

REPORT OF THE WP9 interlaboratory comparison

Round 03/2020

Arsenic 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.goen@fau.de
Author(s) (IPASUM)	Moritz Schäfer (IPASUM), Stefanie Nübler (IPASUM), Karin H. A. Zarrabi (IPASUM)
Approved by:	Thomas Göen (IPASUM)

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1 Summary

Within the framework of the HBM4EU project, an interlaboratory comparison was organised and conducted for the analysis of arsenic in urine. Arsenic corresponds to six biomarkers: total arsenic, As(III), As(V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine.

The study was performed from February to March 2020 and was conducted to assess the comparability and reliability of analytical methods across the participating expert laboratories.

The HBM4EU QAU had selected three expert laboratories for arsenic in urine. The expert laboratories were from three different countries in Europe.

The participation in this interlaboratory comparison for arsenic in urine was mandatory for these laboratories.

In February 2020, two different test samples consisting of 5 mL urine spiked with arsenic at two different concentrations (R3A, R3B) were prepared and sent to the participating expert laboratories for single analysis.

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

Consensus values were calculated by averaging the values obtained by the expert labs when the relative uncertainty of the mean was within 17.5%.

In order to express the proficiency of the laboratories in a numerical way, Z-scores were calculated using the consensus value and a fixed fit-for-purpose relative target standard deviation (FFP-RSD) of 25%.

For **total As, AS(III)_{R3B}, As(V), MMA and DMA**, consensus values could be calculated from the results of all three experts, the obtained Z-scores were all satisfactory and the relative standard deviation (RSD) was in a range from 4.3% to 23.1%.

In case there were only two results for a parameter, the two results were considered comparable when the difference to the mean was $\leq 35\%$. Then, no Z-scores were calculated.

For **As(III)_{R3A}**, no consensus value could be calculated and no Z-scores could be provided, because the relative uncertainty (u) of the mean concentration from three expert labs was too high. However, the results of two expert laboratories (AEL1 and AEL2) were in a good comparable range with a difference from the mean of 1.9%.

Table 1 below gives an overview of the respective number of quantitative results and the consensus/mean values for the two different levels of all arsenic biomarkers.

As matters stand, this third round marked the conclusion of the interlaboratory comparison rounds for arsenic in urine. The final evaluation of the comparability of the respective expert laboratories will take place in due course.

Table 1 Overview of results for arsenic in urine in interlaboratory comparison/round 3

biomarker	participants	quantitative results	consensus/ <i>mean</i> value
total As R3A	3	3	48.600 ng/mL
total As R3B	3	3	31.114 ng/mL
As(III) R3A	3	3	<i>0.508 ng/mL</i>
As(III) R3B	3	3	0.947 ng/mL
As(V) R3A	3	3	0.591 ng/mL
As(V) R3B	3	3	1.041 ng/mL
MMA R3A	3	3	0.600 ng/mL
MMA R3B	3	3	2.588 ng/mL
DMA R3A	3	3	5.068 ng/mL
DMA R3B	3	3	9.780 ng/mL
Arsenobetaine R3A	3	3	41.601 ng/mL
Arsenobetaine R3B	3	3	15.304 ng/mL

2 Introduction

This interlaboratory comparison is intended to assess the comparability and reliability of analytical methods across the participating expert laboratories. Participation in this exercise forms an integral part of quality control, in addition to initial and ongoing in-house method validation.

This study has been organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in these exercises is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the 3rd round of interlaboratory comparison for arsenic 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.

The selection of the most relevant arsenic biomarkers was previously made in WP9, and has been described in Deliverable report 9.5 v2.0. Based on this and in cooperation with the QAU and proven experts in the field, IPASUM – as task leader of Task 9.4 – selected a set of 6 target biomarkers for arsenic to be included in this 3rd interlaboratory comparison (see **Table 2**).

