HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

REPORT OF THE WP9 ICI

Round 04/2019

Chromium in serum

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Table of contents

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 serum.

The study was performed from October 2019 to December 2019.

In total, 46 laboratories were invited for this fourth round, of which 17 laboratories from 12 countries registered.

The participation in this ICI was satisfactory; 15 out of 17 laboratories (88%) submitted their results.

In November 2019, two different test samples consisting of 3 mL serum 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 at FFP = 25%, as described in 5.3.

The evaluation showed that 86% of the results for **Cr_{low}** and 93% of the results for **Cr_{high}** were satisfactory (**Table 1**).

Table 1 Overview of results for chromium in serum in 4th ICI

Number of laboratories with respective results for chromium in serum in 4th ICI				
biomarker	**assigned value**	**satisfactory** (|Z-score| ≤2)	**questionable** (2<|Z-score|<3)	**unsatisfactory** (|Z-score|>3)
Cr_{low}	**5.889 ng/mL**	**13 (86%)**	**1 (7%)**	**1 (7%)**
Cr_{high}	**14.491 ng/mL**	**14 (93%)**	**0**	**1 (7%)**

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 4th round of proficiency testing for chromium in serum, 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 bovine serum of German origin with sodium azide. Animal health conditions are certified. 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 (3 mL each) into tubes with caps (66x11.5 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 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 serum. On the last day of the deadline for submission of results by the participants (December 4, 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 October 21, 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 (December 4, 2019).
Seventeen laboratories (37%) from 12 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**).

15 of the 17 potential participants (88%) performed the assays and submitted their results. All participants reported their results within the stipulated deadline (December 4, 2019).

4.2 Dispatch and instructions

Test materials were dispatched to the participants under ambient conditions on November 6, 2019. Each participant received two test samples spiked with the biomarker at two levels, one of each concentration. Each sample consisted of approximately 3 mL serum.
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 4th ICI for Chromium in serum 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) is described in HBM4EU-SOP-QA-003. In this EQUAS there were no false positives and no ND. Therefore, no further description is given here.

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

6 Results and discussion

6.1 Results submitted by participants

In total, 17 laboratories from 12 countries agreed to participate in this study. Not all participants were able to meet the stipulated deadline, so that in the end, 15 out of 17 participants (88%) 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}** and for **Cr_{high}** no laboratory indicated ND.

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

Methods:

In all cases, the samples were analysed by ICP-MS. For sample preparation, most laboratories used no digestion while the others used an acid digestion. Almost all participating laboratories used a single quad as detection system, the rest used a triple quad. As internal standard, the majority of participating laboratories applied rhodium, germanium or yttrium and for calibration, most candidates used an external calibrant (matrix-based), followed by standard addition and external calibrant (solvent-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**.

Fifteen laboratories out of 17 registered candidate laboratories reported results. In **Cr_{low}**, 86% of the laboratories achieved satisfactory Z-scores. In **Cr_{high}**, 93% of the laboratories have satisfactory Z-scores.

6.4 Conclusions and recommendations

The overall participation in the HBM4EU ICI round 4 was successful. Seventeen laboratories out of the 46 laboratories (37%) in the candidate list confirmed their participation in this ICI.

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

15 of these 17 registered candidate laboratories reported results, representing a participation rate of 88%.

Regarding the quantification of chromium in serum, the participants achieved in **Cr_{low}** 86% and in **Cr_{high}** 93% satisfactory results.

A direct comparison of the overall performance of the laboratories with that of the previous rounds is not entirely possible, because there were some laboratories from the previous rounds that did not participate in this round.

In the 2nd ICI, the percentage of satisfactory Z-scores at the low level was 95% and 100% at the high level. In the third round all participants achieved satisfactory Z-scores at the low and the high level.

