HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

ICI REPORT OF THE WP9 ICI

Round 01/2018

Chromium in whole blood

Version / date of issue	1 / 07-11-2018
Organiser Coordinator	Department of Environmental Sciences Jožef Stefan Institute Jamova 39 1000 Ljubljana SLOVENIA Radmila Milačič, radmila.milacic@ijs.si
Author(s) (Short name of institute)	Radmila Milačič (JSI), Tea Zuliani (JSI), Janja Vidmar (JSI), Katarina Marković (JSI), Stefan Marković (JSI), Ana Drinčić (JSI), Janez Ščančar (JSI), Milena Horvat (JSI)
Approved by:	Thomas Göen (IPASUM)

Table of contents

1 Summary

Within the framework of the HBM4EU project, an Inter-Laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of chromium (Cr) in human whole blood. The study was performed from August 2018 to September 2018.

In total, 8 laboratories from 7 countries participated in this ICI. The participation in the ICI was satisfactory, as 8 out of 8 laboratories submitted their results.

Six test samples consisting of 2.2 mL whole blood each were prepared, corresponding to different concentration levels of the targeted biomarker, and sent to the participating laboratories for analysis. These samples were defined as follows:

- 3 samples at very low Cr concentration, to be below or as close as possible to expected instrumental detection limits.

- 3 samples at high Cr concentration.

Homogeneity and stability assessment of the material confirmed that high Cr concentration level sample was adequately homogeneous and stable. Stability results for very low concentration level were not found all satisfying. As expected, the concentrations determined by the organizer for the very low concentration level sample did not permit to correctly assess the homogeneity and the stability of this material.

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

Assessment scores were calculated for high concentration level samples. As a global overview, the proportion of satisfying results (-2 < Z-score < 2) was 100 %.

As expected, scores associated to the very low concentration level sample were not calculated due to the high number of non-quantified samples (“<LOQ”) and to the high variability between laboratories.

2 Introduction

Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are efficient tools to assess the proficiency of laboratories, and the comparability and reliability of the analytical methods used. Participation in ICI/EQUAS is full part of Laboratory Quality Assurance system together with initial and on-going in-house method validation.

This ICI study was 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 be further involved in the analytical characterization of the HBM4EU samples.

This report describes the 1st round for chromium in whole blood and was organised by the Jožef Stefan Institute (JSI), Ljubljana, Slovenia.

2.1 Confidentiality

In this report the identity of the participants and the information provided by them is treated as confidential. However, lab codes of the participants will be disclosed to the HBM-QAU for performance assessments.

3 Control material

3.1 Preparation of control material

The material was prepared on 20th August by mixing two lyophilised Seronorm reference materials "Trace elements in whole blood", containing different concentrations of chromium (High Level (L1) and Low level (L2)), to prepare samples at high chromium concentration. Each vial of the reference material was reconstructed with water, following the instructions of the producer. Reconstructed whole blood samples were gathered in 250 mL Teflon bottle, covered by Teflon cup and gently swirled to avoid formation of foam. The same procedure was applied to prepare very low chromium concentration, using reference material (L1), which was after reconstruction diluted with 0.9% sodium chloride (suprapure). 2.2 mL of the pooled samples with very low and high chromium concentrations were then filled into labelled 4.5 mL cryogenic polyethylene tubes and tightly closed by cups. These samples were immediately deeply frozen at -20° C.

3.2 Homogeneity of control material

For the between bottle homogeneity, five tubes containing whole blood with high chromium concentration were randomly selected from the freezer (-20 °C) on the day when the participants received the samples. Samples were thawed, left to equilibrate to room temperature, gently swirled (three times) to avoid formation of foam and analysed in duplicate by ICP-MS as reported in Appendix 1. The homogeneity was evaluated according to HBM4EU-SOP-QA-002 "Preparation of control materials for Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)", ISO 13528:2015, Fearn et al. (2001) and Thompson, (2000). The results are presented in Appendix 2.

The conclusions are summarized in the table 1 below.

