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## REPORT OF THE WP9 EQUAS

**Round 04/2019**

**PAH metabolites in urine**

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## Table of contents

Table of contents .....	2
1 Summary .....	3
2 Introduction .....	6
2.1 Confidentiality .....	7
3 Control material .....	8
3.1 Preparation of control material .....	8
3.2 Homogeneity of control material .....	8
3.3 Stability of control material .....	8
4 Organisational details .....	9
4.1 Participants .....	9
4.2 Dispatch and instructions .....	9
5 Data evaluation .....	10
5.1 False positives and <LOQ .....	10
5.2 Assigned value .....	10
5.3 Target standard deviation ( $\sigma_T$ ) .....	11
5.4 ICI/EQUAS standard deviation .....	11
5.5 Z-scores .....	11
5.6 Proxy-Z-scores .....	12
6 Results and discussion .....	13
6.1 Results submitted by participants .....	13
6.2 Assigned values and (target) standard deviations .....	13
6.3 Assessment of laboratory performance .....	14
6.4 Conclusions and recommendations .....	14
7 References .....	27

## Appendices

1 Homogeneity data .....	28
2 Stability data .....	34
3 Copy of letter of invitation .....	40
4 Copy of letter/instructions sent together with test samples .....	42
5 Method information .....	43
6 Assigned values and participant`s performance .....	44
7 Graphical representation of Z-scores .....	55
8 Results of the shipped test samples analysed by the participants .....	73

# 1 Summary

Within the framework of the HBM4EU project, an External Quality Assurance Scheme (EQUAS) was organized and conducted for the analysis of **Polycyclic Aromatic Hydrocarbon metabolites** (PAH met.). PAH met. correspond to eleven biomarkers: 1-naphthol, 2-naphthol, 2-FLUO, 3-FLUO, 9-FLUO, 1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN, 9-PHEN and 1-PYR in urine.

The study was performed from October 2019 to November 2019.

In total, 40 laboratories were invited for this 3<sup>rd</sup> EQUAS/round 4, of which 22 laboratories from ten countries registered.

The participation in this EQUAS was satisfactory; 20 out of the registered 22 laboratories (91%) submitted their results.

In October 2019, twelve different test samples consisting of 5 mL urine spiked with PAH met. at two different concentrations (PAH met.<sub>low</sub> and PAH met.<sub>high</sub>), six of each concentration, were prepared and sent (on dry ice) to the participating expert laboratories for single analysis. Each candidate laboratory got two samples, one of each concentration for single analysis.

Five expert laboratories participated in this round for PAH met. in urine. Four expert laboratories were from Europe (HBM4EU consortium) and also participated as candidates. One expert laboratory was from outside Europe (USA).

Homogeneity assessment of the control materials confirmed that the materials were adequately homogeneous (except for 9-PHEN low level). The stability test demonstrated no significant loss of the biomarkers in the course of this EQUAS.

The proficiency of the laboratories was assessed through Z-scores, which were calculated using the mean concentration as established by expert laboratories as assigned value, and a fixed fit-for-purpose relative target standard deviation (FFP-RSD<sub>R</sub>) of 25%. Assigned values and Z-scores could be determined for nine biomarkers at the low level and for nine biomarkers at the high level.

**Table 1** below gives an overview of the respective results for the low and the high level of all PAH met. biomarkers.

For two biomarkers (2-PHEN and 4-PHEN), Z-scores could only be determined using the consensus value of the ICI as described in SOP HBM4EU-SOP-QA-003.

The percentage of satisfactory Z-scores obtained for each biomarker ranges from 50% (2-FLUO) to 100% (2-PHEN<sub>low</sub>, 3-PHEN and 4-PHEN).

For two biomarkers (9-FLUO, 9-PHEN) no kind of Z-score could be provided.

**Table 2** shows the results of each participating laboratory for each biomarker and also reflects how many PAH met. biomarkers have been analysed per participant (sum) and how many participants have analysed the low and the high level of the respective biomarkers (TOTAL).

**Table 1 Overview of results for PAH met. in urine in 3<sup>rd</sup> EQUAS/round 4**

number of laboratories with respective results for PAH met. in urine in 3 <sup>rd</sup> EQUAS/round 4								
biomarker	participants (+ experts)	quantitative results	evaluation scheme	assigned/ consensus value	satisfactory	questionable	unsatisfactory	proxy-Z- score
1-naphthol low	15 (+2)	15	EQUAS	2.773 ng/mL	12 (80%)	2 (13%)	1 (7%)	0
1-naphthol high	15 (+2)	15	EQUAS	10.623 ng/mL	14 (93%)	1 (7%)	0	0
2-naphthol low	14 (+2)	14	EQUAS	5.339 ng/mL	12 (86%)	1 (7%)	1 (7%)	0
2-naphthol high	14 (+2)	14	EQUAS	9.899 ng/mL	13 (93%)	1 (7%)	0	0
2-FLUO low	8 (+2)	8	EQUAS	0.503 ng/mL	4 (50%)	2 (25%)	2 (25%)	0
2-FLUO high	8 (+2)	8	EQUAS	0.995 ng/mL	4 (50%)	2 (25%)	2 (25%)	0
3-FLUO low	4 (+2)	3	EQUAS	0.170 ng/mL	2 (67%)	0	1 (33%)	1
3-FLUO high	4 (+2)	4	EQUAS	0.401 ng/mL	3 (75%)	0	1 (25%)	0
9-FLUO low	2 (+1)	2	none	-	no Z-scores	no Z-scores	no Z-scores	0
9-FLUO high	2 (+1)	2	none	-	no Z-scores	no Z-scores	no Z-scores	0
1-PHEN low	8 (+2)	8	EQUAS	0.230 ng/mL	6 (75%)	2 (25%)	0	0
1-PHEN high	8 (+2)	8	EQUAS	0.414 ng/mL	7 (87.5%)	1 (12.5%)	0	0
2-PHEN low	9 (+1)	9	ICI	0.125 ng/mL	9 (100%)	0	0	0
2-PHEN high	9 (+1)	9	ICI	0.274 ng/mL	8 (89%)	0	1 (11%)	0
3-PHEN low	9 (+1)	8	EQUAS	0.188 ng/mL	8 (100%)	0	0	1
3-PHEN high	9 (+1)	8	EQUAS	0.353 ng/mL	8 (100%)	0	0	1
4-PHEN low	8 (+1)	7	ICI	0.091 ng/mL	7 (100%)	0	0	1
4-PHEN high	8 (+1)	8	ICI	0.193 ng/mL	8 (100%)	0	0	0
9-PHEN low	4 (+1)	4	none	-	no Z-scores	no Z-scores	no Z-scores	0
9-PHEN high	4 (+1)	4	none	-	no Z-scores	no Z-scores	no Z-scores	0
1-PYR low	20 (+1)	19	EQUAS	0.113 ng/mL	13 (68%)	4 (21%)	2 (11%)	1
1-PYR high	20 (+2)	19	EQUAS	0.253 ng/mL	17 (90%)	1 (5%)	1 (5%)	1

**Table 2 Results of each participating laboratory (Sum and TOTAL = satisfactory results)**

+ = satisfactory, ° = questionable, - = unsatisfactory, NA = not analysed, *NA* = sum of isomers, ND = not detected, no = no Z-score available

1-NAPH			2-NAPH		2-FLUO		3-FLUO		9-FLUO		1-PHEN		2-PHEN		3-PHEN		4-PHEN		9-PHEN		1-PYR		Sum
Lab	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	low	high	
QR/113	+	+	+	+	+	+	+	+	no	no	+	+	+	+	+	+	+	+	NA	NA	+	+	18
QR/118	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	°	+	5
QR/122	+	+	+	+	-	-	NA	NA	NA	NA	+	+	+	+	+	+	+	+	no	no	+	+	14
QR/123	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	2
QR/124	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	6
QR/126	+	+	+	+	°	°	NA	NA	NA	NA	+	+	+	+	+	+	+	+	no	no	+	+	14
QR/127	+	+	+	+	+	+	ND	+	NA	NA	+	+	+	+	+	+	+	+	no	no	+	+	17
QR/128	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	°	+	5
QR/129	+	°	+	+	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+	NA	NA	+	+	17
QR/130	NA	NA	NA	NA	°	°	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-	0
QR/140	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND	4
QR/141	+	+	+	+	NA	NA	NA	NA	NA	NA	°	°	+	+	+	+	+	+	NA	NA	+	+	12
QR/143	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	2
QR/201	-	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	°	+	4
QR/202	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	2
QR/207	°	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	3
QR/210	+	+	°	+	+	+	NA	NA	NA	NA	°	+	+	+	+	+	+	NA	NA	NA	+	+	12
QR/217	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	2
QR/219	+	+	+	+	-	-	-	-	no	no	+	+	+	+	ND	ND	+	+	ND	no	°	+	11
QR/221	°	+	-	-	NA	NA	NA	NA	NA	NA	NA	NA	+	-	+	+	ND	+	NA	NA	-	°	5
<b>TOTAL</b>	<b>12</b>	<b>14</b>	<b>12</b>	<b>13</b>	<b>4</b>	<b>4</b>	<b>2</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>7</b>	<b>9</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>7</b>	<b>8</b>	<b>0</b>	<b>0</b>	<b>13</b>	<b>17</b>	

## 2 Introduction

Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to assess the proficiency of laboratories, 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 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 on the HBM4EU website. Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the 3<sup>rd</sup> EQUAS/round 4 for PAH met. 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 PAH met. biomarkers was previously done by WP9 and was described in Deliverable report 9.2 v1.1. Based on this and according to the results of the two previous ICI/EQUAS rounds, a set of eleven target biomarkers was compiled to be included in this 3<sup>rd</sup> EQUAS/round 4 (see **Table 3**). For 1.2-DHN and 3-BaP, the number of experts and participants was too small in the two previous ICI/EQUAS rounds, so that it was decided by the QAU to exclude these biomarkers from further proficiency tests.

**Table 3 PAH met. biomarkers in urine included in this 3<sup>rd</sup> EQUAS/round 4**

Abbreviation	Target biomarker
1-naphthol	1-hydroxynaphthalene
2-naphthol	2-hydroxynaphthalene
2-FLUO	2-hydroxyfluorene
3-FLUO	3-hydroxyfluorene
9-FLUO	9-hydroxyfluorene
1-PHEN	1-hydroxyphenanthrene
2-PHEN	2-hydroxyphenanthrene
3-PHEN	3-hydroxyphenanthrene
4-PHEN	4-hydroxyphenanthrene
9-PHEN	9-hydroxyphenanthrene
1-PYR	1-hydroxypyrene

For this 3<sup>rd</sup> EQUAS/round 4, expert laboratories were selected according to the selection criteria described in HBM4EU-SOP-QA-001 and in agreement with the QAU.

The selection criteria included:

- Number of years of experience with the biomarker/matrix combination of interest.
- Application of highly sensitive and selective analytical techniques for the analysis.
- Application of isotope-labelled standards for quantification.
- Availability of in-house validation reports, data on ongoing intra-laboratory performance, ISO17025 accreditation for the biomarker of interest.
- Success rate in inter-laboratory comparisons, external quality assessment schemes or at least comparative results in application studies.

WP9 EQUAS Report, Round 04/2019	Version: 1	Date: 20-12-2019	Page: 7
PAH met. in urine, Round 4			

EQUAS does not require a certain number of participants because the performance evaluation is not based on the participants' results but on assigned values and tolerance ranges as derived from the analysis data from the expert laboratories.

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). The expert-assigned values were calculated by averaging the values obtained by the expert labs.

## 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, partly burdened material was used. It consists of human urine (from smokers) with the addition of sodium azide. The samples are clear and free of precipitates as much as possible (filtration). The eleven different stock solutions (1-naphthol, 2-naphthol, 2-FLUO, 3-FLUO, 9-FLUO, 1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN, 9-PHEN and 1-PYR) were diluted into two different concentrations and the addition to the native control material resulted in the intended concentration in control material (PAH met.<sub>low</sub>, PAH met.<sub>high</sub>). The two spiked control materials were aliquoted (5 mL each) into vials with caps (82 x 13 mm, polypropylene, Sarstedt). The tubes were stored in a freezer ( $\leq -18$  °C) until transportation. The two different concentrations (PAH met.<sub>low</sub>, PAH met.<sub>high</sub>) were measured by IPASUM using the analytical method mentioned in **Appendix 5**. The measured concentrations are shown in 3.2 and 3.3 of this report.

### 3.2 Homogeneity of control material

Six tubes of each concentration (deviation from EQUAS SOP due to pursuit of schedule) of the control material (PAH met.<sub>low</sub>, PAH met.<sub>high</sub>) were randomly selected from the freezer ( $\leq -18$  °C). The thawed samples were re-homogenised by vortex shaking and analysed in duplicate by IPASUM (analysis method see **Appendix 5**).

The homogeneity was evaluated according to ISO 13528:2015, Fearn et al [2001] and Thompson [2000] as well as HBM4EU-SOP-QA-002. The results are presented in **Appendix 1**. The conclusion is that no outliers are detected, homogeneity is adequate and the method is suitable (except for 2-PHEN at low and high level, 9-PHEN at low level and 1-PYR at low level).

### 3.3 Stability of control material

Stability testing was done according to HBM4EU-SOP-QA-002. On the day of preparation of the control materials, randomly selected test samples of PAH met.<sub>low</sub> and PAH met.<sub>high</sub> were stored at -80 °C. The assumption is that under these conditions, the biomarkers are stable in urine. On the last day of the deadline for submission of analysis results by the participants (November 19, 2019), six test samples of both materials stored at -80 °C, and six samples of PAH met.<sub>low</sub> and PAH met.<sub>high</sub> randomly selected from the -18 °C freezer were thawed and re-homogenised by vortex shaking. Next, all 24 samples were analysed by IPASUM using the analytical method mentioned 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 3<sup>rd</sup> EQUAS/round 4, IPASUM conducted a survey to find expert laboratories for PAH metabolites in urine willing to participate in the project. Then, IPASUM evaluated their eligibility and selected five expert laboratories in agreement with the QAU and according to HBM4EU-SOP-QA-001. Four expert laboratories were from Europe (HBM4EU consortium) and also participated as candidates. One expert laboratory was from outside Europe (USA).

A list of 40 candidate laboratories from different countries eligible for the analysis of PAH met. had been compiled by the Work Package (WP) Task 9.2 leaders and made available to the institution organising the respective EQUAS.

Invitation letters were sent by e-mail to all 40 candidate laboratories on October 7, 2019 (see **Appendix 3**). It was indicated that participation would be free of charge and that those who subscribed to the EQUAS would receive a kit containing the test materials needed for analysis. The condition for participation was that the test results had to be submitted within the stipulated deadline (November 19, 2019).

Twenty-two laboratories from ten countries out of the 40 laboratories (55%) in the revised candidate list indicated their interest in participating in this EQUAS and sent their registration form to IPASUM, with their agreement to abide by the conditions for participation. These laboratories received an individual laboratory code to report their measurement results.

20 of the 22 potential participants (91%) from ten countries performed the assays and submitted their results.

Nineteen participants reported their results within the stipulated deadline (November 19, 2019), while one participant reported with a delay (see **Appendix 8**; QR/221 on November 23, 2019).

### 4.2 Dispatch and instructions

Test materials were dispatched on dry ice to the participants on October 22, 2019. Each participant received two test samples spiked with the biomarker at two levels, one of each concentration. Each sample consisted of approximately 5 mL urine.

Moreover, a letter with instructions on sample handling (instruction letter, see **Appendix 4**), a sample receipt form to be sent back to IPASUM upon receipt of the test material as well as a result submission form and a method information form (see **Appendix 5**) 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 HBM4EU and to report results following the instructions given.

## 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, the expert laboratories, 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 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 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. The LOQ is considered not adequate (too high) if:

- 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) quantitative determination is feasible by the majority of laboratories

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

### 5.2 Assigned value

For EQUAS studies, the concentration established by expert laboratories is used as the assigned value as described in SOP HBM4EU-SOP-QA-001.

The HBM4EU QAU selected five expert laboratories for PAH met. in urine. Four expert laboratories were from Europe (HBM4EU consortium) and also participated as candidates. One expert laboratory was from outside Europe (USA). Some of the expert laboratories did not cover all eleven biomarkers, but this was not an exclusion criterion. The expert laboratories received the same control material and instructions as the participants. Each expert laboratory analysed each of the six control samples per level (PAH met.<sub>low</sub>, PAH met.<sub>high</sub>) as single analysis. Upon receipt of their results and method information, the acceptability of the results for establishment of the expert value was verified. The expert value was determined as described in HBM4EU-QA-001.

The individual means and standard deviations for each expert laboratory were calculated. Then, the mean of all individual means of the five expert laboratories (mean-of-means, mom) and the standard deviation of the mom (SD<sub>mom</sub>) were calculated. The relative uncertainty of the mean of the means is given by:

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

with  $u$  = relative uncertainty of the mean of the mean concentrations from the expert labs

RSD = relative standard deviation of the mean of the mean concentrations

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

The mean-of-means derived from the expert laboratories is considered suitable for use as assigned value in EQUAS studies if  $u \leq 0.7 \cdot \sigma_T$  ( $\sigma_T = 25\%$  as described in 5.3).

If  $u > 0.7 \cdot \sigma_T$ , the individual means are checked for outliers. When an individual expert mean is identified as an outlier, it is rejected from the data set and the relative uncertainty is recalculated. If the condition  $u \leq 0.7 \cdot \sigma_T$  is still not met, then the uncertainty of the expert-derived mean is too high to be used as assigned value. In this case, no EQUAS assessment of participants' performance is possible for the applicable biomarker. This is also the case if the number of (remaining) individual expert means is less than three.

Only if the EQUAS assessment of the participants' performance is not possible, is the consensus value of the ICI used as assigned value and calculated as described in SOP HBM4EU-SOP-QA-003. In brief, the consensus value and its uncertainty were calculated from the results submitted by the participants using robust statistics to minimize the influence of outliers. Two criteria were evaluated before calculating performance's score:

- if the uncertainty was not significant ( $u \leq 0.3 \sigma_T$ ), Z-scores were then calculated, otherwise another kind of performance's scores was calculated taking into account the uncertainty.
- if the uncertainty did not meet the following criteria:  $u \leq 0.7 \sigma_T$ , no kind of score was provided.

### 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 assigned value was used as target standard deviation. This was the default indicated in HBM4EU-SOP-QA-003 and considered appropriate based on the outcome of the 1<sup>st</sup> round.

### 5.4 ICI/EQUAS standard deviation

To gain insight into the actual inter-laboratory variability of the biomarker analysis in this study, the robust relative standard deviation (RSD<sub>R</sub>) was calculated based on the participants' results, as described in HBM4EU-SOP-QA-003. For this, the results of the expert laboratories were not included.

### 5.5 Z-scores

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

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

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

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 4**.

**Table 4: Classification of Z-scores**

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

## 5.6 Proxy-Z-scores

'Proxy-Z-scores' are used 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). If no LOQ was specified, zero was used.