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

Table 3 Performance of the candidate laboratories for chromium in serum

Lab. code	LOQ [ng/mL]	Cr_{low}	Cr_{high}
QR/101	1.000	**satisfactory**	**satisfactory**
QR/111	0.176	**satisfactory**	**satisfactory**
QR/115	0.600	**satisfactory**	**satisfactory**
QR/119	0.200	**unsatisfactory**	**unsatisfactory**
QR/129	0.090	**satisfactory**	**satisfactory**
QR/130	0.150	**satisfactory**	**satisfactory**
QR/143	0.180	**satisfactory**	**satisfactory**
QR/203	0.290	**satisfactory**	**satisfactory**
QR/205	0.010	**questionable**	**satisfactory**
QR/206	1.200	**satisfactory**	**satisfactory**
QR/211	0.100	**satisfactory**	**satisfactory**
QR/212	0.100	**satisfactory**	**satisfactory**
QR/224	0.050	**satisfactory**	**satisfactory**
QR/225	0.050	**satisfactory**	**satisfactory**
QR/301	-	**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: **serum**

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	5.260	5.530	5.395	-0.270	0.073	0.000
2	5.740	5.330	5.535	0.410	0.168	0.023
3	5.410	5.430	5.420	-0.020	0.000	0.001
4	5.290	5.550	5.420	-0.260	0.068	0.001
5	5.280	5.530	5.405	-0.250	0.063	0.000
6	5.360	5.330	5.345	0.030	0.001	0.002
7	5.610	5.180	5.395	0.430	0.185	0.000
8	5.420	4.990	5.205	0.430	0.185	0.032
9	5.520	5.140	5.330	0.380	0.144	0.003
10	5.500	5.300	5.400	0.200	0.040	0.000
				Σ =	0.927	0.063

Lowest:	4.990 µg/kg
Highest	5.740 µg/kg
Grand mean ($\bar{x}$):	5.385 µg/kg
Stdev:	0.176 µg/kg
VC%:	3.3% µg/kg

Outliers: Cochran's test

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

--> C = 0.200
--> 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% =	35.12	RSD% =	22	RSD% =	25
σ_H =	1.891	σ_H =	1.185	σ_H =	1.346

σ_H used: 1.346

Homogeniteit [1]:

s_x =	0.084	
s_w =	0.215	(within sample standard deviation)
s_s =	0.000	(between sample standard deviation)
critical=	0.404	

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: **serum**

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	13.090	13.320	13.205	-0.230	0.053	0.158
2	13.610	13.480	13.545	0.130	0.017	0.003
3	13.220	13.660	13.440	-0.440	0.194	0.027
4	12.890	13.850	13.370	-0.960	0.922	0.054
5	13.980	13.500	13.740	0.480	0.230	0.019
6	13.690	13.570	13.630	0.120	0.014	0.001
7	13.340	13.870	13.605	-0.530	0.281	0.000
8	13.960	13.520	13.740	0.440	0.194	0.019
9	13.750	14.000	13.875	-0.250	0.063	0.074
10	13.630	14.130	13.880	-0.500	0.250	0.077
				Σ =	2.217	0.432

Lowest:	12.890	µg/kg
Highest	14.130	µg/kg
Grand mean ($\bar{x}$):	13.603	µg/kg
Stdev:	0.322	µg/kg
VC%:	2.4%	µg/kg

Outliers: Cochran's test

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

--> C = 0.416
--> 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% =	30.55	RSD% =	22	RSD% =	25
σ_H =	4.156	σ_H =	2.993	σ_H =	3.401

σ_H used: 3.401

Homogeniteit [1]:

s_x =	0.219	
s_w =	0.333	(within sample standard deviation)
s_s =	0.000	(between sample standard deviation)
critical=	1.020	

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 (serum)**	
Storage:	minus 80 °C	minus 18 °C
Biomarker	**Chromium low**	

	t=0 (storage)	t=a (analysis)
dates:	07.10.2019	04.12.2019
values:	5.00	5.49
	5.19	5.83
	5.67	5.03
	5.86	5.48
	6.17	6.01
	5.89	5.77
number=	6	6
average=	5.63	5.60
std dev=	0.448	0.347

analysis date	time (days)	µg/L	n	std dev
07.10.2019	0	5.63	6	0.448
04.12.2019	58	5.60	6	0.347
x0-xa=	**0.03**			

test 'consequential instability	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xav-yav =<0,3σH		
σH=	1.239	1.4075
0,3*σH=	0.372	0.42225
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	1.67
Fcrit=	5.05
Significant difference?	**No significant difference in std detected**
sed^2=	0.40
n=	10
std difference=	0.23
t=	0.12
t-crit=	2.23
Significant difference?	**No statistic instability detected**

