HORIZON2020 Programme
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REPORT OF THE WP9 ICI

Round 01/2018

Cadmium in urine

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Table of contents

1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of cadmium in urine. The study was performed from April 2018 to May 2018.

Nine different test samples consisting of 5 mL urine each (6 of them spiked with two different concentrations of cadmium (Cd_{low}, Cd_{high}), 3 of them containing native control material (Cd_{native})) were prepared and sent to the participating laboratories for analysis.

Homogeneity and stability assessment of the control materials confirmed that the materials were adequately homogeneous and stable.

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

In total, 21 laboratories from 17 countries participated in the ICI. The participation in the ICI was satisfactory; 21 out of 21 laboratories submitted their results.

The evaluation showed that 85 % of the results were satisfactory, 5 % were questionable and 5 % were unsatisfactory. For one laboratory (5 %) no Z-score could be calculated (false negatives, the limit of quantification was too high).

2 Introduction

Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to assess the proficiency of laboratories, and the comparability and reliability of analytical methods. Participation in ICI / EQUAS forms an integral part of quality control, in addition to initial and on-going in-house method validation.

This ICI/EQUAS study has been 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 analyse HBM4EU samples.

This report describes the 1st round for cadmium in urine and was organised by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) at Friedrich-Alexander University of Erlangen-Nuremberg.

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

For control material, surrogate material was used. It consists of human urine with the addition of sodium azide. Absence of cadmium in the native material was approved several times by homogeneity and stability testing. The stock solution (Cadmium ICP standard, $Cd(NO_3)_2$ in HNO_3 2-3 %, 1000 mg/L, Merck) was diluted into two different concentrations and the addition to the native control material resulted in the intended concentration in control material (Cd_{low}, Cd_{high}). The two spiked and the native control materials (Cd_{native} is not part of the evaluation, but only serves to show background information) were each filled with a volume of 5 mL in tubes with caps (82x13 mm, polypropylene, Sarstedt). The tubes were stored in a freezer (≤ -18 °C) until transportation. The three different concentrations (Cd_{native}, Cd_{low}, Cd_{high}) were measured by ICP-MS (see analysis method in Appendix 5). The measured concentrations are shown in Sections 3.2 and 3.3 of this report.

3.2 Homogeneity of control material

Ten tubes of each concentration of the control material (Cd_{native}, Cd_{low}, Cd_{high}) were randomly selected from the freezer (≤ -18 °C). The thawed samples were re-homogenised by vortex shaking and analysed using ICP-MS (see analysis method in Appendix 5). The homogeneity was evaluated according to ISO 13528:2015, Fearn et al [2001] and Thompson [2000]. The results are presented in Appendix 1. The conclusion is that no outliers are detected, the homogeneity is adequate and the method is suitable.

3.3 Stability of control material

In accordance with ISO 13528:2015 and the International Harmonised Protocol for the Proficiency Testing of Analytical Laboratories [Thompson 2006], ten randomly selected test samples from each concentration (Cd_{native}, Cd_{low}, Cd_{high}) were analysed by ICP-MS (see analysis method in Appendix 5). The randomly selected control materials were stored at ≤ -18 °C.

The stability was evaluated using the Excel-sheet “HBM4EU ICI-EQUAS stability test CM v1”. The results are presented in Appendix 2. No clear decrease was detected; minor deviations can be covered by the day-to-day imprecision of the method applied.

4 Organisational details

4.1 Participants

A list of 38 candidate laboratories from different countries eligible for the analysis of cadmium had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organizing the respective ICI. However, as one candidate laboratory on the list was the organiser itself, finally only 37 candidate laboratories were considered. This was in line with the Standard Operation Procedure HBM4EU-SOP-QA-001, according to which a laboratory involved in the organisation of an ICI/ EQUAS for a certain biomarker/ matrix cannot participate in the ICI/ EQUAS studies for that particular biomarker/ matrix combination

Invitation letters were thus sent by e-mail to 37 candidate laboratories on 23rd February 2018 (see Appendix 3). It was indicated that participation would be free of charge, and that those who subscribed to the ICI would receive a kit containing the test materials needed for analysis. The condition for participation was that the test results had to be submitted within the stipulated deadline (1st May 2018).

Twenty-one laboratories from 17 countries out of the 37 laboratories (57%) in the revised candidate list indicated their interest in participating in this ICI and sent their registration form to IPASUM, with their agreement to abide by the conditions for participation. These laboratories received an individual laboratory code to report their measurement results.

