HBM4EU science and policy for a healthy future

HORIZON2020 Programme
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REPORT OF THE WP9 ICI

Round 02/2019

Chromium in urine

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Organiser	Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) Friedrich-Alexander University of Erlangen-Nuremberg Henkestr. 9-11 91054 Erlangen GERMANY
Coordinator	Thomas Göen, thomas.goeen@fau.de
Authors (IPASUM)	Moritz Schäfer, Stefanie Nübler, Karin H. A. Zarrabi
Approved by:	Thomas Göen (IPASUM)

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Appendices

1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of chromium in urine.

The study was performed from March 2019 to April 2019.

In total, 46 laboratories were invited for this second round, of which 25 laboratories from 18 countries registered.

The participation in this ICI was satisfactory; 24 out of 25 laboratories (96%) submitted their results.

In March 2019, two different test samples consisting of 5 mL urine spiked with chromium at two different concentrations (Cr_{low}, Cr_{high}), one of each concentration, were prepared, and one sample of each concentration was 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.

The evaluation of Cr_{low} and Cr_{high} showed that 100% of the results were satisfactory (**Table 1**).

Table 1 Overview of results for Chromium in urine in 2nd ICI

Number of laboratories with respective results for chromium in urine in 2nd ICI				
biomarker	**assigned value**	**satisfactory** (|Z-score| ≤2)	**questionable** (2<|Z-score|<3)	**unsatisfactory** (|Z-score|>3)
Cr_{low}	1.341 ng/mL	24 (100%)	0	0
Cr_{high}	17.088 ng/mL	24 (100%)	0	0

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 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 2nd round of proficiency testing for chromium in urine, which 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 assessment.

3 Control material

3.1 Preparation of control material

For control material, surrogate material was used. It consists of human urine with sodium azide. The stock solution (Chromium ICP standard, ammonium dichromate in H_2O, 1000 mg/L, J.T.Baker) was diluted into two different concentrations and the addition to the native control material resulted in the intended concentration in control material (Cr_{low}, Cr_{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 (Cr_{low}, Cr_{high}) were measured using ICP-MS (see analysis method in **Appendix 5**).

3.2 Homogeneity of control material

Ten tubes of each concentration of the control material (Cr_{low}, Cr_{high}) were randomly selected from the freezer (≤ -18°C). The thawed samples were re-homogenised by vortex shaking and were analysed in duplicate 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 1**. The conclusion is that no outliers are detected, 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 Cr_{low} and six randomly selected test samples of Cr_{high} were stored at -80°C. The assumption is that under these conditions, the biomarker (Cr) is stable in urine. On the last day of the deadline for submission of results by the participants (April 30, 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

A list of 46 candidate laboratories from different countries eligible for the analysis of chromium had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organizing the respective ICI.
Invitation letters were sent by e-mail to all 46 candidate laboratories on March 4, 2019 (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 (April 30, 2019).
Twenty-five laboratories (54%) from 18 countries out of the 46 laboratories in the candidate list indicated their interest in participating in this ICI and sent their registration forms 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**).

Twenty-four of the 25 potential participants (96%) performed the assays and submitted their results. 22 participants reported their results within the stipulated deadline (April 30, 2019), while 2 participants reported with a delay (on May 8, 2019 and on May 14, 2019).

4.2 Dispatch and instructions

Test materials were dispatched to the participants under ambient conditions on March 25, 2019. Each participant received two test samples spiked with the biomarker at two levels, one of each concentration. 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 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

The 2nd ICI for Chromium in urine followed the HBM4EU-QA-SOPs (version 2). There were no deviations from these SOPs.

5 Data evaluation

5.1 False negatives 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 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, 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 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.

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 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, 25 laboratories from 18 countries agreed to participate in this study. Not all participants were able to meet the stipulated deadline due to technical problems, so that in the end, 24 out of 25 participants (96%) 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 **Cr_{low}**, one participant (QR/131) indicated ND and the LOQ was used as input for the ICI evaluation. A proxy-Z-score could be calculated for this participant.

