ICI / EQUAS REPORT

OPFRs/round_02 (2018)

OPFRs in urine

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Organiser	UCT (VSCHT) Prague, Department of Food Analysis and Nutrition Technická 3, Prague 6, 166 28
Coordinator	Jana Hajslova (jana.hajslova@vscht.cz)
Author(s) (Short name of institute)	Darina Lankova (VSCHT), Jana Pulkrabova (VSCHT), Jana Hajslova (VSCHT)
Approved by:	Jana Hajslova (VSCHT)

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1 Summary

Within the frame of the HBM4EU project, an External Quality Assurance Schemes (EQUAS) was organised on the determination of four OPFR biomarkers in urine. This was the 2nd ICI/EQUAS round for this substance group within the HBM4EU program.

In total 17 laboratories were invited for this 2nd ICI/EQUAS and only five laboratories (including three expert laboratories) submitted results. The number of OPFRs covered by the different laboratories varied widely from two to all four target biomarkers.

In November 2018, each participant received one tube for burdened control materials of human urine (low level – level 1), one tube for burdened control materials of human urine (high level – level 2) and one tube for "blank" urine (non spiked). The biomarker concentrations were approximately in the range 2-4 μg/L and 8-15 μg/L for level 1 and level 2, respectively. The concentrations were chosen according to the review of relevant data on the occurrence of OPFRs in urine of European population published mostly during last five years.

A homogeneity assessment showed that both materials were sufficiently homogeneous for EQUAS testing. No issues with stability of testing materials occurred for OPFRs.

The determination of expert value based on results from expert laboratories was not possible for any biomarker. The uncertainty of the expert-derived mean for DPHP and BDCIPP was too high to be used as assigned value. For BCIPP and BCEP the minimum number of expert results has not been reached.

Due to a limited number of obtained results, further evaluation of laboratory performance using Z-scores was not able to be perform. The achieved data are present in the report for further comparison between participants and expert labs.

2 Introduction

Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to access the proficiency of laboratories, and the comparability and reliability of analytical methods. Participation in ICI/EQUAS forms an integral part of quality control, in addition to initial and on-going in-house method validation.

This 2nd ICI/EQUAS study has been organised within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses, following protocols HBM4EU-SOP-QA-001 to 004 which are available through the HBM4EU website (https://www.hbm4eu.eu/online-library/). Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the 2nd ICI/EQUAS for OPFRs in urine, which was conducted as EQUAS and was organised by UCT Prague (University of Chemistry and Technology, Prague; VŠCHT, Vysoká škola chemicko-technologická v Praze), Department of Food Analysis and Nutrition. The analyses for homogeneity and stability testing were performed by partner laboratory IPASUM (Institut und Poliklinik für Arbeits-, Sozial- und Umweltmedizin der Universität Erlangen-Nürnberg).

For this 2nd ICI/EQUAS, expert laboratories had to be selected according to the selection criteria described in HBM4EU-SOP-QA-001 and in agreement with the QAU.

The selection of the most relevant OPFRs was previously done in WP9, and has been described in Deliverable report 9.2 v1.1. Based on this, a set of four target biomarkers was compiled to be included in the EQUAS for OPFR analysis in urine.

EQUAS is similar to ICI but instead of using the consensus value as assigned value, the mean concentration as established from data generated by at least three designated expert laboratories is used. As in an ICI, Z-scores are calculated as a measure of proficiency.

2.1 Confidentiality

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

3 Control material

3.1 Preparation of control material

The human urine was collected from one person during one day. Total of three litres were obtained. Urine was placed into the refrigerator at 7°C overnight. Next day the sediment was centrifuged and filtrated. The whole procedure was repeated twice. Before a spiking procedure, the background concentrations were investigate. The samples were sent to the project partner laboratory IPASUM. In the testing material DPHP has been quantified at mean concentration 0.36 ng/mL.

Before the spiking procedure, the urine was thawed at room temperature (20 °C). Than it was stirred for 30 min in a 3 L beaker using magnetic stir. After that, three aliquots (700 mL in graduated cylinder) were taken into the 1 L baker (one aliquot for "blank" – non spiked, one for urine level 1 and one for urine level 2). Individual OPFR delivered as solids were dissolved with respect to the manufacturers' recommendations. Subsequently, each standard of the biomarker was appropriately diluted into methanol and individually spiked into the urine level 1 and urine level 2 using calibrated Eppendorf Multipette®. During the spiking procedure, the urine was mixed using magnetic stir for the whole time, and when all compounds have been added, subsequent mixing for 30 minutes was done. Total of 10 mL from "blank" urine, level 1 and level 2 urine was placed into the tube and later analysed for the homogeneity testing. For the Round 2 and stability testing, total of 5 mL was placed into the tube from each prepared material ("blank", urine level 1, urine level 2). All tubes were placed into the freezer at -18 °C before analysis / dispatch.