Proxy-Z-scores are indicated in the report as between brackets and are for information. They are not included in the graphical representations of Z-scores of the participants. The interpretation is as follows:

<b>proxy-Z <math>\leq -3</math></b>	based on the LOQ provided, the laboratory should have been able to detect and quantify the biomarker. The result is classified as a false negative (FN) and is interpreted as 'unsatisfactory' performance.
<b><math>-3 \leq \text{proxy-Z} &lt; -2</math></b>	based on the LOQ provided, it is highly likely that the laboratory should have been able to detect and quantify the biomarker. The result is classified as a false negative (FN) and should be interpreted as 'questionable'.
<b><math>-2 \leq \text{proxy-Z} \leq 2</math></b>	<p><b>-2 to 0:</b> based on the assigned value and the LOQ provided, the result cannot be classified as false negative.</p> <p><b>0 to 2:</b> benchmark: the LOQ is in the range of what is analytically feasible*.</p>
<b><math>2 &lt; \text{proxy-Z} &lt; 3</math></b>	benchmark: the LOQ is high compared to what is analytically feasible* and might be high in relation to HBM4EU analysis. The laboratory should consider to lower their LOQ.
<b>proxy-Z <math>\geq 3</math></b>	benchmark: the LOQ is too high compared to what is analytically feasible* and might be too high in relation to HBM4EU analysis. The laboratory should consider to lower their LOQ.

\* the analytical feasibility is derived from the ICI/EQUAS results. When an assigned value can be determined, this means that reliable quantitative determination at a certain low level is feasible.

## 6 Results and discussion

### 6.1 Results submitted by participants

In total, 22 laboratories from ten EU countries agreed to participate in this study. In the end, 20 out of 22 participants submitted their results (91%).

**Appendix 8** gives an overview of results and LOQs submitted by the participants as well as reasons for delayed submission.

#### Results reported as 'not detected' (ND):

For **3-FLUO<sub>low</sub>**, one participant (QR/127) indicated 'not detected' (ND) and the LOQ was used as input for the EQUAS evaluation. A proxy-Z-score could be calculated by using the LOQ value provided by the participant (0.050 ng/mL). Considering this LOQ, it is highly likely that the laboratory should have been able to detect and quantify **3-FLUO<sub>low</sub>**. The result is classified as a false negative (FN) and should be interpreted as 'questionable'.

For **3-PHEN**, one participant (QR/219) indicated ND and the LOQ was used as input for the EQUAS evaluation. A proxy-Z-score could be calculated by using the LOQ value provided by the participant (0.369 ng/mL). For **3-PHEN**, this LOQ might be too high in relation to HBM4EU analysis. The laboratory should consider lowering the LOQ.

For **4-PHEN<sub>low</sub>**, one participant (QR/221) indicated ND and the LOQ was used as input for the EQUAS evaluation. A proxy-Z-score could be calculated by using the LOQ value provided by the participant (0.086 ng/mL).

For **9-PHEN<sub>low</sub>**, one participant (QR/219) indicated ND. However, no proxy Z-score could be calculated, because no EQUAS and no ICI evaluation was possible for **9-PHEN**.

For **1-PYR**, one participant (QR/140) indicated ND and the LOQ (0.200) was used as input for the EQUAS evaluation. Proxy Z-scores could be obtained. The LOQ from QR/140 LOQ might be too high in relation to HBM4EU analysis of **1-PYR**. The laboratory should consider lowering the LOQ.

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

**Reporting sums of isomers:** Some participants reported values for the sum of two isomers instead of a separate value for each isomer. In these cases, the results could not be considered in the evaluation and were treated as 'not analysed' (NA).

**Methods:** Laboratories were asked to provide details on the method used for analysis. Almost all labs performed an enzymatic deconjugation step, mostly using  $\beta$ -glucuronidase/aryl-sulfatase. Most of the participating laboratories analysed the PAH met. biomarkers by using liquid chromatography with mass spectrometric detection. Four laboratories used GC-MS as analytical instrument. The majority of the participants used an isotope dilution (addition to sample before extraction) as calibration followed by matrix-matched (addition to blank matrix before extraction) and solvent standards.

### 6.2 Assigned values and (target) standard deviations

For most of the biomarkers, the results from the expert laboratories were used to calculate the mean-of-means, which was then used as assigned value (**1-naphthol**, **2-naphthol**, **2-FLUO**, **3-FLUO**, **1-PHEN**, **3-PHEN**, **1-PYR**).

For two biomarkers, the consensus value of the ICI was used as assigned value and was calculated as described in SOP HBM4EU-SOP-QA-003 (**2-PHEN** and **4-PHEN**).

For two biomarkers, no criterion (at least three expert labs or at least seven participants) was met to calculate an assigned value (**9-FLUO**, **9-PHEN**).

The assigned (EQUAS)/consensus (ICI) value and its uncertainty, the expert (EQUAS)/robust (ICI) standard deviation, the fit-for-purpose (FFP) relative target standard deviation ( $\sigma_T$ ) and the study  $RSD_R$  for each of the control materials are included in **Appendix 6**.

### 6.3 Assessment of laboratory performance

Twenty laboratories out of 22 registered candidate laboratories reported results. A summary of the number of laboratories with satisfactory, questionable and unsatisfactory Z-scores for the respective levels of each biomarker, the applied evaluation scheme and the number of ND results without proxy-Z-score is given in **Table 1**.

**Table 2** shows the results of each participating laboratory for each biomarker and also reflects how many PAH met. biomarkers have been analysed per participant (sum) and how many participants have analysed the low and the high level of the respective biomarkers (TOTAL).

Z-scores were calculated for the low and the high concentration level of **1-naphthol**, **2-naphthol**, **2-FLUO**, **3-FLUO**, **1-PHEN**, **2-PHEN**, **3-PHEN**, **4-PHEN** and **1-PYR** with graphical representations provided in **Appendix 7**.

In case a laboratory analysed a biomarker and reported '<LOQ value', a proxy-Z-score was calculated. These proxy-Z-scores are indicated in **Appendix 6** as a Z-score between brackets and were not included in the graphs in **Appendix 7**.

The percentage of satisfactory Z-scores obtained for each biomarker ranged from 100% for **2-PHEN<sub>low</sub>**, **3-PHEN** and **4-PHEN** to 50% for **2-FLUO** and was 82% on average for the other biomarkers.

The calculation of expert values or consensus values was not possible for **9-FLUO** and **9-PHEN** due to the limited number of expert labs (smaller than three) and participants (smaller than seven). Thus, in these cases, no Z-scores could be provided.

A direct comparison of the overall performance of the laboratories with that of the previous rounds is not entirely possible, because some laboratories from the previous rounds did not participate in this fourth round. A highly satisfactory performance rate was achieved for **2-PHEN<sub>low</sub>**, **3-PHEN** and **4-PHEN**, while **2-FLUO** was more challenging.

### 6.4 Conclusions and recommendations

The overall participation in the HBM4EU 3<sup>rd</sup> EQUAS/round 4 was successful. Twenty-two laboratories out of the 40 laboratories (55%) in the revised candidate list confirmed their participation in this EQUAS. Twenty of these 22 registered candidate laboratories reported results, representing a participation rate of 91%.

The tables below (**Table 5 to 15**) provide the LOQs and an overview of the performance of the candidate laboratories in this 3<sup>rd</sup> EQUAS/round 4 for PAH met. biomarkers in urine.

Evaluation of laboratory performance was possible for nine biomarkers. The percentage of satisfactory Z-scores for the individual biomarkers was between 50% and 100%.

WP9 EQUAS Report, Round 04/2019	Version: 1	Date: 20-12-2019	Page: 15
PAH met. in urine, Round 4			

In this fourth round, one participating laboratory analysed all eleven biomarkers of PAH met. in urine. The other laboratories measured a number of biomarkers in a scope between one and ten. The number of satisfactory results in this round for all biomarkers was higher compared to the previous round except for **2-FLUO**. In this fourth round as well as in the previous rounds, no evaluation could be established for the biomarkers **9-FLUO** and **9-PHEN**. The reasons were an insufficient number of participating laboratories and no suitable assigned value derived from the expert results.

**Table 5 Performance of the candidate laboratories for 1-naphthol in urine**

Lab.code	LOQ [ng/mL]	1-naphthol <sub>low</sub>	1-naphthol <sub>high</sub>
QR/113	0.015	satisfactory	satisfactory
QR/118	1.400	satisfactory	satisfactory
QR/122	0.100	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	1.000	satisfactory	satisfactory
QR/126	0.025	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128	0.200	satisfactory	satisfactory
QR/129	0.001	satisfactory	questionable
QR/130	-	NA	NA
QR/140	2.000	satisfactory	satisfactory
QR/141	0.500	satisfactory	satisfactory
QR/143	-	NA	NA
QR/201	6.000	unsatisfactory	satisfactory
QR/202	-	NA	NA
QR/207	2.000	questionable	satisfactory
QR/210	0.400	satisfactory	satisfactory
QR/217	-	NA	NA
QR/219	1.340	satisfactory	satisfactory
QR/221	0.806	questionable	satisfactory

NA = not analysed



**Table 6 Performance of the candidate laboratories for 2-naphthol in urine**

<b>Lab.code</b>	<b>LOQ [ng/mL]</b>	<b>2-naphthol<sub>low</sub></b>	<b>2-naphthol<sub>high</sub></b>
QR/113	0.017	satisfactory	satisfactory
QR/118	1.400	satisfactory	satisfactory
QR/122	0.100	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	1.000	satisfactory	satisfactory
QR/126	0.010	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128	0.200	satisfactory	satisfactory
QR/129	0.001	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	0.500	satisfactory	satisfactory
QR/141	0.050	satisfactory	satisfactory
QR/143	-	NA	NA
QR/201	6.000	satisfactory	satisfactory
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.400	questionable	satisfactory
QR/217	-	NA	NA
QR/219	0.013	satisfactory	satisfactory
QR/221	0.511	unsatisfactory	unsatisfactory

NA = not analysed

**Table 7 Performance of the candidate laboratories for 2-FLUO in urine**

<b>Lab.code</b>	<b>LOQ [ng/mL]</b>	<b>2-FLUO<sub>low</sub></b>	<b>2-FLUO<sub>high</sub></b>
QR/113	0.009	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	0.050	unsatisfactory	unsatisfactory
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.010	questionable	questionable
QR/127	0.010	satisfactory	satisfactory
QR/128	-	NA	NA
QR/129	0.003	satisfactory	satisfactory
QR/130	0.020	questionable	questionable
QR/140	-	NA	NA
QR/141	-	NA	NA
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.020	satisfactory	satisfactory
QR/217	-	NA	NA
QR/219	0.013	unsatisfactory	unsatisfactory
QR/221	-	NA	NA

NA = not analysed

**Table 8 Performance of the candidate laboratories for 3-FLUO in urine**

Lab.code	LOQ [ng/mL]	3-FLUO <sub>low</sub>	3-FLUO <sub>high</sub>
QR/113	0.011	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	-	NA	NA
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.050	NA*	NA*
QR/127	0.050	ND	satisfactory
QR/128		NA	NA
QR/129	0.003	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	-	NA	NA
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	-	NA	NA
QR/217	-	NA	NA
QR/219	0.013	unsatisfactory	unsatisfactory
QR/221	-	NA	NA

NA = not analysed; ND = not detected

\* = analysed the sum of 3- and 9-FLUO

**Table 9 Performance of the candidate laboratories for 9-FLUO in urine**

Lab.code	LOQ [ng/mL]	9-FLUO <sub>low</sub>	9-FLUO <sub>high</sub>
QR/113	0.010	no Z-score available	no Z-score available
QR/118	-	NA	NA
QR/122	-	NA	NA
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.050	NA*	NA*
QR/127	-	NA	NA
QR/128		NA	NA
QR/129	-	NA	NA
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	-	NA	NA
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	-	NA	NA
QR/217	-	NA	NA
QR/219	0.013	no Z-score available	no Z-score available
QR/221	-	NA	NA

NA = not analysed; \* = analysed the sum of 3- and 9-FLUO

**Table 10 Performance of the candidate laboratories for 1-PHEN in urine**

Lab.code	LOQ [ng/mL]	1-PHEN <sub>low</sub>	1-PHEN <sub>high</sub>
QR/113	0.003	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	0.010	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.010	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128		NA	NA
QR/129	0.001	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	0.010	questionable	questionable
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.020	questionable	satisfactory
QR/217	-	NA	NA
QR/219	0.013	satisfactory	satisfactory
QR/221	0.174	NA**	NA**

NA = not analysed; \*\* = analysed the sum of 1-Phen and 9-Phen

**Table 11 Performance of the candidate laboratories for 2-PHEN in urine**

Lab.code	LOQ [ng/mL]	2-PHEN <sub>low</sub>	2-PHEN <sub>high</sub>
QR/113	0.004	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	0.010	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.010	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128		NA	NA
QR/129	0.001	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	0.010	satisfactory	satisfactory
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.020	satisfactory	satisfactory
QR/217	-	NA	NA
QR/219	0.013	satisfactory	satisfactory
QR/221	0.084	satisfactory	unsatisfactory

NA = not analysed

**Table 12 Performance of the candidate laboratories for 3-PHEN in urine**

Lab.code	LOQ [ng/mL]	3-PHEN <sub>low</sub>	3-PHEN <sub>high</sub>
QR/113	0.004	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	0.010	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.010	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128		NA	NA
QR/129	0.001	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	0.010	satisfactory	satisfactory
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.020	satisfactory	satisfactory
QR/217	-	NA	NA
QR/219	0.013	ND	ND
QR/221	0.051	satisfactory	satisfactory

NA = not analysed; ND = not detected

**Table 13 Performance of the candidate laboratories for 4-PHEN in urine**

Lab.code	LOQ [ng/mL]	4-PHEN <sub>low</sub>	4-PHEN <sub>high</sub>
QR/113	0.003	satisfactory	satisfactory
QR/118	-	NA	NA
QR/122	0.002	satisfactory	satisfactory
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.010	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128		NA	NA
QR/129	0.003	satisfactory	satisfactory
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	0.010	satisfactory	satisfactory
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.040	NA***	NA***
QR/217	-	NA	NA
QR/219	0.013	satisfactory	satisfactory
QR/221	0.086	ND	satisfactory

NA = not analysed; \*\*\* = analysed the sum of 4-PHEN and 9-PHEN

ND = not detected



**Table 14 Performance of the candidate laboratories for 9-PHEN in urine**

Lab.code	LOQ [ng/mL]	9-PHEN <sub>low</sub>	9-PHEN <sub>high</sub>
QR/113	-	NA	NA
QR/118	-	NA	NA
QR/122	0.010	no Z-score available	no Z-score available
QR/123	-	NA	NA
QR/124	-	NA	NA
QR/126	0.025	no Z-score available	no Z-score available
QR/127	0.010	no Z-score available	no Z-score available
QR/128		NA	NA
QR/129	-	NA	NA
QR/130	-	NA	NA
QR/140	-	NA	NA
QR/141	-	NA	NA
QR/143	-	NA	NA
QR/201	-	NA	NA
QR/202	-	NA	NA
QR/207	-	NA	NA
QR/210	0.040	NA***	NA***
QR/217	-	NA	NA
QR/219	0.025	ND	no Z-score available
QR/221	0.174	NA**	NA**

NA = not analysed; ND = not detected; \*\* = analysed the sum of 1-Phen and 9-Phen

\*\*\* = analysed the sum of 4-PHEN and 9-PHEN

**Table 15 Performance of the candidate laboratories for 1-PYR in urine**

<b>Lab code</b>	<b>LOQ [ng/mL]</b>	<b>1-PYR<sub>low</sub></b>	<b>1-PYR<sub>high</sub></b>
QR/113	0.013	satisfactory	satisfactory
QR/118	0.200	questionable	satisfactory
QR/122	0.020	satisfactory	satisfactory
QR/123	0.010	satisfactory	satisfactory
QR/124	0.020	satisfactory	satisfactory
QR/126	0.025	satisfactory	satisfactory
QR/127	0.010	satisfactory	satisfactory
QR/128	0.100	questionable	satisfactory
QR/129	0.003	satisfactory	satisfactory
QR/130	0.100	unsatisfactory	unsatisfactory
QR/140	0.200	ND	ND
QR/141	0.010	satisfactory	satisfactory
QR/143	0.010	satisfactory	satisfactory
QR/201	0.050	questionable	satisfactory
QR/202	0.020	satisfactory	satisfactory
QR/207	0.200	satisfactory	satisfactory
QR/210	0.050	satisfactory	satisfactory
QR/217	0.010	satisfactory	satisfactory
QR/219	0.050	questionable	satisfactory
QR/221	0.025	unsatisfactory	questionable

ND = not detected

## 7 References

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## Appendix 1. Homogeneity data

	<u>1-naphthol</u>				<u>2-naphthol</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	2.88	2.55	12.93	13.08	6.84	6.91	13.06	13.16
2	2.90	3.20	12.30	13.09	6.80	7.15	12.91	13.25
3	2.93	2.98	13.19	12.93	6.99	7.12	13.19	13.18
4	2.79	2.74	12.14	12.85	6.85	6.94	13.08	13.01
5	2.58	2.65	11.60	11.99	6.54	6.82	12.66	12.78
6	2.71	3.18	11.69	11.70	6.93	6.92	12.37	12.47
7	2.80	2.82	11.78	11.66	6.89	6.87	12.25	12.40
8	2.98	2.88	12.29	12.30	6.68	6.57	12.27	12.30
9	2.61	2.64	12.62	12.40	6.44	6.56	11.82	12.04
10	2.51	2.63	12.14	11.68	6.33	6.40	12.26	11.73
grand mean	2.798		12.318		6.778		12.610	
Cochran's test								
C	0.485		0.379		0.466		0.553	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target $\sigma_{FFP}$ :	0.700		3.080		1.694		3.152	
$s_x$	0.167		0.518		0.224		0.479	
$s_w$	0.151		0.287		0.115		0.159	
$s_s$	0.128		0.477		0.209		0.466	
Critical= $0.3 \sigma_{FFP}$	0.210		0.924		0.508		0.946	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$ ?	Method suited		Method suited		Method suited		Method suited	

## Appendix 1. Homogeneity data (continued)

	<u>2-FLUO</u>				<u>3-FLUO</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.45	0.42	0.87	0.95	0.18	0.16	0.42	0.42
2	0.42	0.43	0.97	1.05	0.15	0.17	0.40	0.41
3	0.43	0.42	0.96	1.17	0.18	0.19	0.41	0.44
4	0.51	0.49	1.05	1.12	0.15	0.15	0.36	0.38
5	0.50	0.47	0.96	1.04	0.16	0.13	0.35	0.36
6	0.51	0.51	1.03	1.15	0.16	0.13	0.36	0.39
7	0.46	0.50	0.93	0.95	0.13	0.15	0.35	0.36
8	0.50	0.47	0.98	1.01	0.15	0.14	0.37	0.38
9	0.48	0.48	1.03	1.04	0.15	0.15	0.35	0.36
10	0.42	0.50	1.06	1.04	0.13	0.15	0.34	0.35
grand mean	0.469		1.018		0.153		0.378	
Cochran's test								
C	0.566		0.523		0.250		0.321	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target $\sigma_{FFP}$ :	0.117		0.255		0.038		0.095	
$s_x$	0.031		0.059		0.015		0.029	
$s_w$	0.024		0.065		0.013		0.012	
$s_s$	0.026		0.038		0.011		0.028	
Critical= $0.3 \sigma_{FFP}$	0.035		0.076		0.011		0.028	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$ ?	Method suited		Method suited		Method suited		Method suited	

