.a,b

Non-Peptide Hormones in Serum: Cortisol and Progesterone

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ABSTRACT

Hormones are chemical messengers that regulate many life functions. Deviations from normal hormone levels can have serious health consequences. Accurate measurement of hormone levels in serum can be beneficial in diagnosing, monitoring, and treating a number of diseases. Two steroid hormones, cortisol and progesterone, were selected by the Organic Analysis Working Group (OAWG) to evaluate its member Institutes' measurement capabilities for this important class of measurand.

Serum concentrations of cortisol range from 30 ng/mL to 230 ng/mL. Serum concentrations pf progesterone in adult females range from 0.15 ng/mL to 25 ng/mL but can rise to ≈ 230 ng/mL during pregnancy. The ability to measure cortisol is indicative of a laboratory's ability to measure steroid hormones at concentration levels similar to cortisol. The ability to measure progesterone is indicative of a laboratory's ability to measure steroid hormones with similar functional groups and concentration levels, such as testosterone.

Pilot studies CCQM-P77.a and CCQM-P77.b on the determination of cortisol and progesterone in human serum were completed in 2006. There was good agreement among the results reported by participants who used isotope dilution/mass spectrometry (ID/MS) with either gas chromatography (GC) or liquid chromatography (LC). In 2007 the OAWG decided to proceed with Key Comparison (KC) CCQM-K63.a Cortisol in Human Serum and CCQM-K63.b Progesterone in Human Serum. Thus, following established OAWG procedure, only results from participants that 1) used an ID/MS-based method, 2) participated in the relevant pilot study, and 3) used a metrologically traceable primary standard were to be eligible for use in calculating the Key Comparison Reference Value (KCRV) for each measurand.

Six laboratories participated in CCQM-K63.a and eight laboratories participated in CCQM-K63.b. The same pooled frozen female serum material was used in both of the KCs. The mean value for the six ID/MS-based cortisol results is 100.4 ng/g with a relative standard deviation (%RSD) of 1.1%. The mean value for the seven ID/MS-based progesterone originally reported results is 2.83 with a %RSD of 1.8%. While a number of the reported results were not eligible to be used in establishing the KCRVs, the KCRVs as estimated from just the eligible results agree very well with these means. The excellent among-participant agreement indicates that ID/MS-based measurement procedures can provide precise and true results for steroid hormones at levels greater than about 1 ng/g. The progesterone results reported by a laboratory using a non-isotopically labelled internal standard was about 11% larger than the KCRV.

INTRODUCTION AND CONDUCT OF STUDY

Hormones are chemical messengers that regulate many life functions, and deviations from normal levels can have serious health consequences. Accurate measurement of levels of hormones in serum can be beneficial in diagnosing, monitoring, and treating a number of diseases. At the CCQM Organic Analysis Working Group (OAWG) meeting of September 2005, pilot studies for the measurement of two steroid hormones were proposed, and these studies were approved at the April 2006 meeting. For the pilot studies two steroid hormones, cortisol and progesterone, were selected to compare measurement capabilities. Cortisol is a steroid hormone that is produced by the adrenal gland, and it is often called the "stress hormone" because it is secreted at higher levels in response to stress. Prolonged elevation of cortisol levels in the bloodstream can have negative effects on thyroid function, metabolism, and immune function. Measurement of cortisol in serum can be used to diagnose problems in the adrenal or pituitary gland, as well as stress-related disorders. Serum concentrations of cortisol range from 30 ng/mL to 230 ng/mL. Progesterone is involved in regulating female reproductive processes, and serum concentrations in adult females range from 0.15 ng/mL to 25 ng/mL but can rise to ≈ 230 ng/mL during pregnancy.

In 2006, pilot studies CCQM-P77.a and CCQM-P77.b were carried out on the determination of cortisol and progesterone in human serum. NIST served as the coordinating laboratory for both studies. A candidate Standard Reference Material (SRM) 971 consisting of two pooled human serum materials (female and male) was used for these pilot studies. Results were discussed at the CCQM OAWG meeting of October/November 2006. For cortisol (CCQM-P77.a), six laboratories submitted results for both female and male serum materials. For progesterone (CCQM-P77.b), five laboratories submitted results for female serum and three laboratories submitted results for the male serum. Except for the very low level of progesterone in the male serum, agreement among the laboratories using isotope dilution/mass spectrometry (ID/MS) was generally good. Therefore the CCQM OAWG decided to proceed with Key Comparisons (KCs) for cortisol and progesterone in serum. The two KCs were designated as CCQM-K63.a Cortisol in Human Serum, and CCQM-K63.b Progesterone in Human Serum. Laboratories could choose to participate in either or both of the KCs. As with the pilot studies, NIST served as the coordinating laboratory.

