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Report of the ICI/EQUAS

Round 02/2020

Anilines (aromatic amines) in urine

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1 Summary

Within the framework of the HBM4EU project, an External Quality Assurance Scheme (EQUAS) and an Inter-laboratory Comparison Investigation (ICI) was organized and conducted for the analysis of aromatic amines (AA) in urine.

The study was performed from Nov 2019 until Jan 2020. In total, 18 laboratories were invited for this 2nd EQUAS/ICI Round, of which eleven laboratories from four countries registered. Ten laboratories submitted results yielding in a participation rate of 91 %.

In November 2019, six different test samples of low and high concentration, consisting of 8 mL urine spiked with aromatic amines were sent on dry ice to the participating expert laboratories for single analysis. Candidate laboratories received six samples, three for the low and high concentration levels, respectively, for single analysis. The HBM4EU QAU selected three expert laboratories for analysis of aromatic amines in urine (at least four per parameter). In order to obtain a sufficient number of laboratories for all six parameters, one candidate was asked to perform analysis for two of the biomarkers as an expert laboratory.

Assessment of the control material according to Thompson (2006) confirmed the adequate homogeneity for all substances except for the low level of Aniline (AN). However, with additional allowance for sampling errors and repeatability, homogeneity for AN was sufficient (Fearn and Thompson, 2001). No significant instability was detected for the aromatic amines investigated.

The performance of the laboratories was assessed by calculating Z-Scores using the assigned value, mean of expert laboratories (EQUAS) or robust mean of all participants (ICI), and a fixed fit-for-purpose target standard deviation of 25 %. Assigned values and thus Z-scores could be calculated for all aromatic amines except for aniline (AN), since the requirements for evaluating the Z-Scores were not met. An overview of the results for the analytes investigated is given in Table 1.

Table 1: Overview of the results for aromatic amines in urine, round 2

Analyte	participants	evaluation scheme	expert labs (after exclusion of outliers)	assigned value	Satisfactory	Questionable	Unsatisfactory
2,4-TDA$_{LOW}$	8	ICI	-	39.7 ng/mL	7 (87.5 %)	0 (0. %)	1 (12.5 %)
2,4-TDA$_{HIGH}$	8	ICI	-	133.8 ng/mL	7 (87.5 %)	0 (0 %)	1 (12.5 %)
2,6-TDA$_{LOW}$	8	ICI	-	45.3 ng/mL	6 (75.0 %)	1 (12.5 %)	1 (12.5 %)
2,6-TDA$_{HIGH}$	8	ICI	-	191.3 ng/mL	7 (87.5 %)	0 (0 %)	1 (12.5 %)
Aniline$_{LOW}$	0	ICI	-	not evaluated	no Z-scores	no Z-scores	no Z-scores
Aniline$_{HIGH}$	0	ICI	-	not evaluated	no Z-scores	no Z-scores	no Z-scores
MDA$_{LOW}$	9	ICI	-	5.6 ng/mL	8 (89.0 %)	0 (0 %)	1 (11.0 %)
MDA$_{HIGH}$	9	ICI	-	92.7 ng/mL	8 (89.0 %)	0 (0 %)	1 (11.0 %)
MOCA$_{LOW}$	8	ICI	-	12.4 ng/mL	7 (87.5 %)	1 (12.5 %)	0 (0 %)
MOCA$_{HIGH}$	8	EQUAS	3	127.2 ng/mL	8 (100.0 %)	0 (0 %)	0 (0 %)
TOL$_{LOW}$	5	EQUAS	3	0.28 ng/mL	5 (100.0 %)	0 (0 %)	0 (0 %)
TOL$_{HIGH}$	5	EQUAS	3	1.36 ng/mL	5 (100.0 %)	0 (0 %)	0 (0 %)

Except for TOL (low and high) and MOCA (high), the uncertainty of the expert-derived mean was too high to be used as assigned value. In these cases the assigned value was determined using the consensus value of the ICI (SOP HBM4EU-SOP-QA-003). Table 2 gives an overview of the laboratories performance for each analyte, both for the low and high concentration level.

Table 2: Results of participating laboratories for corresponding aromatic amines in urine in the low and high concentration range with (+) satisfactory, (o) questionable, (-) unsatisfactory, (NA) not analyzed, (ND) not detected, (NE) not evaluable.

	2,4-TDA		**2,6-TDA**		**AN**		**MDA**		**MOCA**		**TOL**		***SUM of biomarkers analyzed***
Lab	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	
AA_01	+	+	+	+	NE	NE	+	+	+	+	+	+	*6*
AA_03	+	+	o	+	NA	NA	+	+	+	+	NA	NA	*4*
AA_05	NA	NA	NA	NA	ND	NE	NA	NA	NA	NA	NA	NA	*1*
AA_06	NA	NA	NA	NA	NA	NA	+	+	NA	NA	NA	NA	*1*
AA_07	+	+	+	+	NA	NA	+	+	+	+	NA	NA	*4*
AA_10	+	+	+	+	NA	NA	+	+	+	+	+	+	*5*
AA_12	+	+	+	+	NA	NA	+	+	o	+	NA	NA	*4*
AA_16	-	-	-	-	NE	NE	-	-	+	+	+	+	*6*
AA_21	+	+	+	+	NE	NE	+	+	+	+	+	+	*6*
AA_33	+	+	+	+	NE	NE	+	+	+	+	+	+	*6*
SUM	*8*	*8*	*8*	*8*	*5*	*5*	*9*	*9*	*8*	*8*	*5*	*5*	

2 Introduction

Inter-Laboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS) are tools to assess 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 ICI/EQUAS study has been organized within the frame of HBM4EU as part of the Quality Assurance program for biomonitoring analyses. Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will analyze HBM4EU samples.

This report describes the 2nd round for anilines (aromatic amines, AA) in urine and was organized by ABF GmbH. Six anilines were included in this 2nd round of the EQUAS/ICI (Table 3). EQUAS assessment requires at least three expert laboratories (HBM4EU-SOP-QA-001) to calculate the expert assigned value by averaging the values obtained by the expert laboratories. If EQUAS evaluation was not possible, the consensus value – the robust mean of all participants – was used as assigned value.

Table 3: Aromatic amines analyzed in this EQUAS/ICI Round 2

Aromatic amines	Abbreviation
2,4-Diaminotoluene	2,4-TDA
2,6-Diaminotoluene	2,6-TDA
Aniline	AN
4,4'-Methylenedianiline	MDA
4,4'-Methylenebis(2-chloroaniline)	MOCA
o-Toluidine	TOL

3 Confidentiality

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

4 Control materials

4.1 Preparation of control material

For control material, two different pools of human non-smoker urine were adjusted to pH 4.0 and spiked with all aromatic amines investigated for the high concentration level (AA_{High}) and all aromatic amines except AN for the low concentration level (AA_{Low}). For AN_{Low} it was not necessary to fortify the urine pool. Unless otherwise stated, the stock solution contained the following aromatic amines: aniline (AN), o-toluidine (TOL), 2,4-diaminotoluene (2,4-TDA), 2,6-diaminotoluene (2,6-TDA), 4,4'-methylenedianiline (MDA), and 4,4'-methylenebis(2-chloroaniline) (MOCA). Due to poor stability of the free analytes, some of the aromatic amines were spiked as conjugates and the participants were instructed to perform a hydrolysis step.

Control material was aliquoted with a volume of 8 mL in 15 mL falcon tubes (PP, Greiner, Germany) and stored at -20 °C until shipment. Analytical methods used for measurement of homogeneity and stability are described in Appendix III. Measured concentrations are given in Appendix I (Table 6) and Appendix II (Table 7) of this report.

4.2 Homogeneity of control material

For determining the homogeneity, ten tubes per level of each control material (AA_{Low} and AA_{High}) were randomly selected from the freezer (-20 °C). Samples were thawed, homogenized by vortex shaking and analyzed in duplicates using the corresponding method (see Appendix III).

Homogeneity was evaluated according to HBM4EU-SOP-QA-002, Fearn (2001) and Thompson (2006) (Fearn and Thompson 2001, Thompson et al. 2006). Results of the homogeneity testing are given in Appendix I. No outliers were detected. Except for MOCA (low and high) and AN (low) the statistical analysis method used is suited to determine inhomogeneity. Homogeneity according to Thompson (2006) was adequate for all analytes except for AN (low). With additional allowance for sampling errors and repeatability, homogeneity was sufficient for AN with the method described by Fearn and Thompson (2001).

4.3 Stability of control material

Stability testing was performed according to HBM4EU-SOP-QA-002. Therefore, three samples each were randomly selected from the freezer (-20 °C) and analyzed at t = 0 d (day of control material preparation) and t ≥ 56 d. Control samples (AA_{Low} and AA_{High}) were analyzed by ABF with the methods described in Appendix III. Assessment of the stability was done by comparing the means of the triplicates. 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 II. Consequential instability was detected for $2,4\text{-TDA}_{Low}$, $2,6\text{-TDA}_{High}$, MDA_{High}, and $MOCA_{Low}$. However, no significant difference ($\alpha = 0.05$) was observed within the time period investigated.

5 Organizational details

For the organization of the ICI/EQUAS, the HBM4EU consortium conducted a survey to find expert laboratories for the determination of anilines in urine. Three expert laboratories, all from Europe, were selected according to HBM4EU-SOP-QA-001 and in agreement with HBM4EU QAU. For a sufficient number of laboratories for all six parameters, it was necessary to post-recruit a candidate as expert laboratory for the analysis of two biomarkers.