Appendix 2 Stability data (continued)

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material:	**Cr (serum)**	
Storage:	minus 80 °C	minus 18 °C
Biomarker	**Chromium high**	

	t=0 (opslag)	t=a (analyse)
dates:	07.10.2019	04.12.2019
values:	14.03	13.99
	14.30	13.96
	14.34	14.13
	13.75	13.80
	13.94	13.78
	13.79	13.86
number=	6	6
average=	14.03	13.92
std dev=	0.250	0.133

analysis date	time (days)	µg/L	n	std dev
07.10.2019	0	14.03	6	0.250
04.12.2019	58	13.92	6	0.133
x0-xa=	**0.10**			

test 'consequential instability'	Horwitz/Thompson	**Fit-for-purpose (FFP)**
xgem-ygem =<0,3σH		
σH=	3.086	3.50625
0,3*σH=	0.926	1.051875
x0-xa<0,3*σH?	**No consequential instability detected**	**No consequential instability detected**

test 'significant difference':	
F=	3.55
Fcrit=	5.05
Significant difference?	**No significant difference in std detected**
sed^2=	0.20
n=	10
std difference=	0.12
t=	0.91
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 4

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 4th 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.**

We would appreciate the participation of all laboratories in this ICI irrespective of their participation/approval in the previous ICIs.

Calendar:

Registration deadline	November 01, 2019 (we would be pleased to receive your feedback as soon as possible)
Distribution of test samples (projected)	November 04, 2019
Deadline for submission of results (projected)	December 02, 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

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

HBM4EU: Instruction letter ICI study Cr in serum/Round 4

Title of ICI: Chromium in serum

Dear participant,

Thank you for participation in the HBM4EU ICI study Cr in serum/Round 4 for the determination of Chromium in serum.

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 3 mL serum.
The parcel will be shipped on November 6, 2019 under ambient conditions.

Instructions:

- Upon receipt, please check the content for any damage/leakage 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/ 21.10.2019.
- 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 **December 4, 2019**.

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

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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 4