Table 2 Arsenic biomarkers in urine included in this 3rd interlaboratory comparison

Abbreviation	Target biomarker
Total As	Total arsenic
AS(III)	Arsenic (III)
AS(V)	Arsenic (V)
MMA	Monomethylarsonic acid
DMA	Dimethylarsinic acid
Arsenobetaine	Arsenobetaine

For this 3rd interlaboratory comparison, expert laboratories were selected according to the following selection criteria described in HBM4EU-SOP-QA-005 and in agreement with the QAU.

The selection criteria included:

1. Experience in analysis of all selected parameters in (the selected) human matrices at levels expected in the general population (proven experience, papers, reports, etc.)
2. Capacity for analysis (number of samples/time for analysis)
3. Limit of quantification of the method sufficiently low for HBM4EU samples (indicate how the LOQ was determined)
4. Historical data of the successful participation in interlaboratory comparison exercises for the target substance (selected parameters)

The interlaboratory comparison assesses the comparability of analysis results for the same sample analysed by multiple expert laboratories in the same time frame. As measure of proficiency, Z-scores are calculated using the mean value derived from the experts' results as consensus value, and a pre-set target standard deviation (e.g. fit-for-purpose standard deviation). Expert laboratories are requested to apply the same procedure as they will use for analysis of samples in the frame of HBM4EU.

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.

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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 pH was adjusted to 5 with acetic acid. The five different stock solutions (As(III), As(V), MMA, DMA, Arsenobetaine) were diluted into two different concentrations and the addition to the native control material resulted in the intended concentration in control material (R3A, R3B). 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 ($\leq -18\text{ }^{\circ}\text{C}$) until transportation. The two different concentrations (R3A, R3B) were measured using LC-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 (R3A, R3B) were randomly selected from the freezer ($\leq -18\text{ }^{\circ}\text{C}$). The thawed samples were re-homogenised by vortex shaking and analysed in duplicate using LC-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 R3A and six randomly selected test samples of R3B were stored at $-80\text{ }^{\circ}\text{C}$. The assumption is that under these conditions, the biomarker (As) is stable in urine. On the last day of the deadline for submission of results by the participants (March 16, 2020), six test samples of each level (stored at $-80\text{ }^{\circ}\text{C}$) and six samples of each level (stored at $-18\text{ }^{\circ}\text{C}$) were thawed and re-homogenised by vortex shaking. Next, all samples were analysed using LC-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.

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4 Organisational details

4.1 Participants

For the organisation of the 3rd interlaboratory comparison, IPASUM contacted the three selected expert laboratories (all from Europe) and sent them instruction letters by e-mail on February 25, 2019 (see **Appendix 4**). It was indicated that participation would be free of charge and that participants would receive a kit containing the test materials needed for analysis. Test results had to be submitted within the stipulated deadline (March 16, 2020).

The laboratories received an individual laboratory code to report their measurement results (see **Appendix 8**).

All laboratories performed the assays and submitted their results within the stipulated deadline.

4.2 Dispatch and instructions

Test materials were dispatched on dry ice to the participants on February 26, 2020. Each participant received two test samples spiked with the biomarker at different levels, one of each concentration (R3A, R3B). 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 SOPs

For this 3rd interlaboratory comparison, the HBM4EU-QA-SOPs were followed. There were no deviations from the relevant 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 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 a consensus 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.

5.2 Consensus value (from at least 3 labs) and mean value of two labs

The minimum number of expert laboratories required for establishment of a consensus value in these interlaboratory comparisons is three.

The results obtained by the expert laboratories will be used to calculate the mean of all expert values, the respective relative standard deviation, and the relative uncertainty of the mean, which is given by:

$$u = \text{RSD} / \sqrt{N}$$

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

RSD = relative standard deviation of the mean concentration

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

The mean concentration derived from the expert laboratories is considered as acceptable consensus value in interlaboratory comparison studies if $u \leq 0.7 \cdot \sigma_T$ ($\sigma_T = 25\%$).

