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REPORT OF THE WP9 EQUAS

Round 03/2019

Cadmium in urine

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1 Summary

Within the framework of the HBM4EU project, an External Quality Assurance Scheme (EQUAS) was organized and conducted for the analysis of cadmium in urine.

The study was performed from May 2019 to June 2019.

The HBM4EU QAU had selected six expert laboratories for cadmium in urine. Four expert laboratories were from Europe (HBM4EU consortium) and three of them also participated as candidates. Two expert laboratories were from outside Europe (Japan, USA).

In total, 56 laboratories were invited for this second round, of which 42 laboratories from 22 countries registered.

The participation in this EQUAS was successful; 42 out of 42 laboratories (100%) submitted their results.

In Mai 2019, twelve different test samples consisting of 5 mL urine spiked with cadmium at two different concentrations (Cd_{low}, Cd_{high}), six of each concentration, were prepared and sent to the participating expert laboratories for analysis in duplicate. Each candidate laboratory got two samples, one of each concentration for single analysis.

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

The expert assigned values were calculated by averaging the values obtained by the expert labs.

The proficiency of the laboratories was assessed through Z-scores calculated using the mean concentration as established by expert laboratories as assigned value, and a fixed fit-for-purpose relative target standard deviation ($FFP\text{-}RSD_R$) of 25%.

The evaluation of Cd_{low} and Cd_{high} showed that 96% of the results were satisfactory, 2% were questionable and 2% were unsatisfactory (**Table 1**).

Table 1 Overview of results for cadmium in urine in 2nd EQUAS/round 3

Number of laboratories with respective results for cadmium in urine in 2nd EQUAS/round 3				
biomarker	**assigned value**	**satisfactory** (|Z-score| ≤2)	**questionable** (2<|Z-score|<3)	**unsatisfactory** (|Z-score|>3)
Cd_{low}	0.087 ng/ml	40 (96%)	1 (2%)	1 (2%)
Cd_{high}	0.190 ng/ml	40 (96%)	1 (2%)	1 (2%)

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 ongoing in-house method validation.

This 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 3rd round of proficiency testing 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.

For this 2nd EQUAS, expert laboratories had to be selected according to the selection criteria described in HBM4EU-SOP-QA-001 and in agreement with the QAU.

The selection criteria included:

- Number of years of experience with the biomarker/matrix combination of interest.
- Application of highly sensitive and selective analytical techniques for analysis.
- Use of isotope-labelled standards for quantification.
- Availability of in-house validation reports, data on on-going intra-laboratory performance, ISO17025 accreditation for the biomarker of interest.
- Success rate in inter-laboratory comparisons, external quality assessment schemes or at least comparative results in application studies.

EQUAS does not require a certain number of participants, because the performance evaluation is not based on the participants' results but on assigned values and tolerance ranges as derived from the analysis data from the expert laboratories.

The expert-assigned value is the target value based on analysis results obtained from analysis of the control material by at least three expert laboratories (see HBM4EU-SOP-QA-001). The expert-assigned values were calculated by averaging the values obtained by the expert labs.

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 assessment.

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. 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 control materials were aliquoted (5 mL each) into tubes with caps (82x13 mm, polypropylene, Sarstedt). The tubes were stored in a freezer (≤ -18 °C) until transportation. The two different concentrations (Cd_{low}, Cd_{high}) were measured using 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_{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

On the day of preparation of the control materials, six randomly selected test samples of Cd_{low} and six randomly selected test samples of Cd_{high} were stored at -80 °C. The assumption is that under these conditions, the biomarker (Cd) is stable in urine. On the last day of the deadline for submission of results by the participants (January 18, 2019), six test samples of each level (stored at -80 °C) and six samples of each level (stored at -18 °C) were thawed and re-homogenised by vortex shaking. Next, all samples were analysed using ICP-MS (analysis method see **Appendix 5**)

The stability was evaluated according to HBM4EU-SOP-QA-002 and using the Excel-sheet “HBM4EU ICI-EQUAS stability test CM v1”. The results are presented in **Appendix 2**. No consequential instabilities and no statistical differences were detected.

4 Organisational details

4.1 Participants

For the organisation of the 2nd EQUAS, IPASUM invited six selected expert laboratories in agreement with the QAU and according to HBM4EU-SOP-QA-001. Four expert laboratories were from Europe (HBM4EU consortium) and three of them also participated as candidates. Two expert laboratories were from outside Europe (Japan, USA). IPASUM contacted the six selected expert laboratories and sent them invitation letters by e-mail on April 18, 2019 (see **Appendix 3**). It was indicated that participation would be free of charge and that those who subscribed to this EQUAS would receive a kit containing the test materials needed for analysis. Test results had to be submitted within the stipulated deadline (June 11, 2019).

A list of 56 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 EQUAS.

Invitation letters were thus sent by e-mail to 56 candidate laboratories on April 29, 2019 (see **Appendix 3**). It was indicated that participation would be free of charge and that those who subscribed to this EQUAS 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 (June 11, 2019).

Forty-two laboratories from 22 countries out of the 56 laboratories (75%) in the revised candidate list indicated their interest in participating in this EQUAS 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 (see **Appendix 8**).

Forty-two of the 42 potential participants (100%) performed the assays and submitted their results. Thirty-eight participants reported their results within the stipulated deadline (June 11, 2019), while four participants reported with a delay (see **Appendix 8**).