## Appendix 1. Homogeneity data (continued)

	<u>9-FLUO</u>				<u>1-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.55	0.56	1.22	1.26	0.24	0.26	0.45	0.44
2	0.54	0.55	1.25	1.27	0.25	0.26	0.44	0.46
3	0.55	0.54	1.23	1.24	0.25	0.26	0.48	0.50
4	0.52	0.53	1.24	1.25	0.27	0.27	0.52	0.53
5	0.51	0.53	1.20	1.21	0.24	0.26	0.50	0.52
6	0.53	0.53	1.19	1.18	0.27	0.29	0.53	0.53
7	0.50	0.53	1.19	1.20	0.26	0.27	0.52	0.55
8	0.50	0.51	1.14	1.20	0.27	0.27	0.50	0.50
9	0.51	0.50	1.18	1.20	0.26	0.28	0.53	0.55
10	0.52	0.50	1.16	1.14	0.27	0.28	0.54	0.50
grand mean	0.526		1.208		0.264		0.505	
Cochran's test								
C	0.391		0.522		0.200		0.372	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target $\sigma_{FFP}$ :	0.131		0.302		0.066		0.126	
$s_x$	0.018		0.036		0.011		0.034	
$s_w$	0.011		0.016		0.010		0.015	
$s_s$	0.016		0.034		0.008		0.032	
Critical= $0.3 \sigma_{FFP}$	0.039		0.091		0.020		0.038	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$ ?	Method suited		Method suited		Method suited		Method suited	

## Appendix 1. Homogeneity data (continued)

	<u>2-PHEN</u>				<u>3-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.05	0.05	0.14	0.14	0.18	0.17	0.31	0.37
2	0.04	0.06	0.13	0.14	0.17	0.17	0.35	0.39
3	0.05	0.04	0.11	0.10	0.16	0.17	0.33	0.36
4	0.04	0.03	0.10	0.10	0.20	0.19	0.41	0.41
5	0.02	0.04	0.13	0.11	0.18	0.21	0.40	0.44
6	0.02	0.03	0.13	0.14	0.20	0.19	0.42	0.43
7	0.03	0.02	0.13	0.14	0.19	0.21	0.39	0.40
8	0.04	0.04	0.13	0.12	0.17	0.18	0.36	0.38
9	0.04	0.04	0.14	0.13	0.17	0.17	0.38	0.38
10	0.04	0.03	0.13	0.12	0.14	0.17	0.36	0.37
grand mean	0.038		0.126		0.180		0.382	
Cochran's test								
C	0.308		0.364		0.333		0.429	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target $\sigma_{FFP}$ :	0.009		0.031		0.045		0.096	
$s_x$	0.009		0.014		0.016		0.030	
$s_w$	0.008		0.007		0.012		0.020	
$s_s$	0.007		0.013		0.013		0.026	
Critical= $0.3 \sigma_{FFP}$	0.003		0.009		0.013		0.029	
$s_s < \text{critical?}$	Homogeneity insufficient		Homogeneity insufficient		Homogeneity adequate		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$ ?	Method not suited		Method suited		Method suited		Method suited	

## Appendix 1. Homogeneity data (continued)

	<u>4-PHEN</u>				<u>9-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.74	0.73	0.89	0.96	0.22	0.23	0.32	0.31
2	0.72	0.76	0.88	1.00	0.25	0.23	0.30	0.31
3	0.78	0.78	0.97	1.01	0.24	0.25	0.33	0.36
4	0.83	0.88	1.01	0.99	0.26	0.27	0.37	0.37
5	0.73	0.85	0.96	0.94	0.25	0.26	0.37	0.38
6	0.86	0.85	0.95	0.97	0.25	0.27	0.36	0.38
7	0.84	0.87	0.92	0.97	0.24	0.25	0.36	0.35
8	0.82	0.77	0.90	0.98	0.23	0.21	0.34	0.32
9	0.78	0.79	0.94	1.00	0.21	0.24	0.31	0.31
10	0.74	0.81	1.00	0.93	0.20	0.21	0.35	0.32
grand mean	0.797		0.959		0.239		0.341	
Cochran's test								
C	0.531		0.365		0.333		0.300	
Ccrit	0.602		0.602		0.602		0.602	
C < Ccrit?	no outliers detected		no outliers detected		Outliers detected		no outliers detected	
target $\sigma_{FFP}$ :	0.199		0.240		0.060		0.085	
$s_x$	0.045		0.023		0.019		0.026	
$s_w$	0.037		0.044		0.012		0.012	
$s_s$	0.037		0.000		0.017		0.025	
Critical= $0.3 \sigma_{FFP}$	0.060		0.072		0.018		0.026	
$s_s < \text{critical?}$	Homogeneity adequate		Homogeneity adequate		Homogeneity insufficient		Homogeneity adequate	
$s_w < 0.5 \sigma_{FFP}$ ?	Method suited		Method suited		Method suited		Method suited	



## Appendix 1. Homogeneity data (continued)

	<b>1-PYR</b>			
	<b>low [ng/mL]</b>		<b>high [ng/mL]</b>	
	<b>replicate 1</b>	<b>replicate 2</b>	<b>replicate 1</b>	<b>replicate 2</b>
<b>1</b>	0.12	0.09	0.29	0.29
<b>2</b>	0.12	0.08	0.24	0.29
<b>3</b>	0.10	0.11	0.23	0.25
<b>4</b>	0.11	0.09	0.28	0.25
<b>5</b>	0.10	0.10	0.25	0.23
<b>6</b>	0.10	0.09	0.24	0.21
<b>7</b>	0.10	0.10	0.23	0.21
<b>8</b>	0.12	0.11	0.27	0.25
<b>9</b>	0.10	0.09	0.26	0.27
<b>10</b>	0.12	0.10	0.24	0.22
<b>grand mean</b>	0.103		0.250	
<b>Cochran`s test</b>				
<b>C</b>	0.432		0.391	
<b>Ccrit</b>	0.602		0.602	
<b>C &lt; Ccrit?</b>	no outliers detected		no outliers detected	
<b>target <math>\sigma_{FFP}</math>:</b>	0.026		0.063	
<b>s<sub>x</sub></b>	0.006		0.022	
<b>s<sub>w</sub></b>	0.014		0.018	
<b>s<sub>s</sub></b>	0.000		0.018	
<b>Critical=0.3 <math>\sigma_{FFP}</math></b>	0.008		0.019	
<b>s<sub>s</sub> &lt; critical?</b>	Homogeneity adequate		Homogeneity adequate	
<b>s<sub>w</sub> &lt; 0.5*<math>\sigma_{FFP}</math>?</b>	Method not suited		Method suited	

## Appendix 2. Stability data

	<u>1-naphthol</u>				<u>2-naphthol</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	2.470	2.460	10.210	10.160	6.060	6.120	10.540	10.480
2	2.420	2.440	10.820	10.740	5.860	5.960	10.960	11.110
3	2.350	2.360	10.600	10.060	5.680	5.940	10.930	10.310
4	2.550	2.560	10.990	11.130	6.130	6.020	11.360	11.420
5	2.480	2.530	10.540	10.940	6.130	6.180	10.630	10.810
6	2.540	2.620	10.750	10.180	6.040	6.220	10.830	10.150
average	2.468	2.495	10.652	10.535	5.983	6.073	10.875	10.713
stdev	0.075	0.093	0.269	0.459	0.179	0.117	0.290	0.490
difference	-0.027		0.117		-0.090		0.162	
critical=0.3 $\sigma_{FFP}$	0.185		0.799		0.449		0.816	
consequential instability	no		no		no		no	
t	0.545		0.537		1.033		0.696	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

## Appendix 2. Stability data (continued)

	<u>2-FLUO</u>				<u>3-FLUO</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	0.420	0.370	0.830	0.870	0.150	0.130	0.350	0.380
2	0.380	0.450	0.950	0.960	0.150	0.160	0.410	0.410
3	0.490	0.460	1.060	0.970	0.170	0.150	0.370	0.340
4	0.520	0.530	0.980	1.130	0.150	0.160	0.360	0.400
5	0.540	0.570	0.960	0.980	0.170	0.140	0.380	0.380
6	0.490	0.480	1.010	0.960	0.160	0.160	0.400	0.370
average	0.473	0.477	0.965	0.978	0.158	0.150	0.378	0.380
stdev	0.061	0.069	0.077	0.084	0.010	0.013	0.023	0.024
difference	-0.003		-0.013		0.008		-0.002	
critical=0.3 $\sigma_{FFP}$	0.036		0.072		0.012		0.028	
consequential instability	no		no		no		no	
t	0.088		0.286		1.274		0.121	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

## Appendix 2. Stability data (continued)

	<u>9-FLUO</u>				<u>1-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	0.520	0.490	1.070	1.070	0.310	0.310	0.520	0.510
2	0.510	0.510	1.140	1.130	0.330	0.320	0.540	0.550
3	0.490	0.480	1.090	1.030	0.300	0.300	0.550	0.500
4	0.520	0.540	1.150	1.180	0.310	0.330	0.550	0.560
5	0.550	0.520	1.090	1.140	0.320	0.330	0.540	0.550
6	0.540	0.540	1.140	1.050	0.340	0.340	0.550	0.520
average	0.522	0.513	1.113	1.100	0.318	0.322	0.542	0.532
stdev	0.021	0.025	0.034	0.059	0.015	0.015	0.012	0.025
difference	0.008		0.013		-0.003		0.010	
critical=0.3 $\sigma_{FFP}$	0.039		0.084		0.024		0.041	
consequential instability	no		no		no		no	
t	0.620		0.482		0.392		0.892	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

## Appendix 2. Stability data (continued)

	<u>2-PHEN</u>				<u>3-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	0.060	0.080	0.150	0.160	0.180	0.190	0.360	0.350
2	0.050	0.050	0.120	0.140	0.200	0.200	0.380	0.360
3	0.070	0.050	0.200	0.160	0.200	0.200	0.360	0.330
4	0.070	0.050	0.120	0.160	0.190	0.200	0.360	0.350
5	0.070	0.080	0.190	0.180	0.230	0.190	0.360	0.370
6	0.050	0.050	0.210	0.210	0.200	0.220	0.390	0.370
average	0.062	0.060	0.165	0.168	0.200	0.200	0.368	0.355
stdev	0.010	0.015	0.040	0.024	0.017	0.011	0.013	0.015
difference	0.002		-0.003		0.000		0.013	
critical=0.3 $\sigma_{FFP}$	0.005		0.012		0.015		0.028	
consequential instability	no		no		no		no	
t	0.222		0.174		0.000		1.620	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

## Appendix 2. Stability data (continued)

	<u>4-PHEN</u>				<u>9-PHEN</u>			
	low [ng/mL]		high [ng/mL]		low [ng/mL]		high [ng/mL]	
	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C	-80°C	-18°C
1	0.650	0.670	0.830	0.840	0.210	0.220	0.240	0.230
2	0.660	0.700	0.820	0.770	0.220	0.200	0.230	0.230
3	0.670	0.650	0.870	0.860	0.200	0.190	0.230	0.210
4	0.710	0.700	0.820	0.840	0.200	0.210	0.240	0.240
5	0.710	0.710	0.840	0.790	0.190	0.200	0.220	0.230
6	0.700	0.700	0.830	0.810	0.190	0.190	0.230	0.230
average	0.683	0.688	0.835	0.818	0.202	0.202	0.232	0.228
stdev	0.027	0.023	0.019	0.034	0.012	0.012	0.008	0.010
difference	-0.005		0.017		0.000		0.003	
critical=0.3 $\sigma_{FFP}$	0.051		0.063		0.015		0.017	
consequential instability	no		no		no		no	
t	0.347		1.045		0.000		0.659	
tcrit	2.228		2.228		2.228		2.228	
Significant difference	no		no		no		no	

## Appendix 2. Stability data (continued)

	<b>1-PYR</b>			
	<b>low [ng/mL]</b>		<b>high [ng/mL]</b>	
	<b>-80°C</b>	<b>-18°C</b>	<b>-80°C</b>	<b>-18°C</b>
<b>1</b>	0.150	0.100	0.330	0.380
<b>2</b>	0.100	0.150	0.330	0.330
<b>3</b>	0.160	0.110	0.370	0.350
<b>4</b>	0.120	0.150	0.370	0.360
<b>5</b>	0.130	0.160	0.310	0.310
<b>6</b>	0.130	0.160	0.350	0.310
<b>average</b>	0.132	0.138	0.343	0.340
<b>stdev</b>	0.021	0.026	0.024	0.028
<b>difference</b>	-0.007		0.003	
<b>critical=0.3 <math>\sigma_{FFP}</math></b>	0.010		0.026	
<b>consequential instability</b>	no		no	
<b>t</b>	0.481		0.219	
<b>tcrit</b>	2.228		2.228	
<b>Significant difference</b>	no		no	

### **Appendix 3. Copy of letter of invitation**

#### **HBM4EU: Announcement / invitation to participate in ICI / EQUAS study**

##### **OH-PAH/Round 4**

**Title of ICI/EQUAS:** PAH metabolites in urine

Dear Colleagues,

within the frame of HBM4EU the

Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine

Friedrich-Alexander University Erlangen-Nuremberg

Henkestr. 9-11

91054 Erlangen

Germany

announces the 4<sup>th</sup> round of ICI/EQUAS for the determination of PAH metabolites 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.

##### **Test samples**

The matrix will be urine. Accordingly, the participants will receive:

- 2 different materials of urine (1 sample of 5mL each) for determination of PAH metabolites in urine

##### **Target biomarkers**

For the biomarkers potentially present in the test samples, please see the registration form for OH-PAH/Round 4. We would be pleased if your laboratory could analyse as many metabolites as possible.

LOQs should allow the analysis of PAH metabolites in samples of the general population.

##### **Calendar:**

Registration deadline	October 18, 2019
Distribution of test samples (projected)	October 22, 2019
Deadline for submission of results (projected)	November 19, 2019



WP9 EQUAS Report, Round 04/2019	Version: 1	Date: 20-12-2019	Page: 41
PAH met. in urine, Round 4			

## **Registration**

For registration, please find attached a registration form for PAH metabolites 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:**

Coordinators:

- Prof. Dr. Thomas Göen
- Stefanie Nübler
- Moritz Schäfer
- Karin H. A. Zarrabi

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

Email: [ipasum-hbm4eu@fau.de](mailto:ipasum-hbm4eu@fau.de)

## **Appendix 4. Copy of letter/instructions sent together with test samples**

### **HBM4EU: Instruction letter ICI / EQUAS study OH-PAH/Round4**

**Title of ICI/EQUAS:** PAH metabolites in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study OH-PAH in urine/Round 4 for the determination of PAH metabolites in urine.

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

The parcel will be shipped on 22<sup>nd</sup> October 2019 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.
- Store the test samples under frozen (-18°C) conditions until analysis.
- Analyse the samples for the biomarkers indicated in the invitation letter ref/ 07.10.2019.
- 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 **19<sup>th</sup> November 2019**

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

Karin Zarrabi, Stefanie Nübler or Moritz Schäfer

Email: **ipasum-hbm4eu@fau.de**

Tel.: + 49 (0)9131/8526146, /8526145

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 (for the ICI/EQUAS organisers)

WP9 EQUAS Report, Round 04/2019	Version: 1	Date: 20-12-2019	Page: 43
PAH met. in urine, Round 4			

## Appendix 5.

### Method information form of IPASUM in ICI/EQUAS OH-PAH in urine (OH-PAH/Round 4)

Laboratory code	IPASUM	
ISO17025 accredited	No	
<b>SAMPLE PREPARATION</b>		
amount sample extracted	2 mL	
<b>Deconjugation</b>	Yes	
- chemical	-	
- enzymatic	$\beta$ -Glucuronidase/Arylsulfatase / 37 °C / 16 h	
<b>Extraction</b>		
- pH adjustment	-	
- LLE;	-	
- SPE; material	Unmodified polystyrene-divinyl benzene copolymer	
<b>Cleanup</b>	-	
- LLE; solvent(s)		
- SPE; material		
<b>Derivatisation</b>		
- reagent	N-Methyl-N-(trimethylsilyl)trifluoroacetamid (MSTFA)	
<b>INSTRUMENTAL ANALYSIS</b>		
<b>HPLC</b>	-	
- injection volume		
- column stationary phase		
- column L (mm) x ID (mm); dp		
- temperature		
- mobile phase A		
- mobile phase B		
- flow rate		
<b>GC</b>		
- injector	Splitless	
- injection volume	1.2 $\mu$ l	
- column stationary phase	HP-5MS Ultra Inert	
- column L (m) x ID (mm) df ( $\mu$ m)	60 m x 0,25 mm x 0,25 $\mu$ m	
- carrier	Helium	
- flow rate / inlet pressure	1 ml/min / 20.969 psi	
<b>Detection</b>		
MS	Single quad	
other		
<b>Quantification</b>		
Use of internal standard (IS)	Yes	
- isotopic label	Yes	
- other		
- moment of addition	Before deconjugation	
- response normalised to IS	Yes	
<b>Calibration</b>	Matrix-matched (addition to blank matrix before extraction)	
	Multi-level	
<b>Correction for recovery</b>	No	
<b>Identification criteria used</b>		
- retention time tolerance	Yes	
- number of ions/transitions	Yes	
- ion ratio tolerance	No	

## Appendix 6. Assigned values and participant's performance for PAH met. in urine

HBM4EU 04/2019	1-naphthol			
PAH met. (urine)	1-naphthol <sub>low</sub>		1-naphthol <sub>high</sub>	
assigned value from 4 experts	2.773 ng/mL		10.623 ng/mL	
expert standard deviation	0.945 ng/mL		3.056 ng/mL	
uncertainty of assigned value (u)	17.0%		14.4%	
relative target standard deviation (σ <sub>T</sub> )	25%		25%	
0.7 * σ <sub>T</sub>	17.5%		17.5%	
study RSD <sub>R</sub>	23.9%		25%	
laboratory code	value	Z-score	value	Z-score
QR/113	3.350	0.833	11.840	0.458
QR/118	3.460	0.991	12.700	0.782
QR/122	1.532	-1.790	6.059	-1.719
QR/123	NA	NA	NA	NA
QR/124	2.470	-0.437	10.700	0.029
QR/126	2.644	-0.186	10.819	0.074
QR/127	3.801	1.483	13.818	1.203
QR/128	3.380	0.876	12.310	0.635
QR/129	3.940	1.683	17.100	2.439
QR/130	NA	NA	NA	NA
QR/140	2.900	0.183	9.640	-0.370
QR/141	3.450	0.976	12.791	0.816
QR/143	NA	NA	NA	NA
QR/201	6.500	5.376	13.300	1.008
QR/202	NA	NA	NA	NA
QR/207	4.737	2.833	8.483	-0.806
QR/210	4.125	1.950	13.517	1.090
QR/217	NA	NA	NA	NA
QR/219	3.730	1.380	11.600	0.368
QR/221	4.264	2.151	8.516	-0.793

NA = not analysed

**Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)**

<b>HBM4EU 04/2019</b>	<b>2-naphthol</b>			
<b>PAH met. (urine)</b>	<b>2-naphthol<sub>low</sub></b>		<b>2-naphthol<sub>high</sub></b>	
assigned value from 4 experts	5.339 ng/mL		9.899 ng/mL	
expert standard deviation	0.958 ng/mL		2.061 ng/mL	
uncertainty of assigned value (u)	9.0%		10.4%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD <sub>R</sub>	14.4%		15.5%	
Laboratory code	value	Z-score	value	Z-score
QR/113	5.218	-0.091	9.735	-0.066
QR/118	6.060	0.540	11.000	0.445
QR/122	4.108	-0.922	7.215	-1.084
QR/123	NA	NA	NA	NA
QR/124	5.000	-0.254	9.390	-0.206
QR/126	6.147	0.605	12.495	1.049
QR/127	6.195	0.641	10.919	0.412
QR/128	5.650	0.233	10.390	0.199
QR/129	5.740	0.300	12.300	0.970
QR/130	NA	NA	NA	NA
QR/140	5.700	0.270	10.300	0.162
QR/141	5.701	0.271	10.604	0.285
QR/143	NA	NA	NA	NA
QR/201	4.800	-0.404	8.050	-0.747
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	1.377	-2.968	5.250	-1.879
QR/217	NA	NA	NA	NA
QR/219	7.590	1.686	11.000	0.445
QR/221	11.099	4.315	18.161	3.339