A pooled frozen female serum material, K63, was prepared and characterized at NIST for use in both the K63.a and K63.b. The serum was not fortified with any hormones. Each vial contained 5 mL of frozen serum. Homogeneity testing was performed on this material for both cortisol and progesterone, and the results indicated that among-vial variation (< 0.2%) was not statistically significant. This K63 material and the proposal for CCQM-K63.a and .b were approved at the CCQM OAWG meeting of April 2007. For each analyte, four vials of the K63 material and three vials of a material used in CCQM-P77 were provided to each participating laboratory. Measurement results in nanograms per gram (ng/g) of serum for three sets, each set consisting of one vial of K63 material, were requested for each study. Based on the results from the pilot studies, ID/MS-based methods with either gas chromatography (GC) or liquid chromatography (LC) were established to provide acceptable results for the determination of serum cortisol and progesterone. Therefore only results from ID-GC/MS, ID-LC/MS, or ID-LC/MS/MS were included in calculating the Key Comparison Reference Value (KCRV).

LABORATORIES RECEIVING SAMPLES

CENAM, Mexico
KRISS, Korea
LGC, UK
NIM, China
NIST, USA
NMIA, Australia
NMIJ, Japan
PTB, Germany

CENAM and NMIA chose to participate only in CCQM-K63.b. All other laboratories participated in both CCQM-K63.a and CCQM-K63.b.

SCHEDULE

April 2007	Proposal and material approved
August 2007	Finalize participant list
August-September 2007	Send samples
February 2008	Results due
March-April 2008	Discussion of results
June-August 2008	Prepare Draft B Report and distribute to OAWG
August-September 2007	Discussion of Draft B
November 2008	OAWG Fall meeting

CMC CLAIMS

Because the concentration levels of most other serum corticosteroids are significantly lower than that of cortisol, the CMC claims based on measurements of cortisol in this study apply only to serum cortisol. The CMC claims based on measurement of progesterone in this study can be applied to other serum steroid hormones with similar functional groups and concentration levels (greater than 1 ng/g of serum), such as testosterone.

KCRV CRITERIA AND CALCULATIONS

The OAWG has established three criteria for including results in the calculation of the KCRV: (1) participation in an appropriate pilot study, (2) use of a method that has been verified as appropriate for the measurand and of higher metrological order, and (3) use of a primary standard with a metrologically traceable assigned purity – that is, either a Certified Reference Material or a material the purity of which has been suitably assessed by the reporting participant.

When all results meeting all three of the above criteria agree well within their approximate 95% uncertainties, U_{95}, the OAWG has also established that (1) the KCRV would be assigned as the arithmetic mean of the eligible results, and (2) the approximate 95% uncertainty on the KCRV,

U_{95}(KCRV), would be assigned as the standard deviation of the mean (the standard deviation of the values divided by the square-root of the number of values) of the eligible results multiplied by a coverage factor of 2.0. At minimum, "agree well" implies that the reported laboratory mean values overlap within their U_{95} uncertainties and that the U_{95} uncertainties are all of the same magnitude.

RESULTS

Cortisol

The methodologies, primary standards, and internal standards used by the laboratories in CCQM-K63.a Cortisol in Human Serum are summarized in Table 1. The reported results and the assessment of the KCRV criteria are provided in Table 2. All participants used methods based on ID/MS coupled with GC or LC, and they used various deuterium-labeled cortisol materials as internal standards. Results from the six laboratories are in very good agreement, with overlapping values and U_{95} uncertainties of similar magnitude. Five participants satisfied all three criteria for their results to be included in assigning the KCRV and the U_{95}(KCRV).

Given the good agreement among all participants, the KCRV is assigned as the arithmetic mean of the five eligible results: KCRV = 100.5 ng/g. Likewise, the U_{95}(KCRV) is assigned as twice the standard deviation of the mean of the five eligible results: $U_{95}(\text{KCRV}) = 2 * 1.24 / \sqrt{5} = 1.1$ ng/g. The data and the calculations used to assign the KCRV and U_{95}(KCRV) are summarized in Table 3. Figure 1 is an illustration of all of the results, along with lines denoting the KCRV and KCRV ± U_{95}(KCRV) values.