4.2 Dispatch and instructions

Test materials were dispatched to the participants under ambient conditions on May 14, 2019. Each candidate received two test samples spiked with the biomarker at different levels, one of each concentration (Cd_{low}, Cd_{high}). Each sample consisted of approximately 5 mL urine.

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 (expert laboratories: duplicate analysis) using the same procedure as will be used for analysis of samples in the frame of HMB4EU and to report results following the instructions given.

4.3 Deviations from ICI/EQUAS SOPs

For this 2nd EQUAS, the HBM4EU-QA-SOPs were followed. There were no deviations from these SOPs.

5 Data evaluation

5.1 False positives and <LOQ

Classification of false positives and biomarkers reported as "<LOQ-value" or "not detected" (ND) was done as described in HBM4EU-SOP-QA-003.

A result was assigned as false positive if all of the following conditions applied:

1) the biomarker was below the LOQ value as applied by the organiser, the expert laboratories, and the majority of the participants.
2) the biomarker was reported by the participant at a level clearly exceeding the LOQs mentioned under 1.

If a biomarker is reported as "<LOQ-value", AND an assigned value could be established for the biomarker in the control material, a further assessment was done to verify whether this result might be a false negative and to judge whether the LOQ is considered adequate (low enough) for analysis within the frame of HBM4EU. A result is a false negative if the LOQ of a biomarker is well below the assigned value, but the laboratory did not report a quantitative value. The LOQ is considered not adequate (too high) if:

1) the LOQ is substantially above the assigned value
2) the assigned value represents a realistic concentration of real samples in the frame of HBM4EU
3) quantitative determination is feasible by the majority of laboratories

In order to judge "<LOQ" results in a quantitative way, 'proxy-Z-scores' were calculated as described in 5.6.

5.2 Assigned value

For EQUAS studies, the concentration established by expert laboratories is used as the assigned value as described in SOP HBM4EU-SOP-QA-001.

The HBM4EU QAU selected six expert laboratories for cadmium in urine. Four expert laboratories were from Europe (HBM4EU consortium) and also participated as candidates. Two expert laboratories were from outside Europe (Japan, USA). The expert laboratories received six samples of the same control material (Cd_{low}, Cd_{high}). Each expert laboratory analysed the control samples in duplicate (except for QR/112 and QR/115 which performed only single analyses). Upon receipt of their results and method information, the acceptability of the results for establishment of the expert value was verified. The expert value was determined as described in HBM4EU-QA-001.

The individual means and standard deviations for each expert laboratory were calculated. Then, the mean of all individual means of the six expert laboratories (mean-of-means, mom) and the standard deviation of the mom (SD_{mom}) were calculated. The relative uncertainty of the mean of the means is given by:

u = RSD / sqrt(N)

with u = relative uncertainty of the mean of the mean concentrations from the expert labs

RSD = relative standard deviation of the mean of the mean concentrations

N = the number of expert labs (after exclusion of outliers if applicable)

The mean-of-means derived from the expert laboratories is considered suitable for use as assigned value in EQUAS studies if $u \leq 0.7*\sigma_T$ (σ_T = 25% as described in 5.3).

If $u > 0.7*\sigma_T$, the individual means are checked for outliers. When an individual expert mean is identified as an outlier, it is rejected from the data set and the relative uncertainty is calculated again. If the condition $u \leq 0.7*\sigma_T$ is still not met, then the uncertainty of the expert-derived mean is too high to be used as assigned value. In this case, no EQUAS assessment of participants' performance is possible for the applicable biomarker. This is also the case if the number of (remaining) individual expert means is less than three.

Only if the EQUAS assessment of the participants' performance is not possible, is the consensus value of the ICI 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. Two criteria were evaluated before calculating performance's score:

- if the uncertainty was not significant ($u \leq 0.3\sigma_T$), z-scores were then calculated, otherwise another kind of performance's scores was calculated taking into account the uncertainty.
- if the uncertainty did not meet the following criteria: $u \leq 0.7\sigma_T$, no kind of score was provided.

5.3 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD_R) of 25% of the assigned value was used as target standard deviation. This was the default indicated in HBM4EU-SOP-QA-003 and considered appropriate based on the outcome of the 1st round.

5.4 ICI/EQUAS standard deviation

To gain insight into the actual inter-laboratory variability of the biomarker analysis in this study, the robust relative standard deviation (RSD_R) was calculated based on the participants' results, as described in HBM4EU-SOP-QA-003. For this, the results of the expert laboratories were not included.

5.5 Z-scores

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

$$Z = \frac{x - C}{\sigma_T} \qquad (1)$$

with: Z = Z-score for the submitted analysis result;
x = result submitted by the laboratory;
C = expert-assigned value;
σ_T = target standard deviation, here 0.25*C

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 2**.**

Table 2 Classification of Z-scores

$\lvert Z\rvert \leq 2$	satisfactory
$2 < \lvert Z\rvert < 3$	questionable
$\lvert Z\rvert \geq 3$	unsatisfactory

5.6 Proxy-Z-scores

'Proxy-Z-scores' are used to judge "<LOQ" results in a quantitative way (see 5.1). The proxy-Z-scores' are calculated using the LOQ-value as result and equation (1). If no LOQ was specified, zero was used.

proxy-Z-scores are classified as follows:

proxy-Z ≤ -3	false negative. Based on the LOQ provided, the laboratory should have been able to detect and quantify the biomarker. Performance is considered 'unsatisfactory'.
proxy-Z ≥3	the LOQ is considered too high to be fit-for-purpose in the frame of HBM4EU analysis. It also means that the LOQ is too high in comparison with other laboratories. (Note: proxy-Z can only be calculated if an assigned value could be established. If this is the case, this inherently means that reliable quantitative determination at a certain low level is feasible). Performance is considered 'unsatisfactory'.
-3 ≤ proxy-Z < -2	possible false negative. Performance is considered 'questionable'.
2 < proxy-Z ≤ 3	the LOQ is relatively high in relation to HBM4EU analysis and compared to other laboratories. Performance is considered 'questionable'.
-2 ≤ proxy-Z ≤ 2	LOQ is within an acceptable range relative to the assigned value, adequate for HBM4EU analysis, and in line with the LOQs of the majority of the participating laboratories. Performance is considered 'satisfactory'.

