HORIZON2020 Programme
Contract No. 733032 HBM4EU

REPORT OF THE WP9 ICI

Round 01/2018

Cadmium in blood

Version / date of issue	1 / 16-07-2018
Organiser Coordinator	Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) Friedrich-Alexander University of Erlangen-Nuremberg Henkestr. 9-11 91054 Erlangen GERMANY Thomas Göen, thomas.goeen@fau.de
Authors (IPASUM)	Moritz Schäfer (IPASUM), Barbara Schaller (IPASUM), Karin H. A. Zarrabi (IPASUM)
Approved by:	Argelia Castaño (ISCIII), Marta Esteban (ISCIII)

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 blood. The study was performed from April 2018 to May 2018.

Nine different test samples consisting of 3 mL blood 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, 19 laboratories from 16 countries participated in the ICI. The participation in the ICI was satisfactory; 19 out of 19 laboratories submitted their results.

The evaluation showed that 84 % of the results were satisfactory, none were questionable and 11 % 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 bio monitoring 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 blood 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 bovine blood in EDTA solution and sodium azide, of German origin. Animal health conditions are certified. 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 three mL in tubes with caps (57x15.3 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 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 (analysis method see Appendix 5). The homogeneity was evaluated according to ISO 13528:2015, Fearn et al [2001] and Thompson [2000]. The results are presented in Appendix 2. 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 37 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.

Invitation letters were sent by e-mail to all 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).

Nineteen laboratories from 16 countries out of the 37 laboratories (51%) in the 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 19 potential participants, 19 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 3 mL blood 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

One of 19 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 value 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, 19 laboratories from 16 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 and method information submitted by the participants is included in Appendix 8.

One of 19 participating laboratories (QR/108) measured false negatives (three out of three in Cd_{low} and one 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.

Nineteen laboratories out of 19 registered candidate laboratories reported results. Sixteen laboratories have satisfactory Z-scores in Cd_{low} and Cd_{high}. Two laboratories have unsatisfactory 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. Nineteen laboratories out of the 37 laboratories (51%) in the candidate list confirmed their participation in the ICI. All of these 19 registered candidate laboratories reported results, representing a participation rate of 100%.

Regarding the quantification of cadmium in blood, 84 % of the results were satisfactory, none were questionable and 11 % were unsatisfactory. For one laboratory (5 %) no Z-score could be calculated. 89 % of the participating laboratories used ICP-MS and 11 % 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	0.033	-	-	not approved
QR/102	AAS	0.024	+	+	approved
QR/103	ICP-MS	0.150	+	+	approved
QR/104	ICP-MS	0.250	+	+	approved
QR/105	ICP-MS	0.250	+	+	approved
QR/106	ICP-MS	0.025	+	+	approved
QR/107	ICP-MS	0.025	+	+	approved
QR/108	ICP-MS	0.150	ND*	ND*	not approved
QR/109	ICP-MS	0.100	-	-	not approved
QR/111	ICP-MS	0.157	+	+	approved
QR/112	ICP-MS	0.050	+	+	approved
QR/113	ICP-MS	0.200	+	+	approved
QR/114	ICP-MS	0.100	+	+	approved
QR/115	ICP-MS	0.018	+	+	approved
QR/116	ICP-MS	0.034	+	+	approved
QR/117	ICP-MS	0.002	+	+	approved
QR/118	ICP-MS	0.202	+	+	approved
QR/119	AAS	0.094	+	+	approved
QR/121	ICP-MS	0.120	+	+	approved

* ND = not detected/ not assessable

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:	blood	**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	0.04	0.01	0.03	0.03	0.00	0.00
2	0.01	0.01	0.01	0.00	0.00	0.00
3	0.00	0.01	0.01	-0.01	0.00	0.00
4	0.04	0.01	0.03	0.03	0.00	0.00
5	0.00	0.01	0.01	-0.01	0.00	0.00
6	0.01	0.01	0.01	0.00	0.00	0.00
7	0.01	0.01	0.01	0.00	0.00	0.00
8	0.01	0.01	0.01	0.00	0.00	0.00
9	0.02	0.01	0.02	0.01	0.00	0.00
10	0.00	0.01	0.01	-0.01	0.00	0.00
Lowest:		0.00 µg/kg		Σ =	0.00	0.00
Highest		0.04 µg/kg				
Grand mean ($\bar{x}$):		0.01 µg/kg				
Stdev:		0.01 µg/kg				
VC%:		88.0% µg/kg				