Progesterone

The methodologies, primary standards, and internal standards used by the laboratories in CCQM-K63.b Progesterone in Human Serum are summarized in Table 4. Table 5 is a list of the reported results and the assessment of the KCRV criteria. KRISS revised their results following discovery of a calculation error after reviewing the Draft A report. All participants except CENAM used methods based on ID/MS coupled with GC or LC, and all participants used progesterone-$^{13}C_2$ as an internal standard. CENAM used a similar measurement technology but with cortisol-d_3 as the internal standard; thus they did not use a true "isotope dilution" method.

The results from the seven laboratories using an ID/MS method are in good agreement; the result from CENAM is approximately 11% larger. The U_{95} uncertainties for six of the seven ID/MS-based results are of about the same magnitude; the U_{95} uncertainties reported by KRISS are about two-fold larger. Only two participants (NIST and NMIJ) met all three criteria for their results to be used in assigning the KCRV and the U_{95}(KCRV).

Given the good agreement among the seven ID/MS-based results, the KCRV is assigned as the arithmetic mean of the two eligible results: KCRV = 2.83 ng/g. Likewise, the U_{95}(KCRV) is assigned as twice the standard deviation of the mean of the two eligible results: $U_{95}(\text{KCRV}) = 2 * 0.031 / \sqrt{2} = 0.04$ ng/g. The data and the calculations used to assign the KCRV and U_{95}(KCRV) are summarized in Table 6. Figure 2 is an illustration of all of the results, along with lines denoting the KCRV and KCRV ± U_{95}(KCRV) values.

Uncertainty
Each laboratory calculated its own uncertainties. The components included in each of the uncertainty calculations are detailed in Table 7.

Degrees of Equivalence
The Degree of Equivalence, D_i, between the i^{th} reported value and the KCRV is the difference between the reported value and the KCRV: $D_i = \text{Value}_i - \text{KCRV}$. Following current OAWG procedure, the uncertainty of D_i, $u(D_i)$ is estimated as $u(D_i) = \sqrt{[u^2(\text{KCRV}) + u^2(\text{Value}_i)]}$. By convention, the expanded uncertainty of the D_i at the 95 % level of confidence, $U_{95}(D_i)$, is twice $u(D_i)$: $U_{95}(D_i) = 2*u(D_i)$.

Figures 3 and 4 report the D_i and $U_{95}(D_i)$ of the originally reported results for CCQM–K63.a and –K63.b, respectively.

CONCLUSIONS

For both the CCQM-K63.a Cortisol in Human Serum and CCQM-K63.b Progesterone in Human Serum, agreement among the laboratories using an ID/MS-based method was excellent. The percent relative standard deviation among the eligible results is 1.3% or less for both of these non-peptide hormones.

Table 1
Summary of Methodologies, Primary Standards, and Internal Standards for CCQM-K63.a Cortisol in Human Serum

		Primary Standard		
Lab	Method	Supplier	Purity Assessment	Internal Standard
KRISS	ID-LC/MS	SRM 921, NIST	Certified	Cortisol-d_3
LGC	ID-LC/MS/MS	SRM 921, NIST	Certified	Cortisol-d_3
NIM	ID-LC/MS/MS	Dr. Ehrenstorfer, Germany	Self	Cortisol-d_4
NIST	ID-LC/MS/MS	SRM 921, NIST	Certified	Cortisol-d_3
NMIJ	ID-GC/MS	NMIJ-purified material	Self	Cortisol-d_2
PTB	ID-GC/MS	SRM 921, NIST	Certified	Cortisol-d_4

Table 2
Summary of Results and KCRV Use Criteria for CCQM-K63.a Cortisol in Human Serum

	Results, ng/g			KCRV Criteria			
Lab	Lab Mean	u_c	U_{95}	Participated in Pilot	Used ID/MS	Traceable Purity	Use in KCRV
KRISS	101.663	1.022	2.411	Yes	Yes	Yes	Yes
LGC	100.4	1.5	3.0	No	Yes	Yes	No
NIM	100.46	0.91	1.83	Yes	Yes	Yes	Yes
NIST	100.48	1.07	2.13	Yes	Yes	Yes	Yes
NMIJ	98.47	1.06	2.11	Yes	Yes	Yes	Yes
PTB	101.31	0.97	2.0	Yes	Yes	Yes	Yes

Mean (SD) of all ID/MS values = 100.46 (1.10) ng/g; %RSD = 1.1 %

Table 3
KCRV Assessment for CCQM-K63.a Cortisol in Human Serum. All values in ng/g.