NA = not analysed

# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	2-FLUO			
PAH met. (urine)	2-FLUO <sub>low</sub>		2-FLUO <sub>high</sub>	
assigned value from 3 experts	0.503 ng/mL		0.995 ng/mL	
expert standard deviation	0.048 ng/mL		0.087 ng/mL	
uncertainty of assigned value (u)	5.5%		5.0%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD <sub>R</sub>	40.6%		43.9%	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.553	0.397	1.064	0.280
QR/118	NA	NA	NA	NA
QR/122	0.910	3.239	1.781	3.162
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	0.807	2.422	1.666	2.701
QR/127	0.542	0.313	1.055	0.243
QR/128	NA	NA	NA	NA
QR/129	0.607	0.830	1.150	0.625
QR/130	0.126	-2.997	0.277	-2.886
QR/140	NA	NA	NA	NA
QR/141	NA	NA	NA	NA
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	0.494	-0.069	0.955	-0.159
QR/217	NA	NA	NA	NA
QR/219	1.270	6.106	1.800	3.240
QR/221	NA	NA	NA	NA

NA = not analysed

# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	3-FLUO			
PAH met. (urine)	3-FLUO <sub>low</sub>		3-FLUO <sub>high</sub>	
assigned value from 3 experts	0.170 ng/mL		0.401 ng/mL	
expert/robust standard deviation	0.030 ng/mL		0.044 ng/mL	
uncertainty of assigned value (u)	10.1%		6.4%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD <sub>R</sub>	75.4%		42.1%	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.179	0.224	0.398	-0.032
QR/118	NA	NA	NA	NA
QR/122	NA	NA	NA	NA
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	NA*	NA*	NA*	NA*
QR/127	ND	(-2.822)	0.337	-0.638
QR/128	NA	NA	NA	NA
QR/129	0.212	0.993	0.584	1.825
QR/130	NA	NA	NA	NA
QR/140	NA	NA	NA	NA
QR/141	NA	NA	NA	NA
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	NA	NA	NA	NA
QR/217	NA	NA	NA	NA
QR/219	0.451	6.622	0.734	3.322
QR/221	NA	NA	NA	NA

NA = not analysed

\* = analysed the sum of 3- and 9-FLUO

ND = not detected; proxy Z-score between brackets (x.x) was calculated using the LOQ as result (see 5.6) and is just for information

**Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)**

HBM4EU 04/2019	9-FLUO			
PAH met. (urine)	9-FLUO <sub>low</sub>		9-FLUO <sub>high</sub>	
assigned/consensus value	X		X	
no ICI/EQUAS evaluation due to	only 2 experts, < 7 participants		only 2 experts, < 7 participants	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.667	X	11.303	X
QR/118	NA	NA	NA	NA
QR/122	NA	NA	NA	NA
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	NA*	NA*	NA*	NA*
QR/127	NA	NA	NA	NA
QR/128	NA	NA	NA	NA
QR/129	NA	NA	NA	NA
QR/130	NA	NA	NA	NA
QR/140	NA	NA	NA	NA
QR/141	NA	NA	NA	NA
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	NA	NA	NA	NA
QR/217	NA	NA	NA	NA
QR/219	0.529	X	0.777	X
QR/221	NA	NA	NA	NA

NA = not analysed

\*= analysed the sum of 3- and 9-FLUO

X = no Z-score available



**Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)**

<b>HBM4EU 04/2019</b>	<b>1-PHEN</b>			
<b>PAH met. (urine)</b>	<b>1-PHEN<sub>low</sub></b>		<b>1-PHEN<sub>high</sub></b>	
<b>assigned value from 4 experts</b>	<b>0.230 ng/mL</b>		<b>0.414 ng/mL</b>	
<b>expert standard deviation</b>	<b>0.057 ng/mL</b>		<b>0.105 ng/mL</b>	
<b>uncertainty of assigned value (u)</b>	<b>12.4%</b>		<b>12.7%</b>	
<b>relative target standard deviation (<math>\sigma_T</math>)</b>	<b>25%</b>		<b>25%</b>	
<b><math>0.7 * \sigma_T</math></b>	<b>17.5%</b>		<b>17.5%</b>	
<b>study RSD<sub>R</sub></b>	<b>24.3%</b>		<b>16.6%</b>	
<b>Laboratory code</b>	<b>value</b>	<b>Z-score</b>	<b>value</b>	<b>Z-score</b>
QR/113	0.163	-1.159	0.295	-1.146
QR/118	NA	NA	NA	NA
QR/122	0.256	0.463	0.458	0.425
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	0.225	-0.083	0.539	1.212
QR/127	0.292	1.084	0.443	0.284
QR/128	NA	NA	NA	NA
QR/129	0.249	0.335	0.470	0.545
QR/130	NA	NA	NA	NA
QR/140	NA	NA	NA	NA
QR/141	0.350	2.094	0.642	2.208
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	0.350	2.094	0.592	1.724
QR/217	NA	NA	NA	NA
QR/219	0.307	1.345	0.493	0.767
QR/221	NA**	NA**	NA**	NA**

NA = not analysed

\*\* = analysed the sum of 1-Phen and 9-Phen

# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	2-PHEN			
PAH met. (urine)	2-PHEN <sub>low</sub>		2-PHEN <sub>high</sub>	
consensus value ICI	0.125 ng/mL		0.274 ng/mL	
robust standard deviation	0.026 ng/mL		0.059 ng/mL	
uncertainty (u)	0.010 ng/mL		0.023 ng/mL	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
study RSD <sub>R</sub>	16.6%		14.5%	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.108	-0.588	0.232	-0.581
QR/118	NA	NA	NA	NA
QR/122	0.122	-0.134	0.263	-0.142
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	0.122	-0.134	0.280	0.088
QR/127	0.14	0.436	0.294	0.282
QR/128	NA	NA	NA	NA
QR/129	0.187	1.926	0.394	1.667
QR/130	NA	NA	NA	NA
QR/140	NA	NA	NA	NA
QR/141	0.11	-0.514	0.261	-0.175
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	0.142	0.500	0.302	0.393
QR/217	NA	NA	NA	NA
QR/219	0.169	1.355	0.215	-0.812
QR/221	0.108	-0.578	0.653	5.254

NA = not analysed

# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	3-PHEN			
PAH met. (urine)	3-PHEN <sub>low</sub>		3-PHEN <sub>high</sub>	
assigned value from 3 experts	0.188 ng/mL		0.353 ng/mL	
expert standard deviation	0.016 ng/mL		0.027 ng/mL	
uncertainty of assigned value (u)	4.9%		4.4%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD <sub>R</sub>	11.6%		13.3%	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.180	-0.156	0.322	-0.347
QR/118	NA	NA	NA	NA
QR/122	0.206	0.391	0.372	0.224
QR/123	NA	NA	NA	NA
QR/124	NA	NA	NA	NA
QR/126	0.226	0.817	0.436	0.436
QR/127	0.191	0.071	0.374	0.243
QR/128	NA	NA	NA	NA
QR/129	0.125	-1.336	0.263	-1.016
QR/130	NA	NA	NA	NA
QR/140	NA	NA	NA	NA
QR/141	0.180	-0.163	0.333	-0.222
QR/143	NA	NA	NA	NA
QR/201	NA	NA	NA	NA
QR/202	NA	NA	NA	NA
QR/207	NA	NA	NA	NA
QR/210	0.187	-0.014	0.336	-0.188
QR/217	NA	NA	NA	NA
QR/219	ND	(3.865)	ND	(0.187)
QR/221	0.256	1.456	0.522	1.922

NA = not analysed

ND = not detected ; (proxy Z-score, only for information)

**Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)**

<b>HBM4EU 04/2019</b>	<b>4-PHEN</b>			
<b>PAH met. (urine)</b>	<b>4-PHEN<sub>low</sub></b>		<b>4-PHEN<sub>high</sub></b>	
<b>consensus value ICI</b>	<b>0.091 ng/mL</b>		<b>0.193 ng/mL</b>	
<b>robust standard deviation</b>	<b>0.032 ng/mL</b>		<b>0.041 ng/mL</b>	
<b>uncertainty (u)</b>	<b>0.015 ng/mL</b>		<b>0.018 ng/mL</b>	
<b>relative target standard deviation (<math>\sigma_T</math>)</b>	<b>25%</b>		<b>25%</b>	
<b>study RSD<sub>R</sub></b>	<b>35.3%</b>		<b>21.0%</b>	
<b>Laboratory code</b>	<b>value</b>	<b>Z-score</b>	<b>value</b>	<b>Z-score</b>
<b>QR/113</b>	0.056	<b>-1.288</b>	0.123	<b>-1.368</b>
<b>QR/118</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/122</b>	0.069	<b>-0.792</b>	0.157	<b>-0.700</b>
<b>QR/123</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/124</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/126</b>	0.136	<b>1.671</b>	0.219	<b>0.496</b>
<b>QR/127</b>	0.108	<b>0.642</b>	0.169	<b>-0.473</b>
<b>QR/128</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/129</b>	0.078	<b>-0.461</b>	0.208	<b>0.283</b>
<b>QR/130</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/140</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/141</b>	0.077	<b>-0.498</b>	0.188	<b>-0.105</b>
<b>QR/143</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/201</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/202</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/207</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/210</b>	NA <sup>***</sup>	<b>NA<sup>***</sup></b>	NA <sup>***</sup>	<b>NA<sup>***</sup></b>
<b>QR/217</b>	NA	<b>NA</b>	NA	<b>NA</b>
<b>QR/219</b>	0.122	<b>1.156</b>	0.271	<b>1.504</b>
<b>QR/221</b>	ND	<b>(-0.145)</b>	0.210	<b>0.321</b>

NA = not analysed

\*\*\* = analysed the sum of 4-PHEN and 9-PHEN

ND = not detected ; (proxy Z-score, only for information)

# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	9-PHEN			
PAH met. (urine)	9-PHEN <sub>low</sub>		9-PHEN <sub>high</sub>	
assigned/consensus value	X		X	
no ICI/EQUAS evaluation due to	only results from 2 experts, not enough participants for ICI evaluation		only results from 2 experts, not enough participants for ICI evaluation	
Laboratory code	value	Z-score	value	Z-score
QR/113	NA	X	NA	X
QR/118	NA	X	NA	X
QR/122	0.109	X	0.122	X
QR/123	NA	X	NA	X
QR/124	NA	X	NA	X
QR/126	0.280	X	0.242	X
QR/127	0.054	X	0.06	X
QR/128	NA	X	NA	X
QR/129	NA	X	NA	X
QR/130	NA	X	NA	X
QR/140	NA	X	NA	X
QR/141	NA	X	NA	X
QR/143	NA	X	NA	X
QR/201	NA	X	NA	X
QR/202	NA	X	NA	X
QR/207	NA	X	NA	X
QR/210	NA <sup>***</sup>	X	NA <sup>***</sup>	X
QR/217	NA	X	NA	X
QR/219	ND	X	0.601	X
QR/221	NA <sup>**</sup>	X	NA <sup>**</sup>	X

NA = not analysed; ND = not detected

X = no Z-score available

\*\* = analysed the sum of 1-PHEN and 9-PHEN

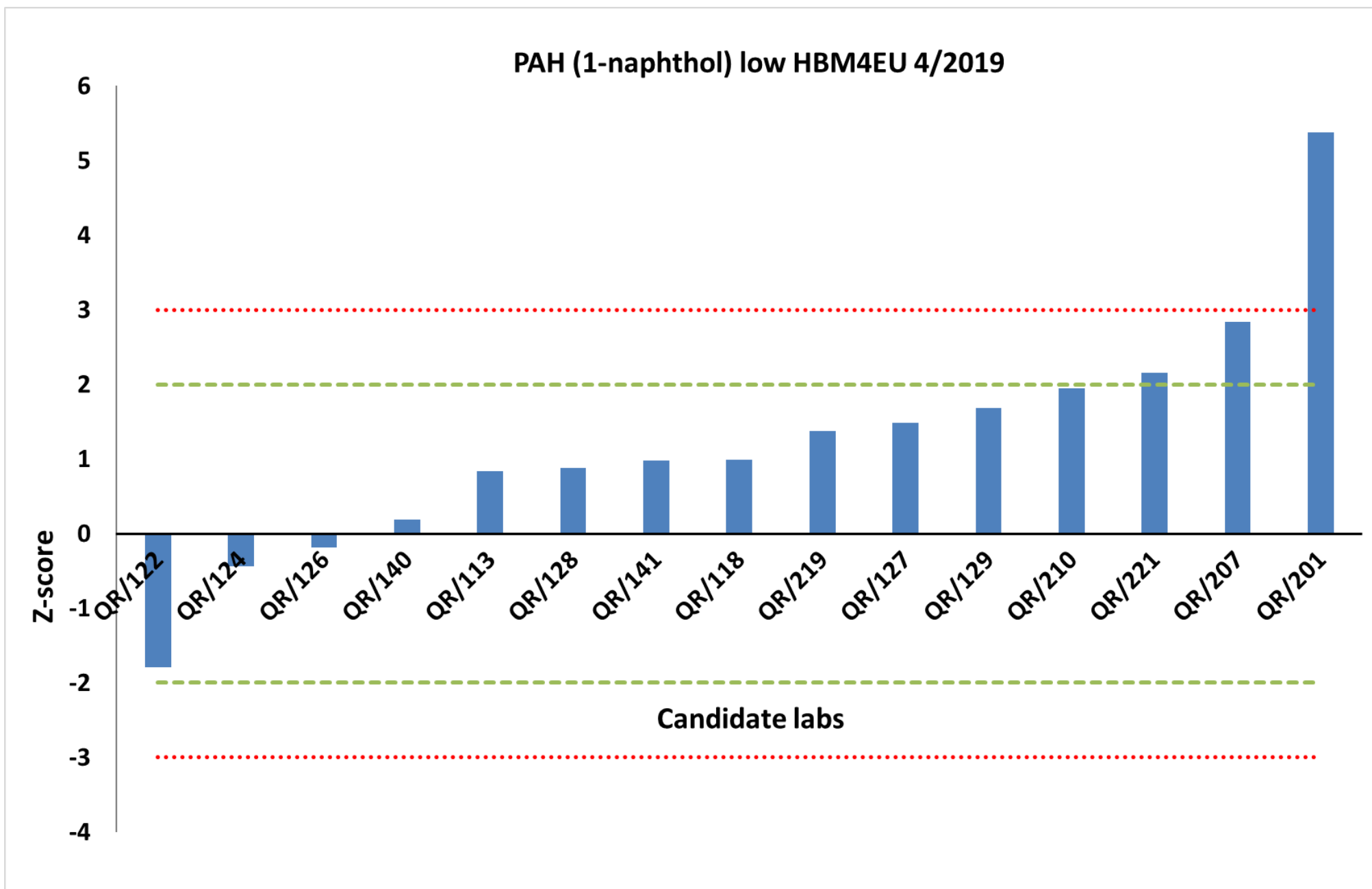
\*\*\* = analysed the sum of 4-PHEN and 9-PHEN

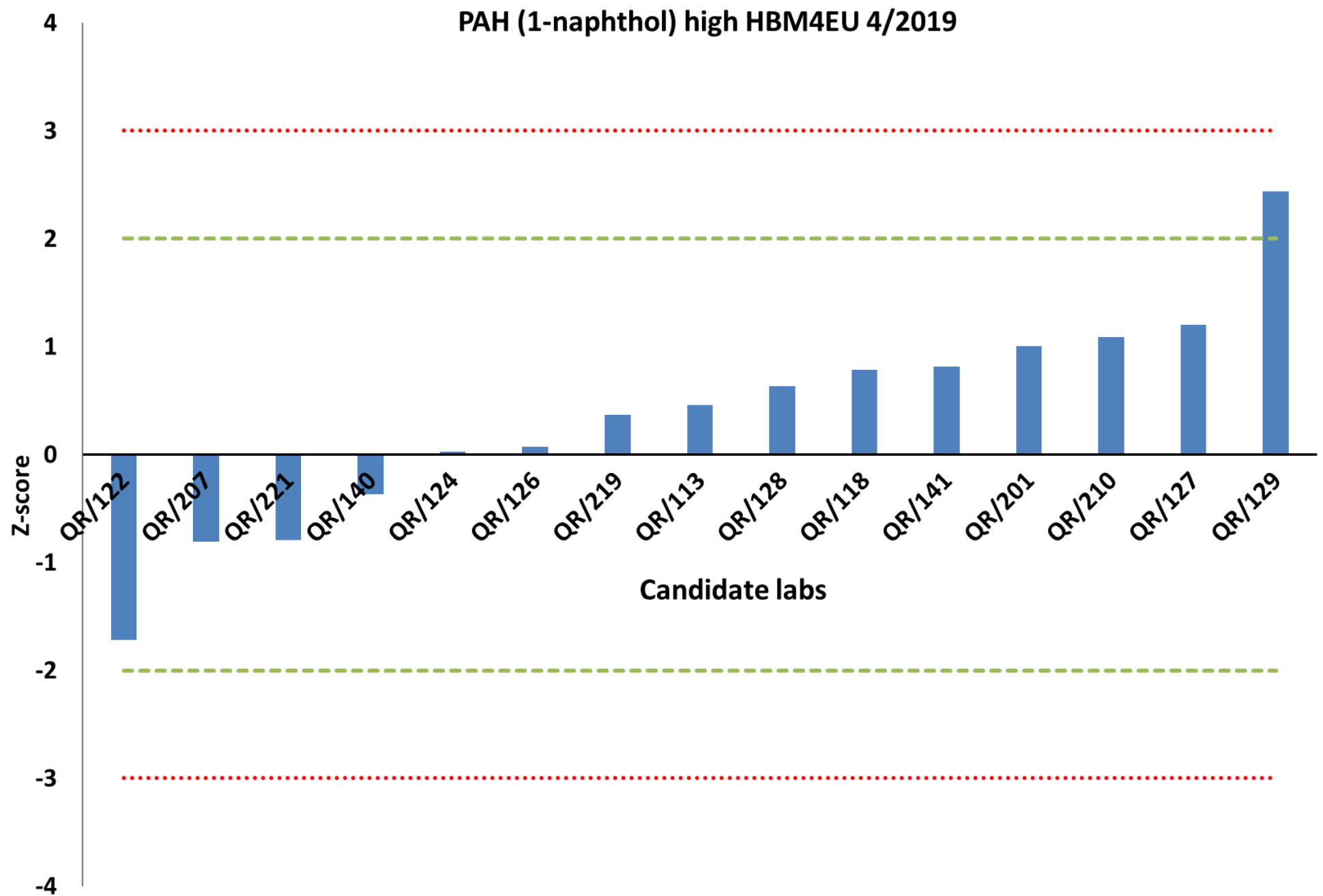
# Appendix 6. Assigned values and participant's performance for PAH met. in urine (continued)