Only if $u > 0.7 \cdot \sigma_T$, were the results of the expert laboratories checked for outliers. If an individual expert value is identified as an outlier, it is rejected from the data set and the relative uncertainty is calculated again when N is still ≥ 3 . If the condition $u \leq 0.7 \cdot \sigma_T$ is still not met, then the comparability of the results of the remaining expert laboratories is considered unsatisfactory.

In case there are only results from two expert labs a mean value can be calculated using the results of these two experts.

Then the comparability of the results of the two expert laboratories is evaluated using the reproducibility limit ($= 2.8 \cdot \sigma_T = 70\%$). Thus, the results are considered comparable when the difference to the mean is $\leq 35\%$. In that case the calculation of Z-scores cannot be applied.

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 consensus value was used as target standard deviation.

5.4 Relative standard deviation

To gain insight into the actual inter-laboratory variability of the biomarker analysis in this study, the relative standard deviation (RSD) was calculated based on the participants' results.

5.5 Z-scores

The quantitative results from all participating expert laboratories are used to calculate a consensus value based on the participants' results (see 5.2).

This consensus value (A) is then used to calculate the Z-scores of the participants' mean results (x) using a target standard deviation (σ_T) of 25%.

The Z-score (Z) is calculated as follows:

$$Z = \frac{x - A}{\sigma_T}$$

Z-scores are classified as presented in **Table 3**.

Table 3 Classification of Z-scores

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

6 Results and discussion

6.1 Results submitted by participants

In total, three laboratories from three European countries participated as experts in this study. All submitted their results. Laboratories were also asked to provide LOQs.

Appendix 7 gives an overview of results and LOQs submitted by the participants.

Results indicated as 'not detected' (ND, see Appendix 7):

False positive results: No participant detected a false positive result.

Methods: The method details provided by the laboratories are included in **Appendix 8**.

For the sample pretreatment, all laboratories used no extraction, no cleanup and no derivatisation. The volume of urine used for the analysis varied from 0.200 to 0.300 mL. All samples were analysed by HPLC and single quad mass spectrometry. The injection volume varied between 25 and 100 µL. Two laboratories used an external calibrant (solvent based) and one laboratory used standard addition.

6.2 Consensus/mean values and (target) standard deviations

The consensus or mean value and its uncertainty, the relative standard deviation as derived from the participant's data, and the fit-for-purpose (FFP) target standard deviation (25%) for each of the control materials are included in **Appendix 6**.

6.3 Assessment of laboratory performance

The three participating expert laboratories reported results.

A summary of the number of quantitative results and the respective consensus/mean values is given in **Table 1**.

For **As(III)_{R3A}**, no Z-scores could be provided, because the relative uncertainty (u) of the mean concentration from three expert labs was too high. Thus, no final evaluation of the performance was possible. However, the results of two expert laboratories (AEL1 and AEL2) were in a comparable range with a difference from the mean of 1.9%. The difference from the mean of AEL1 and AEL3 as well as of AEL2 and AEL3 was also calculated, but it was higher than for AEL1 and AEL2.

6.4 Conclusions and recommendations

The participation in the 3rd HBM4EU interlaboratory comparison for arsenic was successful. All selected expert laboratories reported results, representing a participation rate of 100%.

The evaluation of laboratory performance and comparability was not fully possible for all six biomarkers. Z-scores could not be calculated for **As(III)_{R3A}**. For all other biomarkers, all expert laboratories obtained satisfactory results and were thus in a good comparable range.

For **As(III)_{R3A}** the relative uncertainty (u) of the mean concentration from three expert labs was too high.

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All in all, the three participating expert laboratories were in a comparable range for **total As**, **As(III)_{R3B}**, **As(V)**, **MMA**, **DMA** and **Arsenobetaine**. The results of two expert laboratories (AEL1 and AEL2) showed a good comparability for all levels of the six arsenic biomarkers considering the difference from the mean and the reproducibility limit.