Lab	Lab Mean	u_c	U_{95}
KRISS	101.663	1.022	2.411
NIM	100.46	0.91	1.83
NIST	100.48	1.07	2.13
NMIJ	98.47	1.06	2.11
PTB	101.31	0.97	2.0
Mean	100.48		
SD	1.24	1.01	u_{pooled}
%RSD	1.23 %		

Number of eligible results = N = 5; KCRV = Arithmetic Mean = 100.5 ng/g
u(KCRV) = SD / √N = 1.24 / √5 = 0.55 ng/g
k = 2; U_{95} = k * u(KCRV) = 2 * 0.55 = 1.1 ng/g

Table 4
Summary of Methodologies, Primary Standards, and Internal Standards for
CCQM-K63.b Progesterone in Human Serum

		Primary Standard		
Lab	Method	Supplier	Purity assessed by	Internal Standard
CENAM	GC/MS	Sigma	Self	Cortisol-d_3
KRISS	ID-LC/MS/MS	Sigma #065k0171	NIST	Progesterone-$^{13}C_2$
LGC	ID-LC/MS/MS	Candidate LGC RM 1891	Self	Progesterone-$^{13}C_2$
NIM	ID-GC/MS	Dr. Ehrenstorfer, Germany	Supplier	Progesterone-$^{13}C_2$
NIST	ID-LC/MS/MS	Sigma #065k0171	Self	Progesterone-$^{13}C_2$
NMIA	ID-GC/MS & ID-LC/MS/MS	Dr. Ehrenstorfer, Germany	Self	Progesterone-$^{13}C_2$
NMIJ	ID-GC/MS	NMIJ-purified material	Self	Progesterone-$^{13}C_2$
PTB	ID-GC/MS	Sigma # 065k0171	Supplier	Progesterone-$^{13}C_2$

Table 5
Summary of Results and KCRV-Use Criteria for CCQM-K63.b Progesterone in Human Serum

	Results, ng/g			KCRV Criteria			
Lab	Lab Mean	u_c	U_{95}	Participated in Pilot	Used ID/MS	Traceable Purity	Use in KCRV?
CENAM	3.15	0.12	0.23	No	No	Yes	No
KRISS [a]	2.891	0.0846	0.1861	No	Yes	Yes	No
KRISS [b]	2.880	0.084	0.185	No	Yes	Yes	No
LGC	2.74	0.04	0.09	No	Yes	Yes	No
NIM	2.88	0.04	0.08	Yes	Yes	No	No
NIST	2.806	0.035	0.070	Yes	Yes	Yes	Yes
NMIA	2.799	0.038	0.076	No	Yes	Yes	No
NMIJ	2.85	0.031	0.063	Yes	Yes	Yes	Yes
PTB	2.834	0.038	0.085	Yes	Yes	No	No

[a] KRISS original result; [b] KRISS revised result, reported after receiving the draft A report.

Mean (SD) of all original ID/MS values = 2.829 (0.052) ng/g; %RSD = 1.8 %

Table 6
KCRV Assessment for CCQM-K63.b Progesterone in Human Serum. All values in ng/g.

Lab	Lab Mean	u_c	U_{95}
NIST	2.806	0.035	0.070
NMIJ	2.85	0.031	0.063
Mean	2.828		
SD	0.031	0.033	u_{pooled}
%RSD	1.10 %		

Number of eligible results = N =2; KCRV = Mean = 2.828 ng/g
u(KCRV) = SD / √N = 0.031 / √2 = 0.022 ng/g
k = 2; U_{95} = k * u(KCRV) = 2 * 0.022 = 0.044 ng/g

Table 7-CENAM-b
Uncertainty Budget for CENAM, CCQM-K63.b Progesterone in Human Serum

Reproducibility between subsamples and day of analysis	3.1%
Repeatability between samples	0.4%
Calibration curve	1.9%
Difference of SRM for quantified and reported value	0.6%
Standard solutions; gravimetric preparation including purity of standard	0.2%

Combined Standard Uncertainty (ng/g)	0.12
Coverage Factor (k)	2
Expanded Uncertainty (U) (ng/g)	0.23