6 Results and discussion

6.1 Results submitted by participants

In total, 42 laboratories from 22 countries agreed to participate in this study. In the end, 42 out of 42 participants (100%) submitted their results. Laboratories were also asked to provide LOQs.

Appendix 8 gives an overview of results and LOQs submitted by the participants as well as reasons for delayed submission.

Results indicated as `not detected´ (ND, see **Appendix 8)**:

For **Cd_{low}**, two participants (QR/103 and QR/216) indicated ND and their LOQs were used as input for the EQUAS evaluation. Proxy-Z-scores could be calculated for both laboratories.

For **Cd_{high}**, one participant (QR/216) indicated ND and the LOQ was used as input for the EQUAS evaluation. Proxy-Z-scores could be calculated for this laboratory.

False positive results: No participant detected a false positive in Cd_{low} and Cd_{high}.

Methods:

In almost all cases the samples were analysed by ICP-MS followed by AAS. For sample preparation, most laboratories used no digestion while the others used acid digestion. Almost all participating laboratories used a single quad as detection system, the rest used a triple quad or quadropol. As internal standard, the majority of participating laboratories applied indium or iridium and for calibration, most candidates used an external calibrant (solvent-based), followed by standard addition and external calibrant (matrix-based). In this round, all expert labs and all participants were asked to provide information on the removal of molybdenum oxide interferences. Twenty-four percent removed molybdenum oxide interferences, 44% didn't and 32% didn't provide any information on it.

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 control materials are included in **Appendix 6**.

Graphical representations of the expert values and the mean-of-means for Cd_{low} and for Cd_{high} are shown in **Appendix 9**.

For this report, the results from all expert laboratories were included in the calculation of the mean-of-means, which was then used as assigned value. For the control material Cd_{low}, the assigned value was 0.087 ng/mL and u was $\pm$ 3.2%. For the control material Cd_{high} the assigned value was 0.190 ng/mL and u was $\pm$ 2.0%.

6.3 Assessment of laboratory performance

Z-scores were calculated and graphical representations of the Z-scores for Cd_{low} and Cd_{high} are provided in **Appendix 7**.

Forty-two laboratories out of 42 registered candidate laboratories reported results.

In **Cd_{low}** and in **Cd_{high}**, 40 participants have satisfactory Z-scores, one participant has questionable Z-scores and one participant has unsatisfactory Z-scores (see **Appendix 6**).

6.4 Conclusions and recommendations

The overall participation in the HBM4EU EQUAS Round 3 was successful. Forty-two laboratories out of the 56 laboratories (75%) in the revised candidate list confirmed their participation in this EQUAS. Forty-two of all 42 registered laboratories reported results, representing a participation rate of 100%.

Regarding the quantification of cadmium in urine, the participants achieved in Cd_{low} and in Cd_{high} 96% satisfactory, 2% questionable and 2% unsatisfactory results.

A direct comparison of the overall performance of the laboratories with that of the first and second round is not entirely possible, because there were new laboratories participating in this second EQUAS round and also some laboratories from the other rounds did not participate in this second EQUAS round.

Five additional laboratories reported results in this EQUAS round compared to the first EQUAS round. The percentage of satisfactory Z-scores at both levels was 85% in the first ICI round, 70% in the first EQUAS and 96% in this second EQUAS round.

Table 3 below gives an overview of the performance of the individual laboratories in this round for cadmium in urine.