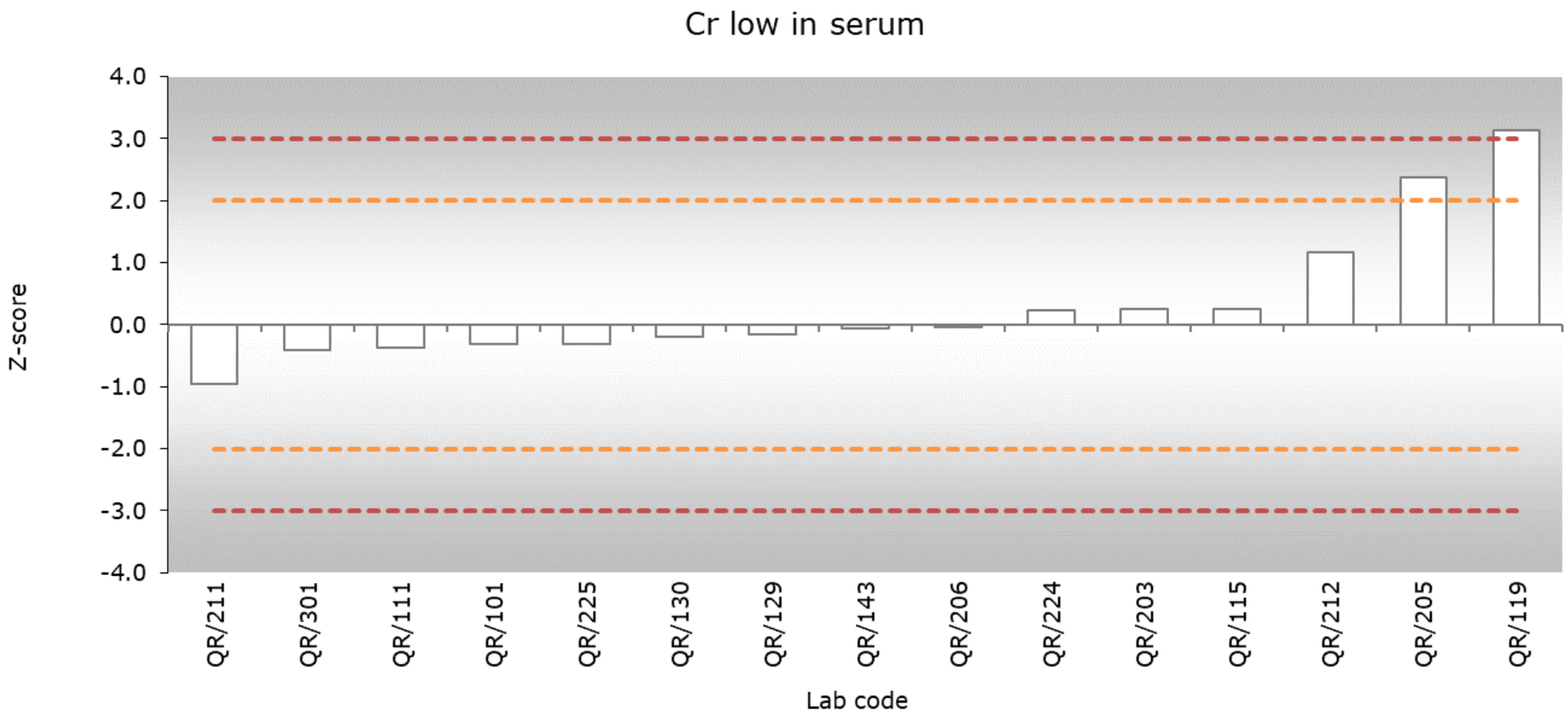
Title of ICI: Chromium in serum

Laboratory code	**IPASUM Erlangen - Germany**
ISO17025 accredited	**no**
SAMPLE PREPARATION	
amount sample	**0.2 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	**1:20 with H_20, HNO_3, C_2H_6O and Triton-X-solution**
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	**-**