As matters stand, this third round marked the conclusion of the interlaboratory comparison rounds for arsenic in urine. The final evaluation of the comparability of the respective expert laboratories will take place in due course. Consequently, the final evaluation will not be included in this report, but in a separate document yet to be finalised.

7 References

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Appendix 1 Homogeneity data

	<u>total As</u>				<u>As(III)</u>			
	R3A [ng/mL]		R3B [ng/mL]		R3A [ng/mL]		R3B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	48.9	47.5	30.7	31.5	0.44	0.41	0.60	0.80
2	48.3	47.0	30.0	30.2	0.49	0.42	0.80	0.70
3	48.6	48.6	30.0	30.7	0.42	0.40	0.80	0.70
4	48.2	48.0	30.9	31.3	0.48	0.44	0.80	0.60
5	48.4	47.2	31.0	30.6	0.41	0.34	0.80	0.80
6	48.3	47.3	30.7	31.1	0.42	0.47	0.80	1.00
7	48.4	48.2	30.8	31.2	0.51	0.46	0.60	0.70
8	47.7	47.7	30.9	30.2	0.49	0.44	0.70	0.70
9	47.9	47.5	30.9	30.8	0.34	0.40	0.80	0.90
10	48.3	46.6	30.5	30.6	0.39	0.41	0.80	0.70
grand mean	47.930		30.712		0.429		0.755	
Cochran's test								
C	0.313		0.286		0.202		0.235	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP} :	11.983		7.678		0.107		0.189	
s_x	0.355		0.330		0.039		0.076	
s_w	0.679		0.322		0.035		0.091	
s_s	0.000		0.239		0.031		0.039	
Critical= $0.3 \sigma_{FFP}$	3.595		2.303		0.032		0.057	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$?	Method suited		Method suited		Method suited		Method suited	

Appendix 1 Homogeneity data (continued)

	<u>As(V)</u>				<u>MMA</u>			
	R3A [ng/mL]		R3B [ng/mL]		R3A [ng/mL]		R3B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.33	0.31	0.80	0.80	0.61	0.53	2.30	2.50
2	0.31	0.28	1.00	1.00	0.59	0.54	2.40	2.20
3	0.29	0.36	0.90	0.90	0.54	0.60	2.30	2.00
4	0.29	0.24	0.80	0.90	0.53	0.66	2.30	2.50
5	0.34	0.35	0.90	1.00	0.58	0.65	2.30	2.10
6	0.29	0.23	1.00	0.90	0.60	0.52	2.30	2.70
7	0.28	0.26	0.80	0.80	0.65	0.51	2.40	2.60
8	0.31	0.24	0.90	1.00	0.52	0.67	2.30	2.70
9	0.33	0.35	1.00	0.80	0.54	0.61	2.30	2.70
10	0.27	0.33	0.80	0.90	0.56	0.66	2.10	2.90
grand mean	0.300		0.895		0.584		2.395	
Cochran's test								
C	0.226		0.444		0.230		0.599	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP} :	0.075		0.224		0.146		0.599	
s_x	0.032		0.069		0.019		0.134	
s_w	0.033		0.067		0.070		0.266	
s_s	0.021		0.049		0.000		0.000	
Critical= $0.3 \sigma_{FFP}$	0.022		0.067		0.044		0.180	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$?	Method suited		Method suited		Method not suited		Method suited	

Appendix 1 Homogeneity data (continued)