From the 21 potential participants, 21 performed the assays and submitted their results.

4.2 Dispatch and instructions

Test materials were dispatched to the participants under ambient conditions on 3rd April 2018. Each participant received nine vials consisting of 5 mL urine each. Six vials were spiked with the biomarker, while three vials contained native control material. Moreover, a letter with instructions on sample handling (instruction letter, see Appendix 4), a sample receipt form to be sent back to IPASUM upon receipt of the test material as well as a result submission form and a method information form were sent to the participants by e-mail. The latter form was used to extract relevant information related to the analytical method used for quantification.

Participants were asked to perform a single analysis of each sample using the same procedure as will be used for analysis of samples in the frame of HMB4EU and to report results following the instruction given.

5 Data evaluation

5.1 False negatives and false positives

Three of 21 participating laboratories measured false negatives in control material Cd_{low} and Cd_{high}.

5.2 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.

5.3 Target standard deviation

For calculation of the Z-scores, Fit-for-purpose (FFP = 25 %) target standard deviation was used because the criteria applied to the first round were not that strict.

5.4 ICI standard deviation

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

5.5 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 1.

Table 1: 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, 21 laboratories from 17 countries agreed to participate in this study. All of them submitted results. However, as this was the first round, not all participants were able to meet the stipulated deadline due to technical problems.

An overview of results, method information and urine properties (creatinine, osmolality, specific gravity) submitted by the participants is included in Appendix 8.

Two of 21 participating laboratories (QR/101 and QR/108) measured false negatives (one out of three in Cd_{low}). The participant`s LOQ is below [assigned value – 3*target standard deviation]. That`s the reason why the value “0” is used as input for the mentioned control materials for calculation of the Z-score.

One of 21 participating laboratories (QR/110) measured false negatives (three out of three in Cd_{low} and three out of three in Cd_{high}). The participant’s LOQ is not below [assigned value – 3*target standard deviation]. This is the reason why no Z-score can be calculated for this participant. The method LOQ is too high to be fit-for-purpose.

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 target standard deviation for each of the analytes/ control materials are included in Appendix 6.

6.3 Assessment of laboratory performance

Z-scores were calculated for all quantified biomarkers and a graphical representation of the Z-scores is provided in Appendix 7.

Twenty-one laboratories out of 21 registered candidate laboratories reported results. Eighteen laboratories have satisfactory Z-scores in Cd_{low} and Cd_{high}. Two laboratories have questionable results (QR/108 in Cd_{low} and QR/101 in Cd_{high}). One laboratory has unacceptable Z-scores and for one laboratory no Z-score could be calculated (see appendix 6).

6.4 Conclusions and recommendations

The overall participation in the HBM4EU ICI Round 1 was successful. Twenty-one laboratories out of the 37 laboratories (56%) in the revised candidate list confirmed their participation in the ICI. All of these 21 registered candidate laboratories reported results, representing a participation rate of 100%.

Regarding the quantification of cadmium in urine, 85 % of the results were satisfactory, 5 % were questionable and 5 % were unsatisfactory. For one laboratory (5 %) no Z-score could be calculated. 90 % of the participating laboratories used ICP-MS and 10 % used AAS as analytical instrument.

An overview of the analytical methods applied and the overall performance of the individual laboratories is given in the table below.

Overview of analytical methods applied and overall performance of the individual laboratories

Lab. code	Analytical method	LOQ	Cd_{low} + ($\|Z\| \leq 2$), ∘ ($2 < \|Z\| < 3$), - ($\|Z\| \geq 3$)	Cd_{high} + ($\|Z\| \leq 2$), ∘ ($2 < \|Z\| < 3$), - ($\|Z\| \geq 3$)	Result
QR/101	ICP-MS	0.022	+	∘	questionable**
QR/102	AAS	0.024	+	+	approved
QR/103	ICP-MS	0.050	+	+	approved
QR/104	ICP-MS	0.160	+	+	approved
QR/105	ICP-MS	0.100	+	+	approved
QR/106	ICP-MS	0.025	+	+	approved
QR/107	ICP-MS	0.008	+	+	approved
QR/108	ICP-MS	0.030	∘	+	questionable**
QR/109	ICP-MS	0.050	+	+	approved
QR/110	ICP-MS	1.250	ND*	ND*	not approved
QR/111	ICP-MS	0.066	+	+	approved
QR/112	ICP-MS	0.050	+	+	approved
QR/113	ICP-MS	0.100	+	+	approved
QR/114	ICP-MS	0.070	+	+	approved
QR/115	ICP-MS	0.010	+	+	approved
QR/116	ICP-MS	0.033	+	+	approved
QR/117	ICP-MS	0.003	+	+	approved
QR/118	ICP-MS	0.003	+	+	approved
QR/119	AAS	0.095	-	-	not approved
QR/120	ICP-MS	0.050	+	+	approved
QR/121	ICP-MS	0.048	+	+	approved