Table 3 Performance of the candidate laboratories for cadmium in urine

Lab. code	LOQ [ng/mL]	Cd_{low}	Cd_{high}
QR/101	0.180	**satisfactory**	**satisfactory**
QR/102	0.028	**unsatisfactory**	**unsatisfactory**
QR/103	0.050	**satisfactory**	**satisfactory**
QR/104	0.160	**satisfactory**	**satisfactory**
QR/105	0.050	**satisfactory**	**satisfactory**
QR/106	0.025	**satisfactory**	**satisfactory**
QR/107	0.008	**satisfactory**	**satisfactory**
QR/109	0.050	**questionable**	**satisfactory**
QR/110	0.025	**satisfactory**	**satisfactory**
QR/111	0.028	**satisfactory**	**satisfactory**
QR/112	0.020	**satisfactory**	**satisfactory**
QR/113	0.040	**satisfactory**	**satisfactory**
QR/114	0.050	**satisfactory**	**satisfactory**
QR/115	0.040	**satisfactory**	**satisfactory**
QR/116	0.033	**satisfactory**	**satisfactory**
QR/117	0.002	**satisfactory**	**satisfactory**
QR/118	0.004	**satisfactory**	**satisfactory**
QR/119	0.033	**satisfactory**	**satisfactory**
QR/120	0.010	**satisfactory**	**satisfactory**
QR/121	0.059	**satisfactory**	**satisfactory**
QR/124	0.050	**satisfactory**	**satisfactory**
QR/129	0.017	**satisfactory**	**satisfactory**
QR/130	0.010	**satisfactory**	**satisfactory**
QR/143	0.100	**satisfactory**	**satisfactory**
QR/200	0.020	**satisfactory**	**satisfactory**
QR/201	0.250	**satisfactory**	**satisfactory**
QR/202	0.050	**satisfactory**	**satisfactory**
QR/203	0.057	**satisfactory**	**satisfactory**
QR/204	0.050	**satisfactory**	**satisfactory**
QR/205	0.010	**satisfactory**	**satisfactory**
QR/206	0.050	**satisfactory**	**satisfactory**
QR/207	0.067	**satisfactory**	**satisfactory**
QR/208	0.100	**satisfactory**	**satisfactory**
QR/210	0.050	**satisfactory**	**satisfactory**
QR/211	0.100	**satisfactory**	**satisfactory**
QR/212	0.100	**satisfactory**	**satisfactory**
QR/213	0.050	**satisfactory**	**satisfactory**
QR/215	0.001	**satisfactory**	**satisfactory**
QR/216	0.060	**satisfactory**	**questionable**
QR/300	0.020	**satisfactory**	**satisfactory**
QR/301	0.050	**satisfactory**	**satisfactory**
QR/302	0.042	**satisfactory**	**Satisfactory**

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

Analyte: Cadmium

Preparation of control material: low

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD FFP (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[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	0.050	0.055	0.053	-0.005	0.000	0.000
2	0.059	0.052	0.056	0.007	0.000	0.000
3	0.068	0.050	0.059	0.018	0.000	0.000
4	0.050	0.051	0.051	-0.001	0.000	0.000
5	0.049	0.048	0.049	0.001	0.000	0.000
6	0.075	0.058	0.067	0.017	0.000	0.000
7	0.052	0.050	0.051	0.002	0.000	0.000
8	0.060	0.053	0.057	0.007	0.000	0.000
9	0.051	0.050	0.051	0.001	0.000	0.000
10	0.056	0.054	0.055	0.002	0.000	0.000
				Σ =	0.001	0.000

Lowest:	0.048	µg/kg
Highest	0.075	µg/kg
Grand mean ($\bar{x}$):	0.055	µg/kg
Stdev:	0.007	µg/kg
VC%:	12.5%	µg/kg

Outliers: Cochran's test

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

--> C = 0.434
--> 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% =	70.11	RSD% =	22	RSD% =	25
σ_H =	0.038	σ_H =	0.012	σ_H =	0.014

σ_H used: 0.014

Homogeniteit [1]:

s_x =	0.005	
s_w =	0.006	(within sample standard deviation)
s_s =	0.003	(between sample standard deviation)
critical=	0.004	

s_s < critical? → ACCEPT: Homogeneity adequate
s_w < 0.5*σ_H? → ACCEPT: Method suited

Appendix 1 Homogeneity data (continued)

Homogeneity

Version HBM4EU v1

Control material: **urine**

Analyte: **Cadmium**

Preparation of control material: high

10 randomly chosen test samples, analysed in duplicate

Target standard deviation:
Fit-for-purpose RSD **FFP** (25% is default value)
if you want to use Horwitz/Thompson,
then delete FFP from cell H5

[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	0.171	0.154	0.163	0.017	0.000	0.000
2	0.161	0.163	0.162	-0.002	0.000	0.000
3	0.161	0.151	0.156	0.010	0.000	0.000
4	0.174	0.155	0.165	0.019	0.000	0.000
5	0.162	0.150	0.156	0.012	0.000	0.000
6	0.157	0.159	0.158	-0.002	0.000	0.000
7	0.169	0.159	0.164	0.010	0.000	0.000
8	0.155	0.147	0.151	0.008	0.000	0.000
9	0.148	0.153	0.151	-0.005	0.000	0.000
10	0.140	0.140	0.140	0.000	0.000	0.000
				Σ =	0.001	0.001

Lowest:	0.140	µg/kg
Highest	0.174	µg/kg
Grand mean ($\bar{x}$):	0.156	µg/kg
Stdev:	0.009	µg/kg
VC%:	5.9%	µg/kg

Outliers: Cochran's test

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

--> C = 0.331
--> 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% =	59.83	RSD% =	22	RSD% =	25
σ_H =	0.094	σ_H =	0.034	σ_H =	0.039

σ_H used: 0.039

Homogeniteit [1]:

s_x =	0.008	
s_w =	0.007	(within sample standard deviation)
s_s =	0.006	(between sample standard deviation)
critical=	0.012	

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 (urine)**
Storage: minus 80 °C / minus 18 °C

Biomarker **Cadmium low**

	t=0 (storage)	t=a (analysis)
dates:	03.05.2019	14.06.2019
values:	0.093	0.094
	0.090	0.088
	0.089	0.097
	0.091	0.097
	0.093	0.088
	0.094	0.091
number=	6	6
average=	0.092	0.093
std dev=	0.002	0.004

analysis date	time (days)	µg/L	n	std dev
03.05.2019	0	0.092	6	0.002
14.06.2019	42	0.093	6	0.004
x0-xa=	**-0.001**			

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.020	0.022916667
0,3*σH=	0.006	0.006875
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	4.422
Fcrit=	5.050
Significant difference?	**No significant difference in std detected**
sed^2=	0.00
n=	10
std difference=	0.00
t=	0.45
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: **Cd (urine)**
Storage: minus 80 °C / minus 18 °C

