ICI REPORT OF THE WP9 ICI

Round 01/2018

Chromium in urine

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1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of chromium (Cr) in human urine. The study was performed from August 2018 to September 2018.

In total, 10 laboratories from 8 countries participated in this ICI. The participation in the ICI was satisfactory, as 10 out of 10 laboratories submitted their results.

Six test samples consisting of 2.2 mL urine each were prepared, corresponding to different concentration levels of the targeted biomarker, and sent to the participating laboratories for analysis. These samples were defined as follows:

- 3 samples at low Cr concentration, to be below or as close as possible to expected instrumental detection limits.

- 3 samples at high Cr concentration.

Homogeneity and stability assessment of the material confirmed that low and high Cr concentration level samples were adequately homogeneous and stable.

Laboratory results were rated using z-scores in accordance with ISO 13528 and ISO 17043. The default standard deviation applied for proficiency assessment (i.e. target standard deviation) was set to FFP = 25 %, as described in 5.3.

Assessment scores were calculated for high and low concentration level samples. As a global overview, the proportion of satisfying results (-2 < Z-score < 2) was 100 %.

2 Introduction

Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are efficient tools to assess the proficiency of laboratories, and the comparability and reliability of the analytical methods used. Participation in ICI/EQUAS is full part of Laboratory Quality Assurance system together with initial and on-going in-house method validation.

This ICI study was organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will be further involved in the analytical characterization of the HBM4EU samples.

This report describes the 1st round for chromium in urine and was organised by the Jožef Stefan Institute (JSI), Ljubljana, Slovenia.

2.1 Confidentiality

In this report the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the participants will be disclosed to the HBM-QAU for performance assessments.

3   Control material

3.1   Preparation of control material

The material was prepared on 20th August by mixing two lyophilised Seronorm reference materials "Trace elements in urine", containing different concentrations of chromium (Low Level (L1) and High level (L2)), to prepare samples at high chromium concentration. Each vial of the reference material was reconstructed with water, following the instructions of the producer. Reconstructed urine samples were gathered in 250 mL Teflon bottle, covered by Teflon cup and gently swirled to avoid formation of foam. The same procedure was applied to prepare low chromium concentration, using material L1, which was after reconstruction diluted with 0.2% sodium chloride (suprapure). 2.2 mL of the pooled samples with low and high chromium concentrations were then filled into labelled 4.5 mL cryogenic polyethylene tubes and tightly closed by cups. These samples were immediately deeply frozen at -20° C.

3.2   Homogeneity of control material

For the between homogeneity of the material, five tubes containing urine with high chromium concentration and seven tubes containing urine with low chromium concentration were randomly selected from the freezer (-20 °C) on the day when the participants received the samples. Five/seven aliquots used for the between homogeneity study were applied due to limited number of the participating laboratories. Samples were thawed, left to equilibrate to room temperature, gently swirled (three times) to avoid formation of foam and analysed in duplicate by ICP-MS as reported in Appendix 5. The homogeneity was evaluated according to HBM4EU-SOP-QA-002 "Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)", ISO 13528:2015, Fearn et al. (2001) and Thompson, (2000). The results are presented in Appendix 2.

The conclusions are summarized in the table 1 below.

Table 1: conclusions associated to the homogeneity test

	Concentration Level	HOMOGENEITY CRITERIA		
		$s_s < 0.3 * \sigma H$	$s_w < 0.5 * \sigma_H$	Outliers ?
Cr	High Level (H)	ACCEPT	ACCEPT	NO
	Low level (L)	ACCEPT	ACCEPT	NO

3.3   Stability of control material

In accordance with HBM4EU-SOP-QA-002 "Preparation of control materials for Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)" and with ISO 13528:2015, six randomly selected test samples containing urine with high chromium concentration and three randomly selected test samples containing urine with low chromium concentration were randomly selected from the freezer (-20 °C) on the day when the participating laboratories should finish with the analysis. Samples were analysed according to the analytical procedure described in Appendix 1.

The stability was evaluated using the Excel-sheet "HBM4EU ICI-EQUAS stability test CM v1". The results are presented in Appendix 3.