ABF sent the invitation letters (see Appendix III) and a registration form (Appendix V) by e-mail on September 27, 2019. It was indicated that participation would be free of charge and that the participants receive test materials needed for analysis.

Eleven laboratories signed in for the participation giving their agreement to abide by the conditions for participation. Each laboratory received an individual lab code in order to report their measurement results.

All participating laboratories including the expert labs had to submit test results within the stipulated deadline (January 14, 2020). Ten of eleven laboratories performed the assays and submitted within the deadline agreed.

6 Dispatch and instructions

Control material was dispatched on dry ice to the participants on November 26, 2019. The expert laboratories received twelve, the other participants six samples (8 mL each) of two different levels of the test material. Moreover, an instruction letter on sample handling (Appendix VI), a sample receipt form to be sent back to ABF as well as a result submission and a method information form (example see Appendix III) were sent to the participants by e-mail. The latter form was used to obtain relevant information on the analytical method used for quantification.

7 Data evaluation

7.1 False positives and <LOQ

Classification of false positives and biomarkers reported as "<LOQ value" or "not detected" (ND) was as described in HBM4EU-SOP-QA-003.

A result is assigned as false positive, if (1) the reported concentration is below the LOQ of the organizer, the expert laboratories, and the majority of the participants and (2) a concentration for a biomarker is reported that was not present in the control material.

If a biomarker is reported as '<LOQ' *and* an assigned value for the biomarker can be determined in the control material, further assessment is done to verify whether this result might be a false negative and the LOQ is considered adequate (low enough) for analysis within the frame of HBM4EU.

Results are classified as false negative, when no numerical value is reported for a biomarker present in the test material, although the laboratories LOQ is well below the assigned value.

LOQ is considered insufficient (too high), if (1) the LOQ is well above the assigned value, (2) the assigned value represents a realistic concentration of real samples within the framework of HBM4EU, and (3) quantitative determination is feasible by the majority of laboratories.
In order to judge results <LOQ in a quantitative way, 'proxy-Z-scores' need to be calculated (see chapter 7.5).

7.2 Assigned value

For EQUAS assessment, the concentration determined by the expert laboratories is used as assigned value. Therefore, at least three expert laboratories must provide assessable results. For each expert laboratory the individual means and standard deviations were calculated. The mean of all individual means (mean-of-means, mom) and its standard deviation (SD_{mom}) were calculated with the relative uncertainty given in Equation 1.

$$u = \frac{SD_{mom}}{\sqrt{n}} \qquad \text{Equation 1}$$

u uncertainty of the mean of the mean concentrations from the expert labs
SD_{mom} standard deviation of the mean of the mean concentrations
n number of expert laboratories (after exclusion of outliers if applicable)

The mean of all expert laboratories is considered suitable as assigned value for EQUAS studies, if $u \leq 0.7\ \sigma_T$ (σ_T see section 7.3). When $u > 0.7\ \sigma_T$, individual means are checked for outliers. Extreme outliers that are outside the range of ± 50 % of the median are excluded from the data set. If exclusion of outliers doesn't yield into reliable results and u is still $> 0.7\ \sigma_T$, 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.
When no EQUAS assessment of the participants' performance is possible or the number of expert laboratories is not sufficient for EQUAS assessment, the consensus value is used as the assigned value and calculated as described in HBM4EU-SOP-QU-003. In brief, the consensus value and its uncertainty is calculated from the results submitted by the participants using robust statistic in order to minimize the influence of outliers. The robust mean is taken as consensus value. The uncertainty of the consensus values is calculated according to Equation 2. For calculating Z-scores two criteria were evaluated:
(1) If $u \leq 0.3\ \sigma_T$, the uncertainty of the consensus value may be considered negligible, otherwise another performances score must be calculated taken into account the uncertainty.
(2) If $u \leq 0.7\ \sigma_T$, the uncertainty of the consensus value is within acceptable limits and Z-scores can be provided.

$$u = 1.25\ \frac{\hat{\sigma}}{\sqrt{n}} \qquad \text{Equation 2}$$

u uncertainty of the consensus value
$\hat{\sigma}$ standard deviation of the participants' results
n number of results used for calculating the consensus value

7.3 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose target standard deviation (FFP) of 25 % of the assigned value is used as target standard deviation (σ_T). This was the default standard indicated in HBM4EU-SOP-QA-003.

7.4 Z-scores

Z-scores are calculated according to SOP HBM4EU-SOP-QA-003 using Equation 3, if the biomarker is stable and the uncertainty of the consensus value is negligible or Equation 4, if the biomarker is stable and the uncertainty of the consensus value is not negligible.

$$Z = \frac{x-C}{\sigma_T}$$ Equation 3

Z Z-score for submitted analysis result
x result submitted by the laboratory
C expert assigned value and consensus value, respectively
σ_T target standard deviation, here 0.25 C

$$Z' = \frac{x-C}{\sqrt{\sigma_T^2+u^2}}$$ Equation 4

Z' Z-score for submitted analysis result
x result submitted by the laboratory
C expert assigned value and consensus value, respectively
σ_T target standard deviation, here 0.25 C
u uncertainty of the consensus value

In accordance with ISO 13528 and ISO 17043, Z-scores are classified as presented in Table 4.

Table 4: Classification of Z-Scores

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

7.5 Proxy-Z-scores

In order to judge results <LOQ in a quantitative way, 'proxy-Z-scores' need to be calculated with the LOQ given by the participant using Equation 3 (chapter 7.4). If no LOQ is specified, zero will be used. Proxy-Z-Scores are for information only and are therefore indicated in brackets.

Calculated proxy-Z-Scores can be interpreted as follows (HBM4EU-SOP-QA-003):

proxy-Z ≤ -3 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.

-3 ≤ proxy-Z < -2 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'.

-2 ≤ proxy-Z ≤ 2 -2 to 0: Based on the assigned value and the LOQ provided, the result cannot be classified as false negative.
0 to 2: The LOQ is in the range of what is analytically feasible[1].

2 < proxy-Z < 3 The LOQ is high compared to what is analytically feasible[1] and might be high in relation to HBM4EU analysis. The laboratory should consider to lower their LOQ.

proxy-Z ≥3 The LOQ is too high compared to what is analytically feasible[1] and might be too high in relation to HBM4EU analysis. The laboratory should consider to lower their LOQ.

8 Results and Discussion

8.1 Participants' Results

In total, eleven laboratories from four countries agreed to participate in this study, of which ten submitted results. Appendix VII (Table 8-Table 9) gives an overview of the results submitted by the participants.
For AN (low), one participant (AA_05) submitted results as 'not detected' (ND). Since the requirements for evaluating the Z-Scores for AN were not met, proxy-Z-Scores could not be calculated. No participant detected a false positive or negative result.

8.2 Assigned values and (target) standard deviations

For **MOCA** (high) and **TOL** (low and high), the results from the expert laboratories were used for calculating the mean, which was used as the assigned value.
In case of **2,4-TDA** (low and high), **2,6-TDA** (low and high), **MDA** (low and high), and **MOCA** (low), the consensus value of the ICI was used as assigned value, since uncertainty of the expert laboratories was too high ($u > 0.7\ \sigma_T$) to calculate an assigned value.
For **AN** (low and high), EQUAS requirements after exclusion of outliers did not meet the criterion ($u > 0.7\ \sigma_T$) and a consensus value could not be calculated due to the lack of participants. Thus, no assigned value could be determined.
Assigned consensus value (EQUAS or ICI) and its uncertainty, standard deviation (SD), fit-for-purpose (FFP) relative target standard deviation (σ_T) and study RSD_R of each analyte are given in Appendix VIII.

8.3 Assessment of laboratory performance

An overview of the number of laboratories with satisfactory, questionable, and unsatisfactory Z-scores for the respective aromatic amines as well as the evaluation scheme applied can be found in Table 1, Chapter 1. More detailed results of the laboratory performance for the respective biomarker in the low and high concentration level is given in Table 2, Chapter 1.

[1] 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.

Z-Scores could be calculated for the low and high concentration level of 2,4-TDA, 2,6-TDA, MDA, MOCA, and TOL. Graphical evaluation of the laboratories' performance is given in Appendix IX (Figure 1-Figure 5).

In general, a satisfactory performance was achieved for TOL (100 % for TOL_{Low} and TOL_{High}) and MOCA (100 % for $MOCA_{Low}$ and 88 % for $MOCA_{High}$). Higher variations within the data set were observed for 2,6-TDA_{Low} with 75 % satisfactory results. For AN (low and high), no Z-Scores could be provided, since a calculation of an assigned value was not possible due to the high variability of the results derived from the expert laboratories and an insufficient number (N < 7) of participating laboratories for Z-score calculation.