Outliers: Cochran's test

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

--> C =	0.409	
--> 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% =	88.06	RSD% =	22	RSD% =	25
σ_H =	0.01	σ_H =	0.00	σ_H =	0.00
σ_H used:	0.00				

Homogeniteit [1]:

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

Homogeneity

Version HBM4EU v1

Control material:	**blood**	**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	0.21	0.24	0.23	-0.03	0.00	0.00
2	0.23	0.24	0.24	-0.01	0.00	0.00
3	0.22	0.24	0.23	-0.02	0.00	0.00
4	0.28	0.24	0.26	0.04	0.00	0.00
5	0.26	0.24	0.25	0.02	0.00	0.00
6	0.25	0.24	0.25	0.01	0.00	0.00
7	0.25	0.24	0.25	0.01	0.00	0.00
8	0.23	0.24	0.24	-0.01	0.00	0.00
9	0.21	0.24	0.23	-0.03	0.00	0.00
10	0.22	0.24	0.23	-0.02	0.00	0.00
				Σ =	0.01	0.00

Lowest:	0.21	µg/kg
Highest	0.28	µg/kg
Grand mean ($\bar{x}$):	0.24	µg/kg
Stdev:	0.02	µg/kg
VC%:	6.8%	µg/kg

Outliers: Cochran's test

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

--> C =	0.320	
--> 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% =	56.17	RSD% =	22	RSD% =	25
σ_H =	0.13	σ_H =	0.05	σ_H =	0.06
σ_H used:	0.06				

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.02	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.02	
s_s < critical?	**→ ACCEPT: Homogeneity adequate**	
s_w < 0.5*σ_H?	**→ ACCEPT: Method suited**	

Homogeneity

Version HBM4EU v1

Control material:	blood	**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	0.54	0.51	0.53	0.03	0.00	0.00
2	0.51	0.51	0.51	0.00	0.00	0.00
3	0.50	0.51	0.51	-0.01	0.00	0.00
4	0.51	0.51	0.51	0.00	0.00	0.00
5	0.49	0.51	0.50	-0.02	0.00	0.00
6	0.47	0.51	0.49	-0.04	0.00	0.00
7	0.50	0.51	0.51	-0.01	0.00	0.00
8	0.47	0.51	0.49	-0.04	0.00	0.00
9	0.54	0.51	0.53	0.03	0.00	0.00
10	0.52	0.51	0.52	0.01	0.00	0.00
				Σ =	0.01	0.00

Lowest:	0.47	µg/kg
Highest	0.54	µg/kg
Grand mean ($\bar{x}$):	0.51	µg/kg
Stdev:	0.02	µg/kg
VC%:	3.4%	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% =	50.12	RSD% =	22	RSD% =	25
σ_H =	0.25	σ_H =	0.11	σ_H =	0.13
σ_H used:	0.13				

Homogeniteit [1]:

s_x =	0.01	
s_w =	0.02	(within sample standard deviation)
s_s =	0.00	(between sample standard deviation)
critical=	0.04	
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 (B)**
Storage: <-18°C

Biomarker **native**

	t=0 (storage)	t=a (analysis)
dates:	22.02.2018	21.03.2018
values:	0.04	0.01
	0.01	0.00
	0.00	0.01
	0.04	0.01
	0.00	0.01
	0.01	0.01
	0.01	0.00
	0.01	0.02
	0.02	0.01
	0.00	0.00
number=	10	10
average=	0.01	0.01
std dev=	0.015	0.006

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.01	10	0.015
21.03.2018	27	0.01	10	0.006

x0-xa= **0.01**

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.003	0.0035
0,3*σH=	0.001	0.00105
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':	
F=	5.667
Fcrit=	3.179
Significant difference?	**Significant difference in std detected**
sed^2=	0.01
n=	18
std difference=	0.01
t=	1.16
t-crit=	2.10
Significant difference?	**No statistic instability detected**