For **Cr_{high}**, no laboratory indicated ND.

False positive results: No participant detected a false positive in Cr_{low} and Cr_{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. As internal standard, the majority of participating laboratories applied germanium, yttrium or rhodium and for calibration, most candidates used an external calibrant (solvent-based), followed by standard addition and external calibrant (matrix-based).

6.2 Assigned values and (target) standard deviations

The assigned value and its uncertainty (u), the robust relative standard deviation of the participant's data (study RSD_R), and the fit-for-purpose (FFP) target standard deviation for each of the control materials are included in Appendix 6.

6.3 Assessment of laboratory performance

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

Twenty-four laboratories out of 25 registered candidate laboratories reported results. All laboratories (100%) have satisfactory Z-scores in **Cr_{low}** and in **Cr_{high}**.

6.4 Conclusions and recommendations

The overall participation in the HBM4EU ICI Round 2 was successful. Twenty-five laboratories out of the 46 laboratories (54%) in the candidate list confirmed their participation in the ICI. Twenty-four of these 25 registered candidate laboratories reported results, representing a participation rate of 96%.

Regarding the quantification of chromium in urine, the participants achieved in **Cr_{low}** and in **Cr_{high}** 100% satisfactory results.

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

Fourteen additional laboratories reported results in this second round compared to the first ICI round. The percentage of satisfactory Z-scores at both levels was 100% in the first round and is also 100% in this second round.

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

Table 3 Performance of the candidate laboratories for chromium in urine

Lab. code	LOQ [ng/mL]	Cr_{low}	Cr_{high}
QR/104	1.200	**satisfactory**	**satisfactory**
QR/105	1.000	**satisfactory**	**satisfactory**
QR/107	0.028	**satisfactory**	**satisfactory**
QR/110	0.500	**satisfactory**	**satisfactory**
QR/111	0.241	**satisfactory**	**satisfactory**
QR/112	0.100	**satisfactory**	**satisfactory**
QR/113	0.050	**satisfactory**	**satisfactory**
QR/115	0.600	**satisfactory**	**satisfactory**
QR/118	0.108	**satisfactory**	**satisfactory**
QR/130	0.250	**satisfactory**	**satisfactory**
QR/131	1.479	**satisfactory**	**satisfactory**
QR/143	0.180	**satisfactory**	**satisfactory**
QR/200	0.050	**satisfactory**	**satisfactory**
QR/202	0.200	**satisfactory**	**satisfactory**
QR/203	0.330	**satisfactory**	**satisfactory**
QR/204	0.500	**satisfactory**	**satisfactory**
QR/205	0.080	**satisfactory**	**satisfactory**
QR/207	0.386	**satisfactory**	**satisfactory**
QR/211	0.250	**satisfactory**	**satisfactory**
QR/212	0.050	**satisfactory**	**satisfactory**
QR/224	0.050	**satisfactory**	**satisfactory**
QR/225	0.050	**satisfactory**	**satisfactory**
QR/226	0.100	**satisfactory**	**satisfactory**
QR/227	0.500	**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: Chromium

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	1.350	1.380	1.365	-0.030	0.001	0.000
2	1.340	1.350	1.345	-0.010	0.000	0.000
3	1.270	1.360	1.315	-0.090	0.008	0.002
4	1.420	1.390	1.405	0.030	0.001	0.002
5	1.370	1.330	1.350	0.040	0.002	0.000
6	1.260	1.480	1.370	-0.220	0.048	0.000
7	1.300	1.340	1.320	-0.040	0.002	0.001
8	1.360	1.460	1.410	-0.100	0.010	0.003
9	1.310	1.400	1.355	-0.090	0.008	0.000
10	1.380	1.280	1.330	0.100	0.010	0.001
				Σ =	0.090	0.009

Lowest:	1.260 µg/kg
Highest	1.480 µg/kg
Grand mean ($\bar{x}$):	1.357 µg/kg
Stdev:	0.058 µg/kg
VC%:	4.3% µg/kg

