HBM4EU science and policy for a healthy future

HORIZON2020 Programme
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REPORT OF THE WP9

INTERLABORATORY COMPARISON

Round 03/2020

Acrylamides in urine

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1 Summary

Within the framework of the HBM4EU project, an interlaboratory comparison was organized and conducted for the analysis of acrylamides (AM) in urine.

Acrylamides correspond to two biomarkers: N-Acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA) and N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA).

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

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

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

In March 2020, two different test samples consisting of 2 mL urine spiked with acrylamides 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_R) of 25%.

Table 1 below gives an overview of the respective number of quantitative results, the consensus values and the performance of the laboratories for the two different levels of all AM biomarkers.

All expert laboratories obtained satisfactory Z-scores for both levels of each of the AM biomarkers.

The final evaluation of the comparability of the respective expert laboratories can, however, only take place upon completion of all interlaboratory comparison rounds.

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

biomarker	participants	quantitative results	consensus value	satisfactory	questionable	unsatisfactory
AAMA R3A	5	5	12.681 ng/mL	5 (100%)	0	0
AAMA R3B	5	5	101.924 ng/mL	5 (100%)	0	0
GAMA R3A	5	5	12.681 ng/mL	5 (100%)	0	0
GAMA R3B	5	5	27.664 ng/mL	5 (100%)	0	0

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 acrylamides 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 acrylamide 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 2 target biomarkers for acrylamides to be included in this 3rd interlaboratory comparison (see **Table 2**).

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

Abbreviation	Target biomarker
AAMA	**N-Acetyl-S-(2-carbamoyl-ethyl)cysteine**
GAMA	**N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine**

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.

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 two different stock solutions (AAMA and GAMA) were diluted into two different concentrations and the addition to the native control material resulted in the intended concentration in control material (AM_{R3A}, AM_{R3B}). The two spiked control materials were aliquoted (5 mL each) into tubes with caps (57x15.3 mm, polypropylene, Sarstedt). The tubes were stored in a freezer (≤ -18 °C) until transportation. The two different concentrations (AM_{R3A}, AM_{R3B}) were measured using ICP-MS (see analysis method in **Appendix 5**). The measured concentrations are shown in Sections 3.2 and 3.3 of this report.

3.2 Homogeneity of control material

Ten tubes of each concentration of the control material (AM_{R3A}, AM_{R3B}) were randomly selected from the freezer (≤ -18 °C). The thawed samples were re-homogenised by vortex shaking and analysed in duplicate using the method shown 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 °C. The assumption is that under these conditions, the biomarker (AM) is stable in urine. On the last day of the deadline for submission of results by the participants (June 08, 2020), 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 the method shown in **Appendix 5**

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

4 Organisational details

4.1 Participants

For the organisation of the 3rd interlaboratory comparison, IPASUM contacted the five selected expert laboratories (all from Europe) and sent instruction letters to them by e-mail on May 20, 2020 (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 (June 8, 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 (see **Appendix 8**).

4.2 Dispatch and instructions

Test materials were dispatched to the participants under ambient conditions on May 26, 2020. Each participant received two test samples spiked with the biomarker at different levels, one of each concentration (AM_{R3A}, AM_{R3B}). Each sample consisted of approximately 2 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

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:

$u = RSD / \sqrt{N}$

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 $u \leq 0.7 * \sigma_T$ (σ_T = 25%).

Only if $u > 0.7 * \sigma_T$, are 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. If the condition $u \leq 0.7 * \sigma_T$ is still not met, then the comparability of the results of the remaining expert laboratories is considered unsatisfactory.

5.3 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD) 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

$\lvert Z \rvert \leq 2$	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, five laboratories from three European countries participated as experts in this study. All submitted their results. Laboratories were also asked to provide LOQs.

Appendix 8 gives an overview of results and LOQs submitted by the participants.

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

Methods:

In almost all cases the samples were analysed by UPLC, followed by HPLC. For sample preparation, all laboratories used no extraction, no clean-up, no derivatisation and no digestion. Almost all participating laboratories used a triple quad or quadrupole as detection system. Most candidates used an external calibrant (matrix-based), followed by external calibrant (solvent-based).

6.2 Consensus values and (target) standard deviations

The consensus 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

All five participating expert laboratories reported results.

A summary of the number of quantitative results, the respective consensus values and the performance of the laboratories for the two different levels of all AM biomarkers is given in **Table 1**.

For **AAMA** and **GAMA**, all participants obtained satisfactory Z-scores (see **Appendix 6**).

6.4 Conclusions and recommendations

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

The LOQ requirements were fully met by all participants.