8.4 Conclusion and recommendations

The HBM4EU EQUAS/ICI Round 2 was performed successfully, however, the total number of participating labs was quite low compared to other HBM4EU EQUAS/ICI programs. Ten of Eleven registered laboratories reported results. Table 5 gives an overview of the laboratories' performance and the LOQ for the corresponding aromatic amines in urine. Evaluation of laboratory performance was possible for five aromatic amines: 2,4-TDA, 2,6-TDA, MDA, MOCA, and TOL. Percentage of satisfactory Z-scores was between 75 % and 100 %. Except for TOL (low and high) and MOCA (high), the uncertainty within the three expert laboratories was too high ($u > 0.7\ \sigma_T$) for EQUAS assessment so that the consensus value (ICI) was calculated. The submitted results of AN were not sufficient for an evaluation and therefore this analyte does not appear suitable for an EQUAS/ICI. The general reason for the problems emerged is an insufficient number of participating laboratories and no suitable assigned value derived from the expert results.

Table 5: Performance of the participating laboratories for aromatic amines in urine

Laboratory code	LOQ [ng/mL]	2,4-TDA$_{Low}$	2,4-TDA$_{High}$
AA_01	25.0	Satisfactory	Satisfactory
AA_03	1.0	Satisfactory	Satisfactory
AA_05	NA	NA	NA
AA_06	NA	NA	NA
AA_07	0.3	Satisfactory	Satisfactory
AA_10	1.0	Satisfactory	Satisfactory
AA_12	0.6	Satisfactory	Satisfactory
AA_16	0.05	Unsatisfactory	Unsatisfactory
AA_21	0.2	Satisfactory	Satisfactory
AA_33	1.0	Satisfactory	Satisfactory

		2,6-TDA$_{Low}$	2,6-TDA$_{High}$
AA_01	25.0	Satisfactory	Satisfactory
AA_03	1.0	Questionable	Satisfactory
AA_05	NA	NA	NA
AA_06	NA	NA	NA
AA_07	0.3	Satisfactory	Satisfactory
AA_10	1.0	Satisfactory	Satisfactory
AA_12	0.6	Satisfactory	Satisfactory
AA_16	0.1	Unsatisfactory	Unsatisfactory
AA_21	0.2	Satisfactory	Satisfactory
AA_33	1.0	Satisfactory	Satisfactory

		AN$_{Low}$	AN$_{High}$
AA_01	0.2	NE	NE
AA_03	0.5	NA	NA
AA_05	2.0	ND	NE
AA_06	NA	NA	NA
AA_07	NA	NA	NA
AA_10	NA	NA	NA
AA_12	NA	NA	NA
AA_16	0.1	NE	NE
AA_21	2.0	NE	NE
AA_33	1.0	NE	NE

Table 5 continued

Laboratory code	LOQ [ng/mL]	MDA_{Low}	MDA_{High}
AA_01	0.1	Satisfactory	Satisfactory
AA_03	0.1	Satisfactory	Satisfactory
AA_05	NA	NA	NA
AA_06	1.0	Satisfactory	Satisfactory
AA_07	0.3	Satisfactory	Satisfactory
AA_10	0.5	Satisfactory	Satisfactory
AA_12	1.0	Satisfactory	Satisfactory
AA_16	0.03	Unsatisfactory	Unsatisfactory
AA_21	0.05	Satisfactory	Satisfactory
AA_33	0.1	Satisfactory	Satisfactory

		$MOCA_{Low}$	$MOCA_{High}$
AA_01	10.0	Satisfactory	Satisfactory
AA_03	0.1	Satisfactory	Satisfactory
AA_05	NA	NA	NA
AA_06	NA	NA	NA
AA_07	0.3	Satisfactory	Satisfactory
AA_10	1.3	Satisfactory	Satisfactory
AA_12	1.3	Questionable	Satisfactory
AA_16	0.05	Satisfactory	Satisfactory
AA_21	0.2	Satisfactory	Satisfactory
AA_33	1.0	Satisfactory	Satisfactory

		TOL_{Low}	TOL_{High}
AA_01	0.01	Satisfactory	Satisfactory
AA_03	0.05	NA	NA
AA_05	NA	NA	NA
AA_06	NA	NA	NA
AA_07	NA	NA	NA
AA_10	0.2	Satisfactory	Satisfactory
AA_12	NA	NA	NA
AA_16	0.1	Satisfactory	Satisfactory
AA_21	0.05	Satisfactory	Satisfactory
AA_33	0.2	Satisfactory	Satisfactory

With $|Z| \leq 2$ – Satisfactory, $2 < |Z| < 3$ – Questionable, $|Z| \geq 3$ - Unsatisfactory.
NA – not analyzes. ND – not detected. NE – not evaluable.

9 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 test materials for ICI / EQUAS’

HBM4EU-SOP-QA-003 ‘Evaluation of ICI / EQUAS results’

HBM4EU-SOP-QA-004 ‘Reporting of ICI / EQUAS studies’

ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing

ISO 13528, 2015, Statistical methods for use in proficiency testing by interlaboratory comparison.

Fearn, T. and M. Thompson (2001). *A new test for ‘sufficient homogeneity’.* Analyst 126(8): 1414-1417.

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Appendix

I Homogeneity data

Table 6: Homogeneity data of 2,4-TDA, 2,6-TDA, Aniline, MDA, MOCA, and o-Toluidine for low and high concentration range.

	2,4-TDA				**2,6-TDA**				**Aniline**			
	Low [ng/mL]		High [ng/mL]		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	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	41.5	40.7	140.0	128.0	43.6	45.6	213.0	186.0	1.4	1.3	10.1	9.6
	36.6	35.5	140.0	140.0	47.4	45.4	217.0	206.0	1.6	1.4	10.4	9.8
	39.4	42.3	145.0	133.0	44.5	45.0	198.0	215.0	1.9	1.6	9.9	9.7
	36.2	36.6	132.0	130.0	44.8	44.7	203.0	211.0	2.0	1.5	9.6	9.7
	38.7	35.5	133.0	128.0	45.0	47.9	190.0	214.0	1.9	1.5	9.7	9.5
	36.6	40.7	150.0	137.0	45.5	47.3	198.0	213.0	1.7	1.4	9.5	9.5
	35.6	41.6	137.0	133.0	47.7	43.6	205.0	208.0	1.5	1.4	9.5	9.4
	37.0	39.1	130.0	140.0	46.1	46.0	200.0	205.0	1.3	1.2	9.5	9.5
	35.8	38.5	142.0	132.0	50.7	45.6	210.0	196.0	1.3	1.2	9.9	9.6
	37.5	37.3	139.0	125.0	46.5	47.2	202.0	209.0	1.4	1.7	10.2	9.5
Grand mean	38.1		135.7		46.0		205.0		1.5		9.7	
Cochran's test												
C	0.4		0.2		0.4		0.3		0.3		0.4	
C_{crit}	0.6		0.6		0.6		0.6		0.6		0.6	
Detection of outliers ($C > C_{crit}$)	no outliers detected		no outliers detected		no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP}	9.5		33.9		11.5		51.2		0.4		2.4	
s_x	1.7		4.2		1.1		3.3		0.2		0.2	
s_w	2.1		6.7		1.8		10.7		0.2		0.3	
s_s	0.9		0.0		0.0		0.0		0.1		0.1	
$C_1 = 0.3\ \sigma_{FFP}$	2.9		10.2		3.5		15.4		0.1		0.7	
$s_s < C_1$?	Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate		No, homogeneity inadequate		Yes, homogeneity adequate	
$s_w < 0.5\ \sigma_{FFP}$?	Yes, method suitable		Yes, method suitable		Yes, method suitable		Yes, method suitable		No, method not suitable		Yes, method suitable	
									With additional allowance for sampling errors and repeatability			
s_{sam}^2									0.0			
$C_2 = F_1 \sigma_{all}^2 + F_2 s_{an}^2$									0.1			
$s_{sam}^2 < C_2$?									Yes, homogeneity adequate			

Table 6 continued

	MDA				**MOCA**				**o-Toluidin**			
	Low [ng/mL]		High [ng/mL]		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	Replicate 1	Replicate 2	Replicate 1	Replicate 2
	7.3	6.3	108.0	112.0	9.1	10.2	110.0	91.1	0.29	0.29	1.44	1.35
	6.9	6.7	107.0	103.0	17.5	10.6	112.0	105.0	0.29	0.31	1.34	1.39
	6.3	6.9	96.9	106.0	10.0	11.9	99.3	122.0	0.30	0.30	1.42	1.40
	7.4	5.9	105.0	106.0	10.0	13.9	89.0	130.0	0.29	0.29	1.37	1.44
	6.1	6.8	95.7	112.0	8.6	11.3	126.0	122.0	0.29	0.30	1.42	1.42
	6.7	6.9	109.0	111.0	11.0	8.1	115.0	122.0	0.29	0.29	1.41	1.35
	6.3	7.1	101.0	114.0	10.1	12.1	121.0	97.3	0.29	0.29	1.37	1.35
	5.9	6.7	105.0	106.0	7.3	9.2	112.0	122.0	0.29	0.29	1.39	1.38
	6.6	7.2	102.0	112.0	9.3	11.6	135.0	125.0	0.29	0.29	1.49	1.40
	6.2	6.2	109.0	108.0	8.8	8.7	103.0	161.0	0.29	0.31	1.38	1.41
Grand mean	6.6		106.4		10.5		116.0		0.29		1.40	
Cochran's test												
C	0.4		0.4		0.5		0.5		0.52		0.29	
C_{crit}	0.6		0.6		0.6		0.6		0.60		0.60	
Detection of outliers ($C > C_{crit}$)	no outliers detected		no outliers detected		no outliers detected		no outliers detected		no outliers detected		no outliers detected	
target σ_{FFP}	1.7		26.6		2.6		29.0		0.07		0.35	
s_x	0.2		2.7		1.7		10.2		0.00		0.03	
s_w	0.5		5.7		2.2		18.4		0.01		0.04	
s_s	0.0		0.0		0.6		0.0		0.00		0.00	
$C_1 = 0.3\ \sigma_{FFP}$	0.5		8.0		0.8		8.7		0.02		0.10	
$s_s < C_1$?	Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate		Yes, homogeneity adequate	
$s_w < 0.5\ \sigma_{FFP}$?	Yes, method suitable		Yes, method suitable		No, method not suitable		No, method not suitable		Yes, method suitable		Yes, method suitable	

II Stability data

Table 7: Stability data of 2,4-TDA, 2,6-TDA, Aniline, MDA, MOCA, and o-Toluidine for low and high concentration range.