Table 2: conclusions associated to the stability test

		STABILITY CRITERIA		
		X-Y < 0.3*σH		Fischer's test
	Concentration Level	Horwitz/Thompson (22%)	Fit-for-purpose (25%)	
Cr	High Level (H)	ACCEPT	ACCEPT	ACCEPT
	Low Level (L)	ACCEPT	ACCEPT	ACCEPT

4 Organisational details

4.1 Participants

A list of 16 candidate laboratories from different countries previously identified as potential candidates for the analysis of chromium had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organizing the respective ICI.

Invitation letters were sent by e-mail to all those 16 candidate laboratories on 28th June 2018 (see Appendix 3), indicating that participation would be free of charge. In the invitation letter it was indicated that the shipment of samples will be performed on 21st August 2018.

Ten laboratories out of 16 laboratories (62.5%) indicated their interest in participating in this ICI and sent their registration form to JSI, with their agreement to abide by the conditions for participation. These laboratories received an individual laboratory code to report their measurement results.

The deadline to submit the test results was initially fixed to 14th September 2018 but was further extended to 30th September 2018. From the 10 potential participants, 10 performed the assays and submitted results.

4.2 Dispatch and instructions

Six test materials consisting of 2.2 mL urine were shipped on 21th August 2018 to participants under frozen conditions on dry ice by DHL in styropore boxes by DHL post to the participants. The amount of dry ice was around 3 kg per each box. Moreover, a letter with instruction on sample handling (Instruction letter, see Appendix 5), a sample receipt form to be sent back to JSI upon receipt of the test material, as well as submission form and a method relevant information related to the analytical method used for quantification were sent to the participants by e-mail the 20th August 2018.

Participants were asked to perform for each sample a single analysis using the same procedure they will routinely use in HMB4EU and to report results according to the instructions given above.

5 Data evaluation

5.1 Assigned value

For ICI studies, the consensus value is used as assigned value and calculated as described in SOP HBM4EU-SOP-QA-003. In brief, the consensus values and its uncertainty were calculated from the results submitted by the participants using robust statistics to minimize the influence of outliers.

5.2 Target standard deviation

For calculation of the Z-scores, Fit-for-purpose (FFP = 25 %) target standard deviation was used as a default value for this first round, in lack of prior information on interlaboratory performance within the HBM4EU laboratories.

5.3 ICI standard deviation

To gain insight in the actual variability of the biomarker analysis in this study, the robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003.

Z-scores

Z-scores were calculated according to SOP HBM4EU-SOP-QA-003.

In accordance with ISO 13528 and ISO 17043 and the deliverable D 9.4 "*The Quality Assurance/Quality Control Scheme in the HBM4EU project*, Z-scores are classified as presented in Table 3.

Table 3: Classification of Z-scores

$\lvert Z \rvert \leq 2$	Satisfactory
$2 < \lvert Z \rvert < 3$	Questionable
$\lvert Z \rvert \geq 3$	Unsatisfactory

6 Results and discussion

6.1 Results submitted by participants

In total 10 laboratories from 8 countries agreed to participate in this study. All 10 participants submitted results.

Laboratories were also asked to provide details on the method used for analysis. All laboratories performed the measurement on the diluted urine samples. Chromium concentrations were determined by inductively coupled plasma mass spectrometers (ICP-MS). This information is compiled in Appendix 6. An overview of results submitted by the participants is included in Appendix 7.

6.2 Assigned values and (target) standard deviations

The assigned value and its uncertainty, the relative standard deviation as derived from the participant's data, and the fit-for-purpose (FFP) target standard deviation for each of the analytes/control materials are included in Appendix 7.

The robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003 and was compared to the FFP target standard deviation, in order to evaluate whether the FFP fitted well with the variability actually observed. All these observations are presented in Appendix 7.

6.3 Assessment of laboratory performance

Z-scores calculated for all target biomarkers and analysed sample are reported in Appendix 7. Graphical representations of the Z-scores are provided in Appendix 8. A summary of number of laboratories that reported results and the number of acceptable/questionable/unacceptable scores are presented in Table 4.