Biomarker **Cadmium high**

	t=0 (opslag)	t=a (analyse)
dates:	03.05.2019	14.06.2019
values:	0.205	0.192
	0.197	0.212
	0.190	0.190
	0.198	0.186
	0.193	0.195
	0.193	0.191
number=	6	6
average=	0.196	0.194
std dev=	0.005	0.009

analysis date	time (days)	µg/L	n	std dev
03.05.2019	0	0.196	6	0.005
14.06.2019	42	0.194	6	0.009
x0-xa=	**0.002**			

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	0.043	0.049
0,3*σH=	0.013	0.0147
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	2.981
Fcrit=	5.050
Significant difference?	**No significant difference in std detected**
sed^2=	0.01
n=	10
std difference=	0.00
t=	0.39
t-crit=	2.23
Significant difference?	**No statistic instability detected**

Appendix 3 Copy of letter of invitation

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study Cd/Round 3

Title of ICI/EQUAS: Cadmium in blood and urine

Dear Colleagues,

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 3rd 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.

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

- 2 different materials of blood (1 sample of 3 mL each) for determination of cadmium in blood and/or
- 2 different materials of urine (1 sample of 5 mL 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:

Registration deadline	May 13, 2019
Distribution of test samples (projected)	May 14-15, 2019
Deadline for submission of results (projected)	June 14, 2019

Registration
For registration, please find attached a registration form each for Cadmium in blood and for 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
- Stefanie Nübler
- Moritz Schäfer
- Karin H. A. Zarrabi

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

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

HBM4EU: Instruction letter ICI / EQUAS study Cd in urine/Round 3

Title of ICI/EQUAS: Cadmium in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study Cd in urine/Round 3 for the determination of Cadmium in urine.

You will receive a parcel containing 2 test samples spiked with the biomarker at 2 levels, 1 of each concentration. Each sample consists of approximately 5 mL urine.

The parcel will be shipped on 14.05.2019 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/29.04.2019.
- 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 deadline for submission of analysis results and method details is June 11, 2019.

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

Karin Zarrabi, Stefanie Nübler or Moritz Schäfer

Email: ipasum-hbm4eu@fau.de

Tel.: + 49 (0)9131/85 26 146, /85 26 145

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Henkestrasse 9-11
91054 Erlangen
Germany

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

Appendix 5 Method information form in ICI / EQUAS study Cd/Round 3

Title of ICI/EQUAS: Cadmium in urine

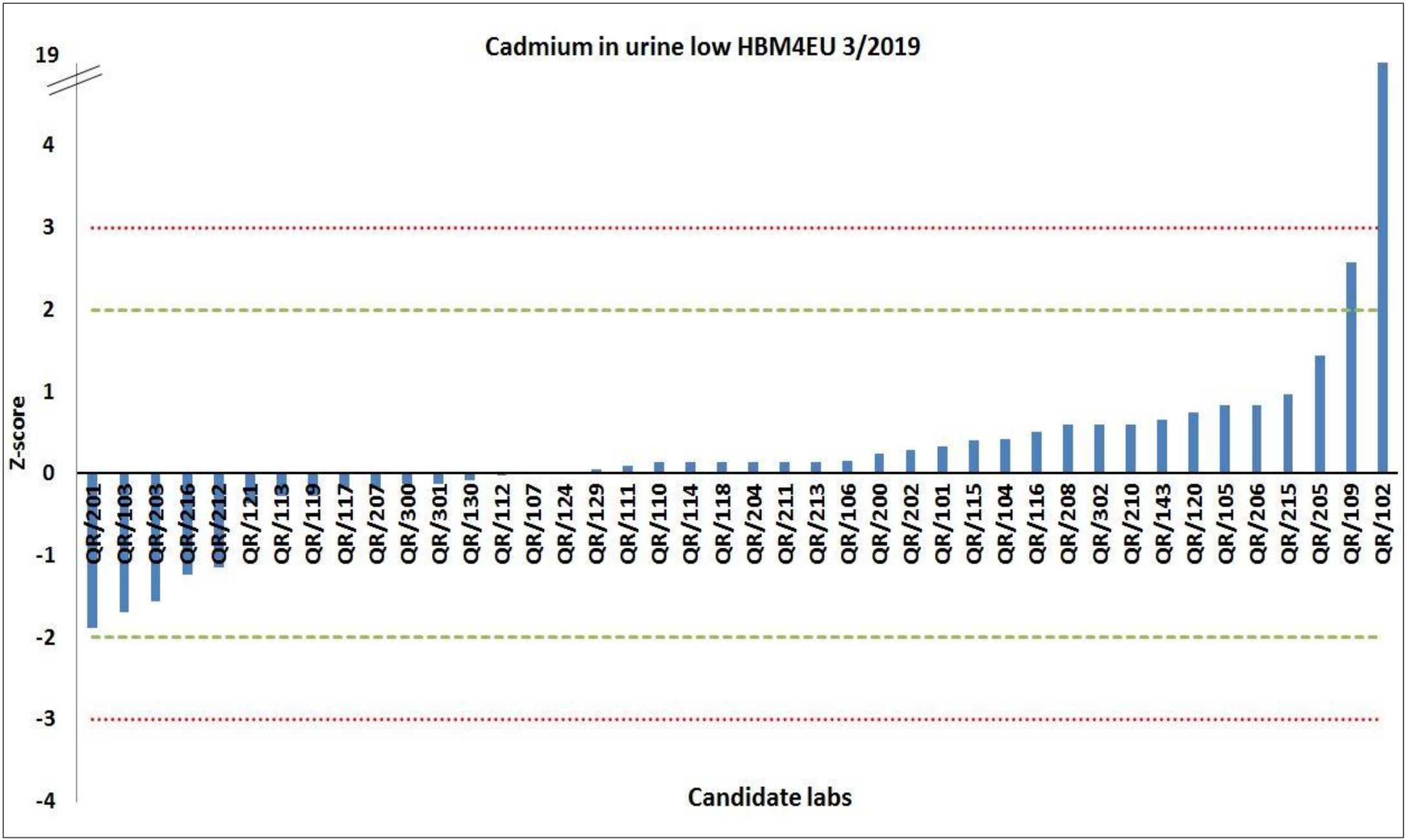
Laboratory code	**IPASUM**	
ISO17025 accredited	**no**	
SAMPLE PREPARATION		
amount sample	0.400	ml
Extraction		
- pH adjustment		
- LLE; solvent(s) / time / shaking		
- SPE; material		
Digestion	**no**	
INSTRUMENTAL ANALYSIS		
AAS	**no**	
Wavelength		
Background compensation		
Matrix modifier		
Dilution factor		
Other remarks		
ICP	**yes**	
Dilution	**1:10**	
Nebulizer	**glass cyclonic**	
Reagent gas	**Argon**	
Masses monitored	**Cd^{114}**	
Detection		
MS	triple quad	
OES		
Quantification		
Use of internal standard (IS)	yes	
- response normalised to IS	yes	
Calibration	external calibrant (matrix based)	
	multi level	
Correction for recovery	no	
Removed interferences with molybdenum oxides?	no	
Identification criteria used		
- ion ratio tolerance	-	
- other	-	