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Cd (B)**
Storage: <-18°C

Biomarker **low**

	t=0 (opslag)	t=a (analyse)
dates:	22.02.2018	21.03.2018
values:	0.21	0.19
	0.23	0.23
	0.22	0.25
	0.28	0.19
	0.26	0.20
	0.25	0.24
	0.25	0.20
	0.23	0.20
	0.21	0.21
	0.22	0.22
number=	10	10
average=	0.24	0.21
std dev=	0.023	0.021

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.24	10	0.023
21.03.2018	27	0.21	10	0.021

x0-xa= **0.02**

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	0.052	0.059
0,3*σH=	0.016	0.0177
x0-xa<0,3*σH?	**Consequential instability detected**	**Consequential instability detected**

test 'significant difference':	
F=	1.207
Fcrit=	3.179
Significant difference?	**No significant difference in std detected**
sed^2=	0.02
n=	18
std difference=	0.01
t=	2.32
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 (B)
Storage:	<-18°C
Biomarker	high

	t=0 (opslag)	t=a (analyse)
dates:	22.02.2018	21.03.2018
values:	0.54	0.47
	0.51	0.47
	0.50	0.50
	0.51	0.47
	0.49	0.50
	0.47	0.52
	0.50	0.49
	0.47	0.51
	0.54	0.51
	0.52	0.49
number=	10	10
average=	0.51	0.49
std dev=	0.025	0.018

analysis date	time (days)	µg/L	n	std dev
22.02.2018	0	0.51	10	0.025
21.03.2018	27	0.49	10	0.018
x0-xa=	**0.01**			

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	0.111	0.12625
0,3*σH=	0.033	0.037875
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	1.811
Fcrit=	3.179
Significant difference?	**No significant difference in std detected**
sed^2=	0.02
n=	18
std difference=	0.01
t=	1.24
t-crit=	2.10
Significant difference?	**No 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 of 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

Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine
Friedrich-Alexander University Erlangen-Nuremberg
Henkestr. 9-11
91054 Erlangen
Germany

Email: ipasum-hbm4eu@fau.de

Barbara.schaller@fau.de

Tel.: + 49 (0)9131/8522374

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

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

Title of ICI/EQUAS: Cadmium in blood

Dear participant,

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

You will receive a parcel containing 9 test samples. Each sample consists of approximately 3 mL blood. 3 vials will contain raw control material, 6 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 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
Barbara.schaller@fau.de
Moritz.Schaefer@fau.de

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 blood

Laboratory code	IPASUM Erlangen - Germany	
ISO17025 accredited	no	
SAMPLE PREPARATION		
amount sample	0.2	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	1:20 with H_20, NH_4, EDTA and Triton-X-solution	
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	