Table 4: Summary of ICI results for Cr

	Low Level	High Level
No of reported quantitative results	30	30
No of reported LOQ	10	10
No of acceptable score	10	10
No of questionable score	0	0
No of unacceptable score	0	0

Assessment scores were calculated for chromium in low and high level samples. All results were satisfying (-2 < Z-score < 2). The summary of participant's scores are included in the Appendix 7.

6.4 Conclusions and recommendations

The overall participation in the HBM4EU ICI Round 1 was successful. Ten laboratories out of ten registered candidate laboratories reported results, representing a participation rate of 100%.

All the submitted results were satisfactory. These results indicate that the participating laboratories are competent for the analysis of Cr in urine samples.

7 References

Analytical Methods Committee, 1989a, Robust statistics - How not to reject outliers Part 1. Basic concepts, Analyst, 114, 1693-1697.

Analytical Methods Committee, 1989b, Robust statistics - How not to reject outliers Part 2. Interlaboratory trials, Analyst, 114, 1699-1702

HBM4EU-SOP-QA-001 "Organisation of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) of interlaboratory studies"

HBM4EU-SOP-QA-002 "Preparation of test materials for ICI / EQUAS"

HBM4EU-SOP-QA-003 "Evaluation of ICI / EQUAS results"

HBM4EU-SOP-QA-004 "Reporting of ICI / EQUAS studies"

ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing

ISO 13528, 2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

Official Methods of Analysis Program Manual, 2002, Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis. Association of Analytical Communities International. http://www.aoac.org/vmeth/Manual_Part_6.pdf.

Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386.

Thompson M., Ellison R. and Wood, R., 2006, The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, Pure Appl. Chem, 78(1), 145-196.

Appendix 1: ICP-MS method information JSI

Title of ICI: Chromium in urine

Laboratory code	JSI	
ISO17025 accredited	no	
SAMPLE PREPARATION		
Amount of sample	1 mL (digestion), 0.2 mL (dilution)	g or mL
Digestion (if applied)	Yes, in addition diluted samples were also analysed	
INSTRUMENTAL ANALYSIS		
AAS	no	
Wavelength		
Background correction		
Matrix modifier		
Dilution factor		
Calibration	external calibrant (matrix based) standard addition external calibrant (solvent based)	
	single level / multi level	
Correction for recovery	no	
Other remarks		
ICP-OES	no	
Nebulizer		
Wavelength		
Dilution factor		
Quantification		
Use of internal standard(s)	no	
Response normalised to IS	no	
Calibration	external calibrant (matrix based) standard addition external calibrant (solvent based)	
	single level / multi level	
Correction for recovery	no	
Other remarks		
ICP-MS	no	
MS (single quadrupole/QQQ/Q-TOF)	ICP-MS quadrupole	
Nebulizer	Miramist	
Reagent gas	Helium	
Masses monitored	*m/z* 52	
Dilution factor	5 times (digested samples), 10 times (diluted samples)	
Quantification		
Use of internal standard(s)	Yes/no	
Response normalised to IS	no	
Calibration	external calibrant (matrix based)	
	multi level	
Correction for recovery	no	

Further remarks/observations:

Appendix 2: Homogeneity study

Homogeneity

Version HBM4EU v1

Control material:		**Urine**		Target standard deviation:			
				Fit-for-purpose RSD	**FFP**	(25% is default value)	
Analyte:		**Cr**		if you want to use Horwitz/Thompson,			
				then delete FFP from cell H5			
Preparation of control material:		mix of RMs					

10 randomly chosen test samples, analysed in duplicate

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in
  relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2		x_t	w_t	w_t^2	$(x_t - \bar{x})^2$
1	27,7	27,2		27,5	0,6	0,3	2,0
2	25,7	26,8		26,3	-1,1	1,1	0,1
3	24,0	24,7		24,3	-0,7	0,5	2,9
4	26,5	25,5		26,0	1,0	1,0	0,0
5	25,7	26,5		26,1	-0,8	0,7	0,0
6							
7							
8							
9							
10							