Tables 3 to 8 provide the LOQs and an overview of the performance of the candidate laboratories in this 3rd round for acrylamides in urine.

Evaluation of laboratory performance was possible for all biomarkers. The percentage of satisfactory Z-scores for all individual biomarkers was 100%.

The final evaluation of the comparability of the respective expert laboratories can, however, only take place upon completion of all interlaboratory comparison rounds

Table 4 Performance of the candidate laboratories for AAMA in urine

Lab. code	LOQ [ng/mL]	$AAMA_{R3A}$	$AAMA_{R3A}$
ACL1	1.000	**satisfactory**	**satisfactory**
ACL2	2.000	**satisfactory**	**satisfactory**
ACL4	3.200	**satisfactory**	**satisfactory**
ACL5	5.000	**satisfactory**	**satisfactory**
ACL6	5.000	**satisfactory**	**satisfactory**

Table 4 Performance of the candidate laboratories for GAMA in urine

Lab. code	LOQ [ng/mL]	$GAMA_{R3B}$	$GAMA_{R3B}$
ACL1	1.000	**satisfactory**	**satisfactory**
ACL2	3.000	**satisfactory**	**satisfactory**
ACL4	1.000	**satisfactory**	**satisfactory**
ACL5	5.000	**satisfactory**	**satisfactory**
ACL6	5.000	**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

	AAMA				GAMA			
	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	43.30	47.20	107.40	114.50	18.50	15.90	39.00	37.00
2	43.70	46.60	115.50	105.40	17.20	17.40	36.20	40.60
3	45.50	43.60	106.40	110.40	16.20	18.50	38.70	39.30
4	40.00	41.70	110.40	107.40	16.40	16.90	39.80	37.40
5	44.80	43.80	103.30	119.50	17.00	16.50	36.70	38.60
6	43.60	44.20	104.30	115.50	18.00	16.80	35.60	36.40
7	44.10	45.70	103.30	110.40	15.20	15.40	34.80	37.30
8	44.20	44.00	105.40	105.40	18.50	16.40	35.00	36.90
9	43.20	44.80	109.40	112.50	17.40	16.20	37.20	35.00
10	45.30	44.00	105.40	105.40	18.00	16.20	38.40	36.30
grand mean	44.165		108.860		16.930		37.310	
Cochran`s test								
C	0.397		0.420		0.292		0.366	
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}:	11.041		27.215		4.233		9.328	
s_x	1.255		2.285		0.640		1.198	
s_w	1.384		5.592		1.076		1.625	
s_s	0.786		0.000		0.000		0.338	
Critical=0.3 σ_{FFP}	3.312		8.165		1.270		2.798	
s_s < critical?	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
s_w< 0.5*σ_{FFP}?	method suited		method suited		method suited		method suited	

Appendix 2 Stability data

	AAMA				GAMA			
	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	43.954	42.240	117.900	120.000	17.522	17.397	42.872	43.742
2	45.768	48.188	113.900	101.800	18.640	19.385	38.647	38.522
3	45.668	38.207	116.900	114.900	16.652	16.652	38.647	47.097
4	41.635	42.341	120.000	102.800	20.504	19.137	37.901	36.286
5	37.603	41.433	101.800	112.900	19.261	17.522	34.670	38.522
6	43.147	43.450	107.900	106.900	16.900	17.024	42.499	41.878
average	42.962	42.643	113.077	109.884	18.246	17.853	39.206	41.008
stdev	3.056	3.249	6.938	7.213	1.494	1.135	3.072	3.999
difference	0.319		3.192		0.394		-1.802	
critical=0.3 σ_{FFP}	3.222		8.481		1.368		2.940	
consequential instability	no		no		no		no	
t	0.175		0.781		0.514		0.875	
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 ACRYLAMIDE biomarkers as an expert laboratory

Title: Acrylamides 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 **acrylamides 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 2 mL each**) for determination of acrylamides in urine

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

- **N-Acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA)**
- **N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA)**

LOQs should allow the analysis of acrylamides in samples of the general population.
The LOQ requirements are as follows:

AAMA: 5.0 µg/L or lower
GAMA: 5.0 µg/L or lower

Calendar: projected dates

Distribution of test samples for round 1	03-02-2020
Deadline for submission of results for round 1	19-02-2020
Report for round 1	25-02-2020
Distribution of test samples for round 2	02-03-2020
Deadline for submission of results for round 2	17-03-2020

Report for round 2	23-03-2020
Distribution of test samples for round 3	18-03-2020
Deadline for submission of results for round 3	02-04-2020
Report for round 3	09-04-2020
Letters of approval and certificates sent to participants	21-04-2020

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
- Karin H. A. Zarrabi
- Johannes Müller

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

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 acrylamides 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 Acrylamides in urine/Round 3

Dear participant,

Thank you for participation in the HBM4EU interlaboratory comparison for the determination of **Acrylamides 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 **2 mL urine**.
The parcel will be shipped on May 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:

  - **N-Acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA)**
  - **N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA)**

- Thaw the samples and re-homogenise them according to your own procedure.

- Analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU.

- Carry out a single analysis for each sample.

- For **submission of results and method information** use the **forms provided**.

- The deadline for submission of analysis results and method details is June 08, 2020

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

Stefanie Nübler, Karin Zarrabi, or Johannes Müller

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Prof. Dr. Thomas Göen

Appendix 5 HBM4EU Method information form for participation in interlaboratory comparison

Acrylamides in urine/Round 1-3

Laboratory code	**IPASUM**	
ISO17025 accredited	no	
SAMPLE PREPARATION		
amount sample extracted	1	mL
Extraction	no	
- pH adjustment		
- LLE;		
- SPE; material		
Cleanup	no	
- LLE; solvent(s)		
- SPE; material		
Evaporation of sample to	yes	
- amount of sample	0.1	mL
- reconstituted in (amount/solvent)	0.1 / methanol	mL
Derivatisation	no	
- reagent		
Digestion	no	
INSTRUMENTAL ANALYSIS		
LC/HPLC/other		
- injection volume	5	µL
- column stationary phase	XBridge BEH HILIC	
- column L (mm) x ID (mm); dp	150 x 3.0; 2.5	
- temperature	25°C	
- mobile phase A	5mM NH_4-acetate / H_2O	
- mobile phase B	5mM NH_4-acetate / CH_3CN	
- mobile phase C		
- flow rate	0.6	mL/min
Detection		
MS	triple quad	
other		
Quantification		
Use of internal standard (IS)	yes	
- response normalised to IS	yes	
Calibration	external calibrant (matrix based)	
	multi level	
Correction for recovery	no	
Identification criteria used		
- retention time tolerance	0.2 min deviation from reference standard	
- number of ions/transitions	1	
- ion ratio tolerance	% relative deviation from reference standard	

Appendix 6 Consensus values and participant's performance

HBM4EU 03/2020	AAMA (urine)			
control material	$AAMA_{R3A}$		$AAMA_{R3B}$	
consensus value from five experts	39.314 ng/mL		101.924 ng/mL	
expert standard deviation	5.541 ng/mL		15.942 ng/mL	
uncertainty of assigned value (u)	6.3%		7.0%	
study RSD	14.1%		15.6%	
laboratory code	value	Z-score	value	Z-score
ACL1	43.050	0.380	119.230	0.679
ACL2	44.000	0.477	110.000	0.317
ACL4	35.278	-0.411	81.208	-0.813
ACL5	42.643	0.339	109.884	0.312
ACL6	31.600	-0.785	89.300	-0.495

HBM4EU 03/2020	GAMA (urine)			
control material	$GAMA_{R3A}$		$GAMA_{R3B}$	
consensus value from five experts	12.681 ng/mL		27.664 ng/mL	
expert standard deviation	3.794 ng/mL		8.114 ng/mL	
uncertainty of assigned value (u)	13.4%		13.1%	
study RSD	29.9%		29.3%	
laboratory code	value	Z-score	value	Z-score
ACL1	9.930	-0.868	22.880	-0.692
ACL2	8.700	-1.256	22.000	-0.819
ACL4	15.223	0.802	29.830	0.313
ACL5	17.853	1.631	41.008	1.930
ACL6	11.700	-0.309	22.600	-0.732

Appendix 8 Results and LOQs and reasons for delayed submission

HBM4EU 3/2020 AAMA in urine [ng/mL]				
Lab.code	**R3A**	**R3B**	**LOQ**	**delayed reporting**
ACL1	43.050	119.230	1.000	
ACL2	44.000	110.000	2.000	
ACL4	35.278	81.208	3.200	
ACL5	42.643	109.884	5.000	
ACL6	31.600	89.300	5.000	

HBM4EU 3/2020 GAMA in urine [ng/mL]				
Lab.code	**R3A**	**R3B**	**LOQ**	**delayed reporting**
ACL1	9.930	22.880	1.000	
ACL2	8.700	22.000	3.000	
ACL4	15.223	29.830	1.000	
ACL5	17.853	41.008	5.000	
ACL6	11.700	22.600	5.000	