3.2 Homogeneity of control material

The homogeneity of the control material was tested according to HBM4EU-QA-002. Ten tubes of control material at level 1 and level 2 were randomly selected from the freezer and send to IPASUM for analysis. The GC-MS/MS based method for the detection of OPFR metabolites in human urine after solid phase extraction and derivatization with pentafluorobenzylbromide was used (Fromme et al. 2014).

The mean concentrations and relative standard deviations (RSDr) as obtained during a homogeneity testing are presented in **Table 1**. The statistical evaluation of materials level 1 and level 2 for each of the biomarkers is provided in **Appendix 1**. It was concluded that homogeneity was adequate for all quantified biomarkers in both levels.

Table 1: Concentration of OPFRs as obtained during homogeneity testing (for details see Appendix 1).

Biomarker	Level 1 (low)		Level 2 (high)	
	Mean (ng/mL)	RSDr (%)	Mean (ng/mL)	RSDr (%)
BCPP	5.025	13	19.464	7
BCEP	3.460	7	19.458	2
DPHP	3.122	4	6.834	7
BDCPP	3.692	7	8.569	7

3.3 Stability of control material

The stability of the control material was tested according to HBM4EU-QA-002. At the day of preparation of the control materials, randomly selected test urine samples of level 1 and level 2 were stored at -80°C. After the deadline of submission of analysis results by the participants six test

samples of both materials stored at -80°C and six samples of both materials randomly selected from the -18°C in freezer, where the ICI samples were stored, were selected for analysis by IPASUM. For the analysis the previously described methods were used (see 3.2 Homogeneity of control material). The stability was evaluated using the Excel-sheet "HBM4EU ICI-EQUAS stability test CM v1". The results are presented in **Appendix 2**. In summary no troubles with the stability was found. Only exception was BCEP at level 2 for which the statistical difference in the stability between stored samples have found. Nevertheless, the difference between the results is within the day-to-day precision of the analytical procedure, so it can be concluded as no indication of instability.

4 Organisational details

4.1 Participants

For the organisation of the 2nd ICI/EQUAS, IPASUM conducted a survey to find expert laboratories for the analysis of OPFRs in urine willing to participate in the project. Then, IPASUM evaluated their eligibility and selected expert laboratories in agreement with the QAU and according to HBM4EU-SOP-QA-001.

IPASUM contacted the selected expert laboratories and sent them invitation letters by e-mail. It was indicated that participation would be free of charge, and that those who subscribed to this EQUAS would receive a kit containing the test materials needed for analysis. Then the registration forms of expert labs were sent to UCT Prague, who sent them the test materials. The final number of expert labs was three all from HBM4EU consortium.

Participants of this 2nd ICI/EQUAS were laboratories from the HBM4EU consortium (including linked-third parties) that had been included as candidate laboratories for analyses in the frame of the HBM4EU project through WP9 (Task 9.2, Deliverable 9.3). Invitation letters (**Appendix 3**) and registration forms (**Appendix 4**) were sent by e-mail on 08/11/2018 to 17 laboratories. For registration, each participant was asked to provide which of four biomarkers were included in their scope. The participants were informed that the participation will be free of charge. The deadline for registration was 19/11/2018. Out of 17 invited laboratories, only six labs (including expert labs) agreed to participate from which only five performed the assays and submitted results.

4.2 Dispatch and instructions

Test materials were dispatched on 11/12/2018. Each participant received one tube of burdened control materials of human urine (low level – level 1), one tube of burdened control materials of human urine (high level – level 2) and one tube for "blank" urine (non spiked). Each sample consisted of approximately 5 mL urine.

Moreover, a letter with instructions on sample handling (**Appendix 5**), a sample receipt form to be sent back to UCT Prague upon receipt of the test material (**Appendix 6**) as well as a result submission form and a method information form (**Appendix 7**) 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.