HBM4EU 04/2019	1-PYR			
PAH met. (urine)	1-PYR <sub>low</sub>		1-PYR <sub>high</sub>	
assigned value from 4 (low)/5 (high) experts	0.113 ng/mL		0.253 ng/mL	
expert standard deviation	0.010 ng/mL		0.070 ng/mL	
uncertainty of assigned value (u)	3.9%		11.1%	
relative target standard deviation ( $\sigma_T$ )	25%		25%	
$0.7 * \sigma_T$	17.5%		17.5%	
study RSD <sub>R</sub>	21.4%		19.2%	
Laboratory code	value	Z-score	value	Z-score
QR/113	0.103	-0.355	0.213	-0.626
QR/118	0.200	3.090	0.330	1.223
QR/122	0.122	0.319	0.259	0.104
QR/123	0.135	0.786	0.298	0.716
QR/124	0.146	1.176	0.308	0.875
QR/126	0.167	1.920	0.345	1.460
QR/127	0.151	1.353	0.275	0.352
QR/128	0.180	2.381	0.350	1.539
QR/129	0.150	1.318	0.314	0.970
QR/130	0.017	-3.397	0.036	-3.430
QR/140	ND	(3.090)	ND	(-0.835)
QR/141	0.114	0.041	0.247	-0.091
QR/143	0.108	-0.160	0.222	-0.492
QR/201	0.185	2.558	0.336	1.318
QR/202	0.168	1.956	0.321	1.080
QR/207	0.152	1.388	0.230	-0.360
QR/210	0.139	0.928	0.283	0.479
QR/217	0.162	1.743	0.292	0.621
QR/219	0.170	2.027	0.329	1.207
QR/221	0.207	3.338	0.434	2.869

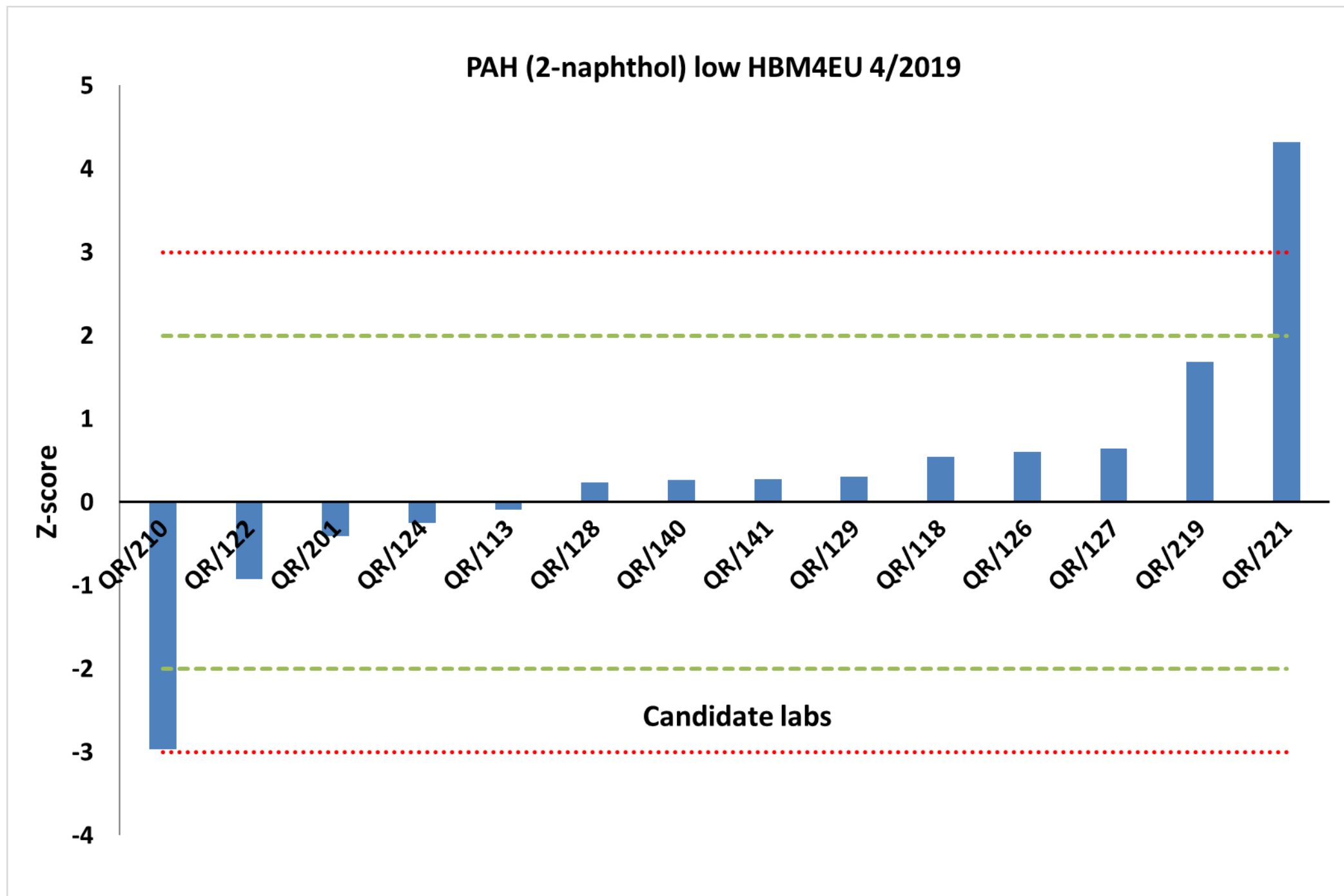
ND = not detected ; (proxy Z-score, only for information)

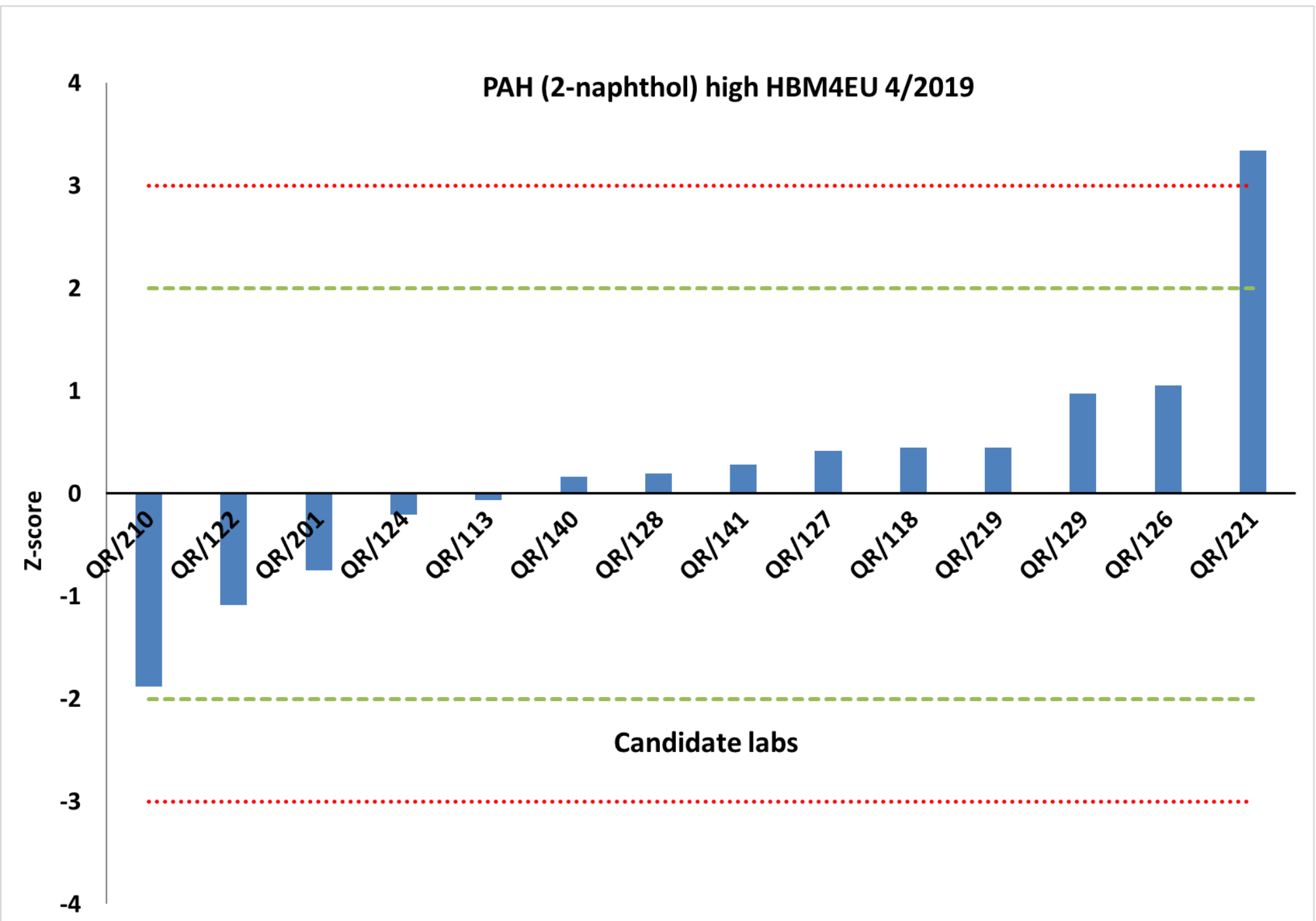
## Appendix 7. Graphical representation of the Z-scores

