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HORIZON2020 Programme  
Contract No. 733032 HBM4EU

# REPORT OF THE WP9 INTERLABORATORY COMPARISON

## Round 01/2020

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

The study was performed from January to February 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 February 2020, two different test samples consisting of 2 mL urine spiked with acrylamides at two different concentrations ( $AM_{R1A}$ ,  $AM_{R1B}$ ) 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<sub>R</sub>) of 25%.

Fehler! Verweisquelle konnte nicht gefunden werden. 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 acrylamides in urine in interlaboratory comparison/round 1**

biomarker	participants	quantitative results	consensus value	satisfactory	questionable	unsatisfactory
AAMA R1A	5	5	23.846 ng/mL	5 (100%)	0	0
AAMA R1B	5	5	107.101 ng/mL	5 (100%)	0	0
GAMA R1A	5	5	8.708 ng/mL	5 (100%)	0	0
GAMA R1B	5	5	27.628 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 1<sup>st</sup> 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 1<sup>st</sup> interlaboratory comparison (see **Table 2**).

**Table 2 Acrylamide biomarkers in urine included in this 1<sup>st</sup> interlaboratory comparison**

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

For this 1<sup>st</sup> 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 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 ( $AM_{R1A}$ ,  $AM_{R1B}$ ). 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 ( $\leq -18$  °C) until transportation. The two different concentrations ( $AM_{R1A}$ ,  $AM_{R1B}$ ) 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 ( $AM_{R1A}$ ,  $AM_{R1B}$ ) were randomly selected from the freezer ( $\leq -18$  °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 R1A and six randomly selected test samples of R1B 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 (February 19, 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 1<sup>st</sup> interlaboratory comparison, IPASUM contacted the five selected expert laboratories (all from Europe) and sent announcement letters to them by e-mail on January 17, 2020 (see **Appendix 3**). 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 (February 19, 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 February 3, 2020. Each participant received two test samples spiked with the biomarker at different levels, one of each concentration (AM<sub>R1A</sub>, AM<sub>R1B</sub>). 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 1<sup>st</sup> 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:

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

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  $u > 0.7 \cdot \sigma_T$ , 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  $u \leq 0.7 \cdot \sigma_T$  is still not met, then the comparability of the results of the remaining expert laboratories is considered unsatisfactory.

### 5.3 Target standard deviation ( $\sigma_T$ )

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD<sub>R</sub>) 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 ( $\sigma_T$ ) 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, 5 laboratories from 3 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 1<sup>st</sup> 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 4 participants, while ACL1 should try to lower their LOQ for AAMA.

**Tables 3 to 8** provide the LOQs and an overview of the performance of the candidate laboratories in this 1<sup>st</sup> 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]	AAMAR <sub>1A</sub>	AAMAR <sub>1A</sub>
ACL1	10.000	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]	GAMAR <sub>1B</sub>	GAMAR <sub>1B</sub>
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.  
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- [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

	<u>AAMA</u>				<u>GAMA</u>			
	R1A [ng/mL]		R1B [ng/mL]		R1A [ng/mL]		R1B [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	23.40	23.40	107.40	114.50	10.20	10.50	39.00	37.00
2	24.90	25.60	115.50	105.40	12.20	11.00	36.20	40.60
3	22.50	22.60	106.40	110.40	11.30	9.50	38.70	39.30
4	22.70	24.20	110.40	107.40	11.70	9.90	39.80	37.40
5	25.20	23.20	103.30	119.50	11.00	10.80	36.70	38.60
6	22.70	23.10	104.30	115.50	9.90	9.30	35.60	36.40
7	24.10	22.10	103.30	110.40	12.20	12.80	34.80	37.30
8	23.50	24.00	105.40	105.40	11.50	11.60	35.00	36.90
9	23.50	22.60	109.40	112.50	10.60	10.50	37.20	35.00
10	23.30	22.80	105.40	105.40	12.00	9.50	38.40	36.30
grand mean	23.470		108.860		10.900		37.310	
Cochran's test								
C	0.327		0.420		0.416		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 $\sigma_{FFP}$ :	5.868		27.215		2.725		9.328	
$s_x$	0.778		2.285		0.808		1.198	
$s_w$	0.782		5.592		0.867		1.625	
$s_s$	0.547		0.000		0.526		0.338	
Critical=0.3 $\sigma_{FFP}$	1.760		8.165		0.818		2.798	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \cdot \sigma_{FFP}$ ?	Method suited		Method suited		Method suited		Method suited	