Table 7-KRISS-a
Uncertainty Budget for KRISS, CCQM-K63.a Cortisol in Human Serum

			K63.a	SRM 971
Mean (ng/g)			101.663	88.442
Source of uncertainty	Calibration standard mixture	Uncertainty of purity of standard compound (0.2%, ng/g)	0.203	0.177
		Repeatability (0.71%, ng/g)	0.725	0.630
	Measurement uncertainty (0.68%, ng/g) (include uncertainties of measurement of isotope ratio of sample, weighing sample taken for analysis and weighing IS solution added to the sample)		0.691	0.611
Combined uncertainty (1.01%, ng/g) *k* (95%)			1.022 2.36	0.889 2.36
Expanded uncertainty (2.37%, ng/g)			2.411	2.098

Table 7-KRISS-b
Uncertainty Budget for KRISS, CCQM-K63.b Progesterone in Human Serum

			Original Report		Revised Report	
			K63.b	SRM 971	K63.b	SRM 971
Mean (ng/g)			2.891	1.887	2.880	1.887
Source of uncertainty	Calibration standard mixture	Uncertainty of purity of standard compound (0.7%, ng/g)	0.020	0.013	0.020	0.013
		Repeatability (2.66%, ng/g)	0.077	0.050	0.077	0.050
	Measurement uncertainty (1%, ng/g) (include uncertainties of measurement of isotope ratio of sample, weighing sample taken for analysis and weighing IS solution added to the sample)		0.029	0.019	0.029	0.019
Combined uncertainty (2.93%, ng/g) *k* (95%)			0.085 2.2	0.055 2.2	0.084 2.2	0.055 2.2
Expanded uncertainty (6.43%, ng/g)			0.186	0.121	0.185	0.121

Table 7-LGC-a
Uncertainty Budget for LGC, CCQM-K63.a Cortisol in Human Serum

Parameter	Percent contribution to total uncertainty
The measured isotope amount ratio of sample blend / the measured isotope amount ratio of calibration blend	85%
Mass fraction of analyte in primary standard	2%
Blend to blend variation – the standard deviation of the 9 aliquots mass fractions, divided by square root of 9.	12%
Mass of spike added to the sample to prepare the sample blend	0.04%
Mass of sample added to the spike to prepare the sample blend	0.04%
Mass of primary standard solution added to the spike to prepare calibration blend	0.2%
Mass of spike added to primary standard solution to prepare calibration blend	0.04%
Combined standard uncertainty	1.5%
Combined expanded uncertainty (k=2)	3%

Table 7-LGC-b
Uncertainty Budget for LGC, CCQM-K63.b Progesterone in Human Serum

Parameter	Percent contribution to total uncertainty
The measured isotope amount ratio of sample blend / the measured isotope amount ratio of calibration blend	46%
Mass fraction of analyte in primary standard	2%
Blend to blend variation – the standard deviation of the 6 aliquots mass fractions, divided by square root of 6.	51%
Potential difference between M+2 progesterone peak in sample and calibration blend	0.5%
Mass of spike added to the sample to prepare the sample blend	0.09%
Mass of sample added to the spike to prepare the sample blend	0.01%
Mass of primary standard solution added to the spike to prepare calibration blend	0.1%
Mass of spike added to primary standard solution to prepare calibration blend	0.09%
Combined standard uncertainty	1.6%
Combined expanded uncertainty (k=2)	3.2%

Table 7-NIM-a
Uncertainty Budget for NIM, CCQM-K63.a Cortisol in Human Serum

Parameter	Source of uncertainty	x_i	$u(x_i)$	$\left(\frac{\partial f}{\partial x_i}\right)u(x_i)$	Degrees of freedom (ν_i)	Type	Source of data
Method precision	Between batch precision for the method as a whole (major source)	100.46 ng/g	0.86 ng/g	0.86 ng/g	6	A	Replicate analysis of samples among three batches
Calibration solution	Calibration solution, corrected for purity (major source)	100.23 ng/g	0.28 ng/g	0.28 ng/g	large	B	Supplier's specification
Weight of calibration compound		1.0024 mg	0.0010 mg	0.0010 mg	large	B	Balance calibration certificate
Weight of sample	Balance linearity (minor source)	1.0080 g	0.00017 g	0.00017 g	large	B	Balance calibration certificate
Weight of calibration solution	Balance linearity (minor source)	0.72331 g	0.00012 g	0.00012 g	large	B	Balance calibration certificate

$\left(\frac{U_c(y)}{y}\right)^2$ =(0.86%)^2+(0.5%/1.732)^2+(0.1%)^2+(0.017%)^{2}X2