Test materials were dispatched to the expert laboratories under frozen conditions (with dry ice) conditions on 11/12/2018. each lab received six tubes of burdened control materials of urine (low level – level 1), six tubes of burdened control materials of urine (high level – level 2) and six tubes for "blank" urine (non spiked). Each sample consisted of approximately 5 mL urine.

Participants and expert labs 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 instruction given. The deadline for submitting results was 18/01/2019.

4.3 Deviations from ICI/EQUAS SOPs

For this 2nd ICI/EQUAS, the HBM4EU-QA-SOPs (version 2) were followed. There were no deviations from these SOPs.

5   Data evaluation

5.1   False positives and <LOQ

Classification of false positives and biomarkers reported as "<LOQ-value" or "not detected" is done as described in HBM4EU-SOP-QA-003.

A result was assigned as false positive when the following conditions all applied:

1) the biomarker is below the LOQ value as applied by the organiser, the expert laboratories, and the majority of the participants.

2) the biomarker is reported by the participant at a level clearly exceeding the LOQs mentioned under 1).

When a biomarker is reported as "<LOQ-value", AND an assigned 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 in the frame of HBM4EU. A result is a false negative when the LOQ of a biomarker is well below the assigned value, but the laboratory did not report a quantitative value. The LOQ is considered not adequate (too high) when:

1) the LOQ is substantially above the assigned value

2) the assigned value represents a realistic concentration of real samples in the frame of HBM4EU

3) quantitatively determination is feasible by the majority of the laboratories

In order to judge "<LOQ" results in a quantitative way, 'proxy-Z-scores' are calculated as described in 5.6.

5.2   Assigned value

For EQUAS studies, the concentration as established by expert laboratories is used as assigned value. The expert-assigned value is the target value based on analysis results obtained from analysis of the control material by at least three expert laboratories (see HBM4EU-SOP-QA-001). In brief, using the individual means of the expert laboratories, the mean of the means was calculated and its relative uncertainty. The mean of means is used as assigned value when the relative uncertainty was below $0.7*\sigma_T$. When this condition is not met, and no outliers could be identified, then the uncertainty of the expert-derived mean is considered too high to be used as assigned value. The other requirement to be met is that the number of (remaining) individual expert means had to be at least three.

In case no expert value could be obtained, the consensus value derived from the combined results from both participants and expert laboratories is used as an alternative, but this is subject to a minimum of seven results in total. In this case the consensus value is calculated using robust statistics as described for ICI in HBM4EU-SOP-QA-003.

5.3   Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSDR) of 25% of the assigned value is used as target standard deviation. This was the default indicated in HBM4EU-SOP-QA-003 and considered appropriate based on the outcome of the 1st round.

5.4 ICI/EQUAS standard deviation (RSD$_R$)

To gain insight in the actual interlaboratory variability of each biomarker determination in this study, the robust relative standard deviation (RSD$_R$) is calculated based on the participants' results, as described in HBM4EU-SOP-QA-003. For this the results of the expert laboratories is not included.

5.5 Z-scores

Z-scores are calculated according to SOP HBM4EU-SOP-QA-003.

$$Z = \frac{x - C}{\sigma_T} \qquad (1)$$

with: Z = Z-score for the submitted analysis result;
x = result submitted by the laboratory;
C = expert-assigned value;
σ_T = target standard deviation, here 0.25*C

In accordance with ISO 13528 and ISO 17043 and the deliverable D 9.4 "*The Quality Assurance/Quality Control Scheme in the HBM4EU project*, Z-scores are classified as presented in **Table 2**.

Table 2: Classification of Z-scores

$\lvert Z \rvert \leq 2$	Satisfactory
$2 < \lvert Z \rvert < 3$	Questionable
$\lvert Z \rvert \geq 3$	Unsatisfactory

5.6 Proxy-Z-scores

'Proxy-Z-score' are used here to judge "<LOQ" results in a quantitative way (see 5.1). The proxy-Z-scores' are calculated using the LOQ-value as result and equation (1). When no LOQ is specified, zero is used.

Proxy-Z-scores are classified as follows:

proxy-Z ≤ -3   false negative. Based on the LOQ provided, the laboratory should have been able to detect and quantify the biomarker. Performance is considered 'unsatisfactory'.

proxy-Z ≥3   the LOQ is considered too high to be fit-for-purpose in the frame of HBM4EU analysis. It also means that the LOQ is too high in comparison with other laboratories. (Note: proxy-Z can only be calculated when an assigned value could be established. When this is the case, this inherently means that reliable quantitative determination at a certain low level is feasible). Performance is considered 'unsatisfactory'.