* ND = not detected/ not assessable

** A final evaluation will take place upon completion of all three rounds as an overall assessment of the participant's performance in ICI Round 1, ICI Round 2 and EQUAS.

7 References

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

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

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

[4] HBM4EU-SOP-QA-002 “Preparation of test materials for ICI / EQUAS”

[5] HBM4EU-SOP-QA-003 “Evaluation of ICI / EQUAS results”

[6] HBM4EU-SOP-QA-004 “Reporting of ICI / EQUAS studies”

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

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

[9] 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.

[10] 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.

[11] 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. Homogeneity data

Homogeneity

Version HBM4EU v1

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

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	0.07	0.09	0.08	-0.02	0.00	0.00
2	0.06	0.09	0.08	-0.03	0.00	0.00
3	0.11	0.09	0.10	0.02	0.00	0.00
4	0.11	0.09	0.10	0.02	0.00	0.00
5	0.09	0.09	0.09	0.00	0.00	0.00
6	0.08	0.09	0.09	-0.01	0.00	0.00
7	0.10	0.09	0.10	0.01	0.00	0.00
8	0.09	0.09	0.09	0.00	0.00	0.00
9	0.08	0.09	0.09	-0.01	0.00	0.00
10	0.08	0.09	0.09	-0.01	0.00	0.00
				Σ =	0.00	0.00
Lowest:		0.06 µg/kg				
Highest		0.11 µg/kg				
Grand mean ($\bar{x}$):		0.09 µg/kg				
Stdev:		0.01 µg/kg				
VC%:		12.8% µg/kg				

Outliers: Cochran's test

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

--> C =	0.360	
--> Ccrit=	0.602	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% =	65.19	RSD% =	22	RSD% =	25
σ_H =	0.06	σ_H =	0.02	σ_H =	0.02
σ_H used:	0.02				

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.01	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.01	
s_s < critical?	→ ACCEPT: Homogeneity adequate	
s_w < 0.5*σ_H?	→ NOT ACCEPTED: Method not suited	

Homogeneity

Version HBM4EU v1

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

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	0.19	0.20	0.20	-0.01	0.00	0.00
2	0.18	0.20	0.19	-0.02	0.00	0.00
3	0.19	0.20	0.20	-0.01	0.00	0.00
4	0.18	0.20	0.19	-0.02	0.00	0.00
5	0.20	0.20	0.20	0.00	0.00	0.00
6	0.20	0.20	0.20	0.00	0.00	0.00
7	0.20	0.20	0.20	0.00	0.00	0.00
8	0.18	0.20	0.19	-0.02	0.00	0.00
9	0.21	0.20	0.21	0.01	0.00	0.00
10	0.22	0.20	0.21	0.02	0.00	0.00
				$\Sigma =$	0.00	0.00

Lowest:	0.18	µg/kg
Highest	0.22	µg/kg
Grand mean ($\bar{x}$):	0.20	µg/kg
Stdev:	0.01	µg/kg
VC%:	4.9%	µg/kg

Outliers: Cochran's test

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

--> C =	0.211	
--> Ccrit=	0.602	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% =	57.77	RSD% =	22	RSD% =	25
σ_H =	0.11	σ_H =	0.04	σ_H =	0.05
σ_H used:	0.05				

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.01	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.01	
s_s < critical?	→ ACCEPT: Homogeneity adequate	
s_w < 0.5*σ_H?	→ ACCEPT: Method suited	

Homogeneity

Version HBM4EU v1

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

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	0.44	0.45	0.45	-0.01	0.00	0.00
2	0.45	0.45	0.45	0.00	0.00	0.00
3	0.42	0.45	0.44	-0.03	0.00	0.00
4	0.43	0.45	0.44	-0.02	0.00	0.00
5	0.46	0.45	0.46	0.01	0.00	0.00
6	0.48	0.45	0.47	0.03	0.00	0.00
7	0.45	0.45	0.45	0.00	0.00	0.00
8	0.47	0.45	0.46	0.02	0.00	0.00
9	0.45	0.45	0.45	0.00	0.00	0.00
10	0.47	0.45	0.46	0.02	0.00	0.00
				$\Sigma =$	0.00	0.00