Lowest:		24,0	µg/kg		$\Sigma =$	3,6	5,1
Highest		27,7	µg/kg				
Grand mean ($\bar{x}$):		26,02	µg/kg				
Stdev.		1,15	µg/kg				
VC%:		4,4%	µg/kg				

Outliers: Cochran's test

$C = w^2{}_{max} / \Sigma w_t^2$

--> $C =$ 0,309
--> $Ccrit =$ 0,841   **C < Ccrit → No outliers detected**

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)		Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
$RSD\% =$	27,71	$RSD\% =$	22	$RSD\% =$	25
$\sigma_H =$	7,21	$\sigma_H =$	5,72	$\sigma_H =$	6,51
σH used:	6,51				

Homogeniteit [1]:

$s_x =$	1,12	
$s_w =$	0,60	(within sample standard deviation)
$s_s =$	1,04	(between sample standard deviation)
critical=	1,95	
s_s < critical?	**→ ACCEPT: Homogeneity adequate**	
s_w < 0.5*σH?	**→ ACCEPT: Method suited**	

Homogeneity

Version HBM4EU v1

Control material:		**Urine - low**			**Target standard deviation:**				
					Fit-for-purpose RSD		**FFP**	(25% is default value)	
Analyte:		**Cr**			if you want to use Horwitz/Thompson,				
					then delete FFP from cell H5				
Preparation of control material:		mix of RMs							

10 randomly chosen test samples, analysed in duplicate

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in
relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2		x_t	w_t	$w_t{}^2$	$(x_t - \bar{x})^2$	
1	1,75	1,66		1,70	0,086	0,007	0,001	
2	1,67	1,62		1,64	0,055	0,003	0,001	
3	1,70	1,71		1,71	-0,009	0,000	0,002	
4	1,66	1,71		1,68	-0,055	0,003	0,000	
5	1,63	1,64		1,64	-0,011	0,000	0,001	
6	1,64	1,67		1,65	-0,031	0,001	0,000	
7	1,64	1,68		1,66	-0,037	0,001	0,000	
8								
9								
10								

Lowest:		1,6	µg/kg	$\Sigma =$	0,0	0,0	
Highest		1,7	µg/kg				
Grand mean ($\bar{x}$):		1,67	µg/kg				
Stdev:		0,04	µg/kg				
VC%:		2,2%	µg/kg				

Outliers: Cochran's test

$C = w^2{}_{max} / \Sigma w_t{}^2$

--> C =	0,465	
--> Ccrit=	0,727	C < Ccrit → No outliers detected

Horwitz [3]:

Mean > 120 ppb: CV=2(1-½ log c)			Mean < 120 ppb: σ = 0,22c		FFP (fit-for-purpose)	
RSD% =	41,90		RSD% =	22	RSD% =	25
$\sigma_H =$	0,70		$\sigma_H =$	0,37	$\sigma_H =$	0,42
σ_H used:	0,42					

Homogeniteit [1]:

$s_x =$	0,03	
$s_w =$	0,03	(within sample standard deviation)
$s_s =$	0,02	(between sample standard deviation)
critical=	0,13	
s_s < critical?	→ ACCEPT: Homogeneity adequate	

Appendix 3: Stability study

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine-high**
Storage: Frozen

Biomarker **Cr**

	t=0 (storage)	t=a (analysis)
dates:	21.08.2018	10.09.2018
values:	25,3	27,1
	27,4	26,3
	25,1	27,1
	28,1	27,1
	26,5	26,8
	26,9	26,1
number=	6	6
average=	26,6	26,7
std dev=	1,190	0,442

analysis date	time (days)	µg/L	n	std dev
21.08.2018	0	26,6	6	1,190
10.09.2018	20	26,7	6	0,442

x0-xa= -0,19

test 'consequential instability': Horwitz/Thompson — **Fit-for-purpose (FFP)**

xav-yav=<0,3σH

σH=	5,843	6,64078986
0,3*σH=	1,753	1,99023696
x0-xa<0,3*σH?	No consequential instability detected	No consequential instability detected

test 'significant difference':