-3 ≤ proxy-Z < -2   possible false negative. Performance is considered 'questionable'.

2 < proxy-Z ≤ 3   the LOQ is relatively high in relation to HBM4EU analysis and compared to other laboratories. Performance is considered 'questionable'.

-2 ≤ proxy-Z ≤ 2   LOQ is within an acceptable range relative to the assigned value, adequate for HBM4EU analysis, and in line with the LOQs of the majority of the participating laboratories. Performance is considered 'satisfactory'.

6 Results and discussion

6.1 Results submitted by participants

In total six laboratories including three expert labs agreed to participate in this study and five of them submitted results.

The scope of OPFR biomarkers measured by the laboratories varied substantially: from two to all four target compounds. All participants reported results for DPHP and BDCIPP. Worth to notice, that only one lab is able to reported results for BCEP. The provided LOQs were comparable between participants (**Table 3**).

Table 3: Scope and LOQs (ng/ml) as provided in the method information submitted by the laboratories

Lab code	DPHP	BDCIPP	BCEP	BCIPP	Total
PT2OPFR01	0.05	0.02	NA	0.40	3
PT2OPFR03	0.03	0.09	NA	0.30	3
PT2OPFR04	0.15	0.1	0.10	0.20	4
PT2OPFR05	0.1	0.5	NA	NA	2
PT2OPFR07	0.3	0.01	NA	0.2	3
Total	5	5	1	4	

The overview of all results reported by both expert and candidate laboratories is in the **Table 4**. Except one laboratory (PT2OPFR03) the provided results were comparable.

Table 4: The comparison of results reported by participating laboratories

	DPHP level 1	DPHP level 2	BDCIPP level 1	BDCIPP level 2	BCIPP level 1	BCIPP level 2	BCEP level 1	BCEP level 2
No. of candidates + experts	3+2	3+2	3+2	3+2	2+2	2+2	0+1	0+1
No. of quantitative results	5	5	5	5	4	4	1	1
Study RSD$_R$ for all results (%)	81	87	72	75	57	53	NC	NC
Study RSD$_R$ without outliers (%)	19	18	17	7	8	17	NC	NC
	Results (mean of 6 results reported by experts, 1 result for candidates)							
PT2OPFR03 - expert	11.907	40.237	11.500	41.150	16.742	61.882	NA	NA
PT2OPFR04 - expert	3.428	6.102	3.750	10.207	5.055	17.511	2.805	15.701
PT2OPFR05 - expert	2.259	8.497	3.112	9.252	NA	NA	NA	NA
PT2OPFR01	3.050	10.280	2.930	10.390	6.120	24.850	NA	NA
PT2OPFR07	2.252	8.477	2.325	11.205	5.937	26.339	NA	NA

6.2 Assigned values and (target) standard deviations

Using the individual means of the expert values, the mean of the means was calculated and its relative uncertainty. The mean of the means can be as assigned value used when the relative uncertainty is below 0.7*σT. This condition was not met in case of DPHP and BDCIPP. Using the Grubbs´test, results from one expert lab (PT2OPFR03) were identified as outliers. However, the

number of remaining individual expert means was lower than three, which means that no expert values could be obtained.

Calculation of the consensus value derived from the combined results from both participants and expert laboratories was also not possible, because the number of lab´s results needed for the robust statistic was lower than seven.

6.3 Assessment of laboratory performance

The assessment of laboratory performance was not possible because no assigned values needed for the Z-score calculation were available.

6.4 Conclusions and recommendations

In this HBM4EU 2nd ICI/EQUAS on OPFR biomarkers in urine, 17 laboratories were invited of which five submitted results. The overall participation rate was lower than 30%. Three test materials were provided to each participant ("blank" material, spiked material at low level and spiked material on high level).

Quantitative performance could not be assessed for all four biomarkers due to the low number of results from both expert laboratories and candidates. Nevertheless, four labs provided results, which were in a good agreement.

Firstly, as recommended in the 1st ICI/EQUAS it is necessary to encourage more laboratories to participate within a next round, invite the same laboratories (which have not participate within the Round 1 and Round 2) and expand the current group of all possible candidates including expert laboratories.

7 References

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

HBM4EU-SOP-QA-002 "Preparation of control materials for Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

HBM4EU-SOP-QA-003 "Evaluation of results from Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

HBM4EU-SOP-QA-004 "Reporting of results of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)"

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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.