Lowest:	0.42	µg/kg
Highest	0.48	µg/kg
Grand mean ($\bar{x}$):	0.45	µg/kg
Stdev:	0.01	µg/kg
VC%:	2.9%	µg/kg

Outliers: Cochran's test

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

--> C =	0.281	
--> Ccrit=	0.602	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% =	51.02	RSD% =	22	RSD% =	25
σ_H =	0.23	σ_H =	0.10	σ_H =	0.11

σ_H used: 0.11

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.01	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.03	
s_s < critical?	→ ACCEPT: Homogeneity adequate	
s_w < 0.5*σ_H?	→ ACCEPT: Method suited	

Appendix 2. Stability data

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Cd (U)**
Storage: <-18°C

Biomarker **native**

	t=0 (storage)	t=a (analysis)
dates:	22.02.2018	22.03.2018
values:	0.07	0.13
	0.06	0.14
	0.11	0.14
	0.11	0.12
	0.09	0.13
	0.08	0.12
	0.10	0.12
	0.09	0.13
	0.08	0.12
	0.08	0.14
number=	10	10
average=	0.09	0.13
std dev=	0.016	0.009

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.09	10	0.016
22.03.2018	28	0.13	10	0.009

x0-xa= **-0.04**

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.019	0.02175
0,3*σH=	0.006	0.006525
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':	
F=	3.493
Fcrit=	3.179
Significant difference?	**Significant difference in std detected**
sed^2=	0.01
n=	18
std difference=	0.01
t=	7.16
t-crit=	2.10
Significant difference?	**Statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material:	Cd (U)
Storage:	<-18°C
Biomarker:	low

	t=0 (opslag)	t=a (analyse)
dates:	22.02.2018	22.03.2018
values:	0.19	0.22
	0.18	0.24
	0.19	0.22
	0.18	0.24
	0.20	0.23
	0.20	0.24
	0.20	0.22
	0.18	0.23
	0.21	0.23
	0.22	0.23
number=	10	10
average=	0.20	0.23
std dev=	0.014	0.008

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.20	10	0.014
22.03.2018	28	0.23	10	0.008

x0-xa=	**-0.04**

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	0.043	0.04875
0.3*σH=	0.013	0.014625
x0-xa<0.3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':	
F=	2.750
Fcrit=	3.179
Significant difference?	**No significant difference in std detected**
sed^2=	0.01
n=	18
std difference=	0.01
t=	7.00
t-crit=	2.10
Significant difference?	**Statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: Cd (U)
Storage: <-18°C

Biomarker **high**

	t=0 (opslag)	t=a (analyse)
dates:	22.02.2018	22.03.2018
values:	0.44	0.47
	0.45	0.46
	0.42	0.48
	0.43	0.49
	0.46	0.47
	0.48	0.47
	0.45	0.46
	0.47	0.48
	0.45	0.48
	0.47	0.46
number=	10	10
average=	0.45	0.47
std dev=	0.019	0.010

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.45	10	0.019
22.03.2018	28	0.47	10	0.010

x0-xa=	**-0.02**

test 'consequential instability':	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	0.099	0.113
0,3*σH=	0.030	0.0339
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	3.292
Fcrit=	3.179
Significant difference?	**Significant difference in std detected**
sed^2=	0.02
n=	18
std difference=	0.01
t=	2.96
t-crit=	2.10
Significant difference?	**Statistic instability detected**

Appendix 3. Copy of letter of invitation

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study Cd/Round 1

Title of ICI/EQUAS: Cadmium in blood and urine

Dear Colleague,

within the frame of HBM4EU the

Institute and Outpatient Clinic for Occupational-, Social- and Environmental Medicine

Friedrich-Alexander University Erlangen-Nuremberg

Henkestr. 9-11

91054 Erlangen

Germany

announces the 1st round of ICI/EQUAS for the determination of Cadmium in blood and urine.
The aim of ICI/EQUAS exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other laboratories and/or expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

Participation is mandatory for laboratories analysing samples in the frame of HBM4EU.