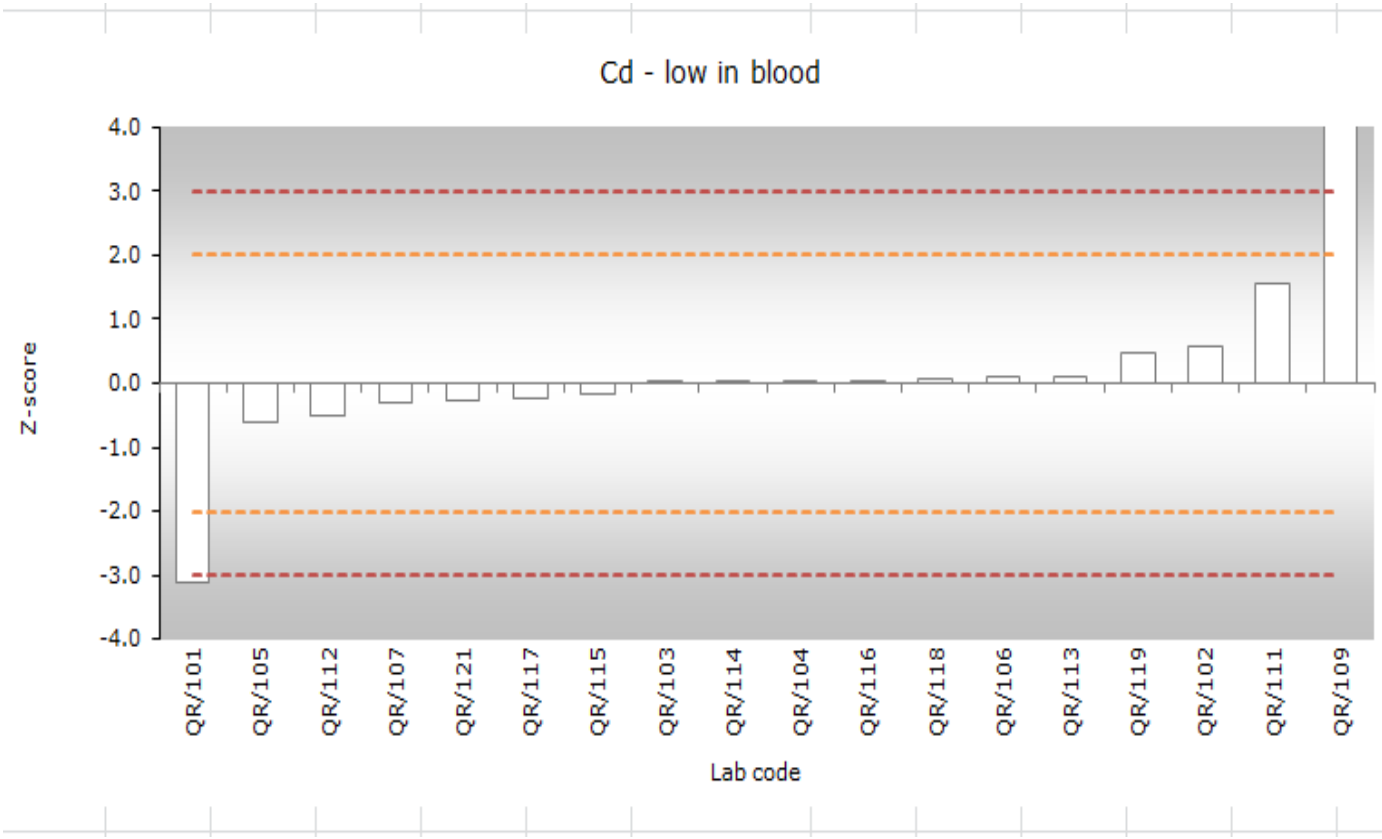
Calibration	external calibrant (matrix-based)	
	multi-level	
Correction for recovery	No	
Identification criteria used		
- ion ratio tolerance	% relative	
- other		

Further remarks/observations:

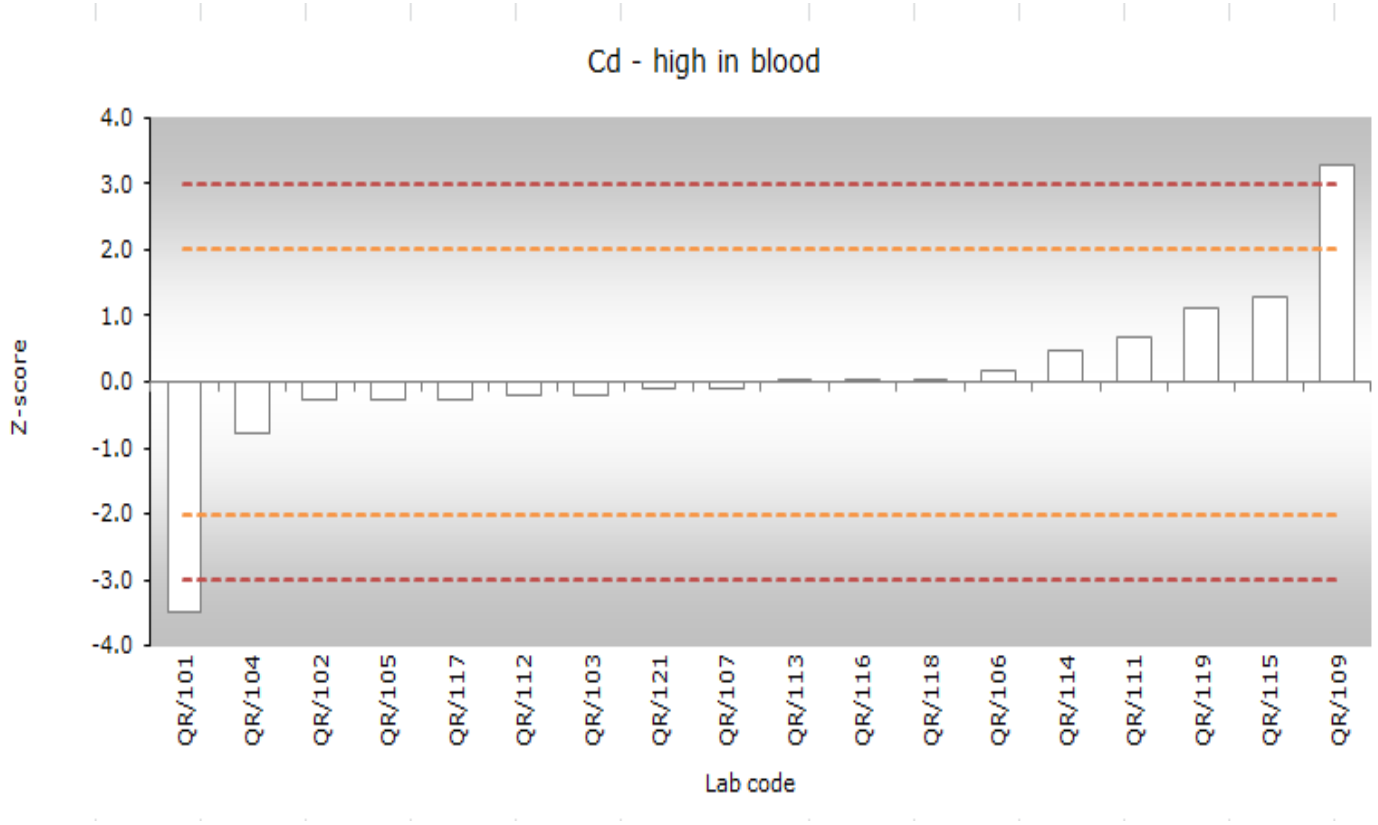
Appendix 6. Assigned values and participant's performance