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Appendix 1: Homogeneity data

	BCPP - level 1		BCEP - level 1		DPP - level 1		BDCPP - level 1	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	5.654	5.147	3.195	3.020	3.220	3.175	3.840	3.580
2	5.736	4.822	3.488	3.548	3.318	3.264	3.600	3.710
3	4.467	5.469	3.588	3.685	3.145	2.981	3.850	4.010
4	3.513	4.941	3.624	3.702	2.985	3.292	3.930	3.970
5	5.072	4.357	3.738	3.557	3.148	3.131	3.650	3.710
6	4.494	5.409	3.553	3.712	3.046	3.101	4.100	3.830
7	5.777	4.508	3.510	3.752	3.130	3.388	3.750	3.540
8	5.543	4.268	3.289	3.574	3.127	3.250	3.740	3.820
9	4.343	4.860	3.390	3.028	2.943	2.935	3.150	**3.580**
10	6.260	5.868	3.122	3.125	2.957	2.911	3.290	3.190
Grand mean	5.025		3.460		3.122		3.692	
Cochran´s test								
C	0.2229		0.3461		0.4425		0.4312	
C crit	0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
σ_T	1.1056		0.7612		0.6869		0.8122	
s_x	0.4983		0.2289		0.1214		0.2405	
s_w	0.6761		0.1377		0.1034		0.1464	
s_s	0.1405		0.2071		0.0969		0.2171	
c crit	0.3317		0.2284		0.2061		0.2437	
Ss < c	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited	

	BCPP - level 2		BCEP - level 2		DPP - level 2		BDCPP - level 2	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	22.001	18.859	19.722	19.360	7.252	6.659	8.997	7.496
2	17.779	17.867	19.834	19.517	6.639	7.273	8.265	8.744
3	19.883	22.459	19.323	19.537	7.048	6.818	8.302	8.787
4	17.940	19.624	19.954	18.857	7.389	7.180	8.785	8.180
5	18.665	21.165	19.390	19.178	6.963	6.393	9.191	9.185
6	19.612	20.735	19.825	19.585	6.930	7.554	8.860	8.399
7	19.982	18.788	18.938	19.073	5.929	5.856	8.381	8.152
8	20.627	17.879	19.672	19.299	6.410	6.885	7.106	7.692
9	19.771	19.971	19.275	19.363	6.532	6.816	9.617	9.203
10	16.647	19.019	19.629	19.835	6.771	7.387	8.680	9.364
Grand mean	19.464		19.458		6.834		8.569	
Cochran´s test								
C	0.2379		0.6718		0.1778		0.5205	
C crit	0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
σ_T	4.2820		4.2808		1.5035		1.8852	
s_x	1.0847		0.2233		0.3999		0.5653	
s_w	1.4407		0.2992		0.3360		0.4655	
s_s	0.3724		0.0715		0.3217		0.4597	
c crit	1.2846		1.2842		0.4511		0.5656	
Ss < c	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited	

Appendix 2: Stability data

Biomarker	BCPP level 1		BCPP level 2		BCEP level 1		BCEP level 2	
time (days)	0	40	0	40	0	40	0	40
	5.654	6.240	18.859	20.290	2.949	2.510	19.517	14.610
	5.736	5.730	20.735	20.660	2.820	2.860	19.323	14.540
	5.777	5.900	19.982	19.120	3.089	2.170	18.857	16.040
	5.543	5.570	19.771	18.540	2.928	2.610	19.178	16.660
	6.260	5.890	19.971	21.430	2.922	2.860	18.938	15.600
	5.868	5.670	19.019	19.570	2.925	2.960	19.073	15.800
Average	5.806	5.833	19.723	19.935	2.939	2.662	19.148	15.542
Std dev	0.248	0.237	0.693	1.061	0.087	0.295	0.246	0.830
x0-xa (difference)	-0.027		-0.212		0.277		3.606	
Test 'consequential instability':								
σH	1.28		4.34		0.65		4.21	
0,3*σH	0.38		1.30		0.19		1.26	
x0-xa<0,3*σH? (consequential instability)	NO		NO		YES		YES	
Test 'significant difference':								
t	0.19		0.41		2.21		10.21	
t-crit	2.23		2.23		2.23		2.23	
Significant difference	NO		NO		NO		YES*	