	<u>DMA</u>				<u>Arsenobetaine</u>			
	R3A [ng/mL]		R3B [ng/mL]		R3A [ng/mL]		R3B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	4.70	4.30	9.40	9.80	43.3	40.5	15.3	14.7
2	5.10	5.20	9.80	9.30	39.6	38.2	15.7	14.3
3	5.00	4.90	9.50	9.60	38.9	39.0	15.3	13.7
4	4.70	5.00	9.50	9.60	42.5	39.2	15.5	13.9
5	5.00	4.90	9.40	10.40	39.6	40.2	15.4	13.8
6	5.10	5.20	9.50	10.20	39.7	38.1	16.3	20.5
7	5.30	5.00	9.60	10.10	43.0	38.8	16.2	17.3
8	4.50	3.50	9.20	9.80	38.3	40.1	15.7	16.7
9	4.90	4.00	9.30	9.80	39.2	40.3	15.4	16.4
10	4.70	4.10	9.40	10.20	41.6	39.4	15.5	16.6
grand mean	4.755		9.670		39.975		15.710	
Cochran's test								
C	0.392		0.292		0.349		0.550	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP} :	1.189		2.418		9.994		3.928	
s_x	0.395		0.158		1.034		1.222	
s_w	0.357		0.414		1.590		1.266	
s_s	0.304		0.000		0.000		0.831	
Critical= $0.3 \sigma_{FFP}$	0.357		0.725		2.998		1.178	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$?	Method suited		Method suited		Method suited		Method suited	

Appendix 2 Stability data

	<u>total As</u>				<u>As(III)</u>			
	<u>R3A [ng/mL]</u>		<u>R3B [ng/mL]</u>		<u>R3A [ng/mL]</u>		<u>R3B [ng/mL]</u>	
	<u>-80°C</u>	<u>-18°C</u>	<u>-80°C</u>	<u>-18°C</u>	<u>-80°C</u>	<u>-18°C</u>	<u>-80°C</u>	<u>-18°C</u>
1	48.9	49.8	31.12	31.53	0.30	0.23	0.77	0.92
2	49.1	48.2	31.55	31.57	0.22	0.25	0.89	0.79
3	48.6	49.3	30.81	30.83	0.24	0.26	0.95	0.85
4	49.4	48.8	31.08	31.16	0.23	0.30	0.75	0.66
5	48.9	49.0	30.93	31.37	0.29	0.25	0.65	0.85
6	49.1	48.7	31.13	31.83	0.22	0.24	0.74	0.90
average	48.980	48.965	31.103	31.382	0.250	0.255	0.792	0.828
stdev	0.289	0.536	0.252	0.350	0.036	0.024	0.109	0.094
difference	0.015		-0.278		-0.005		-0.037	
critical=0.3 σ_{FFP}	3.674		2.333		0.019		0.059	
consequential instability	no		no		no		no	
t	0.060		1.581		0.283		0.623	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

Appendix 2 Stability data (continued)

	<u>As(V)</u>				<u>MMA</u>			
	R3A [ng/mL]		R3B [ng/mL]		R3A [ng/mL]		R3B [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	0.33	0.38	1.20	1.13	0.61	0.58	2.76	2.50
2	0.40	0.34	1.21	1.19	0.53	0.57	2.66	2.58
3	0.35	0.47	1.11	1.34	0.50	0.56	2.49	2.48
4	0.47	0.39	1.24	1.15	0.57	0.63	2.76	2.55
5	0.41	0.36	1.10	1.12	0.59	0.61	2.76	2.76
6	0.48	0.51	1.17	1.12	0.56	0.56	2.69	2.85
average	0.407	0.408	1.172	1.175	0.560	0.585	2.687	2.620
stdev	0.061	0.067	0.056	0.085	0.040	0.029	0.105	0.150
difference	-0.002		-0.003		-0.025		0.067	
critical=0.3 σ_{FFP}	0.031		0.088		0.042		0.202	
consequential instability	no		no		no		no	
t	0.045		0.080		1.242		0.889	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

Appendix 2 Stability data (continued)