Table 1: conclusions associated to the homogeneity test

		HOMOGENEITY CRITERIA		
	Concentration Level	$s_s < 0.3*\sigma H$	$s_w < 0.5*\sigma_H$	***Outliers ?***
Cr	High Level (H)	ACCEPT	ACCEPT	NO

3.3 Stability of control material

In accordance with HBM4EU-SOP-QA-002 "Preparation of control materials for Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)" and with ISO 13528:2015, seven randomly selected test samples containing whole blood with high chromium concentration were randomly selected the day samples were prepared and from the freezer (-20 °C) on the day when the participating laboratories should finish with the analysis. Samples were analysed according to the analytical procedure described in Appendix 1.

The stability was evaluated using the Excel-sheet "HBM4EU ICI-EQUAS stability test CM v1". The results are presented in Appendix 3. As expected, for samples with very low chromium concentrations, the stability criteria were not met. This factor was included in the score calculation as foreseen in the procedure.

Table 2: conclusions associated to the stability test

		STABILITY CRITERIA		
		X-Y < 0.3*σH		Fischer's test
	Concentration Level	Horwitz/Thompson (22%)	Fit-for-purpose (25%)	
Cr	High Level (VL)	ACCEPT	ACCEPT	ACCEPT

4 Organisational details

4.1 Participants

A list of 16 candidate laboratories from different countries previously identified as potential candidates 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 those 16 candidate laboratories on 28th June 2018 (see Appendix 4), indicating that participation would be free of charge. In the invitation letter it was indicated that the shipment of samples will be performed on 21st August 2018.

Ten laboratories out of 16 laboratories (62.5%) indicated their interest in participating in this ICI and sent their registration form to JSI, with their agreement to abide by the conditions for participation. These laboratories received an individual laboratory code to report their measurement results.

The deadline to submit the test results was initially fixed to 14th September 2018 but was further extended to 30th September 2018. From the 10 potential participants, 8 performed the assays and submitted results.

4.2 Dispatch and instructions

Six test materials consisting of 2.2 mL whole blood were shipped on 21th August 2018 to participants under frozen conditions on dry ice by DHL in styropore boxes by DHL post to the participants. The amount of dry ice was around 3 kg per each box. Moreover, a letter with instruction on sample handling (Instruction letter, see Appendix 5), a sample receipt form to be sent back to JSI upon receipt of the test material, as well as submission form and a method relevant information related to the analytical method used for quantification were sent to the participants by e-mail the 20th August 2018.

Participants were asked to perform for each sample a single analysis using the same procedure they will routinely use in HMB4EU and to report results according to the instructions given above.

5 Data evaluation

5.1 Assigned value

For ICI studies, the consensus value is used as assigned value and calculated as described in SOP HBM4EU-SOP-QA-003. In brief, the consensus values and its uncertainty were calculated from the results submitted by the participants using robust statistics to minimize the influence of outliers.

5.2 Target standard deviation

For calculation of the Z-scores, Fit-for-purpose (FFP = 25 %) target standard deviation was used as a default value for this first round, in lack of prior information on inter-laboratory performance within the HBM4EU laboratories.

5.3 ICI standard deviation

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

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 3.

Table 3: 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 8 laboratories from 7 countries agreed to participate in this study. All 8 participants submitted results.

Laboratories were also asked to provide details on the method used for analysis. All laboratories performed the measurement on the diluted whole blood samples. 7 participants reported results that were determined by inductively coupled plasma mass spectrometers (ICP-MS) and one participant used atomic absorption spectrometry (AAS). This information is compiled in Appendix 6. An overview of results submitted by the participants is included in Appendix 7.

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 analytes/control materials are included in Appendix 8.

The robust relative standard deviation was calculated as described in HBM4EU-SOP-QA-003 and was compared to the FFP target standard deviation, in order to evaluate whether the FFP fitted well with the variability actually observed. All these observations are presented in the Appendix 7.

6.3 Assessment of laboratory performance

Z-scores calculated for all target biomarkers and analysed sample are reported in Appendix 6. Graphical representations of the Z-scores are provided in Appendix 8. A summary of number of laboratories that reported results and the number of acceptable/questionable/unacceptable scores are presented in Table 4.