Outliers: Cochran's test

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

--> C = 0.540
--> 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% =	43.22	RSD% =	22	RSD% =	25
σ_H =	0.586	σ_H =	0.298	σ_H =	0.339

σ_H used: 0.339

Homogeniteit [1]:

s_x =	0.032	
s_w =	0.067	(within sample standard deviation)
s_s =	0.000	(between sample standard deviation)
critical=	0.102	

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: Chromium

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	17.200	17.170	17.185	0.030	0.001	0.018
2	17.020	17.170	17.095	-0.150	0.023	0.002
3	17.120	17.010	17.065	0.110	0.012	0.000
4	17.030	17.290	17.160	-0.260	0.068	0.012
5	16.980	16.870	16.925	0.110	0.012	0.016
6	17.000	16.830	16.915	0.170	0.029	0.019
7	16.950	16.920	16.935	0.030	0.001	0.014
8	16.990	17.020	17.005	-0.030	0.001	0.002
9	17.160	17.040	17.100	0.120	0.014	0.002
10	16.970	17.290	17.130	-0.320	0.102	0.006
				Σ =	0.263	0.091

Lowest:	16.830	µg/kg
Highest	17.290	µg/kg
Grand mean ($\bar{x}$):	17.052	µg/kg
Stdev:	0.128	µg/kg
VC%:	0.8%	µg/kg

Outliers: Cochran's test

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

--> C = 0.390
--> 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% =	29.53	RSD% =	22	RSD% =	25
σ_H =	5.035	σ_H =	3.751	σ_H =	4.263

σ_H used: 4.263

Homogeniteit [1]:

s_x =	0.100	
s_w =	0.115	(within sample standard deviation)
s_s =	0.059	(between sample standard deviation)
critical=	1.279	

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

Biomarker **Chromium low**

	t=0 (storage)	t=a (analysis)
dates:	09.05.2019	09.05.2019
values:	1.36	1.38
	1.34	1.32
	1.37	1.36
	1.40	1.36
	1.38	1.34
	1.41	1.43
number=	6	6
average=	1.375	1.364
std dev=	0.025	0.039

analysis date	time (days)	µg/L	n	std dev
09.05.2019	0	1.375	6	0.025
09.05.2019	0	1.364	6	0.039
x0-xa=	**0.01**			

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	0.302	0.343625
0,3*σH=	0.091	0.1030875
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	2.30
Fcrit=	5.05
Significant difference?	**No significant difference in std detected**
sed^2=	0.03
n=	10
std difference=	0.02
t=	0.57
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: **Cr (urine)**
Storage: minus 80 °C minus 18 °C

Biomarker **Chromium high**

	t=0 (opslag)	t=a (analyse)
dates:	09.05.2019	09.05.2019
values:	17.103	17.238
	17.321	17.147
	17.329	17.138
	17.369	17.166
	17.344	17.117
	17.124	16.939
number=	6	6
average=	17.265	17.124
std dev=	0.119	0.100

analysis date	time (days)	µg/L	n	std dev
09.05.2019	0	17.265	6	0.119
09.05.2019	0	17.124	6	0.100
x0-xa=	**0.14**			

test 'consequential instabilit	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	3.798	4.31625
0,3*σH=	1.139	1.294875
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	1.42
Fcrit=	5.05
Significant difference?	**No significant difference in std detected**
sed^2=	0.11
n=	10
std difference=	0.06
t=	2.23
t-crit=	2.23
Significant difference?	**No statistic instability detected**

Appendix 3 Copy of letter of invitation

HBM4EU: Announcement / invitation to participate in ICI study Cr/Round 2

Title of ICI: Chromium in blood, serum 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 2nd ICI round for the determination of chromium in blood, serum and urine. The aim of ICI exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other 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, serum and urine, respectively. Accordingly, the participants will receive:

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

Target biomarkers

The biomarker potentially present in the test samples is chromium.

This ICI is specifically aimed at participants who want to qualify for the occupational chromium study. Consequently, successful participation in the ICI studies will lead to approval and a certificate being issued for the occupational exposure range only.