	<u>DMA</u>				<u>Arsenobetaine</u>			
	R3A [ng/mL]		R3B [ng/mL]		R3A [ng/mL]		R3B [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	5.0	4.6	9.6	9.5	44.6	44.7	17.1	15.3
2	4.6	5.0	9.4	9.1	44.4	46.6	17.3	16.0
3	5.0	4.5	9.2	10.2	46.8	42.4	13.8	15.8
4	5.0	4.5	9.9	9.1	43.8	44.5	15.7	14.3
5	4.7	4.5	10.0	9.1	44.0	42.0	15.6	17.3
6	4.7	5.0	9.9	10.3	43.7	42.3	15.5	15.1
average	4.857	4.675	9.662	9.538	44.542	43.750	15.847	15.630
stdev	0.193	0.236	0.314	0.561	1.164	1.821	1.275	1.029
difference	0.182		0.123		0.792		0.217	
critical=0.3 σ_{FFP}	0.364		0.725		3.341		1.189	
consequential instability	no		no		no		no	
t	1.458		0.470		0.897		0.324	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

Appendix 3 Copy of announcement letter

HBM4EU: Announcement to participate in three rounds of interlaboratory comparisons for ARSENIC biomarkers as an expert laboratory

Title: Arsenic in urine

Dear Colleagues,

within the frame of HBM4EU the

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

announces 3 rounds of interlaboratory comparisons for the determination of **arsenic in urine**. The aim of these exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

Test samples

The matrix will be urine. Accordingly, the participants will receive **in each round**:

- 2 different materials of urine (**2 samples of 5 mL each**) for determination of arsenic in urine

Target biomarkers

Please analyse all of the following target biomarkers in both samples.

- **total arsenic**
- **As (III)**
- **As (V)**
- **Monomethylarsonic acid (MMA)**
- **Dimethylarsinic acid (DMA)**
- **Arsenobetaine**

LOQs should allow the analysis of arsenic in samples of the general population.

The LOQ requirements are as follows:

total arsenic: 1.0 µg/L or lower

As (III), As (V), MMA, DMA and Arsenobetaine: 0.1 µg/L or lower

Calendar: projected dates

Distribution of test samples for round 1	08-01-2020
Deadline for submission of results for round 1	27-01-2020
Report for round 1	31-01-2020
Distribution of test samples for round 2	05-02-2020
Deadline for submission of results for round 2	24-02-2020

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Arsenic in urine, Round 3			

Report for round 2	28-02-2020
Distribution of test samples for round 3	24-02-2020
Deadline for submission of results for round 3	16-03-2020
Report for round 3	20-03-2020
Letters of approval and certificates sent to participants	31-03-2020

Fee

For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participants are responsible for custom clearance and associated costs if applicable and that they will not be reimbursed.

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

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Arsenic in urine, Round 3			

Please complete the following sheet and send it back to ipasum-hbm4eu@fau.de:

Participating laboratory:

name of the institution

address of the laboratory

name of 1st contact person, telephone number and email address

name of 2nd contact person, telephone number and email address

Address for delivery of the test samples:

name of (the contact person and) the institution

address of the laboratory

The above laboratory will participate in the interlaboratory comparisons for arsenic in urine.
I agree with the conditions mentioned in this letter, and that the laboratory will analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU, and submit results before the indicated deadlines.

Name:

Signature:

Date:

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

HBM4EU: Instruction letter interlaboratory comparison Arsenic in urine/Round 3

Dear participant,

Thank you for participation in the HBM4EU interlaboratory comparison for the determination of **Arsenic in urine**.

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

The parcel will be **shipped on February 26, 2020** under frozen 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 as soon as possible**.
- Store the test samples under frozen (-18°C) conditions until analysis.
- Analyse the samples for the biomarkers:
 - **total arsenic**
 - **As (III)**
 - **As (V)**
 - **Monomethylarsonic acid (MMA)**
 - **Dimethylarsinic acid (DMA)**
 - **Arsenobetaine**
- 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** use the **form provided**.
- The **deadline for submission of analysis results** is **March 16, 2020**

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

Stefanie Nübler, Karin Zarrabi, or Moritz Schäfer

Email: ipasum-hbm4eu@fau.de; Tel.: + 49 (0)9131/8526145, /8526146

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

Prof. Dr. Thomas Göen

Appendix 5: Method information form for participation in interlaboratory comparison