Replicate	2,4-TDA				2,6-TDA				Aniline			
	Low [ng/mL]		High [ng/mL]		Low [ng/mL]		High [ng/mL]		Low [ng/mL]		High [ng/mL]	
	t = 0 d	t = 60 d	t = 0 d	t = 60 d	t = 0 d	t = 60 d	t = 0 d	t = 60 d	t = 0 d	t = 56 d	t = 0 d	t = 56 d
1	41.5	41.8	140.0	154.0	43.6	40.3	213.0	229.0	1.4	1.7	10.1	9.9
2	36.6	44.6	140.0	142.0	47.4	47.8	217.0	231.0	1.6	1.6	10.4	9.9
3	39.4	40.2	145.0	152.0	44.5	50.8	198.0	217.0	1.9	1.6	9.9	9.8
Average	39.2	42.2	141.7	149.3	45.2	46.3	209.3	225.7	1.6	1.6	10.1	9.9
SD	2.5	2.2	2.9	6.4	2.0	5.4	10.0	7.6	0.3	0.1	0.3	0.1
Difference (x_0-x_E)	-3.0		-7.7		-1.1		-16.3		0.0		0.3	
c = 0.3 σ_{FFP}	2.9		10.6		3.4		15.7		0.1		0.8	
(x_0-x_E) < c? Consequential instability	Yes		No		No		Yes		No		No	
t	1.6		1.9		0.3		2.3		0.0		1.7	
t_{crit}	2.8		2.8		2.8		2.8		2.8		2.8	
Significant difference	No		No		No		No		No		No	

Table 7 continued

Replicate	MDA				MOCA				o-Toluidine			
	Low [ng/mL]		High [ng/mL]		Low [ng/mL]		High [ng/mL]		Low [ng/mL]		High [ng/mL]	
	t = 0 d	t = 60 d	t = 0 d	t = 60 d	t = 60 d	t = 60 d	t = 0 d	t = 60 d	t = 0 d	t = 56 d	t = 0 d	t = 56 d
1	7.28	6.51	108.0	110.0	9.09	7.06	110.0	123.0	0.3	0.3	1.4	1.4
2	6.89	7.02	107.0	115.0	17.5	7.41	112.0	89.1	0.3	0.3	1.3	1.4
3	6.33	7.11	96.9	116.0	10	8.23	99.3	101.0	0.3	0.3	1.4	1.5
Average	6.8	6.9	104.0	113.7	12.2	7.6	107.1	104.4	0.3	0.3	1.4	1.4
SD	0.5	0.3	6.1	3.2	4.6	0.6	6.8	17.2	0.0	0.0	0.1	0.0
Difference (x_0-x_E)	0.0		-9.7		4.6		2.7		0.0		0.0	
c = 0.3 σ_{FFP}	0.5		7.8		0.9		8.0		0.0		0.1	
(x_0-x_E) < c? Consequential instability	No		Yes		Yes		No		No		No	
t	0.1		2.4		1.7		0.3		1.0		1.4	
t_{crit}	2.8		2.8		2.8		2.8		2.8		2.8	
Significant difference	No		No		No		No		No		No	

III Method information form for the participation in EQUAS/ICI (Round 2)

I.I Method information for measuring homogeneity and stability of AN and TOL

Method information form for participation in ICI/EQUAS Aromatic Amines in urine/Round 2

Laboratory code		
ISO17025 accredited	Yes	
SAMPLE PREPARATION		
amount sample extracted	5 mL	g or mL
Hydrolysis	37 % HCl, 1 mL, 1 h at 80 °C	
Extraction		
- pH adjustment	6.0-6.4	
- LLE;	2x 5 mL Hexane (15 min)	
- SPE; material	--	
Cleanup		
- LLE; solvent(s)	--	
- SPE; material	--	
Derivatisation		
- reagent	PFPA	
INSTRUMENTAL ANALYSIS		
HPLC		
- injection volume	--	
- column stationary phase	--	
- column L (mm) x ID (mm); dp (µm)	--	
- temperature	--	
- mobile phase A	--	
- mobile phase B	--	
- flow rate	--	
GC		
- injector	Split (1:15)	
- injection volume	2 µL	µL
- column stationary phase	RXI-5ms	
- column L (m) x ID (mm) df (µm)	30 m, 0.25 mm ID, 0.25 µm df	
- carrier	He	
- flow rate / inlet pressure	1.1 mL/min, constant flow	

Detection		
MS	single quad	
other	--	
Quantification		
Use of internal standard (IS)	Yes	
- isotopic label	Yes	
- other	--	
- moment of addition	before deconjugationyes	
- response normalised to IS	yes/no	
Calibration	matrix-matched (addition to blank matrix before extraction)	
	multi level	
Correction for recovery	No	
Identification criteria used		
- retention time tolerance	identification via retention time of isotopically labelled std.	
- number of ions/transitions	--	
- ion ratio tolerance	--	

Further remarks/observations:

Date:

Signature:

I.II Method information for measuring homogeneity and stability of 2,4-TDA, 2,6-TDA, MDA, and MOCA

Method information form for participation in ICI/EQUAS Aromatic Amines in urine/Round 2

Laboratory code		
ISO17025 accredited	No	
SAMPLE PREPARATION		
amount sample extracted	0.25 mL	g or mL
Hydrolysis	6 M HCl, 100 µL, 4 h at 80 °C	
Extraction		
- pH adjustment	pH 1.0	
- LLE;	--	
- SPE; material	Strata XC	
Cleanup		
- LLE; solvent(s)	--	
- SPE; material	--	
Derivatisation		
- reagent	--	
INSTRUMENTAL ANALYSIS		
HPLC		
- injection volume	5	µL
- column stationary phase	Gemini C18	
- column L (mm) x ID (mm); dp (µm)	150 x 3 mm; 3 µm	
- temperature	35 °C	
- mobile phase A	NH_4OAc 5 mM; pH 9.2	
- mobile phase B	ACN + 5 mM NH_4OAc	
- flow rate	1	mL/min
GC		
- injector	--	
- injection volume	--	
- column stationary phase	--	
- column L (m) x ID (mm) df (µm)	--	
- carrier	--	
- flow rate / inlet pressure	--	

Detection		
MS	Triple Quad	
other	--	
Quantification		
Use of internal standard (IS)	Yes	
- isotopic label	Yes	
- other	specify	
- moment of addition	before acid hydrolysis	
- response normalised to IS	Yes	
Calibration	matrix-matched (addition to blank matrix before extraction)	
	multi level	
Correction for recovery	No	
Identification criteria used		
- retention time tolerance	Coherence with IS	
- number of ions/transitions	2	
- ion ratio tolerance	% relative/absolute deviation from reference standard	

Further remarks/observations:

Date:

Signature:

IV Copy of invitation letter

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

Anilines/Round 2

Title of ICI/EQUAS: Anilines in urine

Dear Colleague,

within the frame of HBM4EU the

ABF GmbH
Semmelweisstr. 5
82152 Planegg
Germany

announces the 2nd round of ICI/EQUAS for the determination of anilines (aromatic amines) 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 urine. Accordingly, the participants will receive:

- 2 different materials (levels) of urine (3 samples per level of 8 mL each) for determination of anilines/aromatic amines in urine
- expert labs will receive 6 samples per level of 8mL each for the same materials
- The analytes will be spiked into the urine as conjugates. Sample preparation including a hydrolysis step is necessary.

Target biomarkers

For the biomarkers potentially present in the test samples, please see registration form for Anilines/Round 2. We would be pleased if your laboratory could analyse as many metabolites as possible.
LOQs should allow the analysis of aromatic amines in samples of the general population.

Calendar

Registration deadline	18-10-2019
Distribution of test samples (projected)	18-11-2019
Deadline for submission of results (projected)	20-12-2019

Registration

For registration, please find attached a registration form for anilines 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:

- Dr. Nikola Pluym
- Dr. Therese Burkhardt

ABF GmbH
Semmelweisstr. 5
82152 Planegg
Germany

Email: nikola.pluym@abf-lab.com
therese.burkhardt@abf-lab.com

V Copy of registration form

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

Title of ICI/EQUAS: Anilines in urine

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

Parameter	Participation	
	yes	**no**
4,4'-MDA		
MOCA		
aniline		
o-toluidine		
2,4-TDA		
2,6-TDA		

Participating laboratory:

name of the institution

address of the laboratory

name of 1st contact person, telephone number and e-mail address

name of 2nd contact person, telephone number and e-mail address

Address for delivery of the test samples

name of the institution

address of the laboratory

The above laboratory will participate in the ICI/EQUAS study **Anilines/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:

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

nikola.pluym@abf-lab.com

therese.burkhardt@abf-lab.com

The registration deadline is 18-10-2019.