Appendix 6 Assigned values and participant's performance

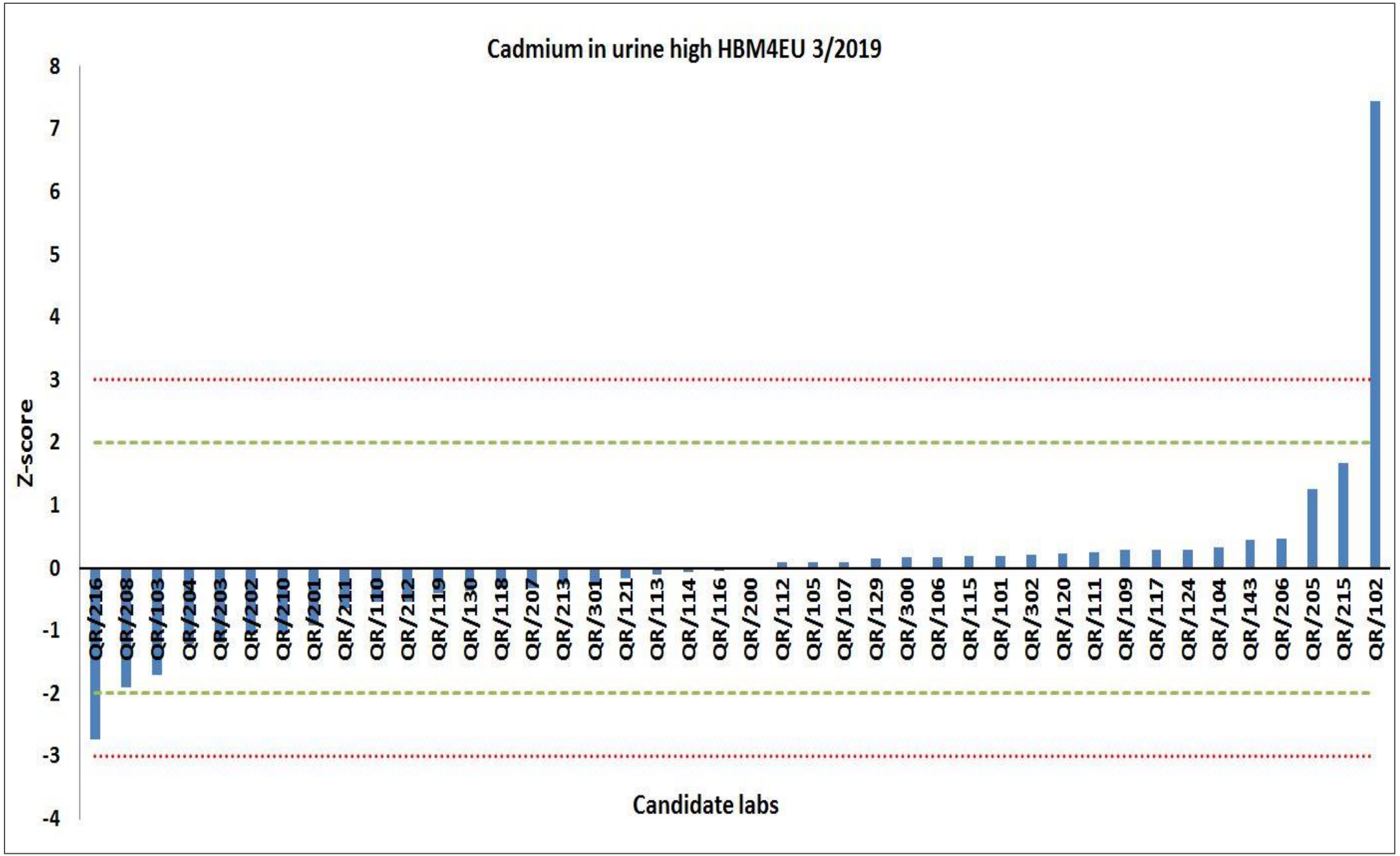
HBM4EU 02/2018 Cd (urine)