Arsenic in urine/Round 1-3

Laboratory code	IPASUM	
ISO17025 accredited	no	
SAMPLE PREPARATION		
amount sample extracted	0.3	mL
Extraction	no	
- pH adjustment	-	
- LLE;	-	
- SPE; material	-	
Cleanup	no	
- LLE; solvent(s)	-	
- SPE; material	-	
Derivatisation	no	
- reagent	-	
Digestion	no	
INSTRUMENTAL ANALYSIS	yes	
LC/HPLC/other	HPLC	
- injection volume	0.050	µL
- column stationary phase	Ion-Exchange	
- column L (mm) x ID (mm); dp	PRP-X100 10 µm 4.1 x 250 mm	
- temperature	ambient	
- mobile phase A	NH ₄ H ₂ PO ₄ + NaNO ₃ + NaOAc + H ₂ O	
- mobile phase B	NH ₄ H ₂ PO ₄ + NaNO ₃ + NaOAc + H ₂ O	
- flow rate	1.2 mL/min	mL/min
ICP	Yes	
Dilution	1:1	
Nebulizer	glass cyclonic	
Reagent gas	argon	
Masses monitored	75	
Cell mode	KED	
Detection		
MS	Single quad	
other	-	
Quantification		
Use of internal standard (IS)	no	
- response normalised to IS	no	
Calibration	external calibrant (solvent based)	
	multi level	
Correction for recovery	no	
Identification criteria used		
- retention time tolerance	-	
- number of ions/transitions	-	
- ion ratio tolerance	-	

Appendix 6 Consensus values and participant's performance

HBM4EU 03/2020	total As (urine)			
control material	R3A		R3B	
consensus value from three experts	48.600 ng/mL		31.114 ng/mL	
expert standard deviation	2.112 ng/mL		2.143 ng/mL	
uncertainty of consensus value (u)	2.5%		4.0%	
study RSD	4.3%		6.9%	
laboratory code	value	Z-score	value	Z-score
AEL 1	48.973	0.031	31.243	0.017
AEL 2	50.500	0.156	33.190	0.267
AEL 3	46.326	-0.187	28.910	-0.283

HBM4EU 03/2020	As(III) (urine)			
control material	R3A		R3B	
<i>mean value (R3A)/ consensus value from three (R3B) experts</i>	<i>0.508 ng/mL</i>		0.947 ng/mL	
expert standard deviation	<i>0.420 ng/mL</i>		0.130 ng/mL	
uncertainty of consensus value (u)	47.8%		7.9%	
study RSD	82.8%		13.7%	
<i>difference of AEL1 and AEL2 from the mean value of these two labs (R3A)</i>	<i>1.9%</i>		na	
<i>difference of AEL2 and AEL3 from the mean value of these two labs (R3A)</i>	<i>57.2%</i>		na	
<i>difference of AEL1 and AEL3 from the mean value of these two labs (R3A)</i>	<i>58.5%</i>		na	
laboratory code	value	Z-score	value	Z-score
AEL 1	0.260	no	0.840	-0.453
AEL 2	0.270	no	0.910	-0.158
AEL 3	0.993	no	1.092	0.611

na: not applicable; no: no Z-score available; instead, the difference from the mean value was calculated

Appendix 6 Consensus values and participant's performance (continued)

HBM4EU 03/2020	As(V) (urine)			
control material	R3A		R3B	
consensus value from three experts	0.591 ng/mL		1.041 ng/mL	
expert standard deviation	0.136 ng/mL		0.182 ng/mL	
uncertainty of consensus value (u)	13.3%		10.1%	
study RSD	23.1%		17.5%	
laboratory code	value	Z-score	value	Z-score
AEL 1	0.480	-0.751	1.170	0.497
AEL 2	0.550	-0.277	1.120	0.305
AEL 3	0.743	1.029	0.832	-0.802