Control material	**Cd (blood) HBM4EU 01/2018**			
Cadmium (blood)	**Cd_{low}**		**Cd_{high}**	
assigned value <ng/mL>	0.2512		0.5356	
uncertainty of assigned value < ng/mL >	0.0081		0.0154	
study RSD_R (%)	10.91		9.78	
Relative target standard deviation (%)	25.04		25.00	
Laboratory code	**Value**	**Z-score**	**value**	**Z-score**
QR/101	0.06	**-3.10**	0.07	**-3.47**
QR/102	0.29	0.57	0.50	-0.28
QR/103	0.25	0.01	0.51	-0.21
QR/104	0.25	0.03	0.43	-0.76
QR/105	0.21	-0.62	0.50	-0.28
QR/106	0.26	0.10	0.56	0.18
QR/107	0.23	-0.30	0.52	-0.10
QR/108	-	-	-	-
QR/109	0.86	**6.77**	0.98	**3.29**
QR/111	0.35	1.57	0.63	0.67
QR/112	0.22	-0.50	0.51	-0.22
QR/113	0.26	0.11	0.54	0.01
QR/114	0.25	0.01	0.60	0.49
QR/115	0.24	-0.18	0.71	1.29
QR/116	0.25	0.04	0.54	0.04
QR/117	0.24	-0.24	0.50	-0.27
QR/118	0.26	0.08	0.54	0.05
QR/119	0.28	0.46	0.69	1.13
QR/121	0.23	-0.27	0.52	-0.12

Appendix 7. Graphical representation of the Z-scores



Lab	Z-score	Z'-score	Z_r-score	Z'_r-score
QR/101	-3.10	-3.08	-3.10	-3.08
QR/105	-0.62	-0.62	-0.62	-0.62
QR/112	-0.50	-0.49	-0.50	-0.49
QR/107	-0.30	-0.30	-0.30	-0.30
QR/121	-0.27	-0.27	-0.27	-0.27
QR/117	-0.24	-0.24	-0.24	-0.24
QR/115	-0.18	-0.18	-0.18	-0.18
QR/103	0.01	0.01	0.01	0.01
QR/114	0.01	0.01	0.01	0.01
QR/104	0.03	0.03	0.03	0.03
QR/116	0.04	0.04	0.04	0.04
QR/118	0.08	0.08	0.08	0.08
QR/106	0.10	0.10	0.10	0.10
QR/113	0.11	0.11	0.11	0.11
QR/119	0.46	0.45	0.46	0.45
QR/102	0.57	0.57	0.57	0.57
QR/111	1.57	1.56	1.57	1.56
QR/109	6.77	6.71	6.77	6.71
0				
0				
0				
0				
0				
0				