Biomarker	BDCPP level 1		BDCPP level 2		DPP level 1		DPP level 2	
time (days)	0	40	0	40	0	40	0	40
	3.840	3.740	9.997	9.470	3.220	3.480	6.639	6.910
	4.010	4.350	9.191	10.160	3.318	2.980	6.393	5.440
	3.930	4.180	9.185	10.010	3.264	4.570	5.929	6.080
	3.970	4.130	9.617	9.920	3.292	3.150	5.856	6.710
	4.100	3.820	9.203	9.610	3.388	2.590	6.410	5.310
	3.580	4.110	9.364	9.660	3.250	3.570	6.532	5.610
Average	3.905	4.055	9.426	9.805	3.289	3.390	6.293	6.010
Std dev	0.181	0.231	0.325	0.266	0.059	0.678	0.324	0.675
x0-xa (difference)	-0.150		-0.379		-0.101		0.283	
Test 'consequential instability':								
σH	0.86		2.07		0.72		1.38	
0,3*σH	0.26		0.62		0.22		0.42	
x0-xa<0,3*σH? (consequential instability)	NO		NO		NO		NO	
Test 'significant difference':								
t	1.25		2.21		0.36		0.93	
t-crit	2.23		2.23		2.23		2.23	
Significant difference	NO		NO		NO		NO	

* the difference between results is within the day-to-day precision of the analytical procedure, so it can be concluded as no indication of instability

Appendix 3: Copy of letter of invitation

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Registration

For registration please find attached a registration form for parameter OPFR in urine. Please send it back to us by mail in case you want to register.
Upon registration, the participant will receive a lab-code to be used for submission of results.

Fee

For partners and linked-third parties of HBM4EU, participation is free of charge. Please note that the participant is responsible for custom clearance and associated costs if applicable.

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:

Dr. Darina Lankova
University of Chemistry and Technology, Prague
Department of Food Analysis and Nutrition
Technicka 3
166 28 Prague 6
Czech Republic

Email: darina.lankova@vscht.cz
Phone: 00420 22044 4312

HBM4EU | science and policy for a healthy future

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study OPFR/Round 2

Title of ICI/EQUAS: OPFR in urine

Dear Colleague,

within the frame of HBM4EU,

Prof. Dr. Jana Hajslova & Dr. Darina Lankova

University of Chemistry and Technology, Prague
Department of Food Analysis and Nutrition
Technicka 3
166 28 Prague 6
Czech Republic

announce the 2nd round of ICI/EQUAS for the determination of OPFR in urine.
The aim of ICI/EQUAS exercises is to provide laboratories with an assessment of their analytical performance and reliability of their data in comparison with other laboratories and/or expert laboratories. This will aid in the quality improvement of analysis in human biomonitoring at each of the laboratories.

Participation is mandatory for laboratories analysing samples in the frame of HBM4EU.
Participants meet the quality criteria of the HBM4EU call if they pass 3 of ICI/EQUAS rounds successfully.

Test samples

The matrix will be human urine. The participants will receive:
-    3 different materials of urine (5 ml each) for determination of OPFR in urine

Target biomarkers

Please see registration form for OPFR/Round 2 for the biomarkers potentially present in the test samples. We would be pleased if your laboratory could participate with the analysis of as most as possible OPFR.
LOQs should allow the analysis of OPFR in samples of the general population.

Calendar:

Deadline registration	19-11-2018
Distribution of test samples	20-11-2018 (projected)
Report	19-12-2018

Appendix 4: Copy of registration form for participation

HBM4EU science and policy for a healthy future

Address for delivery of the test samples

name institution

address of the laboratory

The above laboratory will participate in the ICI/EQUAS study **OPFR/Round 2**.
I agree with the conditions mentioned in the invitation letter, and that the laboratory will analyse the ICI/EQUAS samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU, and submit results before the indicated deadline.

Name: Signature

Date:

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After signing this form, please scan and send the pdf to:

darina.lankova@vscht.cz

Contact information organiser:
Dr. Darina Lankova
University of Chemistry and Technology, Prague
Department of Food Analysis and Nutrition
Technicka 3
166 28 Prague 6
Czech Republic

Email: darina.lankova@vscht.cz
Phone: 00420 22044 4312

HBM4EU science and policy for a healthy future

HBM4EU: Registration form for participation in ICI / EQUAS study OPFR/Round 2.

Title of ICI/EQUAS: OPFR in urine

Please choose the OPFR you want to participate with.
We would appreciate your registration for as much biomarkers as possible.