HBM4EU 03/2020	MMA (urine)			
control material	R3A		R3B	
consensus value from three experts	0.600 ng/mL		2.588 ng/mL	
expert standard deviation	0.027 ng/mL		0.296 ng/mL	
uncertainty of consensus value (u)	2.6%		6.6%	
study RSD	4.5%		11.5%	
laboratory code	value	Z-score	value	Z-score
AEL 1	0.590	-0.067	2.690	0.158
AEL 2	0.580	-0.133	2.820	0.359
AEL 3	0.631	0.207	2.254	-0.516

Appendix 6 Consensus values and participant's performance (continued)

HBM4EU 03/2020	DMA (urine)			
control material	R3A		R3B	
consensus value from three experts	5.068 ng/mL		9.780 ng/mL	
expert standard deviation	1.001 ng/mL		0.807 ng/mL	
uncertainty of consensus value (u)	11.4%		4.8%	
study RSD	19.7%		8.2%	
laboratory code	value	Z-score	value	Z-score
AEL 1	4.620	-0.354	9.540	-0.098
AEL 2	4.370	-0.551	9.120	-0.270
AEL 3	6.215	0.905	10.679	0.368

HBM4EU 03/2020	Arsenobetaine (urine)			
control material	R3A		R3B	
consensus value from three experts	41.601 ng/mL		15.304 ng/mL	
expert standard deviation	4.282 ng/mL		2.229 ng/mL	
uncertainty of consensus value (u)	5.9%		8.4%	
study RSD	10.3%		14.6%	
laboratory code	value	Z-score	value	Z-score
AEL 1	44.300	0.259	16.030	0.190
AEL 2	43.840	0.215	17.080	0.464
AEL 3	36.664	-0.475	12.802	-0.654

Appendix 7 Results and LOQs and reasons for delayed submission

HBM4EU 3/2020 total arsenic in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	48.973	31.243	0.050	
AEL2	50.500	33.190	0.500	
AEL3	46.326	28.910	0.050	

HBM4EU 3/2020 As(III) in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	0.260	0.840	0.100	
AEL2	0.270	0.910	0.100	
AEL3	0.993	1.092	0.100	

HBM4EU 3/2020 As(V) in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	0.480	1.170	0.060	
AEL2	0.550	1.120	0.100	
AEL3	0.743	0.832	0.220	

HBM4EU 3/2020 MMA in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	0.590	2.690	0.070	
AEL2	0.580	2.820	0.200	
AEL3	0.631	2.254	0.240	

HBM4EU 3/2020 DMA in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	4.620	9.540	0.090	
AEL2	4.370	9.120	0.200	
AEL3	6.215	10.679	0.140	

HBM4EU 3/2020 Arsenobetaine in urine [ng/mL]

Lab.code	R3A	R3B	LOQ	delayed reporting
AEL1	44.300	16.030	0.120	
AEL2	43.840	17.080	0.500	
AEL3	36.664	12.802	0.054	

Appendix 8: Method details for determination of arsenic in urine, provided by the laboratories

Lab.code	Pretreatment			
	amount sample extracted	extraction	cleanup	derivatisation
AEL1	0.300 mL	no	no	no
AEL2	n.s.	no	no	no
AEL3	0.200 mL	no	no	no

Lab.code	Instrumental analysis			
	separation	injection volume (µL)	column	detection
AEL1	HPLC	50	4.1 mm x 250 mm; 10 µm	single quad
AEL2	HPLC	25	2.0 mm x 250 mm; 2.7 µm	single quad
AEL3	HPLC	100	4.6 mm x 150 mm; 5.0 µm	single quad

Lab.code	Quantification			Criteria used for identification	
	use of internal standard	calibration	level	retention time tolerance	correction for recovery
AEL1	yes	external calibrant (solvent based)	multi level	no	no
AEL2	yes	standard addition	multi level	+/- 0.1 min	no
AEL3	no	external calibrant (solvent based)	multi level	no	no