control material	Cd_{low}		Cd_{high}	
assigned value from six expert labs	0.087 ng/ml		0.190 ng/ml	
expert standard deviation	0.007 ng/ml		0.019 ng/ml	
uncertainty of assigned value (u)	3.2%		2.0%	
relative target standard deviation (σ_T)	25%		25%	
0.7 * σ_T	17.5%		17.5%	
study RSD_R	12%		13%	
laboratory code	**value**	**Z-score**	**value**	**Z-score**
QR/101	0.094	0.327	0.199	0.189
QR/102	0.494	18.738	0.544	7.450
QR/103	0.050	-1.699	0.109	-1.706
QR/104	0.096	0.419	0.206	0.336
QR/105	0.105	0.833	0.194	0.083
QR/106	0.090	0.154	0.199	0.180
QR/107	0.087	0.004	0.194	0.083
QR/109	0.143	2.582	0.204	0.294
QR/110	0.090	0.143	0.164	-0.548
QR/111	0.089	0.097	0.202	0.252
QR/112	0.087	-0.019	0.194	0.083
QR/113	0.081	-0.272	0.185	-0.106
QR/114	0.090	0.143	0.187	-0.064
QR/115	0.096	0.411	0.199	0.185
QR/116	0.098	0.511	0.188	-0.043
QR/117	0.083	-0.180	0.204	0.294
QR/118	0.090	0.143	0.175	-0.317
QR/119	0.081	-0.272	0.171	-0.401
QR/120	0.103	0.741	0.201	0.231
QR/121	0.079	-0.364	0.182	-0.169
QR/124	0.087	0.004	0.204	0.294
QR/129	0.088	0.051	0.197	0.146
QR/130	0.085	-0.088	0.173	-0.359
QR/143	0.101	0.649	0.211	0.441
QR/200	0.092	0.235	0.190	-0.001
QR/201	0.046	-1.883	0.147	-0.906
QR/202	0.093	0.281	0.139	-1.074
QR/203	0.053	-1.560	0.133	-1.201
QR/204	0.090	0.143	0.132	-1.222
QR/205	0.118	1.431	0.250	1.262
QR/206	0.105	0.833	0.212	0.462
QR/207	0.083	-0.180	0.175	-0.317
QR/208	0.100	0.603	0.100	-1.895
QR/210	0.100	0.603	0.140	-1.053
QR/211	0.090	0.143	0.160	-0.632
QR/212	0.062	-1.146	0.164	-0.548
QR/213	0.090	0.143	0.178	-0.253
QR/215	0.108	0.971	0.270	1.683
QR/216	0.060	-1.238	0.060	-2.737
QR/300	0.084	-0.134	0.198	0.168
QR/301	0.084	-0.134	0.178	-0.253
QR/302	0.100	0.603	0.200	0.210

Appendix 7 Graphical representation of the Z-scores



Appendix 7 Graphical representation of the Z-scores (continued)



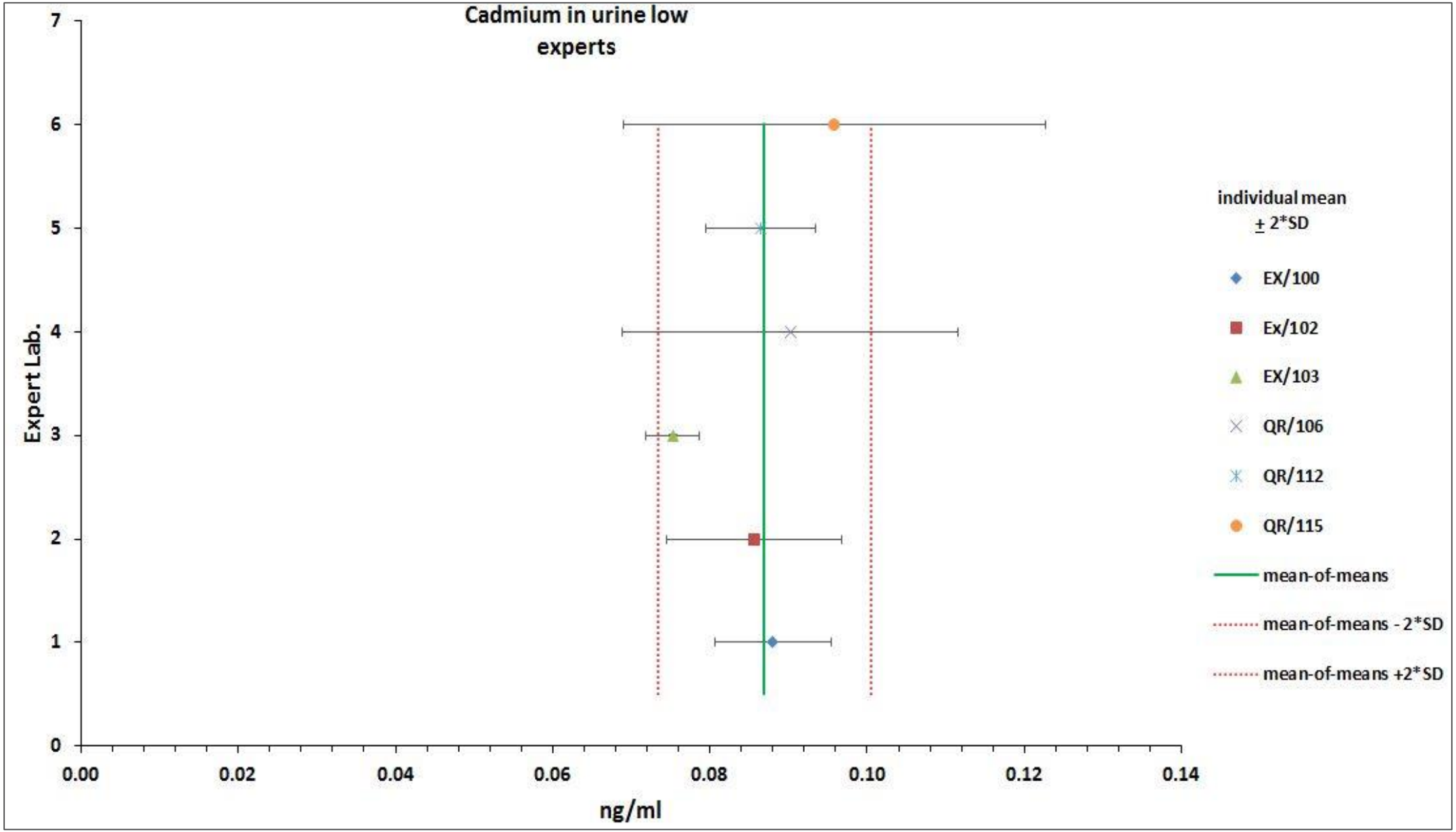
Appendix 8 Results and LOQs and reasons for delayed submission