Test samples
The matrices will be blood and urine, respectively the participants will receive:

- 3 different materials of blood (3 samples of 3mL each) for determination of cadmium in blood and/or
- 3 different materials of urine (3 samples of 5mL each) for determination of cadmium in urine

Target biomarkers
The biomarker potentially present in the test samples is Cadmium.
We recommend at least the following LOQ for the parameters:

LOQ for Cadmium in blood: 0.15 µg/L
LOQ for Cadmium in urine: 0.05 µg/L

Calendar:

Deadline registration	09-03-2018
Distribution of test samples (projected)	23-03-2018
Deadline for reporting the results (projected)	20-04-2018

Registration
For registration, please find attached a registration form for parameter Cadmium in blood and one for parameter Cadmium in urine. Please send them back to us by mail in case you want to register. Upon registration, the participant will receive a lab-code to be used for submission of results.

Fee
For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participant is responsible for custom clearance and associated costs if applicable.

Confidentiality:
All laboratory specific information will be treated confidentially, and will never be disclosed to third parties (government, accreditation bodies) except the HBM4EU QAU, without permission of the laboratory

Contact information organiser:

Coordinators:

- Prof. Dr. Thomas Göen

- Barbara Schaller, MD

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Appendix 4. Copy of letter/instructions sent together with test samples

HBM4EU: Instruction letter ICI / EQUAS study Cd in urine/Round 1

Title of ICI/EQUAS: Cadmium in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study Cd in urine/Round 1 for the determination of Cadmium in urine.

You will receive a parcel containing 9 test samples. Each sample consists of approximately 5 mL urine. 3 vials will contain raw control material, 2x3 are spiked with the biomarker. Only the results of the analysis of the spiked material will be considered for certification.

The parcel will be shipped on 03.04.2018 under ambient conditions.

Instructions:

- Upon receipt, please check the content for any damage/leaking of the containers, complete the sample receipt form and return it to the organiser.
- Store the test samples under frozen (-18°C) conditions until analysis.
- Analyse the samples for the biomarkers indicated in the invitation letter ref/23.02.2018.
- Thaw the samples and re-homogenise them according to your own procedure.
- Analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU.
- Carry out a single analysis for each sample.
- For submission of results and method information use the forms provided the next days by email.
- The deadline for submission of analysis results and method details is 01.05.2018.

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

Barbara Schaller
Moritz Schäfer

Email: ipasum-hbm4eu@fau.de
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Tel.: + 49 (0)9131/8522374

Appendix 5. ICP-MS method information IPASUM

HBM4EU: Method information form for participation in ICI study Cd/Round 1

Title of ICI: Cadmium in urine

Laboratory code	IPASUM Erlangen - Germany	
ISO17025 accredited	no	
SAMPLE PREPARATION		
amount sample	0.4	ml
Extraction	-	
- pH adjustment		
- LLE; solvent(s) / time / shaking		
- SPE; material		
Digestion	-	
INSTRUMENTAL ANALYSIS		
AAS	no	
Wavelength		
Background compensation		
Matrix modifier		
Dilution factor		
Other remarks		
ICP	yes (Nexion3xx PerkinElmer)	
Dilution	1:10 with H_20 and HNO_3	
Nebulizer	PFA-ST	
Reagent gas	argon	
Masses monitored	Cd^{114}	
Detection		
MS	single quad	
OES		

Quantification		
Use of internal standard (IS)	yes (Rh^{103})	
- response normalised to IS	yes	