With regard to the conditions and specifications of the occupational study, we expect participants of this ICI to use suitable analytical methods to obtain an **LOQ < 1 µg/L.**

Participation in this ICI is mandatory for laboratories that want to get approved for the occupational chromium study, irrespective of their participation in the 1st ICI.

Calendar:

Registration deadline	March 15, 2019 (we would be pleased to receive your feedback as soon as possible)
Distribution of test samples (projected)	March 20 – 25, 2019
Deadline for submission of results (projected)	April 24, 2019

Registration

For registration, please find attached a registration form each for chromium in blood, in serum and 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 study Cr in urine/Round 2

Title of ICI: Chromium in urine

Dear participant,

Thank you for participation in the HBM4EU ICI study Cr in urine/Round 2 for the determination of Chromium 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 25 March 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/ 04.03.2019.
- 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 **30 April 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)9131/8526146, /8526145

Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine
Friedrich-Alexander University Erlangen-Nuremberg
Henkestrasse 9-11
91054 Erlangen
Germany

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

Appendix 5 ICP-MS method information IPASUM

HBM4EU: Method information form for participation in ICI / EQUAS study Cr/Round 2

Title of ICI/EQUAS: Chromium in urine

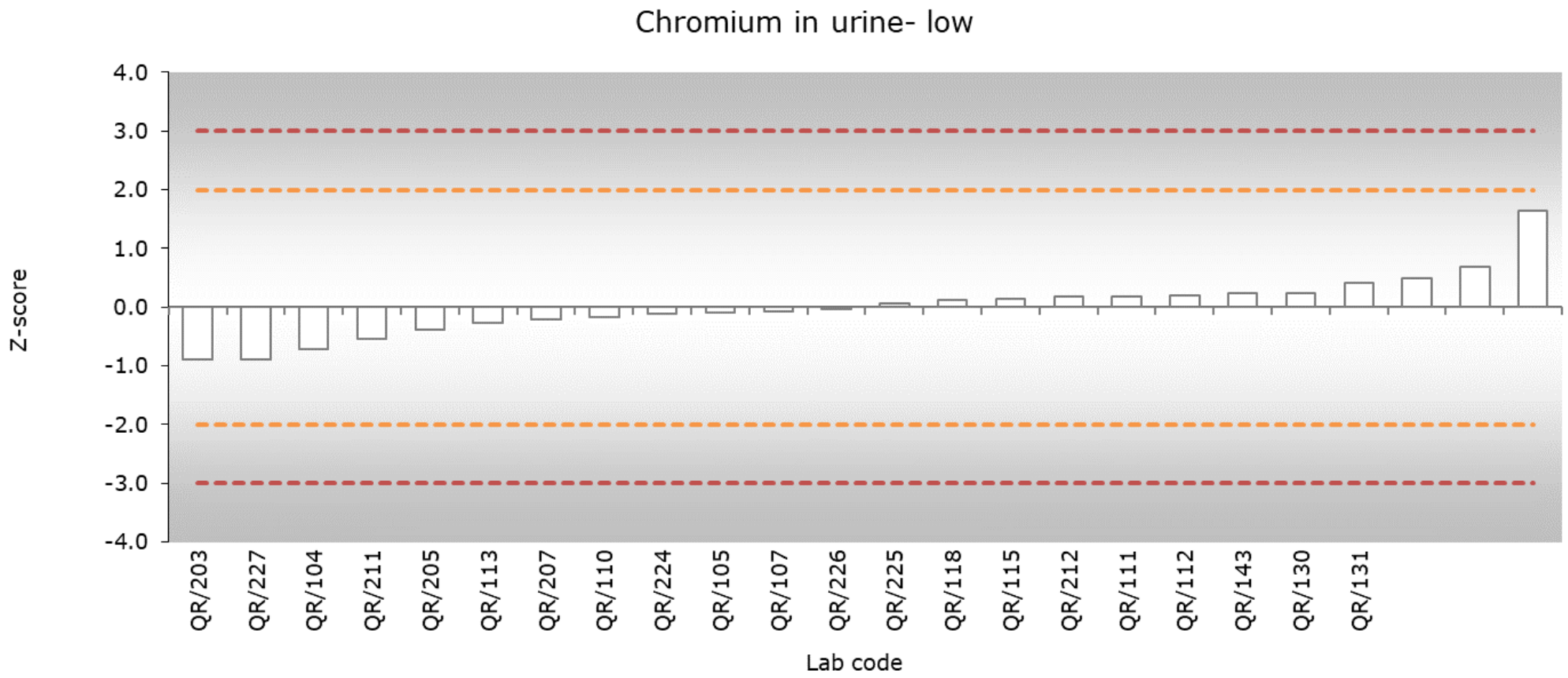
Laboratory code	**IPASUM Erlangen - Germany**
ISO17025 accredited	**no**
SAMPLE PREPARATION	
amount sample	**0.4 mL**
Extraction	**no**
- pH adjustment	
- LLE; solvent(s) / time / shaking	
- SPE; material	
Digestion	**no**
INSTRUMENTAL ANALYSIS	
AAS	**no**
Wavelength	
Background compensation	
Matrix modifier	
Dilution factor	
Other remarks	
ICP	**Yes (ICP-MS Perkin Elmer)**
Dilution	**1:10 with H_20 and HNO_3**
Nebulizer	**glass**
Reagent gas	**Argon**
Masses monitored	**Cr^{52}**
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	**-**