Table 4: Summary of ICI results for Cr

	Low Level	High Level
No of reported quantitative results	7	23
No of reported LOQ	8	8
No of acceptable score	0	8
No of questionable score	0	0
No of unacceptable score	0	0

Assessment scores were calculated for chromium in very low and high level samples. All results were satisfying (-2 < Z-score < 2). The summary of participant's scores is included in the Appendix 7.

The calculation of the score was not achievable for Cr in whole blood at very low concentration level because of unacceptable high variability of the concentrations as well as the limited number of laboratories involved in the reporting of the results. As expected, scores associated to the very low concentration level sample were not calculated due to the high number of non-quantified samples ("<LOQ") and to the high variability between laboratories.

6.4 Conclusions and recommendations

The overall participation in the HBM4EU ICI Round 1 was successful. Eight laboratories out of ten registered candidate laboratories reported results, representing a participation rate of 80%.

All the submitted results were satisfactory. These results indicate that the participating laboratories are competent for the analysis of Cr in whole blood samples.

7 References

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

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

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

HBM4EU-SOP-QA-002 "Preparation of test materials for ICI / EQUAS"

HBM4EU-SOP-QA-003 "Evaluation of ICI / EQUAS results"

HBM4EU-SOP-QA-004 "Reporting of ICI / EQUAS studies"

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

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

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.

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.

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: ICP-MS method information JSI

Title of ICI: Chromium in whole blood

Laboratory code	JSI	
ISO17025 accredited	no	
SAMPLE PREPARATION		
Amount of sample	1 mL (digestion), 0.2 mL (dilution)	g or mL
Digestion (if applied)	Yes, in addition diluted samples were also analysed	
INSTRUMENTAL ANALYSIS		
AAS	no	
Wavelength		
Background correction		
Matrix modifier		
Dilution factor		
Calibration	external calibrant (matrix based) standard addition external calibrant (solvent based)	
	single level / multi level	
Correction for recovery	no	
Other remarks		
ICP-OES	no	
Nebulizer		
Wavelength		
Dilution factor		
Quantification		
Use of internal standard(s)	no	
Response normalised to IS	no	
Calibration	external calibrant (matrix based) standard addition external calibrant (solvent based)	
	single level / multi level	
Correction for recovery	no	
Other remarks		
ICP-MS	no	
MS (single quadrupole/QQQ/Q-TOF)	ICP-MS quadrupole	
Nebulizer	Miramist	
Reagent gas	Helium	
Masses monitored	*m/z* 52	
Dilution factor	5 times (digested samples), 10 times (diluted samples)	
Quantification		
Use of internal standard(s)	Yes/no	
Response normalised to IS	no	
Calibration	external calibrant (matrix based)	
	multi level	
Correction for recovery	no	

Further remarks/observations:

Appendix 2: Homogeneity study

Homogeneity

Version HBM4EU v1

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

10 randomly chosen test samples, analysed in duplicate

[1] ISO 13528:2005
[2] Fearn, T. and M. Thompson, 2001, A New Test for 'Sufficient homogeneity', Analyst, 126, 1414-1417
[3] Thompson M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386

	replicate 1	replicate 2	x_t	w_t	w_t^2	$(x_t - \bar{x})^2$
1	7,28	7,14	7,2	0,1	0,0	0,4
2	7,97	7,33	7,7	0,6	0,4	0,0
3	7,47	7,80	7,6	-0,3	0,1	0,0
4	7,79	7,51	7,6	0,3	0,1	0,0
5	8,18	8,43	8,3	-0,3	0,1	0,2
6	7,66	7,41	7,5	0,2	0,1	0,1
7	8,60	7,86	8,2	0,7	0,6	0,2
8	7,85	7,95	7,9	-0,1	0,0	0,0
9	7,93	8,47	8,2	-0,5	0,3	0,1
10	8,16	7,80	8,0	0,4	0,1	0,0
Lowest:		7,1 µg/kg		Σ =	1,7	1,1
Highest		8,6 µg/kg				
Grand mean ($\bar{x}$):		7,83 µg/kg				
Stdev:		0,40 µg/kg				
VC%:		5,2% µg/kg				