Contact information organiser:

- Nikola Pluym
- Therese Burkhardt

ABF GmbH
Semmelweisstr. 5
82152 Planegg
Germany

E-mail: nikola.pluym@.abf-lab.com
therese.burkhardt@abf-lab.com

VI Copy of instruction letter

HBM4EU: Instruction letter ICI / EQUAS study Anilines/Round 2

Title of ICI/EQUAS: Anilines in urine

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study Anilines in urine/Round 2 for the determination of anilines and aromatic amines in urine.

You will receive a parcel containing 12 test samples. Each sample consists of approximately 8 mL urine spiked with the biomarker.

The parcel will be shipped on 26.11.2019 under frozen conditions.

Instructions:

- Upon receipt, please check the content for any damage/leaking 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.
- Thaw the samples and re-homogenise them according to your own procedure.
- Due to the poor stability of the free analytes we prepared the urine material by spiking with the conjugates wherever these were available. We ask you therefore to perform your complete sample preparation procedure including the hydrolysis step.
- We are aware that some of the laboratories will need to run two methods for quantification of the parameters. Since we were limited in the volume of urine (8 mL per aliquot) we prepared the samples in a manner that shall allow analysis after dilution with respect to the reported LOQs of the most commonly used assays in the literature (we assumed the following LOQs from the literature: 1 µg/L for diamines 2,4-TDA; 2,6-TDA; MDA; MOCA; and LOQs of 50 ng/L for o-toluidine and 500 ng/L for aniline. For these LOQs, for example, a 2 to 2.5-fold dilution should be feasible).
- Analyse the samples for the biomarkers indicated in the invitation letter ref/ 27.09.2019
- 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 14.01.2020.

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

Nikola Pluym

nikola.pluym@abf-lab.com

ABF GmbH
Semmelweisstr. 5
82152 Planegg
Germany

VII Results of the control material analyzed by the participants

Table 8: Results for 2,4-TDA, 2,6-TDA, and AN of all participating laboratories

Lab Code	Sample No.	2,4-TDA [ng/mL]			2,6-TDA [ng/mL]			AN [ng/mL]		
		Low	High	LOQ	Low	High	LOQ	Low	High	LOQ
AA_01	1	41.50	140.00	25.00	43.60	213.00	25.00	1.38	10.06	0.20
	2	36.60	140.00		47.40	217.00		1.61	10.41	
	3	39.40	145.00		44.50	198.00		1.89	9.89	
	4	36.20	132.00		44.80	203.00		2.01	9.59	
	5	38.70	133.00		45.00	190.00		1.89	9.66	
	6	36.60	150.00		45.50	198.00		1.72	9.53	
	7	35.60	137.00		47.70	205.00		1.54	9.52	
	8	37.00	130.00		46.10	200.00		1.31	9.51	
	9	35.80	142.00		50.70	210.00		1.28	9.93	
	10	37.50	139.00		46.50	202.00		1.37	10.23	
AA_12	1	40.69	135.69	0.60	42.43	152.05	0.60	NA	NA	NA
	2	42.43	132.75		44.27	138.63		NA	NA	
	3	43.16	136.98		50.85	140.37		NA	NA	
	4	43.04	138.99		46.73	166.07		NA	NA	
	5	43.76	137.10		48.88	159.09		NA	NA	
	6	42.10	139.82		47.98	173.88		NA	NA	
AA_16	1	115.32	822.99	0.05	189.69	977.29	0.05	2.08	11.33	0.10
	2	115.25	860.80		189.61	915.28		2.34	11.52	
	3	121.09	851.75		175.45	906.20		2.59	12.00	
	4	107.36	862.85		161.72	917.21		2.62	12.75	
	5	117.70	792.12		172.07	846.50		2.24	12.10	
	6	112.51	854.55		166.85	908.94		2.26	12.67	
AA_21	1	38.00	136.77	0.20	44.75	225.06	0.20	24.81	16.92	2.00
	2	37.55	141.52		44.42	226.71		19.80	15.49	
	3	37.56	140.14		43.82	221.03		18.45	17.15	
	4							30.59	24.45	
	5							22.57	20.43	
	6							22.07	13.92	
AA_03	1	19.79	88.95	1.00	10.74	84.65	1.00	NA	NA	0.50
	2	16.57	78.80		13.80	95.27		NA	NA	
	3	24.18	105.76		22.05	88.22		NA	NA	
AA_05	1	NA	NA	NA	NA	NA	NA	ND	7.83	2.00
	2	NA	NA		NA	NA		ND	7.49	
	3	NA	NA		NA	NA		ND	7.83	
AA_06	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
	2	NA	NA		NA	NA		NA	NA	
	3	NA	NA		NA	NA		NA	NA	
AA_07	1	45.71	144.57	0.30	45.76	185.96	0.30	NA	NA	NA
	2	45.58	143.58		44.98	183.18		NA	NA	
	3	45.59	143.96		45.39	183.88		NA	NA	
AA_10	1	46.70	162.00	1.00	46.80	226.00	1.00	NA	NA	NA
	2	48.40	165.00		48.10	230.00		NA	NA	
	3	46.10	164.00		46.30	228.00		NA	NA	
AA_33	1	36.00	99.60	1.00	39.68	150.48	1.00	1.69	5.58	1.00
	2	34.72	110.27		38.55	160.91		1.20	6.36	
	3	34.07	114.74		36.48	168.40		1.45	7.10	

NA – not analyzed, ND – not detected

Table 9: Results for MDA, MOCA, and TOL of all participating laboratories

Lab Code	Sample No.	MDA [ng/mL]			MOCA [ng/mL]			TOL [ng/mL]		
		Low	High	LOQ	Low	High	LOQ	Low	High	LOQ
AA_01	1	7.28	108.00	0.10	9.09	110.00	10.00	0.29	1.44	0.01
	2	6.89	107.00		17.50	112.00		0.29	1.34	
	3	6.33	96.90		10.00	99.30		0.30	1.42	
	4	7.37	105.00		10.00	89.00		0.29	1.37	
	5	6.14	95.70		8.63	126.00		0.29	1.42	
	6	6.67	109.00		11.00	115.00		0.29	1.41	
	7	6.31	101.00		10.10	121.00		0.29	1.37	
	8	5.90	105.00		7.34	112.00		0.29	1.39	
	9	6.61	102.00		9.34	135.00		0.29	1.49	
	10	6.17	109.00		8.82	103.00		0.29	1.38	
AA_12	1	4.50	95.90	1.00	23.35	154.36	1.30	NA	NA	NA
	2	4.62	92.94		23.12	136.49		NA	NA	
	3	5.06	100.64		23.86	135.84		NA	NA	
	4	5.06	99.92		24.36	145.67		NA	NA	
	5	5.25	97.90		23.52	150.29		NA	NA	
	6	5.00	92.69		23.43	146.95		NA	NA	
AA_16	1	2683.72	2076.67	0.03	12.54	134.84	0.05	0.28	1.34	0.10
	2	2588.14	1932.51		13.44	137.27		0.29	1.36	
	3	2639.66	1889.23		12.95	130.39		0.29	1.35	
	4	2885.37	1809.21		12.87	133.55		0.31	1.36	
	5	2899.59	1848.28		12.53	132.59		0.28	1.34	
	6	2714.67	1813.90		12.68	138.26		0.28	1.36	
AA_21	1	4.78	72.59	0.05	11.67	135.49	0.20	0.26	1.24	0.05
	2	4.53	73.99		12.33	135.42		0.27	1.26	
	3	4.83	73.89		12.74	133.20		0.27	1.32	
	4							0.28	1.28	
	5							0.28	1.35	
	6							0.28	1.30	
AA_03	1	1.85	74.38	0.10	4.50	93.78	0.10	NA	NA	0.10
	2	3.13	89.91		5.10	107.60		NA	NA	
	3	4.16	75.76		8.78	96.16		NA	NA	
AA_05	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
	2	NA	NA		NA	NA		NA	NA	
	3	NA	NA		NA	NA		NA	NA	
AA_06	1	4.63	77.00	1.00	NA	NA	NA	NA	NA	NA
	2	5.05	76.10		NA	NA		NA	NA	
	3	4.94	73.40		NA	NA		NA	NA	
AA_07	1	5.85	89.65	0.30	15.98	139.42	0.30	NA	NA	NA
	2	5.92	89.26		16.01	139.94		NA	NA	
	3	5.94	89.04		15.96	138.82		NA	NA	
AA_10	1	6.28	92.30	0.50	17.70	134.00	1.30	0.27	1.26	0.20
	2	5.91	89.40		17.60	129.00		0.29	1.39	
	3	5.88	98.50		16.30	139.00		0.31	1.38	
AA_33	1	5.67	86.17	0.10	12.00	62.22	1.00	0.40	0.91	0.20
	2	6.35	97.75		12.48	97.36		0.32	1.15	
	3	6.29	112.65		12.10	100.65		0.34	1.19	

NA – not analyzed

VIII Assigned values and participants' performance for Anilines in urine

Table 10: Assigned value and participants' performance (mean) for 2,4-Diaminotoluene (2,4-TDA) in urine