F=	7,258
Fcrit=	5,050
Significant difference?	**Significant difference in std detected**
sed√2=	0,90
n=	10
std difference=	0,52
t=	0,37
t-crit=	2,23
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Urine-low**
Storage: Frozen

Biomarker: **Cr**

dates:	t=0 (storage) 21/08/18	t=a (analysis) 10/09/18
values:	1,86	1,75
	1,75	1,61
	1,75	1,83
number=	3	3
average=	1,8	1,7
std dev=	0,062	0,112

analysis date	time (days)	µg/L	n	std dev
21/08/18	0	1,8	3	0,062
10/09/18	20	1,7	3	0,112

x0-xa= **0,06**

test 'consequential instability': Horwitz/Thompson   Fit-for-purpose (FFP)

xav-yav <0,3σH

σH= 0,393   0,446579648
0,3*σH= 0,118   0,13973895

x0-xa<0,3*σH?   No consequential instability detected   No consequential instability detected

test 'significant difference':

F= 3,201
Fcrit= 19,000

Significant difference? **No significant difference in std detected**

sed^2= 0,09
n= 4
std difference= 0,07
t= 0,80
t-crit= 2,78

Significant difference? **No statistic instability detected**

Appendix 4: Copy of letter of invitation

HBM4EU: Invitation letter to participate in ICI study Cr in serum, whole blood and urine/Round 1 and Round 2

Title of ICI: Cr in serum, whole blood and urine

Dear HBM4EU partner,
You are invited to participate in ICI study Cr in serum, whole blood and urine/Round 1 and Round 2. Since Cr ICI was agreed recently, the two rounds Cr ICI 1 and Cr ICI 2 will be performed closely one after another, so that we can evaluate data from two ICI's before the end of this year.
The laboratories which will confirm their participation on Cr ICI (deadline 15.7.2018, will get information about sample shipment, conditions of analysis and reporting of results.
In each Cr ICI you will receive 3 aliquots of samples (2 mL) of serum, whole blood and urine, at two concentration levels (in total 18 samples). Samples will be sent by DHL deeply frozen on dry ICE. After the receipt, you will have 3 weeks to complete the analysis and send the results to the organiser.

The Agenda is the following:

Date	Cr ICI 1st round	Cr ICI 2nd round
Sample shipment (organiser)	21.08.2018	11.09.2018
Submitting of results (participants)	14.09.2018	05.10.2018
Sending of report (organiser)	15.11.2018	15.12.2018

To fasten the procedure, we would like that the participants express their interest to participate on Cr ICI 1 and/or Cr ICI 2 in the attached registration form.

Looking forward to your participation,
Sincerely,

Prof.dr. Radmila Milačič
Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

e-mail: Radmila.milacic@ijs.si Tel.: +396 1 4773560

Appendix 5: Copy of letter/instructions sent together with test samples

HBM4EU: Sample handling before the analysis: ICI study Cr/Round 1
Title of ICI: Cr in serum, whole blood and urine

Dear participant,
Thank you for participation in HBM4EU ICI study Cr in serum, whole blood and urine 1st Round.
You will receive a parcel containing 6 test samples of serum, 6 test samples of whole blood and 6 test samples of urine. For each matrix, 3 are control samples, 3 are samples with biomarker considered for Cr ICI study.
The parcel will be shipped on 21.08.2018 deeply frozen on dry ICE by DHL.

Instructions:
Upon receipt, please check whether samples were received frozen on dry ice. If samples were damaged or are leaking of the containers, please immediately contact the organiser to send you the replacement for damaged samples. Please complete the sample receipt form and return it to the organiser.

Take the samples from dry ice and immediately transfer them to a deep freezer and keep the samples at -20 °C until the analysis.
Before the analysis thaw the samples and leave them to equilibrate to room temperature. Swirl the samples gently (three times) to avoid formation of foam. Do not shake samples. Analyse the samples according to the laboratory standard operational procedure.