$\frac{U_c(y)}{y}$ =0.91%

$U_{95\%} = k_{(95\%,\nu_{eff})}U_c(y)$ =0.91%X2=1.82%

Table 7-NIM-b
Uncertainty Budget for NIM, CCQM-K63.b Progesterone in Human Serum

Mean	2.88 ng/g			
Source of uncertainty	Method precision (major source)	Reference compound purity (major source)	Balance linearity (minor source)	Other systematic error
Relative uncertainty	1.4%	0.5%	0.1%	0.2%
Uncertainty(ng/g)	0.040	0.014	0.0029	0.0058
Type	A	B	B	B

Combined SD uncertainty (u_c), ng/g: 0.04
Coverage Factor (k): 2
Expanded Uncertainty (U) (ng/g): 0.08

Table 7-NIST-a
Uncertainty Budget for NIST, CCQM-K63.a Cortisol in Human Serum

Mean, ng/g	100.48
Type A	
SD, ng/g	0.46
SD of mean, ng/g	0.19
Type B	
1% Uncertainty of volumetric error, ng/g	1.00
0.2% Uncertainty of purity of reference compound, ng/g	0.20
0.1% Uncertainty of weighing, ng/g	0.10
0.2% Other systematic error, ng/g	0.20
Combined standard uncertainty (uc), ng/g	1.07
k Factor	2
Expanded uncertainty (U), ng/g	2.13
Relative expanded uncertainty [a], %	2.1

Table 7-NIST-b
Uncertainty Budget for NIST, CCQM-K63.b Progesterone in Human Serum

Mean, ng/g	2.806
Type A	
SD, ng/g	0.018
SD of mean, ng/g	0.007
Type B	
1% Uncertainty of volumetric error, ng/g	0.028
0.7% Uncertainty of purity of reference compound, ng/g	0.020
0.1% Uncertainty of weighing, ng/g	0.003
Other systematic error, ng/g	0.003
Combined standard uncertainty (uc), ng/g	0.035
k Factor	2
Expanded uncertainty (U), ng/g	0.070
Relative expanded uncertainty [a], %	2.5

[a] Uncertainty of 95% confidence interval.

Table 7-NMIA-b
Uncertainty Budget for NMIA, CCQM-K63.b Progesterone in Human Serum

Parameter	Source of uncertainty	x_i	$u(x_i)$	Degrees of freedom (ν_i)	Source of data
M_{Zc} (g)	Mass of calibration solution added to calibration blend	0.15263	0.000049	Large	Certified balance linearity
M_Y (g)	Mass of internal standard added to sample blend	0.07624	0.000049	Large	Certified balance linearity
M_{Yc} (g)	Mass of internal standard added to calibration blend	0.15317	0.000049	Large	Certified balance linearity
M_X (g)	Mass of sample added to sample blend	1.14460	0.000049	Large	Certified balance linearity
C_Z (ng g^{-1})	Mass fraction of progesterone calibration solution	42.08	0.43	66	Purity/dilution masses/standard biases
R_y	Ratio of progesterone to $^{13}C_2$-progesterone in the internal standard material	0.00903	0.000098	10	Replicate injections at 5 μg.g^{-1}
R_x,R_z	Ratio of progesterone to $^{13}C_2$-progesterone in the sample/standard	250.0	3.5	10	Replicate injections at 5 μg.g^{-1}
Method precision (including R'_B, R'_{Bc})	Precision effects related to ratio measurements, mass measurements and sample variation	1.0000	0.0043	5	Standard deviation of the mean of six independent determinations on the study material over 3 days
B_E, B_M, *and* B_I,	Bias due to extraction, matrix and interference effects	1.0000	0.0078	14	Results from different analytical methods: e.g. LC-MS/MS vs. GC/MS, different ion pairs, solvent versus serum calibration blends etc.

Table 7-NMIJ-ab
Uncertainty Budget for NMIJ, CCQM-K63.b Cortisol in Human Serum and
CCQM-K63.b Progesterone in Human Serum

Uncertainty budget

	Cortisol	Progesterone	d.f	Type	
Measured Value (mean, ng/g)	98.44	2.85			
Uncertainty Components					
Between day Variation (3 days)	0.000	0.784	2	A	
Within day Variation (N = 3)	0.520	0.215	6	A	
Measurement Precision	0.171	0.141	45	A	
Measurement of Calib. Stds	0.854	0.496	35	A	
Unc. of Primary Standard	0.231	0.404		B	(*) Inf.
Preparing Calibrant Solution	0.260	0.300	large	B	
Mass of calibration solution taken for analys	0.117	0.117	large	B	
Mass of sample taken for analysis	0.015	0.015	large	B	
Mass of spiking solution added to calibrant	0.106	0.106	large	B	
Mass of spiking solution added to sample	0.075	0.075	large	B	
Rel. Combined Std. Unc., as %	1.09	1.10			
Combined Std. Unc., ng/g	1.07	0.031			
k	2	2			
Expanded Unc., ng/g	2.14	0.063			