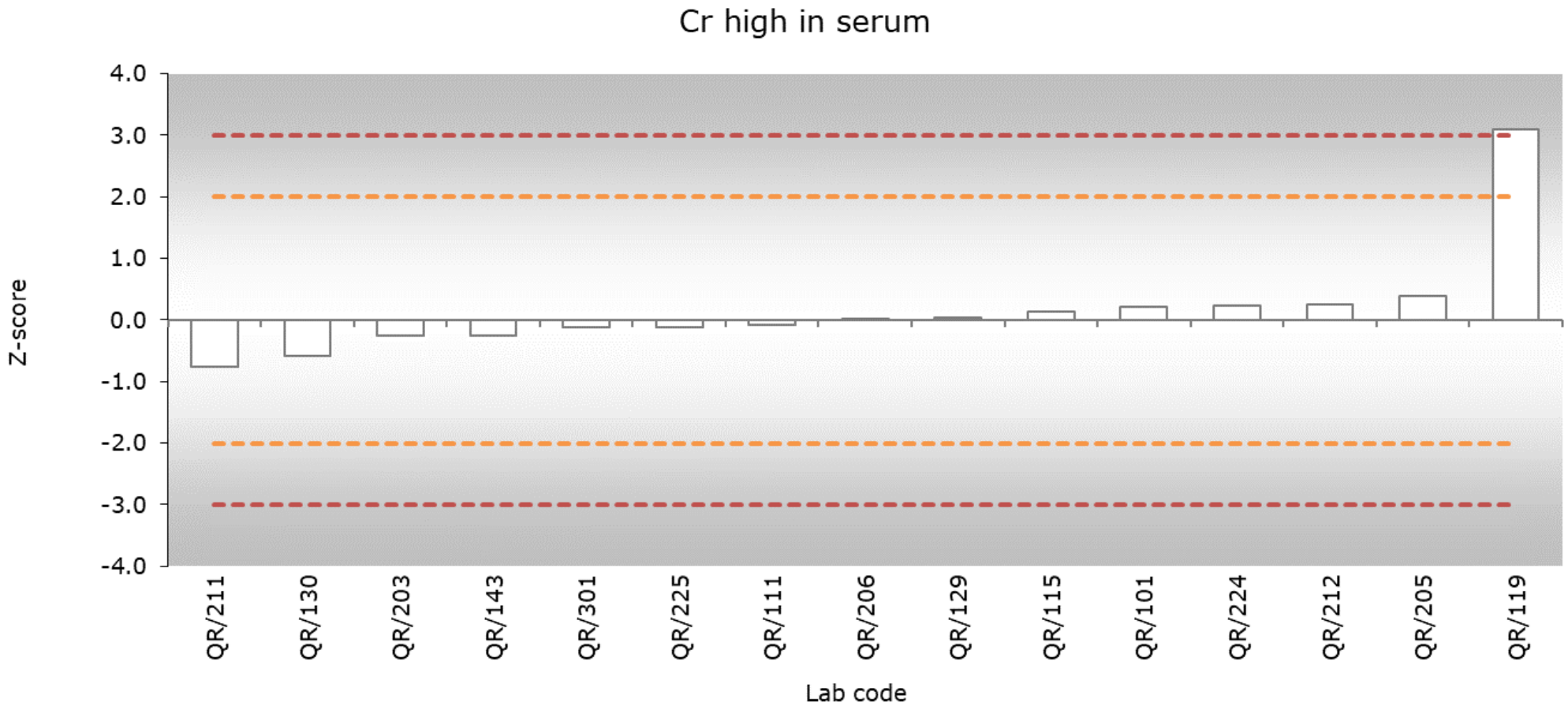
Appendix 6 Assigned values and participant's performance

HBM4EU 04/2019	Cr (serum)			
control material	Cr_{low}		Cr_{high}	
assigned value	5.889 ng/mL		14.491 ng/mL	
uncertainty of assigned value	0.220 ng/mL		0.381 ng/mL	
study RSD_R	11.6%		8.1%	
relative target standard deviation	25%		25%	
laboratory code	value	Z-score	value	Z-score
QR/101	5.414	-0.32	15.232	0.20
QR/111	5.351	-0.37	14.222	-0.07
QR/115	6.270	0.26	15.000	0.14
QR/119	10.500	3.13	25.700	3.09
QR/129	5.670	-0.15	14.600	0.03
QR/130	5.610	-0.19	12.360	-0.59
QR/143	5.800	-0.06	13.600	-0.25
QR/203	6.260	0.25	13.540	-0.26
QR/205	9.381	2.37	15.880	0.38
QR/206	5.830	-0.04	14.540	0.01
QR/211	4.470	-0.96	11.700	-0.77
QR/212	7.600	1.16	15.400	0.25
QR/224	6.226	0.23	15.328	0.23
QR/225	5.420	-0.32	14.080	-0.11
QR/301	5.282	-0.41	14.051	-0.12

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 4-2019 Chromium in serum [ng/mL]				
Lab.code	**low**	**high**	**LOQ**	**delayed reporting**
QR/101	**5.414**	**15.232**	1.000	
QR/111	**5.351**	**14.222**	0.176	
QR/115	**6.270**	**15.000**	0.600	
QR/119	**10.500**	**25.700**	0.200	
QR/129	**5.670**	**14.600**	0.090	
QR/130	**5.610**	**12.360**	0.150	
QR/143	**5.800**	**13.600**	0.180	
QR/203	**6.260**	**13.540**	0.290	
QR/205	**9.381**	**15.880**	0.010	
QR/206	**5.830**	**14.540**	1.200	
QR/211	**4.470**	**11.700**	0.100	
QR/212	**7.600**	**15.400**	0.100	
QR/224	**6.226**	**15.328**	0.050	
QR/225	**5.420**	**14.080**	0.050	
QR/301	**5.282**	**14.051**	-	