	2,4-TDA$_{Low}$	**2,4-TDA$_{High}$**
Assigned value consensus value (low and high)	40.70 ng/mL	138.71 ng/mL
Standard deviation (SD) robust SD (low and high)	8.46 ng/mL	25.82 ng/mL
uncertainty of consensus value (u)	9.2 %	8.2 %
target standard deviation (σ_T, 25 %)	10.17 ng/mL	34.68 ng/mL
Relative target standard deviation (%)	25 %	25 %
0.7 * σ_T	17.5 %	17.5 %
study RSD_R[1]	22.2 %	17.4 %

Laboratory Code	2,4-TDA [ng/mL]	Z-Score (mean)	2,4-TDA [ng/mL]	Z-Score (mean)
AA_01	37.49	-0.3	138.80	0.0
AA_03	20.18	-1.9	91.17	-1.3
AA_05	NA	NE	NA	NE
AA_06	NA	NE	NA	NE
AA_07	45.63	0.5	144.04	0.1
AA_10	47.07	0.6	163.67	0.7
AA_12	42.53	0.2	136.89	0.0
AA_16	114.87	6.8	840.84	19.2
AA_21	37.70	-0.3	139.48	0.0
AA_33	34.93	-0.5	108.20	-0.8

NA – not analyzed, NE – not evaluable. [1] Laboratory AA_16 was excluded for calculating the study RSD_R due to high deviation of the reported results. Study RSD_R is 69.9 % for 2,4-TDA low and 181.5 % for 2,4-TDA high when AA_16 is included.

Table 11: Assigned value and participants' performance for 2,6-Diaminotoluene (2,6-TDA) in urine

	2,6-TDA$_{Low}$	**2,6-TDA$_{High}$**
Assigned value consensus value (low and high)**	45.54 ng/mL	192.89 ng/mL
Standard deviation (SD) robust SD (low and high)	2.37 ng/mL	53.84 ng/mL
uncertainty of consensus value (u)	2.3 %	12.3 %
target standard deviation (σ_T, 25 %)	11.38 ng/mL	48.22 ng/mL
Relative target standard deviation (%)	25 %	25 %
0.7 * σ_T	17.5 %	17.5 %
study RSD_R	25.1 %	25.1 %

Laboratory Code	2,6-TDA [ng/mL]	Z-Score (mean)	2,6-TDA [ng/mL]	Z-Score (mean)
AA_01	46.18	0.1	203.60	0.2
AA_03	15.53	-2.6	89.38	-1.9
AA_05	NA	NE	NA	NE
AA_06	NA	NE	NA	NE
AA_07	45.38	0.0	184.34	-0.2
AA_10	47.07	0.1	228.00	0.7
AA_12	46.86	0.1	155.02	-0.7
AA_16	175.90	11.5	911.90	13.4
AA_21	44.33	-0.1	224.27	0.6
AA_33	38.24	-0.6	159.93	-0.6

NA – not analyzed, NE – not evaluable. [1] Laboratory AA_16 was excluded for calculating the study RSD_R due to high deviation of the reported results. Study RSD_R is 107.6 % for 2,6-TDA low and 136.5 % for 2,6-TDA high when AA_16 is included.

Table 12: Assigned value and participants' performance for Aniline (AN) in urine

	AN$_{Low}$	**AN$_{High}$**
Assigned value	NE	NE

Laboratory Code	Aniline [ng/mL]	Z-Score (mean)	Aniline [ng/mL]	Z-Score (mean)
AA_01	1.60	NE	9.83	NE
AA_03	NA	NE	NA	NE
AA_05	ND	NE	7.71	NE
AA_06	NA	NE	NA	NE
AA_07	NA	NE	NA	NE
AA_10	NA	NE	NA	NE
AA_12	NA	NE	NA	NE
AA_16	2.36	NE	12.06	NE
AA_21	23.05	NE	18.06	NE
AA_33	1.45	NE	6.35	NE

NA – not analyzed, NE – not evaluable, ND – not detected.

Table 13: Assigned value and participants' performance for 4,4'-Methylenedianiline (MDA) in urine

	MDA_{Low}	MDA_{High}
Assigned value consensus value (low and high)	5.60 ng/mL	91.66 ng/mL
Standard deviation (SD) robust SD (low and high)	1.28 ng/mL	15.66 ng/mL
uncertainty of consensus value (u)	9.5 %	7.1 %
target standard deviation (σ_T, 25 %)	1.40 ng/mL	22.92 ng/mL
Relative target standard deviation (%)	25 %	25 %
0.7 * σ_T	17.5 %	17.5 %
study RSD_R[1]	20.1 %	12.4 %

Laboratory Code	MDA [ng/mL]	Z-Score (mean)	MDA [ng/mL]	Z-Score (mean)
AA_01	6.57	0.6	103.86	0.5
AA_03	3.05	-1.7	80.02	-0.5
AA_05	NA	NE	NA	NE
AA_06	4.87	-0.5	75.50	-0.7
AA_07	5.90	0.2	89.32	-0.1
AA_10	6.02	0.3	93.40	0.1
AA_12	4.91	-0.5	96.66	0.2
AA_16	2735.19	1822.7	1894.97	78.7
AA_21	4.71	-0.6	73.49	-0.8
AA_33	6.10	0.3	98.86	0.3

NA – not analyzed, NE – not evaluable. [1] Laboratory AA_16 was excluded for calculating the study RSD_R due to high deviation of the reported results. Study RSD_R is 16261.5 % for MDA low and 656.9 % for MDA high when AA_16 is included.

Table 14: Assigned value and participants' performance for 4,4'-Methylenebis(2-chloroaniline) (MOCA) in urine

	$MOCA_{Low}$	$MOCA_{High}$
Assigned value consensus value (low)/ assigned value from 3 expert labs (high)	13.28 ng/mL	127.22 ng/mL
Standard deviation (SD) robust SD (low)/expert SD (high)	4.45 ng/mL	17.40 ng/mL
uncertainty of consensus value (u)	14.8 %	3.6 %
target standard deviation (σ_T, 25 %)	3.32 ng/mL	31.80 ng/mL
Relative target standard deviation (%)	25 %	25 %
0.7 * σ_T	17.5 %	17.5 %
study RSD_R	39.3 %	16.1 %

Laboratory Code	MOCA [ng/mL]	Z-Score (mean)	MOCA [ng/mL]	Z-Score (mean)
AA_01	10.18	-0.8	112.23	-0.5
AA_03	6.13	-1.9	99.18	-0.9
AA_05	NA	NE	NA	NE
AA_06	NA	NE	NA	NE
AA_07	15.98	0.7	139.39	0.4
AA_10	17.20	1.0	134.00	0.2
AA_12	23.60	2.7	144.93	0.6
AA_16	12.84	-0.1	134.48	0.2
AA_21	12.25	-0.3	67.35	-1.9
AA_33	12.19	-0.3	86.74	-1.3

NA – not analyzed, NE – not evaluable.

Table 15: Assigned value and participants' performance for o-Toluidine (TOL) in urine

	TOL_{Low}		TOL_{High}	
Assigned value from 3 expert labs (low and high)	0.28 ng/mL		1.36 ng/mL	
Standard deviation (SD) expert SD (low and high)	0.01 ng/mL		0.06 ng/mL	
uncertainty of consensus value (u)	1 %		1.1 %	
target standard deviation (σ_T, 25 %)	0.07 ng/mL		0.34 ng/mL	
Relative target standard deviation (%)	25 %		25 %	
0.7 * σ_T	17.5 %		17.5 %	
study RSD_R	9.0 %		8.1 %	

Laboratory Code	o-Toluidine [ng/mL]	Z-Score (mean)	o-Toluidine [ng/mL]	Z-Score (mean)
AA_01	0.29	0.1	1.40	0.1
AA_03	NA	NE	NA	NE
AA_05	NA	NE	NA	NE
AA_06	NA	NE	NA	NE
AA_07	NA	NE	NA	NE
AA_10	0.29	0.0	1.34	0.0
AA_12	NA	NE	NA	NE
AA_16	0.29	0.0	1.35	0.0
AA_21	0.27	-0.2	1.29	-0.2
AA_33	0.35	1.0	1.08	-0.8

NA – not analyzed, NE – not evaluable.