(*) Information on Calibration Solution	Cortisol	Progesterone
Purity of Primary Std., as %	99.2	99.3
Expanded Rel. Unc, as %	0.4	0.7
Rel. Std. Unc., as %	0.23	0.40

Table 7-PTB-a
Uncertainty Budget for PTB, CCQM-K63.a Cortisol in Human Serum

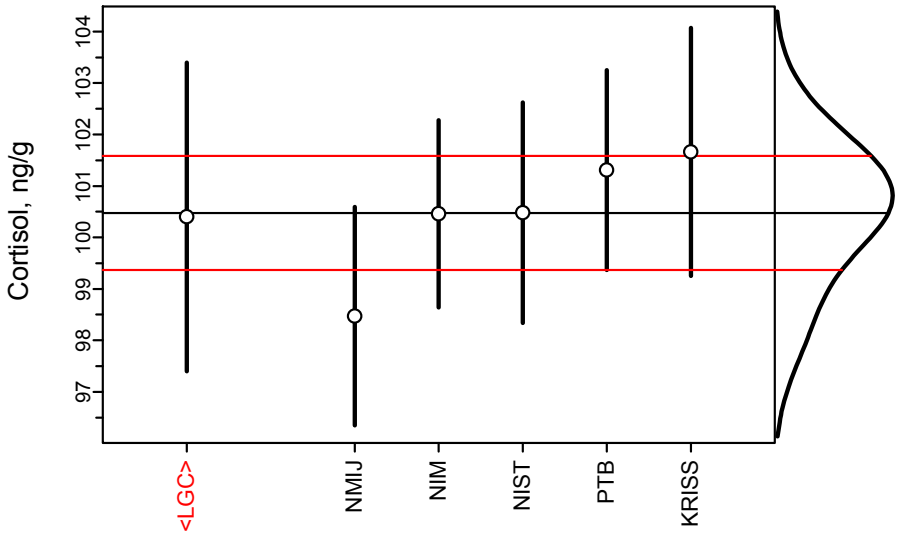
Mean:	101.31 ng/g
Standard deviation of mean:	0.31 ng/g
Combined SD uncertainty:	0.97 ng/g
Expanded Uncertainty:	2.0 ng/g
Rel. Exp. Uncertainty:	2.0%
Number of repetitions:	3
Degrees of freedom:	48 [a]
Coverage factor (95%):	2.1

Table 7-PTB-b
Uncertainty Budget for PTB, CCQM-K63.b Progesterone in Human Serum

Mean:	2.834 ng/g
Standard deviation of mean:	0.023 ng/g
Combined SD uncertainty:	0.038 ng/g
Expanded Uncertainty:	0.085 ng/g
Rel. Exp. Uncertainty:	3.0%
Number of repetitions:	3
Degrees of freedom:	13 [a]
Coverage factor (95%):	2.2

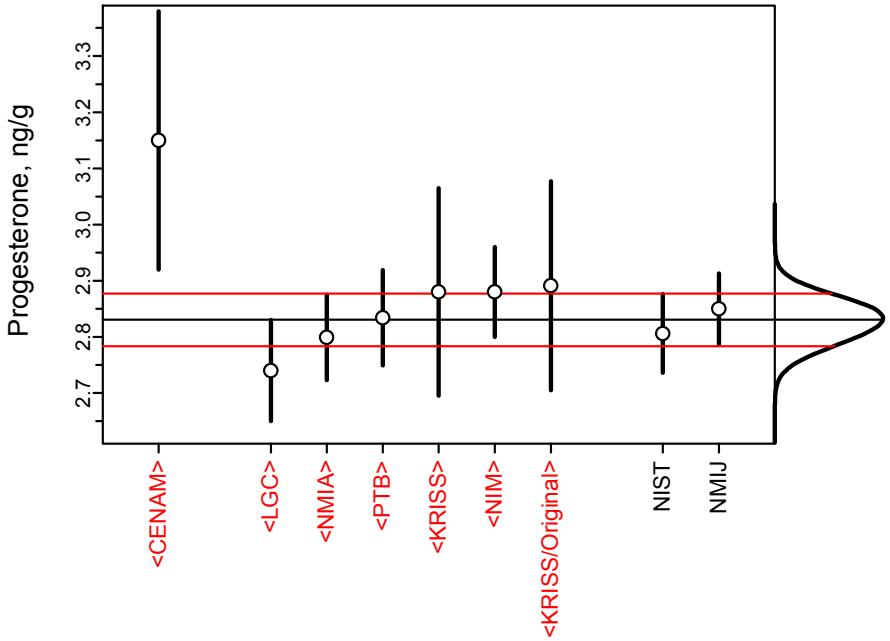
a The number of degrees of freedom resulted from combination using the Satterthwaite-Welch formula.