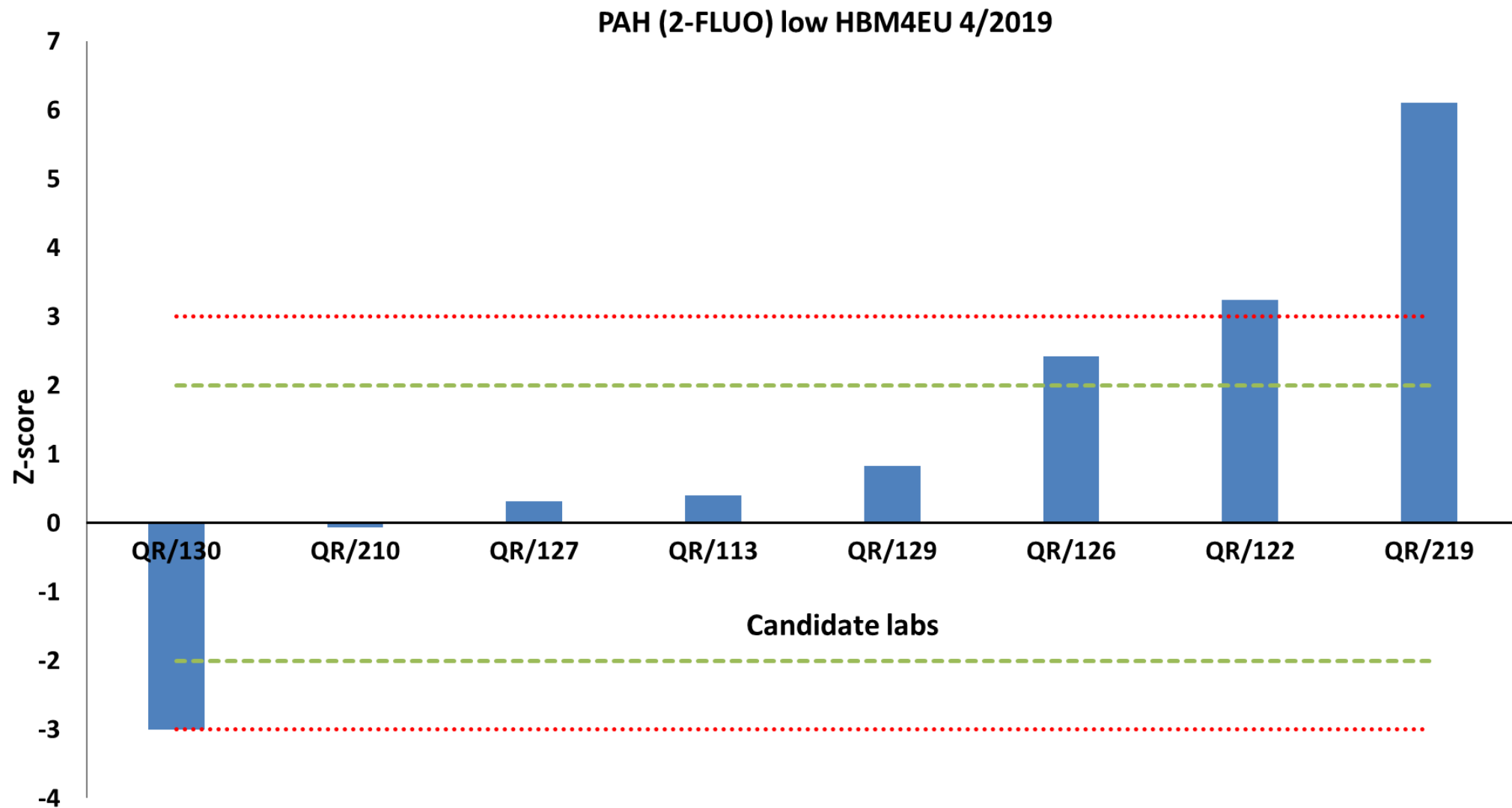


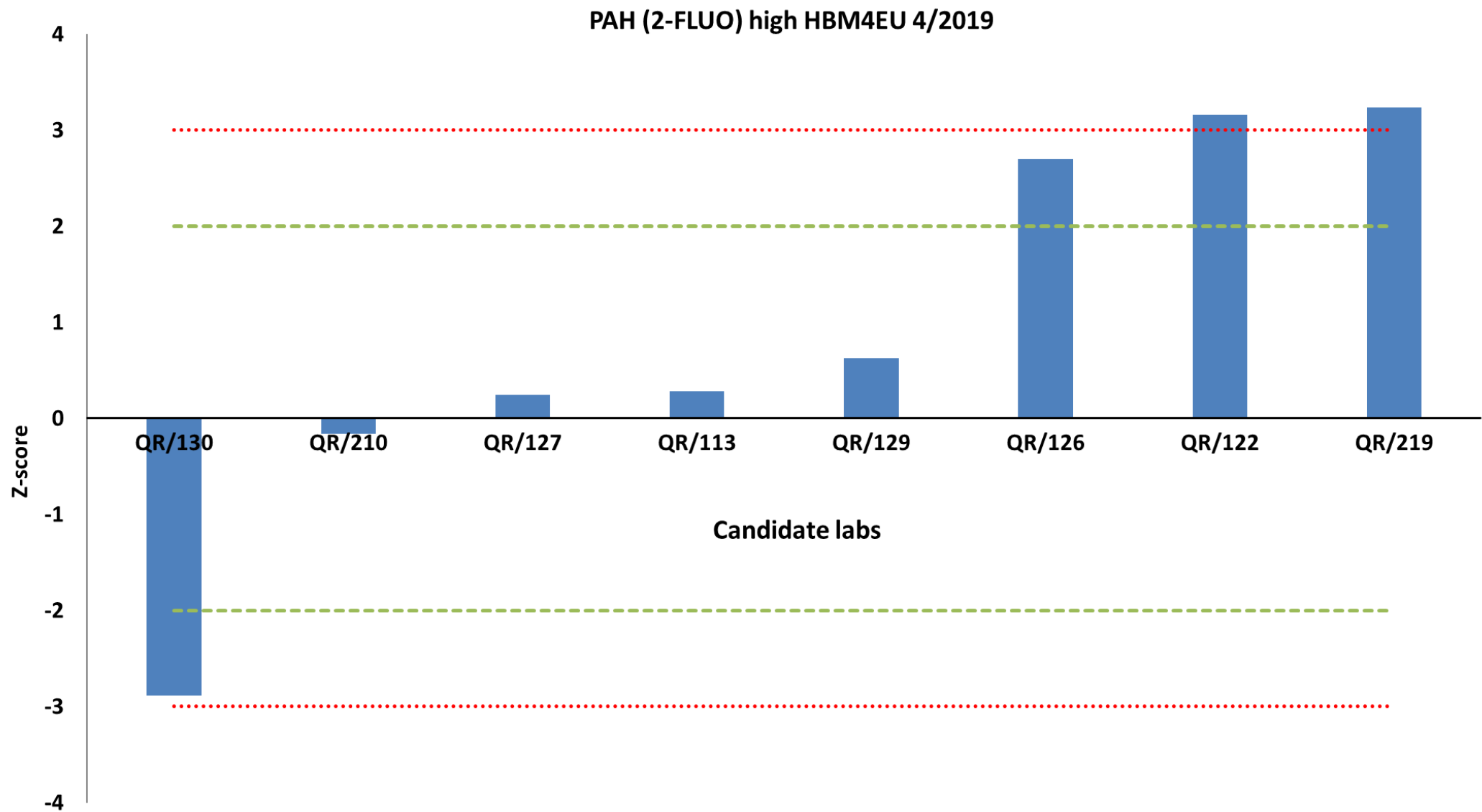


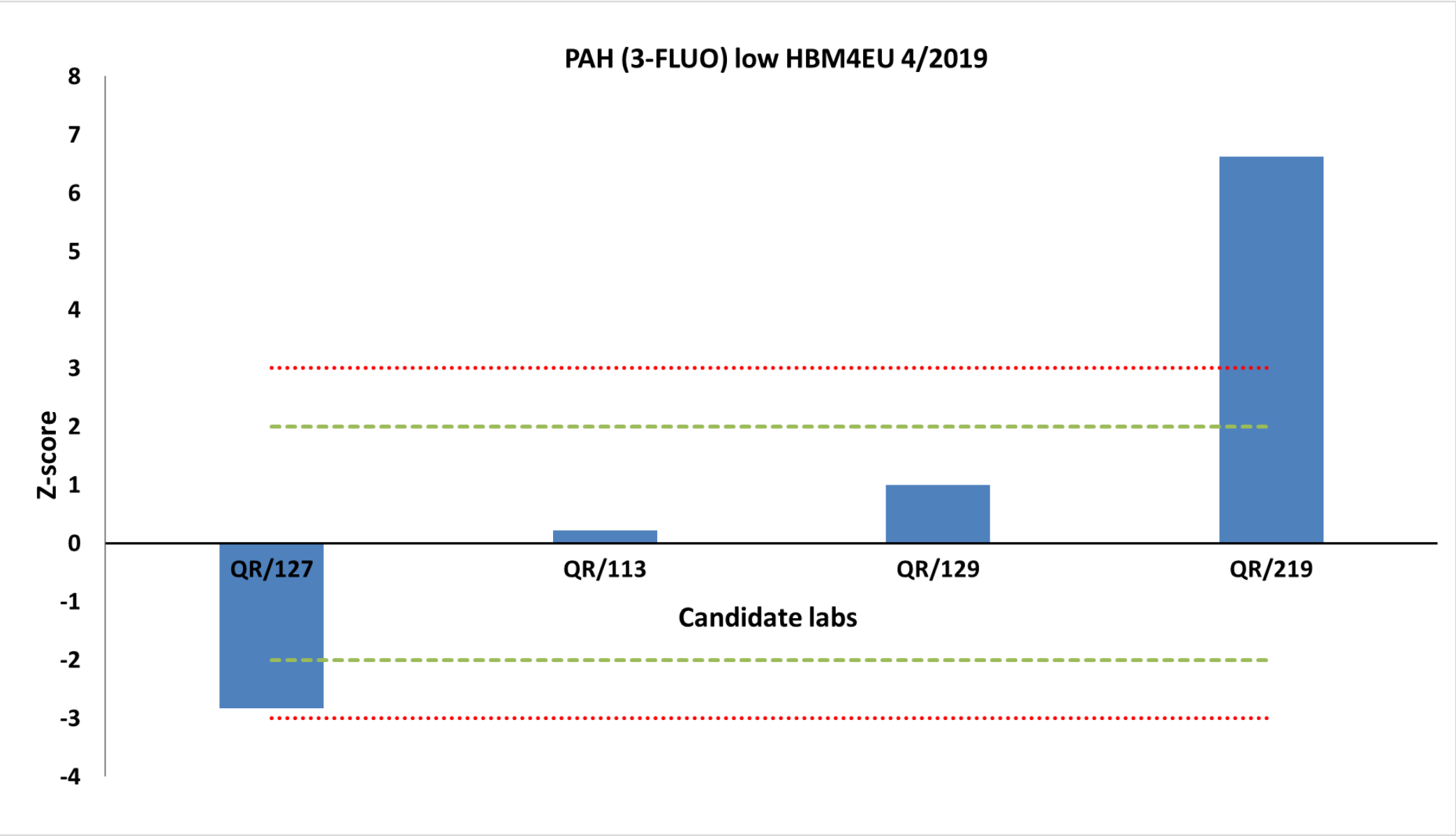


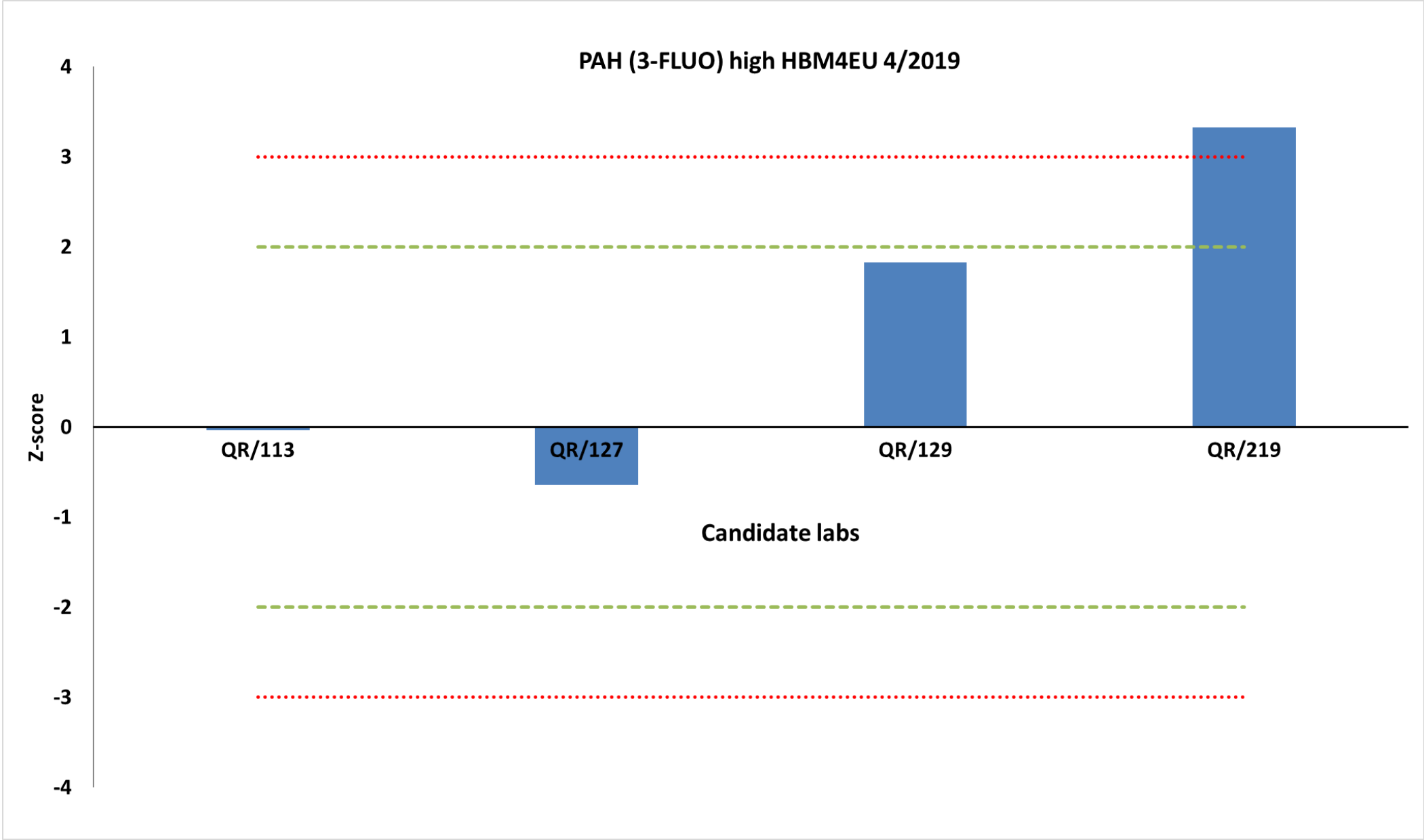




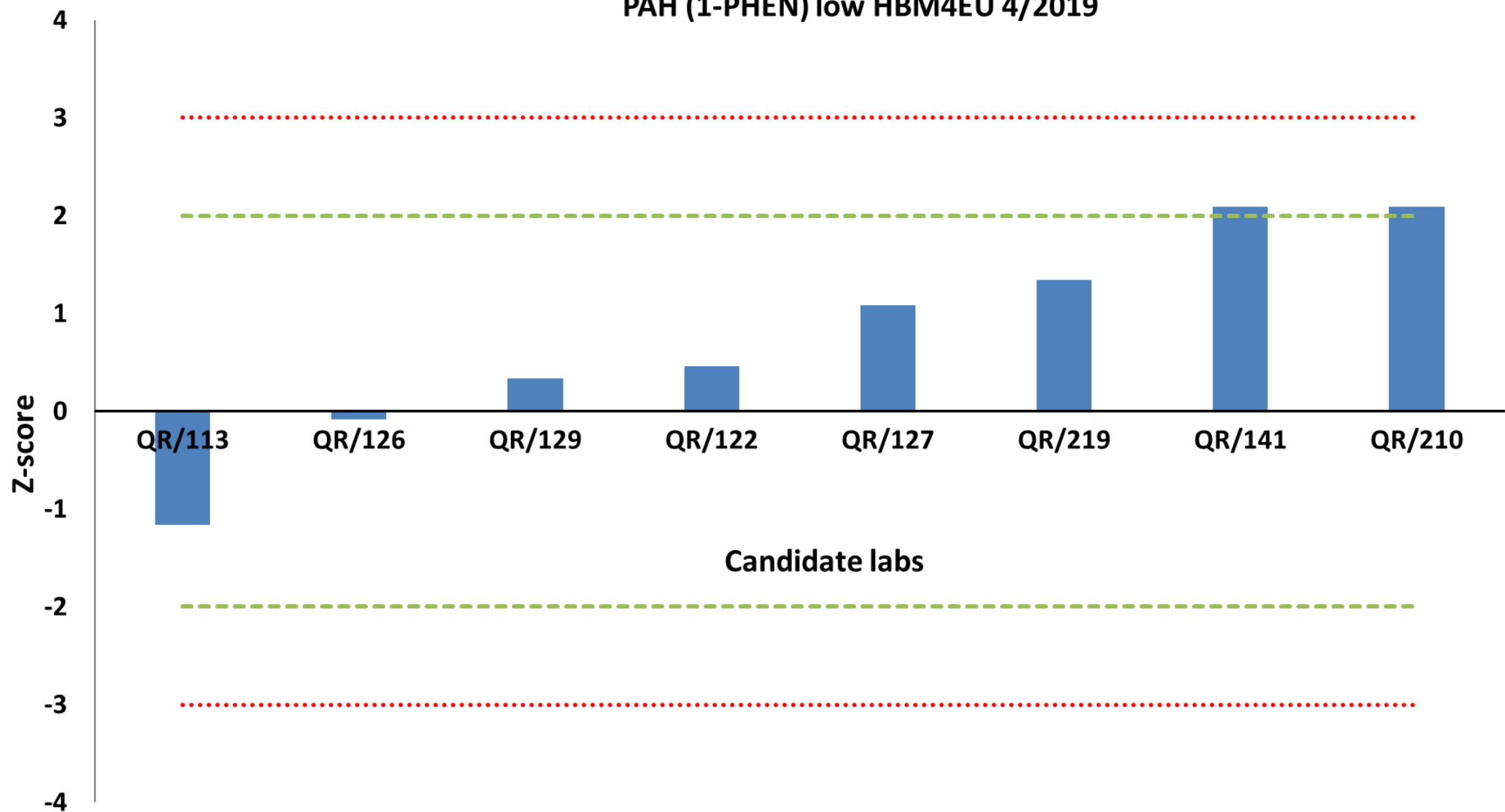


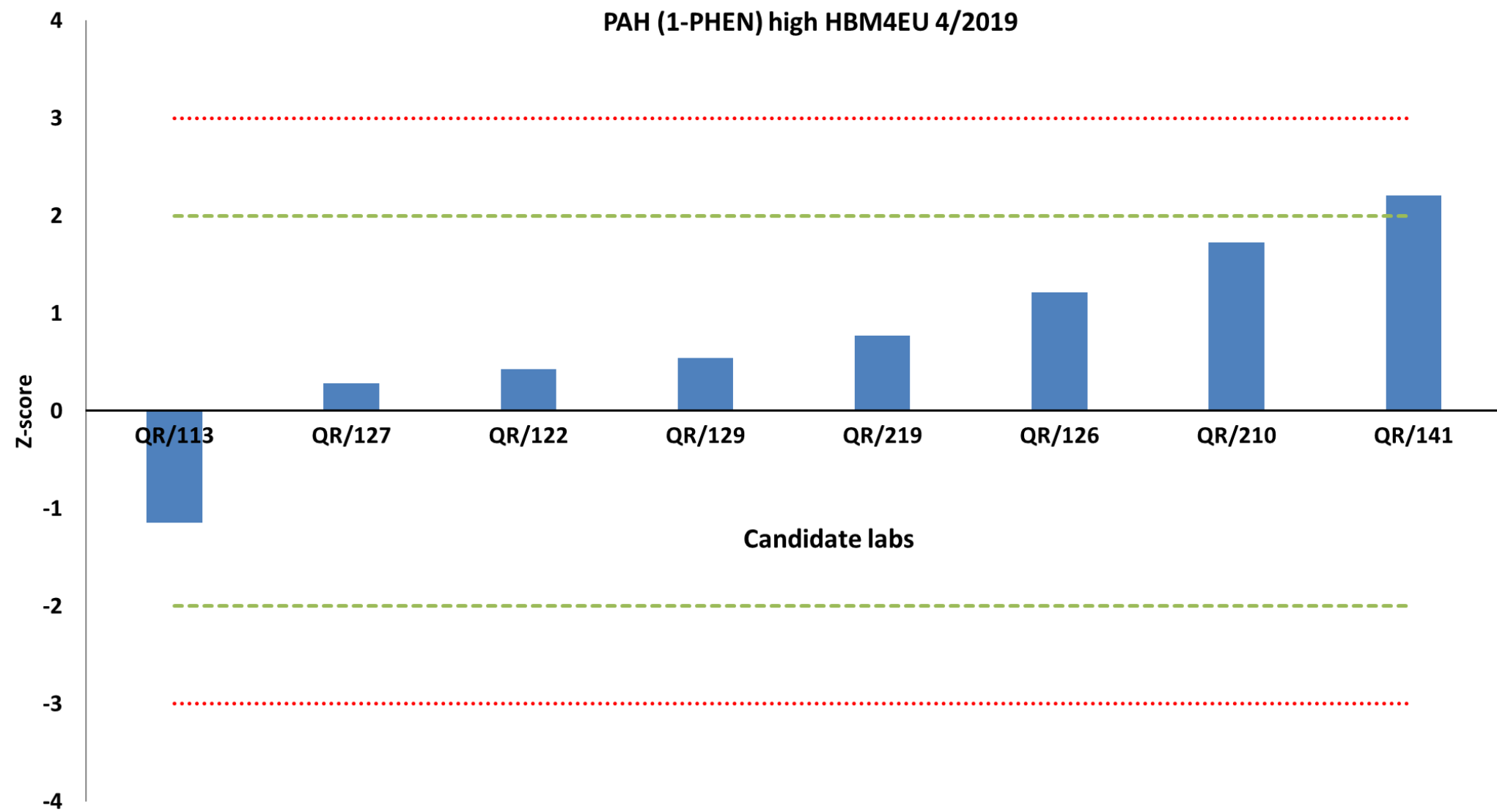




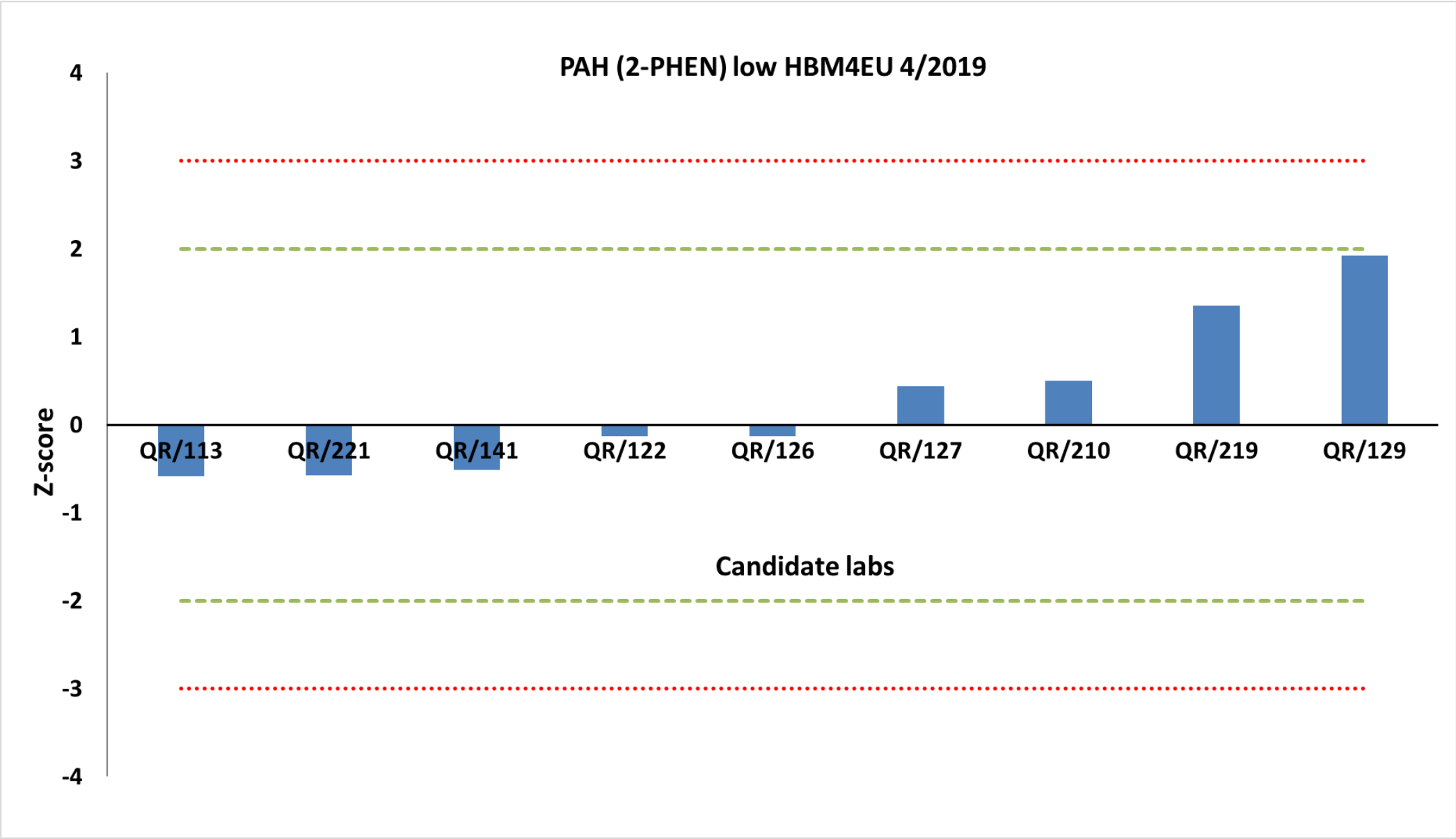


### PAH (1-PHEN) low HBM4EU 4/2019

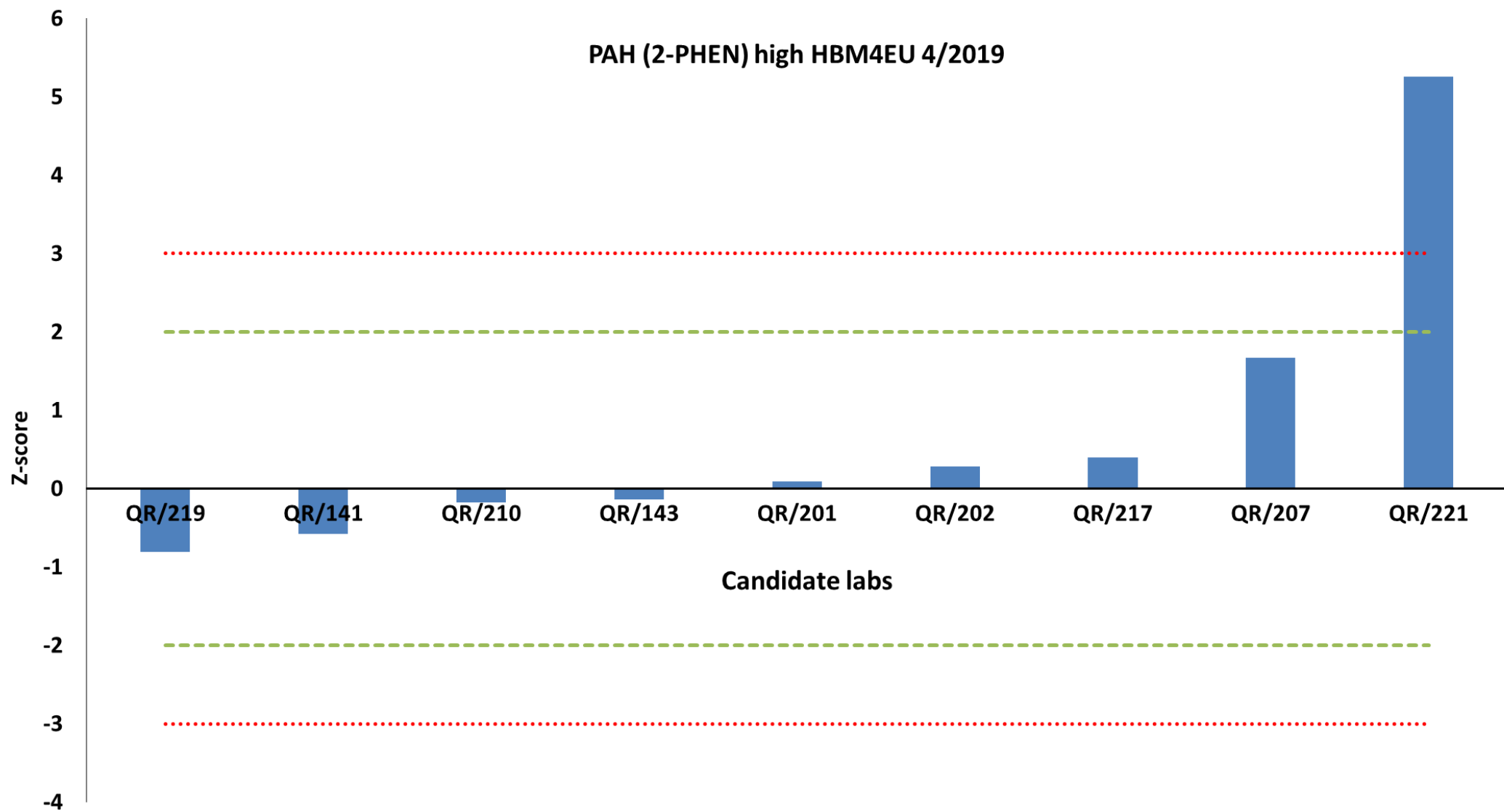




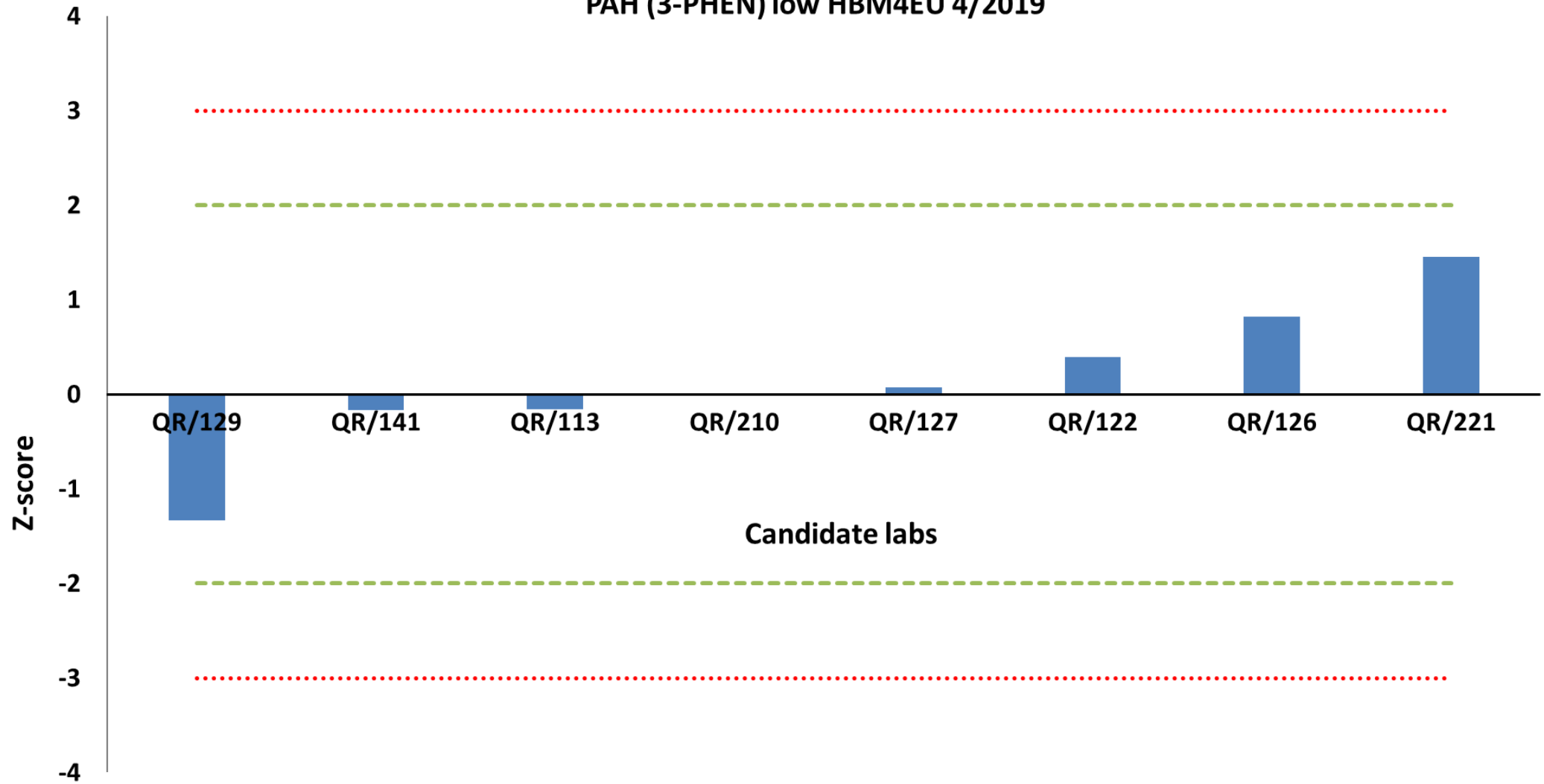


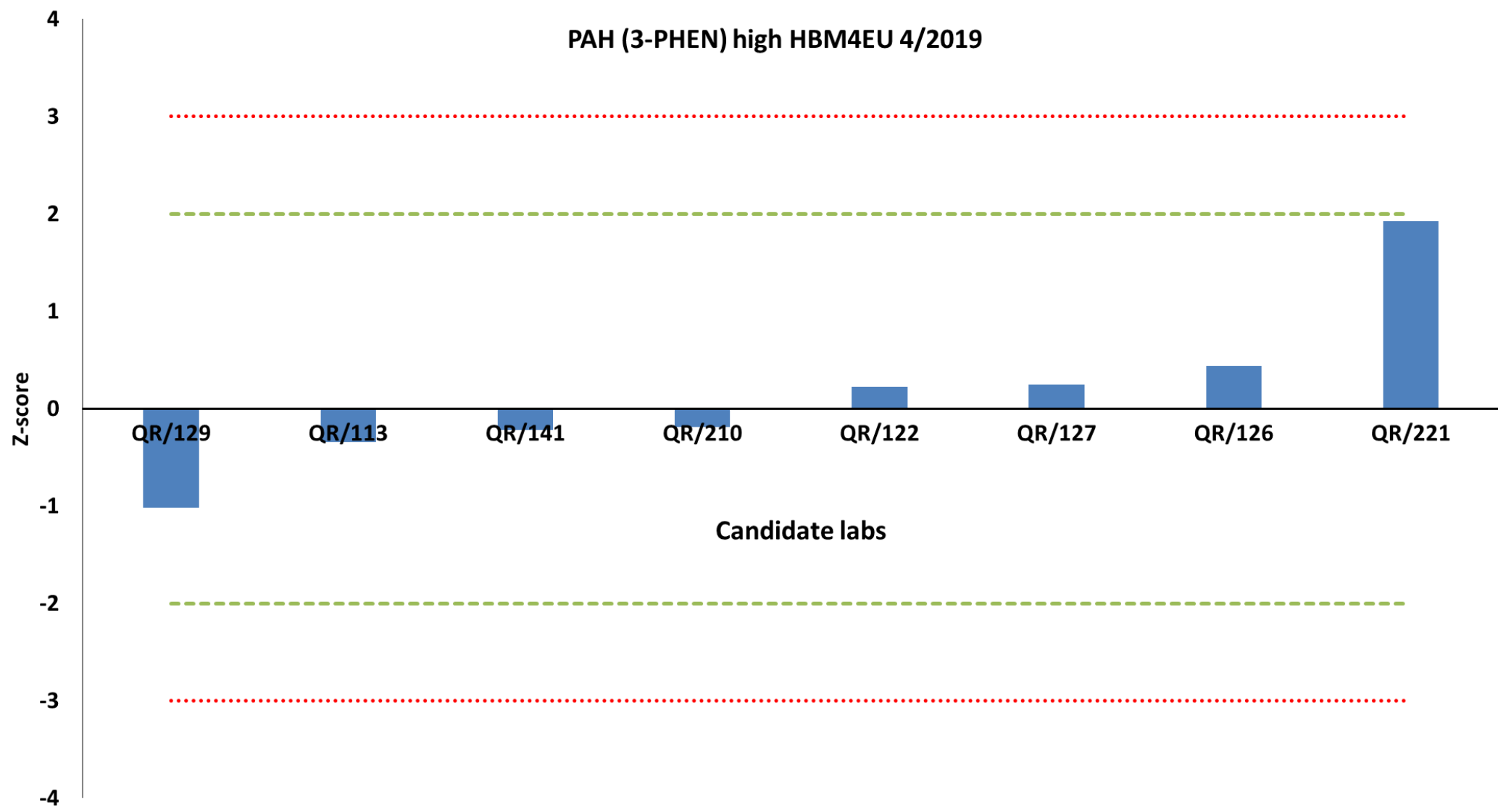


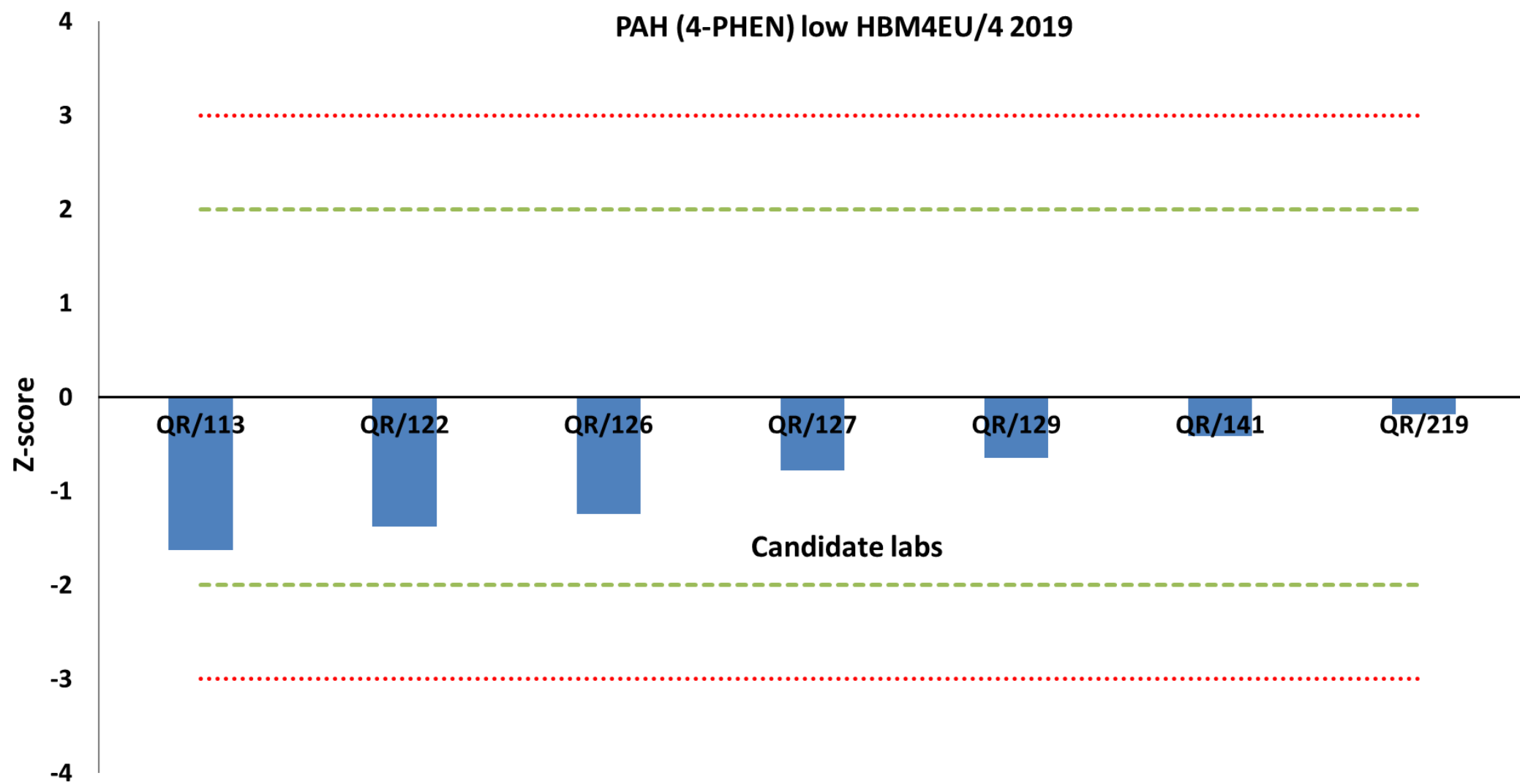
### PAH (2-PHEN) high HBM4EU 4/2019

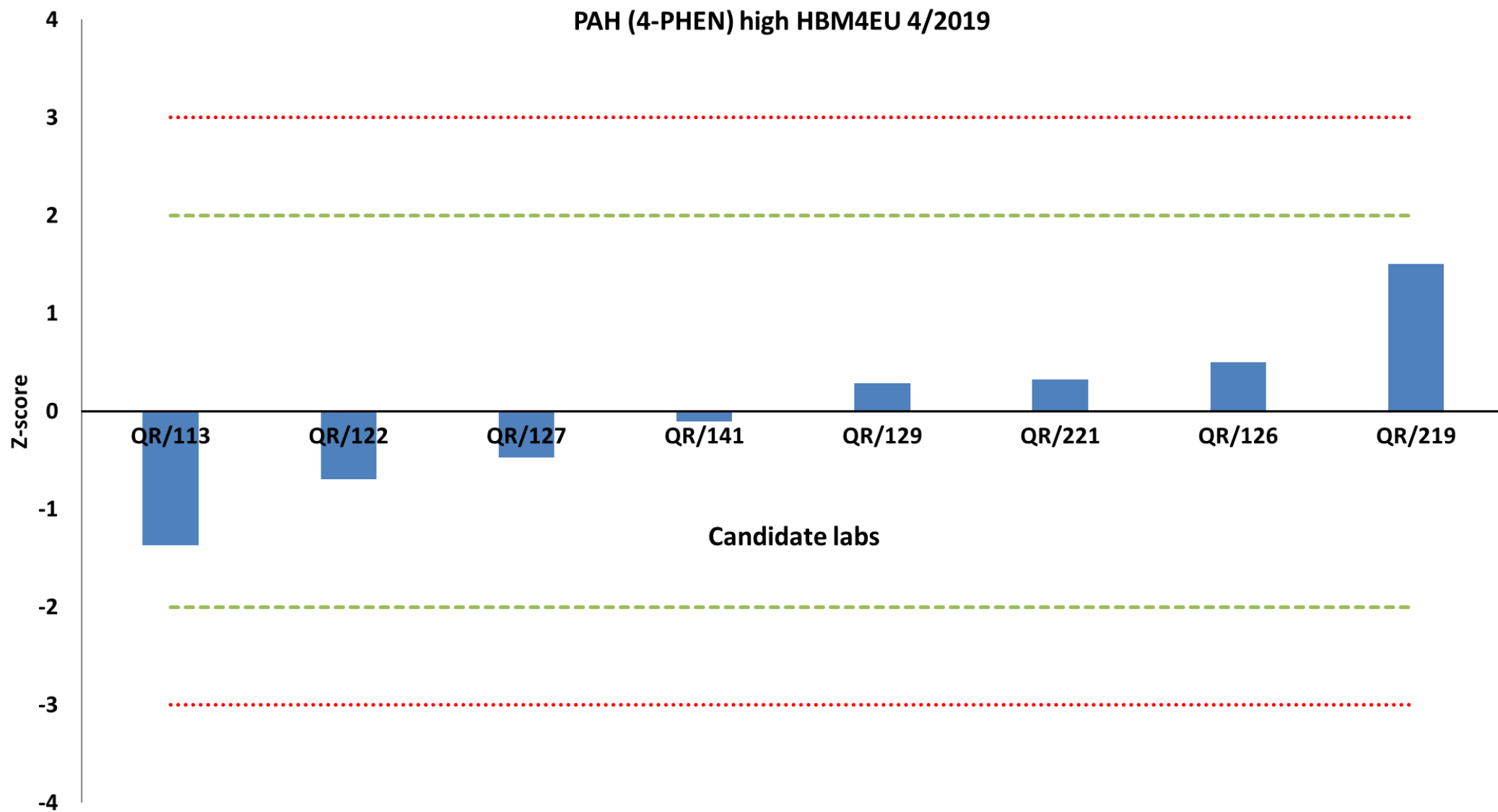


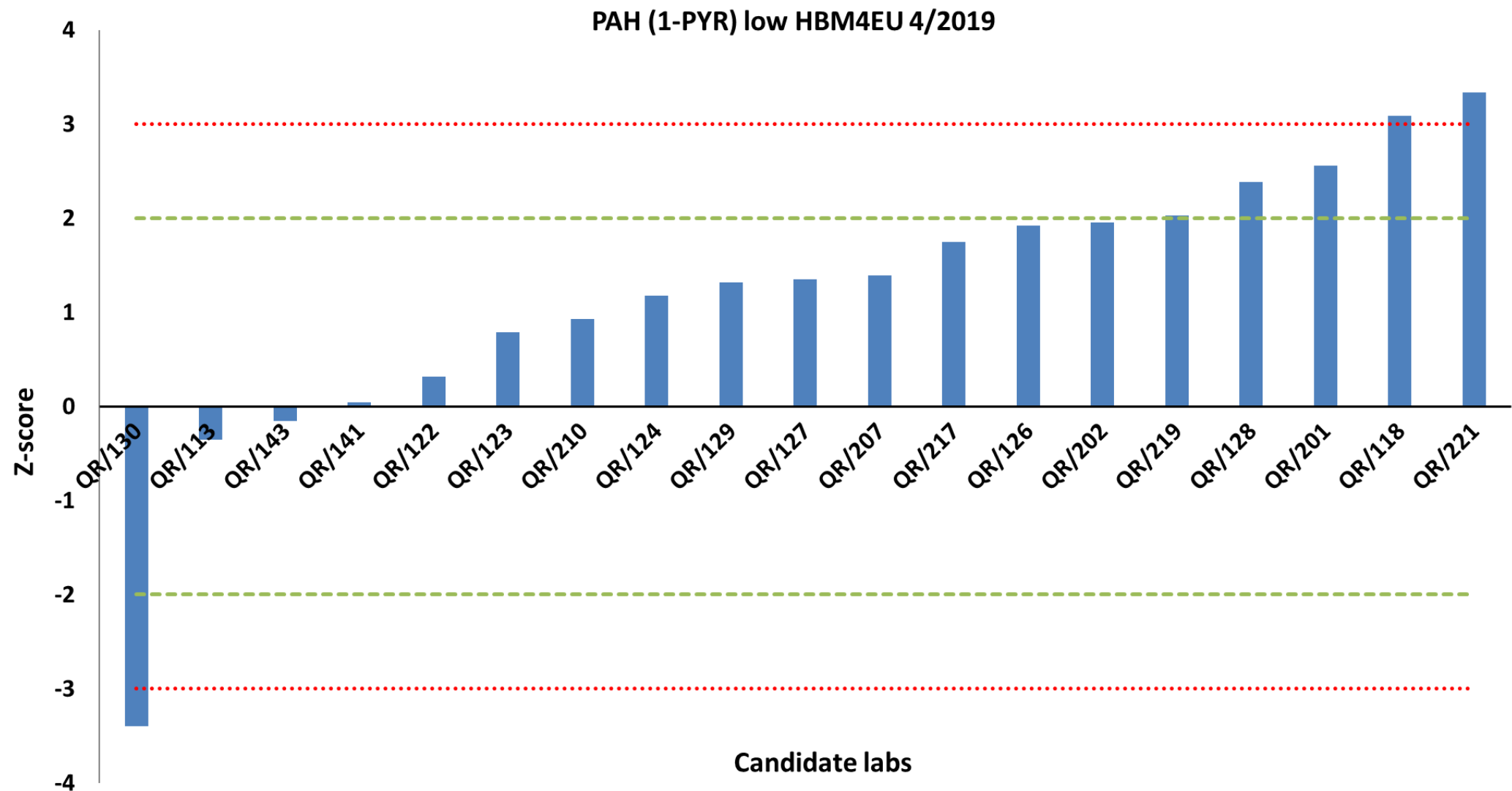
### PAH (3-PHEN) low HBM4EU 4/2019

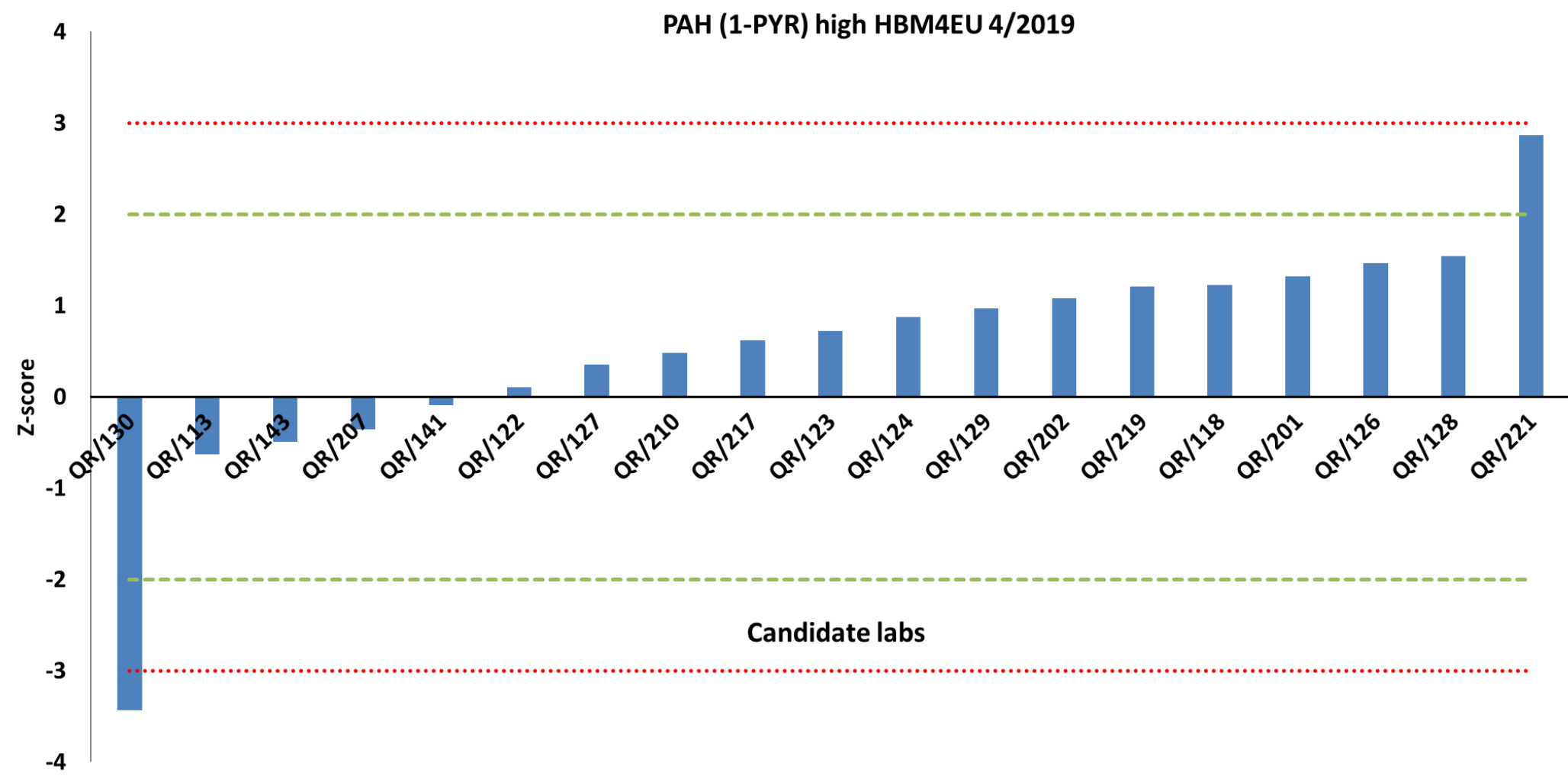














# Appendix 8. Results of the shipped test samples analysed by the participants

1-naphthol in urine [ng/mL]								
Lab.code	low			high			LOQ	delayed reporting
EX/102	2.640	2.670	2.540	11.870	12.140	12.270	0.340	
	2.450	2.480	2.600	12.110	12.040	12.030		
EX/105	3.550	3.650	3.630	12.200	12.300	12.500	0.200	
	3.650	3.680	3.720	12.600	12.600	12.900		
QR/113	3.585	3.298	3.319	12.584	11.312	12.002	0.015	
	3.215	3.199	3.486	11.024	11.528	12.588		
QR/118	3.460			12.700			1.400	
QR/122	1.459	1.846	1.474	6.117	5.923	6.164	0.100	
	1.461	1.522	1.429	5.972	6.031	6.144		
QR/123	NA			NA			-	
QR/124	2.470			10.700			1.000	
QR/126	2.644			10.819			0.025	
QR/127	3.801			13.818			0.010	
QR/128	3.380			12.310			0.200	
QR/129	3.940			17.100			0.001	
QR/130	NA			NA			-	
QR/140	2.900			9.640			2.000	
QR/141	3.450			12.791			0.500	
QR/143	NA			NA			-	
QR/201	6.500			13.300			6.000	
QR/202	NA			NA			-	
QR/207	4.737			8.483			2.000	
QR/210	4.125			13.517			0.400	
QR/217	NA			NA			-	
QR/219	3.730			11.600			1.340	
QR/221	4.264			8.516			0.806	staff shortage

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

2-naphthol in urine [ng/mL]								
Lab.code	low			high			LOQ	delayed reporting
EX/102	6.480	6.380	6.230	12.050	12.250	12.310	0.290	
	6.270	6.490	6.590	12.170	12.290	12.000		
EX/105	5.540	5.570	5.600	10.200	10.300	10.400	0.300	
	5.620	5.690	5.720	10.500	10.600	10.800		
QR/113	5.073	5.293	5.517	9.719	9.502	10.212	0.017	
	5.100	5.134	5.192	9.349	9.438	10.188		
QR/118	6.060			11.000			1.400	
QR/122	3.922	4.960	3.849	7.266	7.142	7.251	0.050	
	3.925	4.068	3.923	7.171	7.213	7.247		
QR/123	NA			NA			-	
QR/124	5.000			9.390			1.000	
QR/126	6.147			12.495			0.010	
QR/127	6.195			10.919			0.010	
QR/128	5.650			10.390			0.200	
QR/129	5.740			12.300			0.001	
QR/130	NA			NA			-	
QR/140	5.700			10.300			0.500	
QR/141	5.701			10.604			0.050	
QR/143	NA			NA			-	
QR/201	4.800			8.050			6.000	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	1.377			5.250			0.400	
QR/217	NA			NA			-	
QR/219	7.590			11.000			4.210	
QR/221	11.099			18.161			0.511	staff shortage

NA = not analysed

**Appendix 8. Results of the shipped test samples analysed by the participants (continued)**

<b>2- hydroxyFLUOrene in urine [ng/mL]</b>								
<b>Lab.code</b>	<b>low</b>			<b>high</b>			<b>LOQ</b>	<b>delayed reporting</b>
<b>EX/102</b>	0.500	0.530	0.480	1.020	1.000	1.070	0.070	
	0.480	0.480	0.520	1.000	1.040	1.000		
<b>EX/105</b>	0.450	0.444	0.457	0.874	0.896	0.889	0.030	
	0.460	0.463	0.469	0.903	0.901	0.923		
<b>QR/113</b>	0.525	0.567	0.554	1.030	1.001	1.094	0.009	
	0.578	0.540	0.551	1.072	1.036	1.152		
<b>QR/118</b>	NA			NA			-	
<b>QR/122</b>	0.928	0.870	0.910	1.781	1.773	1.838	0.050	
	0.903	0.941	0.906	1.711	1.795	1.786		
<b>QR/123</b>	NA			NA			-	
<b>QR/124</b>	NA			NA			-	
<b>QR/126</b>	0.807			1.666			0.010	
<b>QR/127</b>	0.542			1.055			0.010	
<b>QR/128</b>	NA			NA			-	
<b>QR/129</b>	0.607			1.150			0.003	
<b>QR/130</b>	0.126			0.277			0.020	
<b>QR/140</b>	NA			NA			-	
<b>QR/141</b>	NA			NA			-	
<b>QR/143</b>	NA			NA			-	
<b>QR/201</b>	NA			NA			-	
<b>QR/202</b>	NA			NA			-	
<b>QR/207</b>	NA			NA			-	
<b>QR/210</b>	0.494			0.955			0.020	