Appendix 6 Assigned values and participant's performance

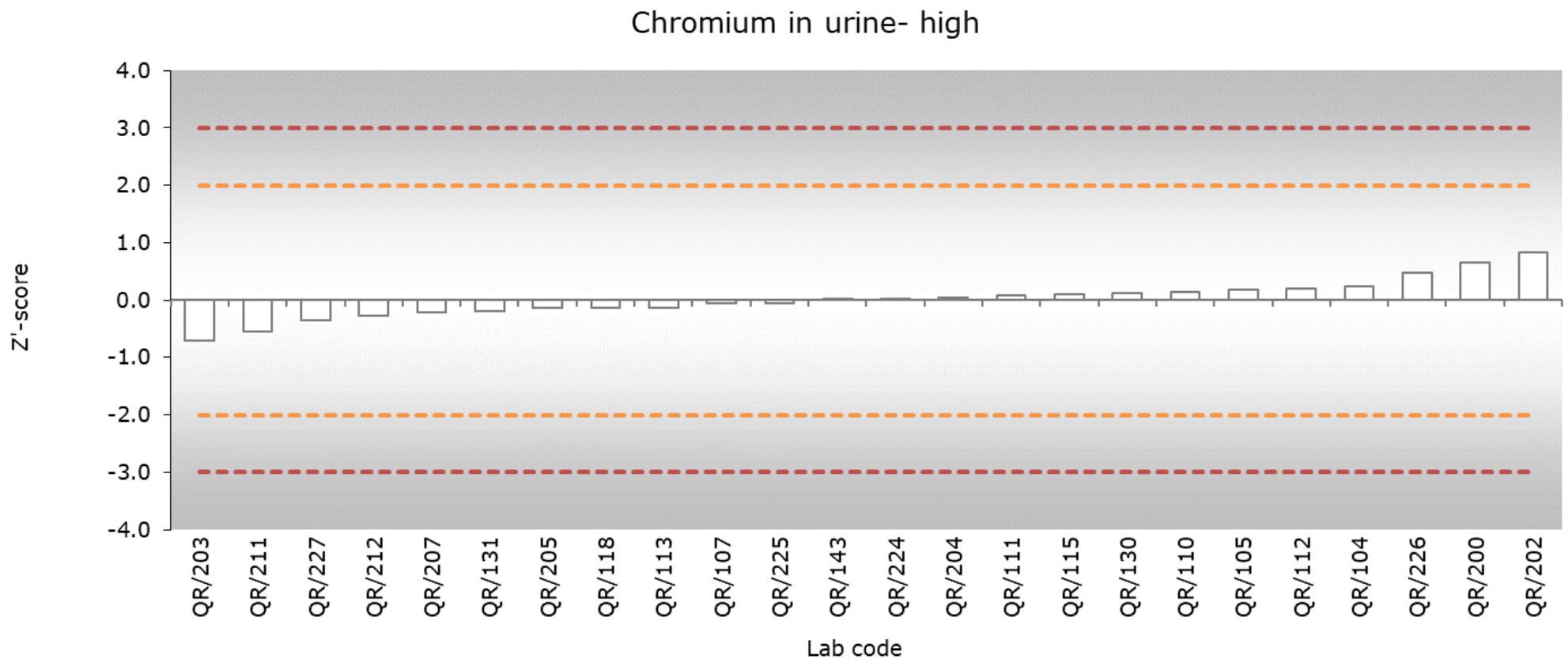
HBM4EU 02/2018	Cr (urine)	
control material	**Cr_{low}**	**Cr_{high}**
assigned value [ng/mL]	**1.341**	**17.088**
uncertainty of assigned value [ng/mL]	**0.032**	**0.278**
study RSD_R (%)	**9**	**6**
relative target standard deviation (%)	**25**	**25**

laboratory code	value	Z-score	value	Z-score
QR/104	1.100	-0.719	18.140	0.246
QR/105	1.310	-0.093	17.840	0.176
QR/107	1.314	-0.081	16.832	-0.060
QR/110	1.285	-0.167	17.707	0.145
QR/111	1.402	0.182	17.433	0.081
QR/112	1.410	0.205	17.980	0.209
QR/113	1.250	-0.272	16.500	-0.138
QR/115	1.390	0.146	17.500	0.097
QR/118	1.380	0.116	16.500	-0.138
QR/130	1.420	0.235	17.600	0.120
QR/131	ND	0.411	16.240	-0.198
QR/143	1.420	0.235	17.100	0.003
QR/200	1.570	0.683	19.900	0.658
QR/202	1.890	1.637	20.600	0.822
QR/203	1.040	-0.898	14.030	-0.716
QR/204	1.507	0.495	17.243	0.036
QR/205	1.212	-0.385	16.475	-0.143
QR/207	1.270	-0.212	16.200	-0.208
QR/211	1.160	-0.540	14.760	-0.545
QR/212	1.400	0.176	15.900	-0.278
QR/224	1.304	-0.111	17.194	0.025
QR/225	1.360	0.06	16.840	-0.06
QR/226	1.330	-0.03	19.100	0.47
QR/227	1.040	-0.90	15.600	-0.35

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 2-2019 Chromium in urine [ng/mL]				
Lab.code	**low**	**high**	**LOQ**	**delayed reporting**
QR/104	1.100	18.140	1.200	
QR/105	1.310	17.840	1.000	
QR/107	1.314	16.832	0.028	
QR/110	1.285	17.707	0.500	no reason given
QR/111	1.402	17.433	0.241	
QR/112	1.410	17.980	0.100	
QR/113	1.250	16.500	0.050	due to problems with shipment
QR/115	1.390	17.500	0.600	
QR/118	1.380	16.500	0.108	
QR/130	1.420	17.600	0.250	
QR/131	ND	16.240	1.479	
QR/143	1.420	17.100	0.180	
QR/200	1.570	19.900	0.050	
QR/202	1.890	20.600	0.200	
QR/203	1.040	14.030	0.330	
QR/204	1.507	17.243	0.500	
QR/205	1.212	16.475	0.080	
QR/207	1.270	16.200	0.386	
QR/211	1.160	14.760	0.250	
QR/212	1.400	15.900	0.050	
QR/224	1.304	17.194	0.050	
QR/225	1.360	16.840	0.050	
QR/226	1.330	19.100	0.100	
QR/227	1.040	15.600	0.500	

ND = not detected / < LOQ