Outliers: Cochran's test

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

--> C = 0,321
--> $Ccrit$ = 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% =	33,20	*RSD%* =	22	*RSD%* =	25
σ_H =	2,60	σ_H =	1,72	σ_H =	1,96
σ_H used:	1,96				

Homogeniteit [1]:

s_x =	0,35	
s_w =	0,29	(within sample standard deviation)
s_s =	0,29	(between sample standard deviation)
critical=	0,59	
s_s < critical?	→ **ACCEPT: Homogeneity adequate**	
s_w < 0.5*σ_H?	→ **ACCEPT: Method suited**	

Appendix 3: Stability data

HBM4EU ICI-EQUAS stability test CM v1

Stability test in frame of ICI-EQUAS

Material: **Whole blood**
Storage: Frozen

Biomarker **Cr**

	t=0 (storage)	t=a (analysis)
dates:	24.08.2018	10.09.2018
values:	7,59	7,77
	8,19	7,82
	7,96	8,29
	8,15	8,20
	8,16	8,14
	7,78	7,97
	8,13	7,93
number=	7	7
average=	7,99	8,02
std dev=	0,232	0,198

analysis date	time (days)	µg/L	n	std dev
24.08.2018	0	7,99	7	0,232
10.09.2018	17	8,02	7	0,198

x0-xa= **-0,02**

test 'consequential instability': Horwitz/Thompson **Fit-for-purpose (FFP)**

xa<y av =<0,3σH

σH=	1,758	1,998202459
0,3*σH=	0,528	0,599460738

x0-xa<0,3*σH? **No consequential instability detected** **No consequential instability detected**

test 'significant difference':

F=	1,375
Fcrit=	4,284
Significant difference?	**No significant difference in std detected**
sed^2=	0,22
n=	12
std difference=	0,12
t=	0,21
t-crit=	2,18
Significant difference?	**No statistic instability detected**

Appendix 4: Copy of letter of invitation

HBM4EU: Invitation letter to participate in ICI study Cr in serum, whole blood, and urine/Round 1 and Round 2

Title of ICI: Cr in serum, whole blood and urine

Dear HBM4EU partner,
You are invited to participate in ICI study Cr in serum, whole blood and urine/Round 1 and Round 2. Since Cr ICI was agreed recently, the two rounds Cr ICI 1 and Cr ICI 2 will be performed closely one after another, so that we can evaluate data from two ICI's before the end of this year.
The laboratories which will confirm their participation on Cr ICI (deadline 15.7.2018, will get information about sample shipment, conditions of analysis and reporting of results.
In each Cr ICI you will receive 3 aliquots of samples (2 mL) of serum, whole blood and urine, at two concentration levels (in total 18 samples). Samples will be sent by DHL deeply frozen on dry ICE. After the receipt, you will have 3 weeks to complete the analysis and send the results to the organiser.

The Agenda is the following:

Date	Cr ICI 1st round	Cr ICI 2nd round
Sample shipment (organiser)	21.08.2018	11.09.2018
Submitting of results (participants)	14.09.2018	05.10.2018
Sending of report (organiser)	15.11.2018	15.12.2018

To fasten the procedure, we would like that the participants express their interest to participate on Cr ICI 1 and/or Cr ICI 2 in the attached registration form.

Looking forward to your participation,
Sincerely,

Prof.dr. Radmila Milačič
Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia

e-mail: Radmila.milacic@ijs.si Tel.: +396 1 4773560

Appendix 5: Copy of letter/instructions sent together with test samples

science and policy
for a healthy future

HBM4EU: Sample handling before the analysis: ICI study Cr/Round 1
Title of ICI: Cr in serum, whole blood and urine

Dear participant,
Thank you for participation in HBM4EU ICI study Cr in serum, whole blood and urine 1st Round.
You will receive a parcel containing 6 test samples of serum, 6 test samples of whole blood and 6 test samples of urine. For each matrix, 3 are control samples, 3 are samples with biomarker considered for Cr ICI study.
The parcel will be shipped on 21.08.2018 deeply frozen on dry ICE by DHL.