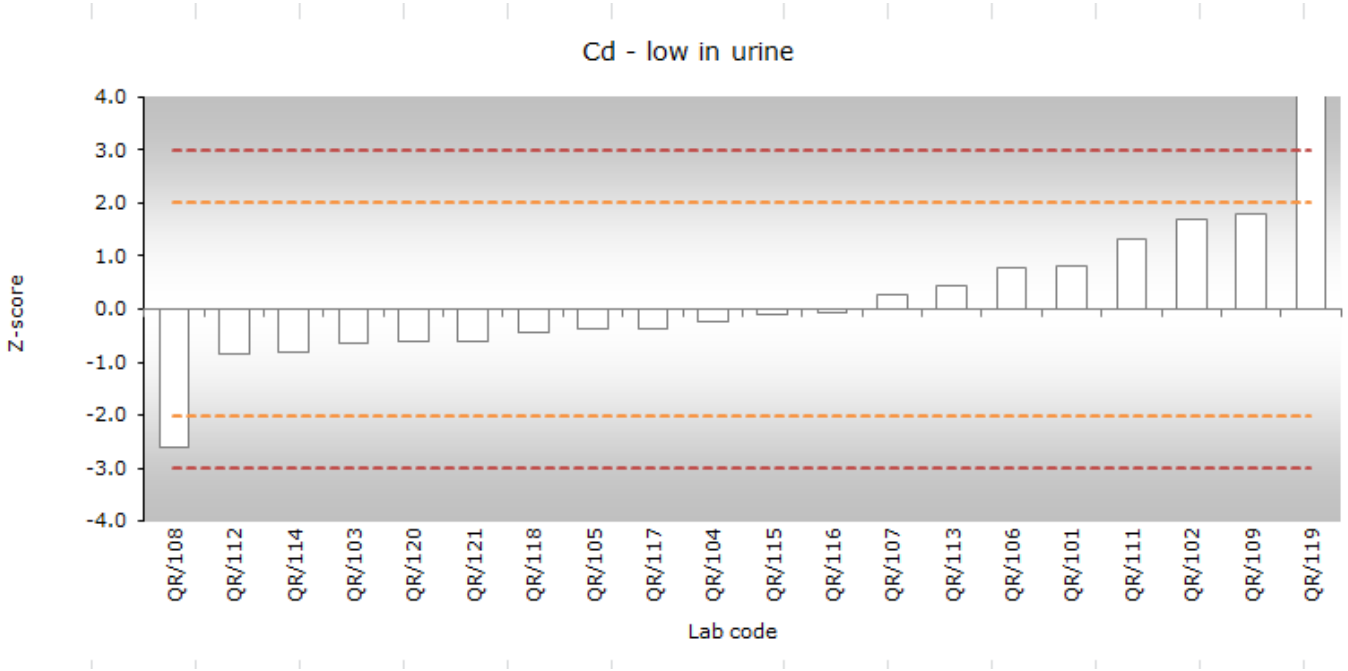
Calibration	external calibrant (matrix based)	
	multi-level	
Correction for recovery	no	
Identification criteria used		
- ion ratio tolerance	% relative	
- other		

Further remarks/observations:

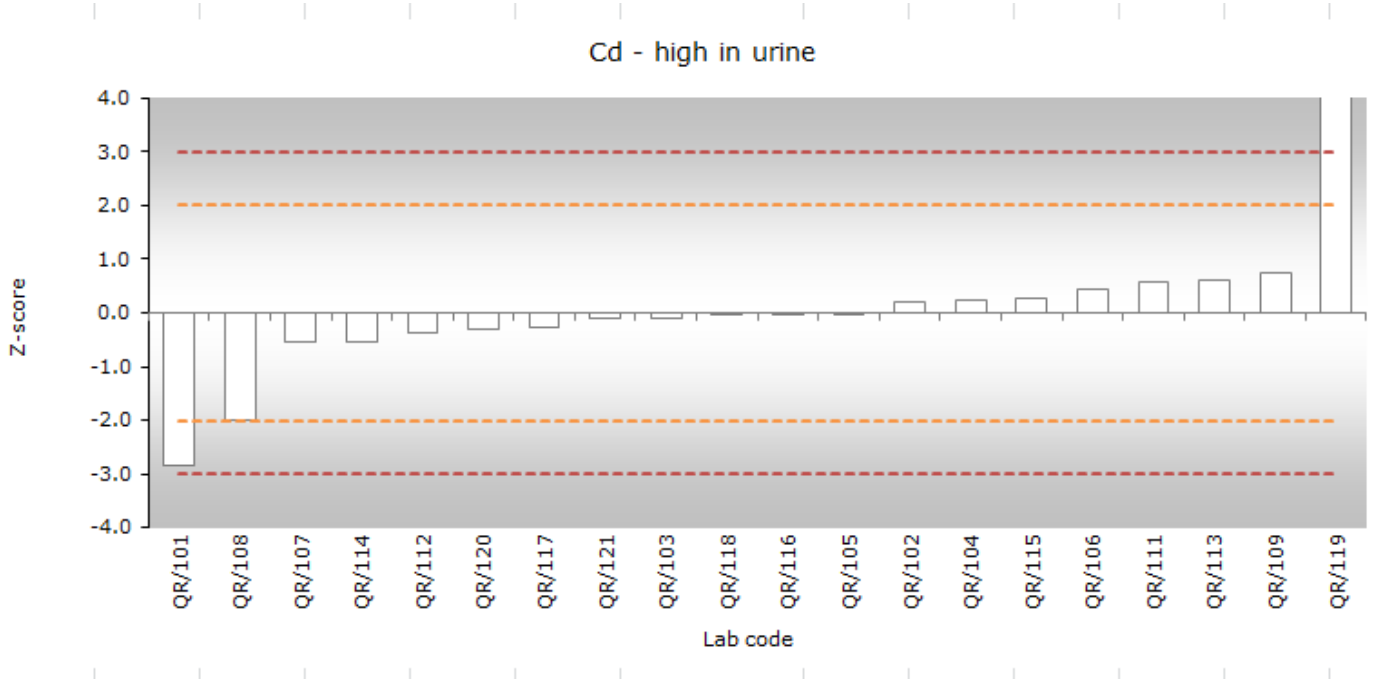
Appendix 6. Assigned values and participant's performance