HBM4EU 3-2019 Cadmium in urine [ng/mL]										
Lab.code	**low**				**high**				**LOQ**	**delayed reporting**
EX/100	0.093	0.089	0.092	0.087	0.194	0.177	0.184	0.186	0.019	
	0.090	0.089	0.085	0.083	0.184	0.184	0.196	0.194		
	0.091	0.083	0.091	0.083	0.188	0.192	0.194	0.188		
EX/102	0.088	0.083	0.080	0.081	0.184	0.183	0.187	0.184	0.050	
	0.080	0.088	0.081	0.090	0.174	0.198	0.199	0.183		
	0.087	0.093	0.096	0.080	0.190	0.195	0.173	0.194		
EX/103	0.077	0.074	0.075	0.079	0.175	0.170	0.172	0.173	0.028	
	0.074	0.076	0.073	0.074	0.170	0.170	0.172	0.180		
	0.077	0.075	0.075	0.074	0.175	0.175	0.177	0.172		
QR/101	0.094				0.199				0.180	X
QR/102	0.494				0.544				0.028	
QR/103	ND				0.109				0.050	
QR/104	0.096				0.206				0.160	
QR/105	0.105				0.194				0.050	
QR/106	0.078	0.101	0.080	0.101	0.183	0.206	0.196	0.204	0.025	XXX
	0.082	0.098	0.081	0.099	0.193	0.203	0.184	0.209		
	0.075	0.097	0.087	0.104	0.192	0.209	0.210	0.194		
QR/107	0.087				0.194				0.008	
QR/109	0.143				0.204				0.050	
QR/110	0.090				0.164				0.025	
QR/111	0.089				0.202				0.028	
QR/112	0.083	0.084	0.089		0.191	0.187	0.195		0.020	
	0.087	0.092	0.084		0.195	0.206	0.190			
QR/113	0.081				0.185				0.040	
QR/114	0.090				0.187				0.050	
QR/115	0.081	0.083	0.093		0.203	0.214	0.208		0.040	
	0.116	0.106	0.096		0.181	0.191	0.196			
QR/116	0.098				0.188				0.033	
QR/117	0.083				0.204				0.002	
QR/118	0.090				0.175				0.004	
QR/119	0.081				0.171				0.033	
QR/120	0.103				0.201				0.010	
QR/121	0.079				0.182				0.059	
QR/124	0.087				0.204				0.050	
QR/129	0.088				0.197				0.017	
QR/130	0.085				0.173				0.010	
QR/143	0.101				0.211				0.100	
QR/200	0.092				0.190				0.020	
QR/201	0.046				0.147				0.250	
QR/202	0.093				0.139				0.050	
QR/203	0.053				0.133				0.057	
QR/204	0.090				0.132				0.050	
QR/205	0.118				0.250				0.010	
QR/206	0.105				0.212				0.050	
QR/207	0.083				0.175				0.067	
QR/208	0.100				0.100				0.100	
QR/210	0.100				0.140				0.050	XXX
QR/211	0.090				0.160				0.100	
QR/212	0.062				0.164				0.100	
QR/213	0.090				0.178				0.050	
QR/215	0.108				0.270				0.001	
QR/216	ND				ND				0.060	XX
QR/300	0.084				0.198				0.020	
QR/301	0.084				0.178				0.050	
QR/302	0.100				0.200				0.042	

NA = not analysed, ND = not detected / < LOQ

X = due to problems with shipment; XX = due to technical problems; XXX = no reason given

Appendix 9 Graphical representation of the expert values and the mean-of-means



Appendix 9 Graphical representation of the expert values and the mean-of-means (continued)