IX Graphical evaluation of Z-Scores

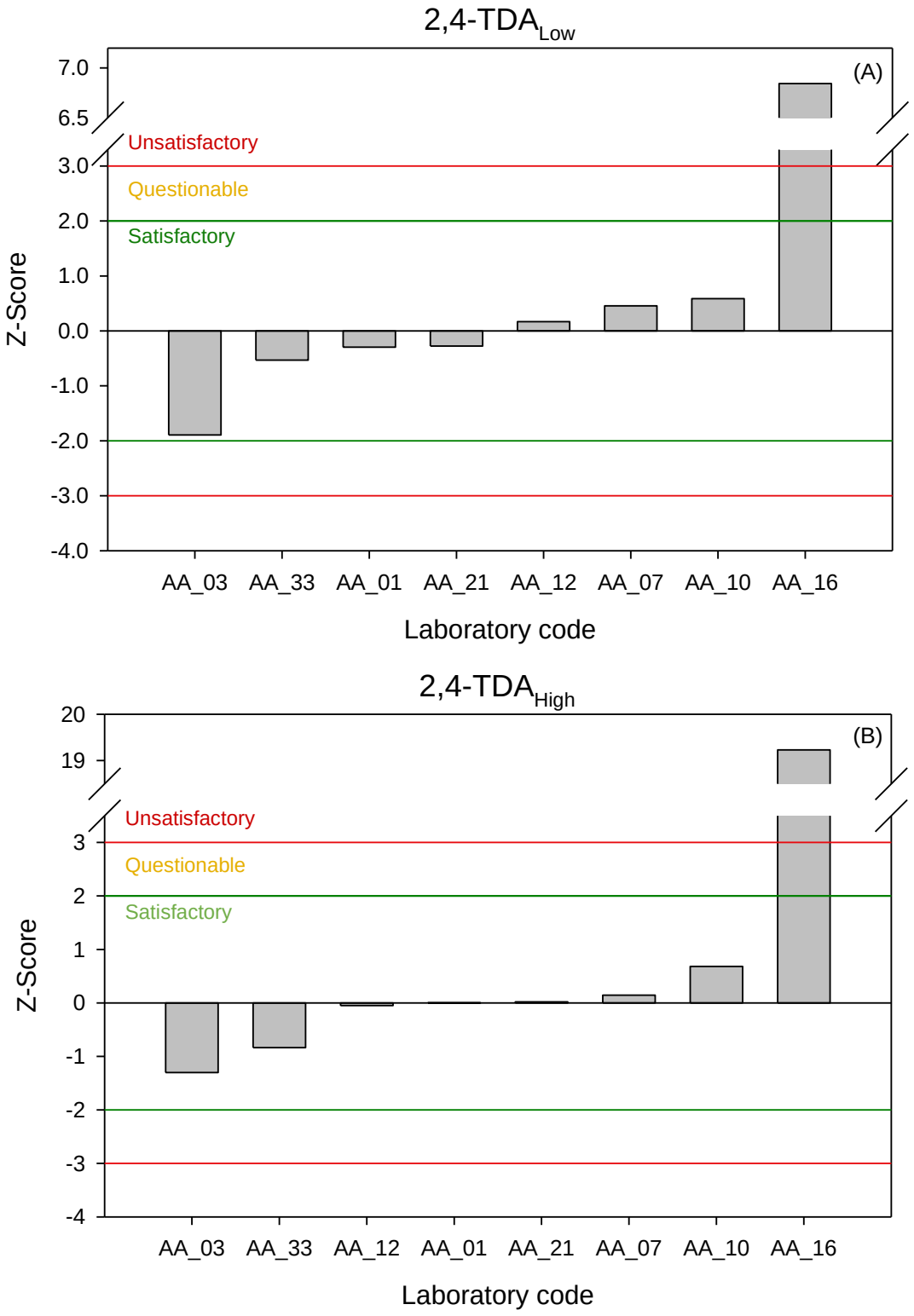

Figure 1: Z-Scores of participating laboratories for the (A) low and (B) high concentration level of 2,4-TDA.

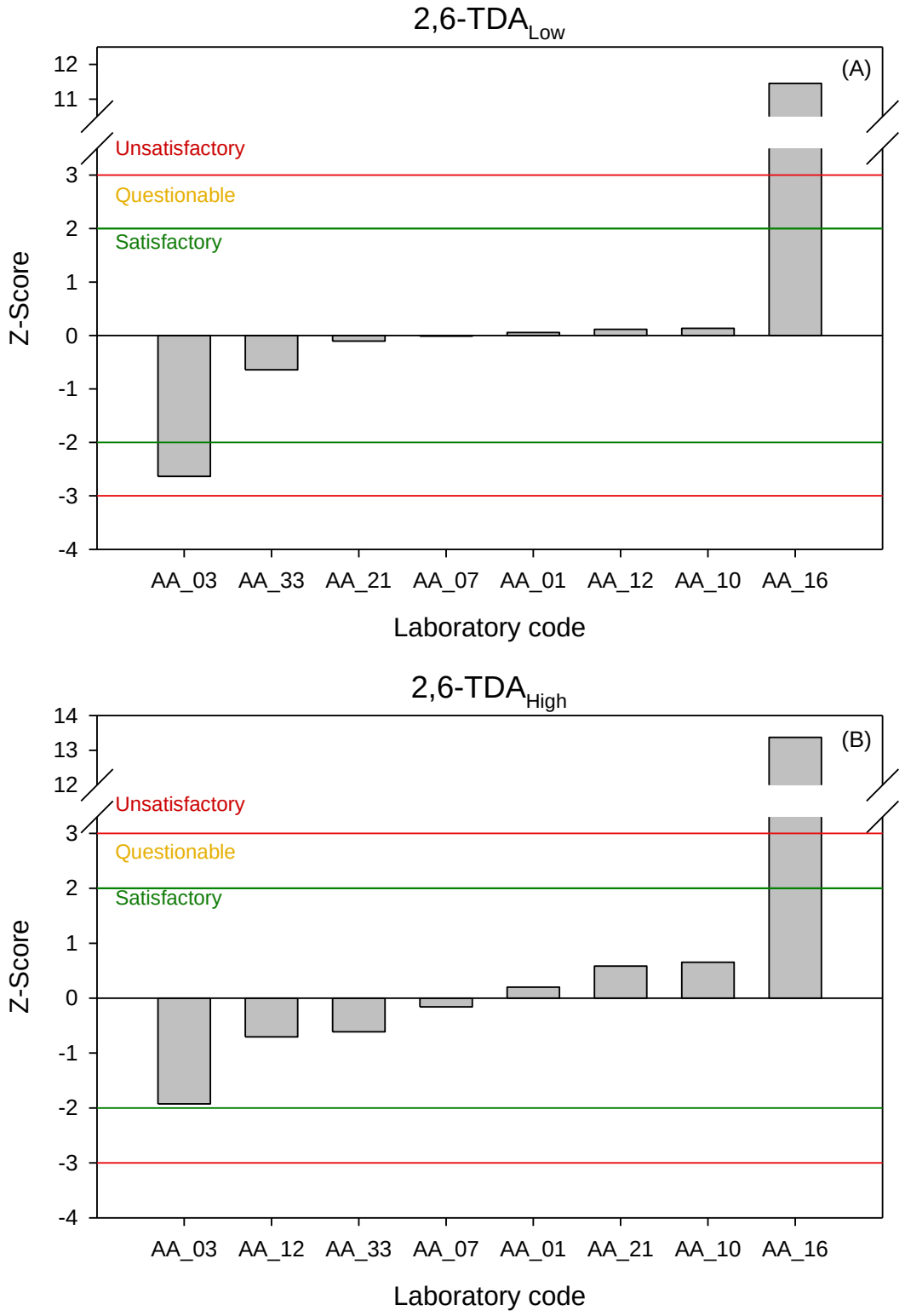

Figure 2: Z-Scores of participating laboratories for the (A) low and (B) high concentration level of 2,6-TDA.

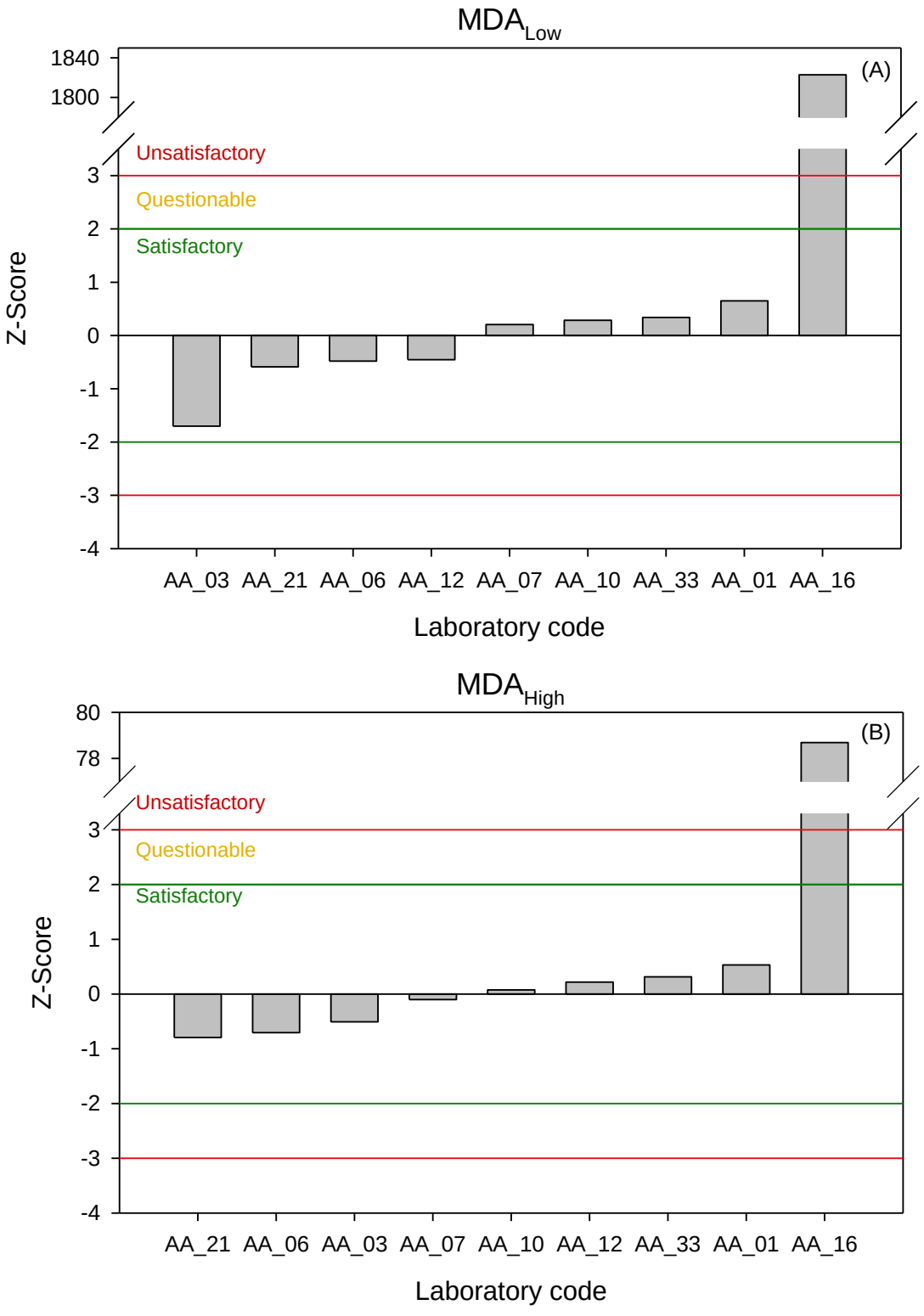

Figure 3: Z-Scores of participating laboratories for the (A) low and (B) high concentration level of MDA.