<b>QR/217</b>	NA			NA			-	
<b>QR/219</b>	1.270			1.800			0.132	
<b>QR/221</b>	NA			NA			-	staff shortage

NA = not analysed;

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

<b>3- hydroxyFLUOrene in urine [ng/mL]</b>								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.130	0.140	0.140	0.340	0.350	0.380	0.060	
	0.130	0.130	0.150	0.360	0.360	0.360		
EX/105	0.191	0.189	0.194	0.437	0.445	0.444	0.030	
	0.193	0.195	0.199	0.448	0.446	0.461		
QR/113	0.165	0.184	0.189	0.384	0.386	0.398	0.011	
	0.176	0.184	0.178	0.404	0.400	0.415		
QR/118	NA			NA			-	
QR/122	NA			NA			-	
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	NA*			NA*			0.050	
QR/127	ND			0.337			0.050	
QR/128	NA			NA			-	
QR/129	0.212			0.584			0.003	
QR/130	NA			NA			-	
QR/140	NA			NA			-	
QR/141	NA			NA			-	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	NA			NA			-	
QR/217	NA			NA			-	
QR/219	0.451			0.734			0.190	
QR/221	NA			NA			-	staff shortage

\* = analysed the sum of 3- and 9-FLUO

ND = not detected; NA = not analysed

**Appendix 8. Results of the shipped test samples analysed by the participants (continued)**

<b>9- hydroxyFLUOrene in urine [ng/mL]</b>								
<b>Lab.code</b>	<b>low</b>			<b>high</b>			<b>LOQ</b>	<b>delayed reporting</b>
<b>EX/102</b>	0.490	0.520	0.500	1.130	1.100	1.160	0.060	
	0.490	0.480	0.510	1.190	1.160	1.140		
<b>EX/105</b>	NA			NA			-	
<b>QR/113</b>	0.653	0.679	0.680	1.269	1.230	1.328	0.010	
	0.677	0.647	0.668	1.279	1.319	1.395		
<b>QR/118</b>	NA			NA			-	
<b>QR/122</b>	NA			NA			-	
<b>QR/123</b>	NA			NA			-	
<b>QR/124</b>	NA			NA			-	
<b>QR/126</b>	NA*			NA*			0.050	
<b>QR/127</b>	NA			NA			-	
<b>QR/128</b>	NA			NA			-	
<b>QR/129</b>	NA			NA			-	
<b>QR/130</b>	NA			NA			-	
<b>QR/140</b>	NA			NA			-	
<b>QR/141</b>	NA			NA			-	
<b>QR/143</b>	NA			NA			-	
<b>QR/201</b>	NA			NA			-	
<b>QR/202</b>	NA			NA			-	
<b>QR/207</b>	NA			NA			-	
<b>QR/210</b>	NA			NA			-	
<b>QR/217</b>	NA			NA			-	
<b>QR/219</b>	0.529			0.777			0.288	
<b>QR/221</b>	NA			NA			-	staff shortage

\* = analysed the sum of 3- and 9-FLUO

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

<b>1-hydroxyPHENanthrene in urine [ng/mL]</b>								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.270	0.300	0.280	0.510	0.540	0.550	0.070	
	0.300	0.310	0.300	0.540	0.550	0.520		
EX/105	0.206	0.197	0.203	0.358	0.368	0.360	0.030	
	0.214	0.209	0.208	0.369	0.366	0.380		
QR/113	0.155	0.163	0.167	0.285	0.287	0.297	0.003	
	0.160	0.175	0.159	0.291	0.298	0.313		
QR/118	NA			NA			-	
QR/122	0.250	0.273	0.255	0.464	0.463	0.468	0.005	
	0.250	0.259	0.251	0.467	0.440	0.444		
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	0.225			0.539			0.010	
QR/127	0.292			0.443			0.010	
QR/128	NA			NA			-	
QR/129	0.249			0.470			0.003	
QR/130	NA			NA			-	
QR/140	NA			NA			-	
QR/141	0.350			0.642			0.010	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	0.350			0.592			0.020	
QR/217	NA			NA			-	
QR/219	0.307			0.493			0.288	
QR/221	NA**			NA**			0.174	staff shortage

\*\* = analysed the sum of 1-PHEN and 9-PHEN

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

2-hydroxyPHENanthrene in urine [ng/mL]								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.030	0.060	0.050	0.150	0.140	0.160	0.040	
	0.040	0.030	0.030	0.130	0.130	0.110		
EX/105	NA			NA			-	
QR/113	0.103	0.109	0.110	0.222	0.219	0.232	0.004	
	0.106	0.110	0.108	0.220	0.246	0.251		
QR/118	NA			NA			-	
QR/122	0.117	0.134	0.119	0.263	0.260	0.273	0.005	
	0.119	0.122	0.121	0.255	0.265	0.264		
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	0.122			0.280			0.010	
QR/127	0.140			0.294			0.010	
QR/128	NA			NA			-	
QR/129	0.187			0.394			0.001	
QR/130	NA			NA			-	
QR/140	NA			NA			-	
QR/141	0.110			0.261			0.010	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	0.142			0.302			0.020	
QR/217	NA			NA			-	
QR/219	0.169			0.215			0.059	
QR/221	0.108			0.653			0.084	staff shortage