Lab	Z-score	Z'-score	Z_r-score	Z'_r-score
QR/101	-3.47	-3.45	-3.47	-3.45
QR/104	-0.76	-0.76	-0.76	-0.76
QR/102	-0.28	-0.28	-0.28	-0.28
QR/105	-0.28	-0.27	-0.28	-0.27
QR/117	-0.27	-0.26	-0.27	-0.26
QR/112	-0.22	-0.21	-0.22	-0.21
QR/103	-0.21	-0.21	-0.21	-0.21
QR/121	-0.12	-0.12	-0.12	-0.12
QR/107	-0.10	-0.10	-0.10	-0.10
QR/113	0.01	0.01	0.01	0.01
QR/116	0.04	0.04	0.04	0.04
QR/118	0.05	0.04	0.05	0.04
QR/106	0.18	0.18	0.18	0.18
QR/114	0.49	0.49	0.49	0.49
QR/111	0.67	0.67	0.67	0.67
QR/119	1.13	1.13	1.13	1.13
QR/115	1.29	1.28	1.29	1.28
QR/109	3.29	3.26	3.29	3.26
0				
0				
0				
0				
0				

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

Lab. code	Cadmium (blood) [ng/mL]										Analytical instrument
	Native-1	***Native-2***	***Native-3***	**Low-1**	**Low-2**	**Low-3**	**High-1**	**High-2**	**High-3**	**LOQ**	
QR/101	*0.046*	*0.039*	*0.044*	0.062	0.045	0.062	0.104	0.055	0.052	0.033	ICP-MS
QR/102	*0.141*	*0.150*	*0.156*	0.263	0.288	0.312	0.510	0.500	0.483	0.024	AAS
QR/103	*ND*	*ND*	*ND*	0.244	0.198	0.314	0.517	0.528	0.476	0.150	ICP-MS
QR/104	*0.041*	*0.046*	*0.014*	0.250	0.240	0.270	0.390	0.400	0.510	0.250	ICP-MS
QR/105	*ND*	*ND*	*ND*	0.219	0.198	0.220	0.474	0.518	0.504	0.250	ICP-MS
QR/106	*0.070*	*0.093*	*0.079*	0.257	0.248	0.268	0.558	0.582	0.539	0.025	ICP-MS
QR/107	*0.035*	*0.029*	*0.033*	0.222	0.230	0.246	0.534	0.502	0.532	0.025	ICP-MS
QR/108	*ND*	*ND*	*ND*	ND	ND	ND	0.431	0.340	ND	0.150	ICP-MS
QR/109	*0.429*	*0.452*	*0.465*	0.679	0.704	0.648	0.918	0.993	1.016	0.100	ICP-MS
QR/111	*0.145*	*0.159*	*0.155*	0.348	0.373	0.330	0.684	0.544	0.648	0.157	ICP-MS
QR/112	*ND*	*ND*	*ND*	0.221	0.217	0.223	0.507	0.508	0.505	0.050	ICP-MS
QR/113	*ND*	*ND*	*ND*	0.259	0.253	0.263	0.525	0.551	0.536	0.200	ICP-MS
QR/114	*ND*	*ND*	*ND*	0.246	0.255	0.255	0.603	0.585	0.615	0.100	ICP-MS
QR/115	*0.022*	*0.023*	*0.026*	0.230	0.239	0.252	0.696	0.703	0.727	0.018	ICP-MS
QR/116	*0.047*	*0.051*	*0.053*	0.252	0.241	0.270	0.543	0.565	0.515	0.034	ICP-MS
QR/117	*0.050*	*0.042*	*0.040*	0.221	0.217	0.271	0.456	0.504	0.540	0.002	ICP-MS
QR/118	*0.079*	*0.089*	*0.090*	0.270	0.251	0.249	0.538	0.520	0.567	0.202	ICP-MS
QR/119	*0.099*	*0.186*	*0.188*	0.293	0.309	0.239	0.533	0.999	0.530	0.094	AAS
QR/121	*ND*	*ND*	*ND*	0.278	0.221	0.205	0.542	0.492	0.526	0.120	ICP-MS