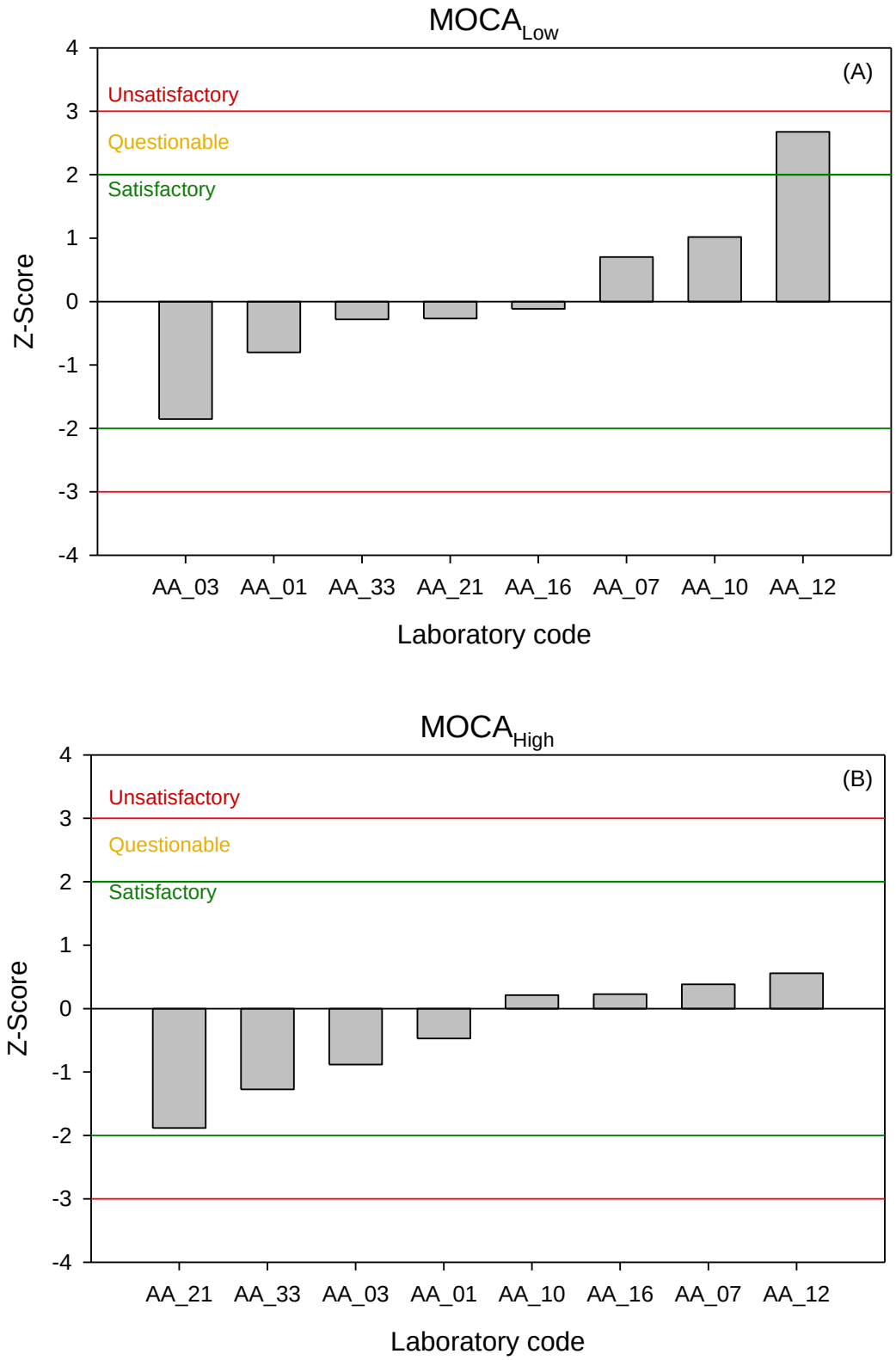

Figure 4: Z-Scores of participating laboratories for the (A) low and (B) high concentration level of MOCA.

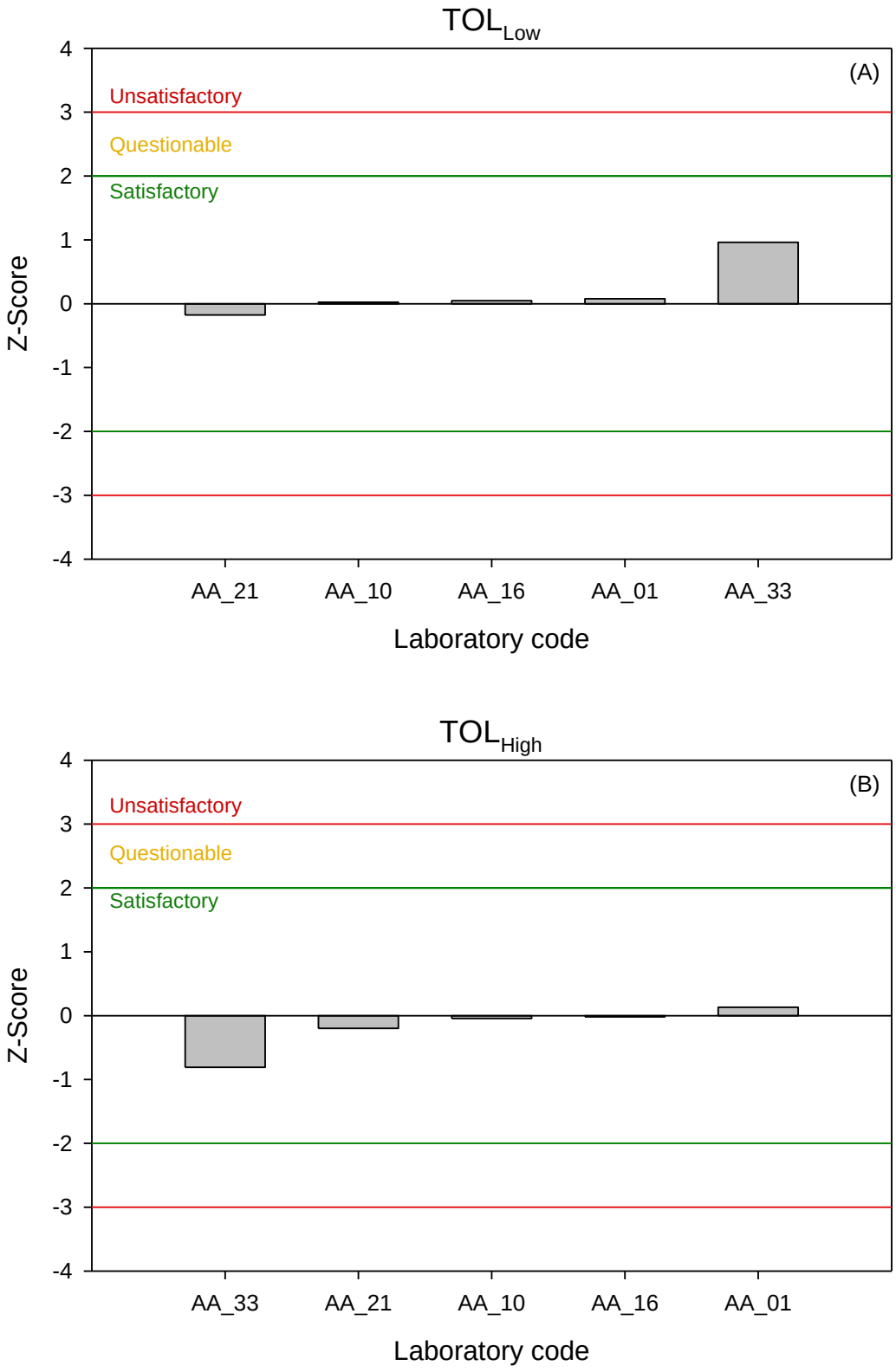

Figure 5: Z-Scores of participating laboratories for the (A) low and (B) high concentration level of TOL.