Parameter	Participation	
	Yes	No
DPHP		
BDCIPP		
BCEP		
BCIPP		
Creatinine determination		

Participating laboratory:

name 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

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

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Please send the filled in result table together with this signed result submission form back to:

darina.lankova@vscht.cz

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

Darina Lankova
Email: darina.lankova@vscht.cz
Phone: 00420 22044 4312

Jana Hajslova
Email: jana.hajslova@vscht.cz
Phone 0042060283424

Contact information organiser:
Dr. Darina Lankova
University of Chemistry and Technology, Prague
Department of Food Analysis and Nutrition
Technicka 3
166 28 Prague 6
Czech Republic

Prof. Ing. Jana Hajslova, CSc.
(for the ICI/EQUAS organisers)

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HBM4EU: Result submission form EQUAS study OPFR/ Round 2.

Title of EQUAS: OPFR in urine

Laboratory name & code:

Contact person:

Institute:

Address:

Country:

For each of the analytes from this ICI/EQUAS round:
- provide the LOQ
- report NA for not analysed/not included in the scope of the method used
- report ND when not detected or detected below LOQ
- report a numerical value when found above LOQ, express to three significant figures (e.g. 0.543)

To be reported voluntarily (for standardization)

Urine determination			
Creatinine (g/L)			
Osmolality (mOsm/kg)			
Specific gravity (g/L)			

Date:

Signature:

Appendix 6: Copy of acknowledgement of receipt sent together with test samples

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

Darina Lankova
Email: darina.lankova@vscht.cz
Phone: 00420 22044 4312

Jana Hajslova
Email: jana.hajslova@vscht.cz
Phone 00420 602 833 424

Contact information organiser:
Dr. Darina Lankova
University of Chemistry and Technology, Prague
Department of Food Analysis and Nutrition
Technicka 3
166 28 Prague 6
Czech Republic

Prof. Ing. Jana Hajslova, CSc.
(for the ICI/EQUAS organisers)

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HBM4EU: Acknowledgement of receipt form ICI/EQUAS study OPFR/Round 2.

Title of ICI/EQUAS: OPFR in serum

Laboratory name:

Contact person:

Contents of parcel:
- 3 tubes with 5 ml urine

Please verify that the items listed below have been received and provide the information requested below.

Date of receipt (dd-mm-yyyy):

Code on tube	Damage/leakage	Remarks

Name:

Signature:

Date:

After signing this form, please scan and send the pdf. to: darina.lankova@vscht.cz

Appendix 7: Copy of method information form for participation in ICI/EQUAS

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Method information form for participation in ICI/EQUAS OPFR in serum
OPFR/Round 2:

Laboratory name & code	
ISO17025 accredited	yes / no
SAMPLE PREPARATION	
amount sample extracted	g or ml
Deconjugation	yes / no
chemical (reagent / pH / temp / time)	
enzymatic (enzyme / pH / temp / time)	
Extraction	
pH adjustment	
LLE (solvent(s) / time / shaking)	
SPE (material)	
Cleanup	
LLE (solvent(s))	
SPE (material)	
Derivatisation	
reagent	
INSTRUMENTAL ANALYSIS	
HPLC	
injection volume	µl
column stationary phase	
column L (mm) x ID (mm); dp (µm)	
temperature	
mobile phase A	
mobile phase B	
flow rate	ml/min
GC	
injector (splitless/PTV/…)	
injection volume	
column stationary phase	
column L (m) x ID (mm) df (µm)	
carrier	
flow rate / inlet pressure	
Detection	
MS (single quad/triple quad/Q-Orbitrap/Q-TOF)	
other	

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Quantification		
Use of internal standard (IS)		
isotopic label yes/no	yes / no	
other	specify	
moment of addition (e.g. before deconjugation, to final extract…)		
response normalised to IS	yes / no	
Calibration	isotope dilution (addition to sample before extraction)	
	isotope dilution (addition to final extract)	
	standard addition (addition to sample before extraction)	
	standard addition (addition to final extract)	
	matrix-matched (addition to blank matrix before extraction)	
	matrix-matched (addition to blank extract)	
	solvent standards	
	single level / multi level	
Correction for recovery	yes / no	
Identification criteria used		
retention time tolerance (min or % deviation from reference standard)		
number of ions/transitions		
ion ratio tolerance (% relative/absolute deviation from reference standard)		

Further remarks/observations:

Date:

Signature: