HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

REPORT OF THE WP9

INTERLABORATORY COMPARISON

Round 02/2020

Acrylamides in urine

Version / date of issue	1 /27.03.2020
Organiser	Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) Friedrich-Alexander University of Erlangen-Nuremberg Henkestr. 9-11 91054 Erlangen GERMANY
Coordinator	Thomas Göen, thomas.goeen@fau.de
Author(s) (IPASUM)	Stefanie Nübler (IPASUM), Moritz Schäfer (IPASUM), Karin H. A. Zarrabi (IPASUM)
Approved by:	Thomas Göen (IPASUM)

Table of contents

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 2 biomarkers: N-Acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA) and N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA),

The study was performed in March 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 (R2A, R2B) 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 2

biomarker	participants	quantitative results	consensus value	satisfactory	questionable	unsatisfactory
AAMA R2A	5	5	40.153 ng/mL	5 (100%)	0	0
AAMA R2B	5	5	204.198 ng/mL	5 (100%)	0	0
GAMA R2A	5	5	11.730 ng/mL	5 (100%)	0	0
GAMA R2B	5	5	52.451 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 2nd 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 4 target biomarkers for acrylamides to be included in this 2nd interlaboratory comparison (see **Table 2**).

Table 2 Acrylamide biomarkers in urine included in this 2nd interlaboratory comparison

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

For this 2nd 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_{R2A}, AM_{R2B}). 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_{R2A}, AM_{R2B}) 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_{R2A}, AM_{R2B}) 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 R2A and six randomly selected test samples of R2B 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 (March 17, 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 2nd interlaboratory comparison, IPASUM contacted the five selected expert laboratories (all from Europe) and sent instruction letters to them by e-mail on March 2, 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 (March 17, 2020).

The laboratories received an individual laboratory code to report their measurement results (see **Appendix 7**).

All laboratories performed the assays and submitted their results. Four participants reported their results within the stipulated deadline (March 17, 2020), while one participant reported with a delay (see **Appendix 7**; ACL2 on March 19).

4.2 Dispatch and instructions

Test materials were dispatched to the participants under frozen conditions on March 4, 2020. Each participant received two test samples spiked with the biomarker at different levels, one of each concentration (AM_{R2A}, AM_{R2B}). 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 2nd 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, 5 laboratories from 3 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 as well as reasons for delayed submission.

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 2nd 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 2nd 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

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			
	R2A [ng/mL]		R2B [ng/mL]		R2A [ng/mL]		R2B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	43.3	47.2	228.7	230.5	18.50	15.90	74.50	73.50
2	43.7	46.6	225.8	231.3	17.20	17.40	77.00	86.00
3	45.5	43.6	221.5	233.5	16.20	18.50	77.00	74.40
4	40.0	41.7	222.4	217.6	16.40	16.90	77.80	75.10
5	44.8	43.8	229.6	228.1	17.00	16.50	81.50	72.00
6	43.6	44.2	229.8	243.3	18.00	16.80	79.10	75.10
7	44.1	45.7	227.2	219.9	15.20	15.40	75.30	76.20
8	44.2	44.0	240.7	254.2	18.50	16.40	75.30	80.30
9	43.2	44.8	232.2	224.7	17.40	16.20	76.70	79.80
10	45.3	44.0	245.3	217.5	18.00	16.20	83.40	77.30
grand	44.165		230.190		16.930		77.365	
Cochran`s								
C	0.397		0.533		0.292		0.328	
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		57.548		4.233		19.341	
s_x	1.255		7.475		0.640		2.235	
s_w	1.384		8.514		1.076		3.708	
s_s	0.786		4.431		0.000		0.000	
Critical=0.3 σ_{FFP}	3.312		17.264		1.270		5.802	
s_s <	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			
	R2A [ng/mL]		R2B [ng/mL]		R2A [ng/mL]		R2B [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	39.6	38.6	228.6	220.2	16.3	16.3	84.0	81.3
2	38.9	42.4	220.2	234.3	16.2	16.1	73.3	76.9
3	40.7	42.4	220.7	216.4	16.1	16.8	81.8	80.1
4	42.3	41.3	218.2	217.2	17.0	16.4	80.2	76.7
5	39.2	41.6	221.1	226.8	16.4	17.0	75.6	77.6
6	41.2	43.2	202.6	213.3	16.7	17.6	78.7	75.4
average	40.317	41.583	218.567	221.367	16.450	16.700	78.933	78.000
stdev	1.314	1.608	8.595	7.813	0.339	0.551	3.960	2.241
difference	-1.267		-2.800		-0.250		0.933	
critical=0.3 σ_{FFP}	3.024		16.393		1.234		5.920	
consequential instability	no		no		no		no	
t	1.494		0.590		0.946		0.502	
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 2

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 March 04, 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 March 17, 2020

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

Stefanie Nübler, Karin Zarrabi, or Johannes Müller

Email: ipasum-hbm4eu@fau.de; Tel.: + 49 (0)9131/85-26145, -26146, -22365

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 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 02/2020	AAMA (urine)			
control material	$AAMA_{R2A}$		$AAMA_{R2B}$	
consensus value from five experts	40.153 ng/mL		204.198 ng/mL	
expert standard deviation	5.736 ng/mL		20.874 ng/mL	
uncertainty of assigned value (u)	6.4%		4.6%	
study RSD	14.3%		10.2%	
laboratory code	value	Z-score	value	Z-score
ACL1	34.400	-0.573	188.700	-0.304
ACL2	45.000	0.483	215.000	0.212
ACL4	43.800	0.363	209.100	0.096
ACL5	44.165	0.400	230.190	0.509
ACL6	33.400	-0.673	178.000	-0.513

HBM4EU 01/2020	GAMA (urine)			
control material	$GAMA_{R2A}$		$GAMA_{R2B}$	
consensus value from five experts	11.730 ng/mL		52.451 ng/mL	
expert standard deviation	3.114 ng/mL		15.111 ng/mL	
uncertainty of assigned value (u)	11.9%		12.9%	
study RSD	26.5%		28.8%	
laboratory code	value	Z-score	value	Z-score
ACL1	8.820	-0.992	40.690	-0.897
ACL2	10.000	-0.590	41.000	-0.873
ACL4	11.700	-0.010	54.900	0.187
ACL5	16.930	1.773	77.365	1.900
ACL6	11.200	-0.181	48.300	-0.317

Appendix 7 Results and LOQs and reasons for delayed submission

HBM4EU 2/2020 AAMA in urine [ng/mL]				
Lab.code	**R2A**	**R2B**	**LOQ**	**delayed reporting**
ACL1	34.400	188.700	1.000	
ACL2	45.000	215.000	2.000	due to coronavirus
ACL4	43.800	209.100	3.200	
ACL5	44.165	230.190	5.000	
ACL6	33.400	178.000	5.000	

HBM4EU 2/2020 GAMA in urine [ng/mL]				
Lab.code	**R2A**	**R2B**	**LOQ**	**delayed reporting**
ACL1	8.820	40.690	1.000	
ACL2	10.000	41.000	3.000	due to coronavirus
ACL4	11.700	54.900	1.000	
ACL5	16.930	77.365	5.000	
ACL6	11.200	48.300	5.000	

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

Lab.code	Pretreatment				
	amount sample extracted [mL]	evaporation of sample to dryness	pH adjustment	extraction / clean-up	derivatisation
ACL1	0.100	yes	no	no	no
ACL2	0.100	-	no	no	no
ACL4	0.500	-	no	no	no
ACL5	1.000	yes	no	no	no
ACL6	0.100	-	no	no	no

Lab.code	Instrumental analysis					
	separation	injection volume (µL)	temperature	flow rate [mL/min]	column	detection
ACL1	UPLC	2.000	30 °C	0.700	150 mm x 3 mm; 1.7 µm	triple quad
ACL2	HPLC	10.000	40 °C	0.250	150 mm x 2.1 mm; 1.8 µm	triple quad
ACL4	HPLC	20.000	40 °C	0.200	150/10 mm x 2.1 mm; 5 µm	Q-TRAP
ACL5	HPLC	5.000	25 °C	0.600	150 mm x 3.0 mm; 2.5 µm	triple quad
ACL6	UHPLC	5.000	50 °C	0.350	100 mm x 2.1 mm; 1.8 µm	triple quad

Lab.code	Quantification		Criteria used for identification		
	use of internal standard	calibration	retention time tolerance	number of ions/transitions	ion ratio tolerance
ACL1	yes	external calibrant (solvent based)	0.3 %	1	-
ACL2	yes	external calibrant (matrix based)	no	no	no
ACL4	yes	external calibrant (matrix based)	no	3	no
ACL5	yes	external calibrant (matrix based)	0.2 min deviation from reference standard	1	realtive
ACL6	yes	external calibrant (solvent based)	no formal criteria	1	no formal criteria