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

<b>3-hydroxyPHENanthrene in urine [ng/mL]</b>								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.180	0.180	0.170	0.360	0.360	0.360	0.230	
	0.170	0.180	0.180	0.370	0.370	0.360		
EX/105	NA			NA			-	
QR/113	0.177	0.172	0.180	0.303	0.312	0.350	0.004	
	0.209	0.160	0.184	0.296	0.334	0.337		
QR/118	NA			NA			-	
QR/122	0.200	0.217	0.202	0.379	0.368	0.378	0.005	
	0.208	0.209	0.200	0.363	0.371	0.375		
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	0.226			0.436			0.010	
QR/127	0.191			0.374			0.010	
QR/128	NA			NA			-	
QR/129	0.125			0.263			0.001	
QR/130	NA			NA			-	
QR/140	NA			NA			-	
QR/141	0.180			0.333			0.010	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	0.187			0.336			0.020	
QR/217	NA			NA			-	
QR/219	ND			ND			0.369	
QR/221	0.256			0.522			0.051	staff shortage

ND = not detected

NA = not analysed



# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

<b>4-hydroxyPHENanthrene in urine [ng/mL]</b>								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.870	0.850	0.820	1.030	1.020	1.020	0.070	
	0.830	0.910	0.860	1.030	1.050	1.050		
EX/105	NA			NA			-	
QR/113	0.053	0.049	0.057	0.119	0.116	0.120	0.003	
	0.064	0.054	0.056	0.122	0.123	0.137		
QR/118	NA			NA			-	
QR/122	0.068	0.073	0.067	0.156	0.158	0.156	0.005	
	0.071	0.066	0.069	0.159	0.161	0.154		
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	0.136			0.219			0.010	
QR/127	0.108			0.169			0.010	
QR/128	NA			NA			-	
QR/129	0.078			0.208			0.003	
QR/130	NA			NA			-	
QR/140	NA			NA			-	
QR/141	0.077			0.188			0.010	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	NA***			NA***			0.040	
QR/217	NA			NA			-	
QR/219	0.122			0.271			0.313	
QR/221	ND			0.210			0.086	staff shortage

\*\*\* = analysed the sum of 4-PHEN and 9-PHEN

ND = not detected

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

<b>9-hydroxyPHENanthrene in urine [ng/mL]</b>								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.160	0.190	0.170	0.250	0.270	0.270	0.110	
	0.180	0.190	0.170	0.250	0.240	0.260		
EX/105	NA			NA			-	
QR/113	NA			NA			-	
QR/118	NA			NA			-	
QR/122	0.101	0.114	0.112	0.116	0.120	0.118	0.005	
	0.102	0.109	0.117	0.133	0.125	0.122		
QR/123	NA			NA			-	
QR/124	NA			NA			-	
QR/126	0.280			0.242			0.025	
QR/127	0.054			0.060			0.010	
QR/128	NA			NA			-	
QR/129	NA			NA			-	
QR/130	NA			NA			0.200	
QR/140	NA			NA			-	
QR/141	NA			NA			-	
QR/143	NA			NA			-	
QR/201	NA			NA			-	
QR/202	NA			NA			-	
QR/207	NA			NA			-	
QR/210	NA***			NA***			0.040	
QR/217	NA			NA			-	
QR/219	ND			0.601			0.313	
QR/221	NA**			NA**			0.174	staff shortage

\*\* = analysed the sum of 1-PHEN and 9-PHEN

\*\*\* = analysed the sum of 4-PHEN and 9-PHEN

ND = not detected

NA = not analysed

# Appendix 8. Results of the shipped test samples analysed by the participants (continued)

1-hydroxyPYRene in urine [ng/mL]								
Lab.code	low			high			LOQ	delayed reporting
EX/102	0.110	0.110	0.120	0.230	0.190	0.220	0.090	
	0.120	0.120	0.130	0.200	0.230	0.190		
EX/105	ND	ND	ND	0.355	0.344	0.367	0.200	
	ND	ND	ND	0.359	0.355	0.377		
QR/113	0.101	0.096	0.108	0.226	0.204	0.214	0.013	
	0.107	0.093	0.112	0.196	0.208	0.231		
QR/118	0.200			0.330			0.200	
QR/122	0.123	0.115	0.122	0.255	0.262	0.262	0.010	
	0.126	0.123	0.122	0.254	0.265	0.258		
QR/123	0.135			0.298			0.010	
QR/124	0.146			0.308			0.020	
QR/126	0.167			0.345			0.025	
QR/127	0.151			0.275			0.010	
QR/128	0.180			0.350			0.100	
QR/129	0.150			0.314			0.003	
QR/130	0.017			0.036			0.010	
QR/140	ND			ND			0.200	
QR/141	0.114			0.247			0.010	
QR/143	0.122	0.109	0.097	0.220	0.211	0.207	0.010	
	0.105	0.096	0.121	0.231	0.229	0.232		
QR/201	0.185			0.336			0.050	
QR/202	0.168			0.321			0.020	
QR/207	0.152			0.230			0.200	
QR/210	0.139			0.283			0.050	
QR/217	0.162			0.292			0.010	
QR/219	0.170			0.329			0.052	
QR/221	0.207			0.434			0.025	staff shortage

ND = not detected