Instructions:
Upon receipt, please check whether samples were received frozen on dry ice. If samples were damaged or are leaking of the containers, please immediately contact the organiser to send you the replacement for damaged samples. Please complete the sample receipt form and return it to the organiser.

Take the samples from dry ice and immediately transfer them to a deep freezer and keep the samples at -20 °C until the analysis.
Before the analysis thaw the samples and leave them to equilibrate to room temperature. Swirl the samples gently (three times) to avoid formation of foam. Do not shake samples. Analyse the samples according to the laboratory standard operational procedure.

Carry out a single analysis for each sample. For submission of results and method information use the forms (attached).
The deadline for submission of results and method details is 18.09.2018.
Provide only one result for each sample bottle in the excel file which will be sent to you by e-mail.

Warning: Handle the samples (human biological material) with care, in spite of negative test for Hepatitis B, Hepatitis C and HIV. The handling of material should be conducted as if the material is infectious.

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

Prof.dr. Radmila Milačič
Department of Environmental Sciences
Jožef Stefan Institute
Jamova 39, 1000 Ljubljana, Slovenia
e-mail: Radmila.milacic@ijs.si
Tel: +386 1 477 3560

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

Appendix 6: Details of analysis methods used by the participants

Laboratory Code	Sample Preparation	Detection Technique	LOQ (ng Cr/mL)
2	dilution	Single quad ICP-MS	1.8
3	dilution	Single quad ICP-MS	0.2
4	dilution	Single quad ICP-MS	0.028
5	dilution	Single quad ICP-MS	2
6	dilution	AAS, Zeeman background correction	2
7	dilution	Single quad ICP-MS	0.4
9	dilution	Single quad ICP-MS	1.12
10	dilution	ICP-MS-QQQ	0.0873

Appendix 7: Assigned values and participant's performance

Control material	**Whole blood**
Parameter	**Cr - high**
Number of participants	8
Number of quantitative results	23
Assigned value ng/mL	7.15
Uncertainty of assigned value ng/mL	0.14
Study RSD_R (%)	4.46
Relative target standard deviation (%)	25.0

Laboratory code	ID sample	Value	Average	Z-Score
2	WB_045	6.94	6.72	-0.28
2	WB_083	6.72		
2	WB_047	6.49		
3	WB_054	7.02	7.12	-0.06
3	WB_062	7.18		
3	WB_071	7.16		
4	WB_039	6.98	6.97	-0.14
4	WB_042	7.02		
4	WB_061	6.91		
5	WB_055	7.402	7.27	0.02
5	WB_080	7.128		
5	WB_081	7.279		
6	WB_052	7.85	8.02	0.44
6	WB_066	8.10		
6	WB_068	8.11		
7	WB_049	6.50	6.53	-0.38
7	WB_070	6.80		
7	WB_074	6.30		
9	WB_044	7.45	7.27	0.07
9	WB_051	NA		
9	WB_062	7.09		

10	WB_037	7.25	7.34	0.07
10	WB_038	7.43		
10	WB_057	7.35		

Control material	**Whole blood**
Parameter	**Cr - low**
Number of participants	8
Number of quantitative results	7
Assigned value ng/mL	-
Uncertainty of assigned value ng/mL	-
Study RSD_R (%)	-
Relative target standard deviation (%)	-

Laboratory code	**ID sample**	**Value**	**Average**	**Z-Score**
2	WB_003	nd	nd	-
2	WB_014	nd		
2	WB_011	nd		
3	WB_025	nd	nd	-
3	WB_026	nd		
3	WB_031	0.237		
4	WB_002	0.465	0.463	-
4	WB_016	0.458		
4	WB_018	0.465		
5	WB_005	nd	nd	-
5	WB_027	nd		
5	WB_029	nd		
6	WB_009	nd	nd	-
6	WB_028	nd		
6	WB_032	nd		
7	WB_007	<loq	<loq	-
7	WB_015	<loq		
7	WB_017	<loq		

9	WB_033	nd	nd	-
9	WB_008	nd		
9	WB_030	nd		
10	WB_001	0.768	0.734	-
10	WB_012	0.696		
10	WB_022	0.739		

Appendix 8: Graphical representation of the Z'i-scores and the Z-scores