## Appendix 2 Stability data

	<u>AAMA</u>				<u>GAMA</u>			
	R1A [ng/mL]		R1B [ng/mL]		R1A [ng/mL]		R1B [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	24.9	26.7	123.0	118.0	10.8	11.0	41.9	33.7
2	24.4	27.8	109.5	128.8	11.0	11.8	36.9	40.0
3	23.8	26.0	125.1	114.6	11.9	11.1	35.5	38.0
4	25.9	24.1	124.1	119.3	8.7	11.8	34.6	38.7
5	25.5	24.1	109.4	116.2	12.0	10.1	34.7	33.4
6	24.4	26.5	115.9	115.2	11.3	10.6	36.0	34.4
average	24.817	25.867	117.833	118.683	10.950	11.067	36.600	36.367
stdev	0.778	1.490	7.254	5.257	1.201	0.668	2.733	2.867
difference	-1.050		-0.850		-0.117		0.233	
critical=0.3 $\sigma_{FFP}$	1.861		8.838		0.821		2.745	
consequential instability	no		no		no		no	
t	1.530		0.232		0.208		0.144	
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

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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

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**Please complete the following sheet and send it back to [ipasum-hbm4eu@fau.de](mailto:ipasum-hbm4eu@fau.de):**

**Participating laboratory:**

name of the institution

address of the laboratory

name of 1<sup>st</sup> contact person, telephone number and email address

name of 2<sup>nd</sup> 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 1**

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 February 03, 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 February 19, 2020

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

Stefanie Nübler, Karin Zarrabi, or Johannes Müller

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

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## Appendix 5 HBM4EU Method information form for participation in interlaboratory comparison

### Acrylamides in urine/Round 1-3

<b>Laboratory code</b>	IPASUM	
ISO17025 accredited	no	
<b>SAMPLE PREPARATION</b>		
amount sample extracted	1	mL
<b>Extraction</b>	no	
- pH adjustment		
- LLE;		
- SPE; material		
<b>Cleanup</b>	no	
- LLE; solvent(s)		
- SPE; material		
<b>Evaporation of sample to</b>	yes	
- amount of sample	0.1	mL
- reconstituted in (amount/solvent)	0.1 / methanol	mL
<b>Derivatisation</b>	no	
- reagent		
<b>Digestion</b>	no	
<b>INSTRUMENTAL ANALYSIS</b>		
<b>LC/HPLC/other</b>		
- 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 <sub>4</sub> -acetate / H <sub>2</sub> O	
- mobile phase B	5mM NH <sub>4</sub> -acetate / CH <sub>3</sub> CN	
- mobile phase C		
- flow rate	0.6	mL/min
<b>Detection</b>		
MS	triple quad	
other		
<b>Quantification</b>		
Use of internal standard (IS)	yes	
- response normalised to IS	yes	
<b>Calibration</b>	external calibrant (matrix based)	
	multi level	
<b>Correction for recovery</b>	no	
<b>Identification criteria used</b>		
- 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 Assigned values and participant's performance

HBM4EU 01/2020	AAMA (urine)			
control material	AAMA <sub>R1A</sub>		AAMA <sub>R1B</sub>	
consensus value from five experts	23.846 ng/mL		107.101 ng/mL	
expert standard deviation	3.126 ng/mL		10.866 ng/mL	
uncertainty of assigned value (u)	5.9%		4.5%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD	13.1%		10.1%	
laboratory code	value	Z-score	value	Z-score
ACL1	27.400	0.596	119.000	0.444
ACL2	24.000	0.026	115.000	0.295
ACL4	24.328	0.081	97.404	-0.362
ACL5	24.700	0.143	109.900	0.105
ACL6	18.800	-0.846	94.200	-0.482

HBM4EU 01/2020	GAMA (urine)			
control material	GAMA <sub>R1A</sub>		GAMA <sub>R1B</sub>	
consensus value from five experts	8.708 ng/mL		27.628 ng/mL	
expert standard deviation	2.356 ng/mL		5.801 ng/mL	
uncertainty of assigned value (u)	12.1%		9.4%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD	27.1%		21.0%	
laboratory code	value	Z-score	value	Z-score
ACL1	6.060	-1.216	22.900	-0.685
ACL2	8.900	0.088	23.000	-0.670
ACL4	11.112	1.104	30.941	0.480
ACL5	10.900	1.007	36.200	1.241
ACL6	6.570	-0.982	25.100	-0.366

## Appendix 8 Results and LOQs and reasons for delayed submission

HBM4EU 1/2020 AAMA in urine [ng/mL]				
Lab.code	R1A	R1B	LOQ	delayed reporting
ACL1	27.400	119.000	10.000	
ACL2	24.000	115.000	2.000	
ACL4	24.328	97.404	3.200	
ACL5	24.700	109.900	5.000	
ACL6	18.800	94.200	5.000	

HBM4EU 1/2020 GAMA in urine [ng/mL]				
Lab.code	R1A	R1B	LOQ	delayed reporting
ACL1	6.060	22.900	1.000	
ACL2	8.900	23.000	3.000	
ACL4	11.112	30.941	1.000	
ACL5	10.900	36.200	5.000	
ACL6	6.570	25.100	5.000	