Control material	Cd (urine) HBM4EU 01/2018			
Cadmium (urine)	Cd_{low}		Cd_{high}	
assigned value <ng/mL>	0.2378		0.4483	
uncertainty of assigned value < ng/mL >	0.0139		0.0160	
study RSD_R (%)	20.94		12.80	
Relative target standard deviation (%)	24.98		25.01	
Laboratory code	value	Z-score	value	Z-score
QR/101	0.29	0.82	0.13	**-2.84**
QR/102	0.34	1.69	0.47	0.19
QR/103	0.20	-0.63	0.44	-0.09
QR/104	0.22	-0.24	0.48	0.25
QR/105	0.22	-0.38	0.45	-0.01
QR/106	0.29	0.79	0.50	0.46
QR/107	0.25	0.26	0.39	-0.54
QR/108	0.08	**-2.59**	0.23	-1.98
QR/109	0.35	1.80	0.53	0.73
QR/110	-	**-**	-	**-**
QR/111	0.32	1.30	0.51	0.59
QR/112	0.19	-0.83	0.41	-0.36
QR/113	0.26	0.45	0.52	0.60
QR/114	0.19	-0.81	0.39	-0.53
QR/115	0.23	-0.10	0.48	0.26
QR/116	0.23	-0.08	0.45	-0.03
QR/117	0.22	-0.38	0.42	-0.27
QR/118	0.21	-0.43	0.45	-0.03
QR/119	1.12	**14.84**	1.30	**7.64**
QR/120	0.20	-0.62	0.41	-0.31
QR/121	0.20	-0.60	0.44	-0.09

Appendix 7. Graphical representation of the Z-scores



Lab	Z-score	Z'-score	Z_r-score	Z'_r-score
QR/108	-2.59	-2.52	-2.59	-2.52
QR/112	-0.83	-0.81	-0.83	-0.81
QR/114	-0.81	-0.79	-0.81	-0.79
QR/103	-0.63	-0.61	-0.63	-0.61
QR/120	-0.62	-0.60	-0.62	-0.60
QR/121	-0.60	-0.59	-0.60	-0.59
QR/118	-0.43	-0.42	-0.43	-0.42
QR/105	-0.38	-0.37	-0.38	-0.37
QR/117	-0.38	-0.37	-0.38	-0.37
QR/104	-0.24	-0.24	-0.24	-0.24
QR/115	-0.10	-0.09	-0.10	-0.09
QR/116	-0.08	-0.08	-0.08	-0.08
QR/107	0.26	0.25	0.26	0.25
QR/113	0.45	0.44	0.45	0.44
QR/106	0.79	0.77	0.79	0.77
QR/101	0.82	0.80	0.82	0.80
QR/111	1.30	1.27	1.30	1.27
QR/102	1.69	1.65	1.69	1.65
QR/109	1.80	1.76	1.80	1.76
QR/119	14.84	14.45	14.84	14.45
0				
0				
0				
0				



Lab	Z-score	Z'-score	Z_r-score	Z'_r-score
QR/101	-2.84	-2.81	-2.84	-2.81
QR/108	-1.98	-1.96	-1.98	-1.96
QR/107	-0.54	-0.54	-0.54	-0.54
QR/114	-0.53	-0.53	-0.53	-0.53
QR/112	-0.36	-0.36	-0.36	-0.36
QR/120	-0.31	-0.31	-0.31	-0.31
QR/117	-0.27	-0.26	-0.27	-0.26
QR/121	-0.09	-0.09	-0.09	-0.09
QR/103	-0.09	-0.09	-0.09	-0.09
QR/118	-0.03	-0.03	-0.03	-0.03
QR/116	-0.03	-0.03	-0.03	-0.03
QR/105	-0.01	-0.01	-0.01	-0.01
QR/102	0.19	0.19	0.19	0.19
QR/104	0.25	0.25	0.25	0.25
QR/115	0.26	0.26	0.26	0.26
QR/106	0.46	0.45	0.46	0.45
QR/111	0.59	0.59	0.59	0.59
QR/113	0.60	0.60	0.60	0.60
QR/109	0.73	0.72	0.73	0.72
QR/119	7.64	7.56	7.64	7.56
0				
0				
0				
0				