Carry out a single analysis for each sample. For submission of results and method information use the forms (attached).
The deadline for submission of results and method details is 18.09.2018.
Provide only one result for each sample bottle in the excel file which will be sent to you by e-mail.

Warning: Handle the samples (human biological material) with care, in spite of negative test for Hepatitis B, Hepatitis C and HIV. The handling of material should be conducted as if the material is infectious.

If you have any questions or need any assistance, please contact:

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Prof. Dr. Thomas Göen
(for the ICI/EQUAS organisers)

Appendix 6: Details of analysis methods used by the participants

Laboratory Code	Sample Preparation	Detection Technique	LOQ (ng Cr/mL)
1	dilution	ICP-MS-QQQ	0.05
2	dilution	Single quad ICP-MS	1.2
3	dilution	Single quad ICP-MS	0.1
4	dilution	Single quad ICP-MS	0.028
5	dilution	Single quad ICP-MS	1
6	dilution	Single quad ICP-MS	0.18
7	dilution	Single quad ICP-MS	0.05
8	dilution	Single quad ICP-MS	0.281
9	dilution	Single quad ICP-MS	0.897
10	dilution	ICP-MS-QQQ	0.386

Appendix 7: Assigned values and participant's performance

Control material	Urine-high
Parameter	Cr
Number of participants	10
Number of quantitative results	30
Assigned value ng/mL	25.6
Uncertainty of assigned value ng/mL	0.6
Study RSD$_R$ (%)	6.1
Relative target standard deviation (%)	25.0

Laboratory code	ID sample	Value	Average	Z-Score
1	U_046	26.4		
1	U_060	27.0	26.8	0.20
1	U_063	27.1		
2	U_059	24.3		
2	U_061	24.0	24.4	-0.18
2	U_036	24.9		
3	U_048	25.8		
3	U_062	25.6	25.6	0.08
3	U_070	25.5		
4	U_038	26.18		
4	U_053	25.82	25.59	0.03
4	U_068	24.76		
5	U_045	23.201		
5	U_082	22.428	22.4	-0.49
5	U_084	21.689		
6	U_040	26.9		
6	U_041	27.7	27.5	0.30
6	U_042	27.8		
7	U_044	24.1		
7	U_074	24.0	23.8	-0.27
7	U_080	23.4		

8	U_051	26.2		
8	U_057	26.2	26.2	0.10
8	U_079	26.1		
9	U_067	27.0		
9	U_066	26.0	26.7	0.17
9	U_064	27.0		
10	U_047	25.4		
10	U_071	25.6	25.6	0.01
10	U_081	25.8		

Control material	Urine-low			
Parameter	Cr			
Number of participants	10			
Number of quantitative results	30			
Assigned value ng/mL	1.707			
Uncertainty of assigned value ng/mL	0.039			
Study RSD$_R$ (%)	5.82			
Relative target standard deviation (%)	25.0			
Laboratory code	ID sample	Value	Average	Z-Score
1	U_011	1.81		
1	U_022	1.76	1.77	0.16
1	U_032	1.75		
2	U_012	1.68		
2	U_024	1.68	1.63	-0.18
2	U_017	1.53		
3	U_005	1.68		
3	U_015	1.68	1.69	-0.03
3	U_030	1.72		
4	U_016	1.60		
4	U_021	1.69	1.65	-0.13
4	U_025	1.66		

5	U_002	1.708		
5	U_004	1.414	1.60	-0.25
5	U_023	1.682		
6	U_009	1.85		
6	U_018	1.95	1.94	0.55
6	U_031	2.02		
7	U_020	1.69		
7	U_028	1.57	1.62	-0.2
7	U_034	1.61		
8	U_008	1.72		
8	U_010	1.85	1.78	0.18
8	U_027	1.78		
9	U_026	2.12		
9	U_029	1.92	2.05	0.81
9	U_007	2.12		
10	U_001	1.66		
10	U_003	1.66	1.67	-0.09
10	U_014	1.68		

Appendix 8: Graphical representation of the Z'i-scores and the Z-scores

Cr in Urine-low

Cr in Urine