Figure 1. CCQM-K63.a Cortisol in Human Serum



Open circles and vertical thick lines represent the reported values and their associated U_{95} uncertainties. The five values to the right (with black participant labels) are used to assign the KCRV; the value to the left (in brackets and with a red participant label) was excluded from the KCRV calculation on the basis of eligibility criteria discussed in Section KCRV CRITERIA AND CALCULATIONS. The black horizontal line represents the KCRV, 100.48 ng/g; the two red horizontal lines represent KCRV ± U_{95}(KCRV), 100.48 ± 1.10 ng/g. The black curve at the right-hand edge is the normal-kernel probability density function for the values used to assign the KCRV.

Figure 2: CCQM-K63.b Progesterone in Human Serum



Format as above. The two values to the right are used to assign the KCRV ± U_{95}(KCRV) of 2.83 ± 0.04 ng/g. The seven values to the left were excluded from the KCRV calculation on the basis of eligibility criteria discussed in Section KCRV CRITERIA AND CALCULATIONS. The value to the far left was obtained using a non-isotopically labeled internal standard.

Figure 3. Degrees of Equivalence for CCQM-K63.a Cortisol in Human Serum

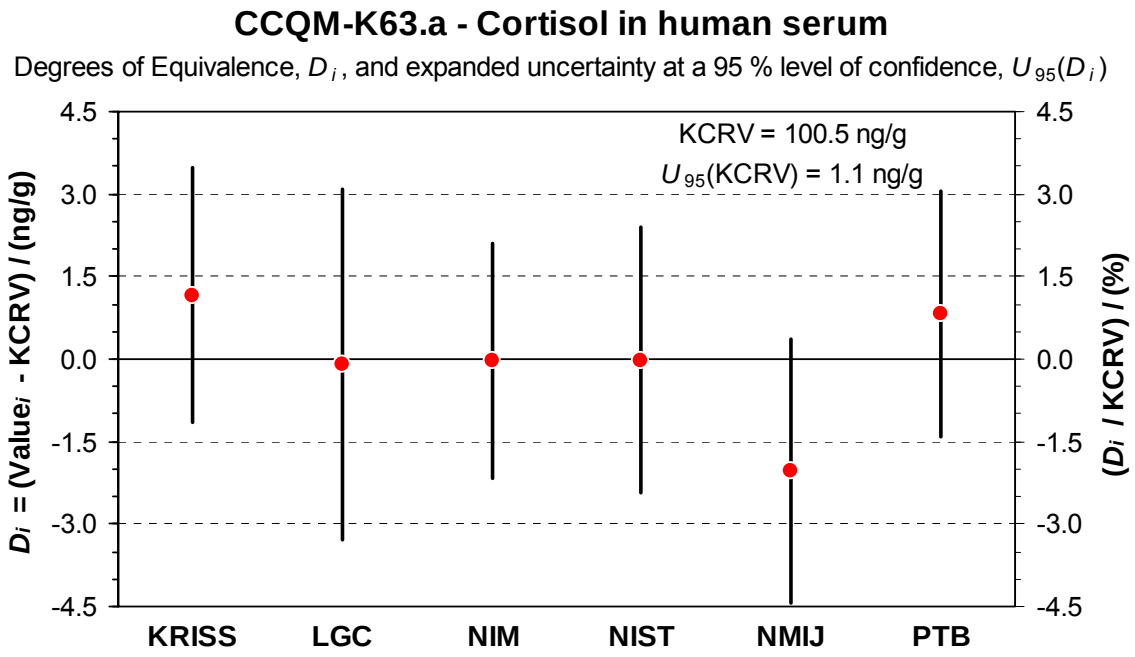


Figure 4. Degrees of Equivalence for CCQM-K63.b Progesterone in Human Serum