Appendix 8. Results of the shipped test samples and analytical instrument used by the participants

Cadmium in urine [ng/mL]														
Lab. code	***Native -1***	***Native -2***	***Native -3***	**Low -1**	**Low -2**	**Low -3**	**High -1**	**High -2**	**High -3**	**LOQ**	**Analytical instrument**	**Creatinine [g/L]**	**Osmolality [mOsm/kg]**	**Specific gravity [g/L]**
QR/101	*0.760*	*0.260*	*ND*	0.630	ND	0.230	0.040	0.120	0.230	0.022	ICP-MS	-	-	-
QR/102	*0.164*	*0.192*	*0.174*	0.514	0.327	0.174	0.514	0.519	0.377	0.024	AAS	-	-	-
QR/103	*0.086*	*0.074*	*0.071*	0.199	0.222	0.180	0.440	0.433	0.443	0.050	ICP-MS	0.408	_	1.011
QR/104	*0.180*	*0.150*	*0.012*	0.230	0.260	0.180	0.420	0.590	0.420	0.160	ICP-MS	0.440	-	-
QR/105	*0.147*	*0.109*	*0.103*	0.215	0.215	0.215	0.449	0.460	0.433	0.100	ICP-MS	-	-	-
QR/106	*0.092*	*0.092*	*0.096*	0.439	0.213	0.203	0.537	0.476	0.485	0.025	ICP-MS	0.530	-	-
QR/107	*0.210*	*0.191*	*0.189*	0.278	0.217	0.265	0.386	0.372	0.405	0.008	ICP-MS	-	-	-
QR/108	*0.070*	*0.076*	*0.083*	0.131	0.120	ND	0.250	0.117	0.311	0.030	ICP-MS	-	-	-
QR/109	*0.254*	*0.283*	*0.222*	0.348	0.341	0.346	0.492	0.561	0.537	0.050	ICP-MS	0.410	-	-
QR/110	*ND*	*ND*	*ND*	ND	ND	ND	ND	ND	ND	1.250	ICP-MS	0.393	-	1.090
QR/111	*0.152*	*0.169*	*0.183*	0.249	0.415	0.282	0.537	0.489	0.518	0.066	ICP-MS	-	-	-
QR/112	*0.090*	*0.094*	*0.093*	0.189	0.186	0.190	0.408	0.414	0.402	0.050	ICP-MS	-	-	-
QR/113	*0.140*	*0.130*	*0.130*	0.261	0.268	0.265	0.525	0.487	0.535	0.100	ICP-MS	-	-	-
QR/114	*0.114*	*0.100*	*0.108*	0.203	0.183	0.183	0.388	0.403	0.375	0.070	ICP-MS	-	-	-
QR/115	*0.118*	*0.115*	*0.120*	0.207	0.247	0.242	0.455	0.488	0.490	0.010	ICP-MS	-	-	1.011
QR/116	*0.154*	*0.144*	*0.138*	0.223	0.241	0.235	0.452	0.449	0.435	0.033	ICP-MS	-	-	-
QR/117	*0.100*	*0.100*	*0.119*	0.218	0.214	0.213	0.428	0.412	0.415	0.003	ICP-MS	-	-	1.012
QR/118	*0.120*	*0.103*	*0.117*	0.191	0.230	0.215	0.442	0.444	0.450	0.003	ICP-MS	-	-	-
QR/119	*1.185*	*1.175*	*0.923*	1.220	1.112	1.028	1.209	1.315	1.390	0.095	AAS	-	-	-
QR/120	*0.104*	*0.119*	*0.099*	0.189	0.199	0.215	0.446	0.377	0.416	0.050	ICP-MS	0.419	463	1.007
QR/121	*0.113*	*0.100*	*0.194*	0.209	0.203	0.194	0.433	0.443	0.439	0.048	ICP-MS	-	-	-