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ICI / EQUAS REPORT

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BFRs in serum

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1 Summary

Within the frame of the HBM4EU project, an External Quality Assurance Scheme (EQUAS) was organised on the determination of 10 BFR biomarkers in serum. This was the 3rd ICI/EQUAS round for this substance group within the HBM4EU program.

In total 28 candidate laboratories were invited for this 3rd ICI/EQUAS and 14 laboratories submitted results.

In June 2019, each participant received one tube of burdened control materials of serum (level 1, low = L1), one tube of burdened control materials of serum (level 2, high = L2) and one tube of "blank" serum (non-spiked). The biomarker concentrations were mostly in the range of 0.09-2.6 ng/mL and 0.3-9.5 ng/mL for level 1 and level 2, respectively. The concentrations were chosen according to the review of relevant data on the occurrence of BFRs in serum of the European population published mostly during the last five years.

A homogeneity assessment showed that both materials were sufficiently homogeneous for the EQUAS testing. The stability test demonstrated no significant loss of the biomarkers during the course except for DP-syn at level 2, for which a statistic instability was detected.

The proficiency of the laboratories was assessed through Z-scores calculated using the expert-assigned values, which were based on results obtained from the analysis of the control material by at least three expert laboratories selected by HBM4EU QAU. The expert-assigned values were calculated by averaging the values obtained by the expert labs for BDE-47, BDE-153 and BDE-209. When the expert assigned-value couldn't be calculated (the number of participating expert labs was lower than three, or after the removal of outlier value the number of remaining experts was lower than three), then the consensus value based on the combined results of participants and expert laboratories was used as assigned value. This approach was used for α-HBCD, γ-HBCD, DP-syn and DP-anti. In the case of TBBPA, DBDPE and 2,4,6-TBP no assigned value could be determined due to a limited number of obtained results both from experts and participants.

Laboratory results were rated using Z-scores in accordance with ISO 13528 and ISO 17043. A fixed fit-for-purpose relative target standard deviation (FFP-RSDR) of 25% was applied for proficiency assessment. **Table 1** presents a global overview of the proportion of satisfying results (-2 < Z-scores < 2). As mentioned above, in the case of TBBPA, DBDPE and 2,4,6-TBP no assessment of laboratories' performance was done.

Table 1: Percentages of satisfying results and number of successful labs for 3rd ICI/EQUAS evaluation

Biomarker	BDE 47		BDE 153		BDE 209		Syn-DP		Anti-DP		α-HBCD		γ-HBCD		TBBPA		DBDPE		2,4,6-TBP	
Control material	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high	L1 low	L2 high
% of satisfying z-scores	100	100	92	85	60	60	88	88	88	x	86	100	86	100	x	x	x	x	x	x
No. of successful labs	13	13	11	11	6	6	7	7	7	x	6	7	6	7	x	x	x	x	x	x

2 Introduction

Interlaboratory 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 3rd ICI/EQUAS study has been organised within the frame of HBM4EU as a part of the Quality Assurance program for biomonitoring analyses, following protocols HBM4EU-SOP-QA-001 to 004 which are available through the HBM4EU website (https://www.hbm4eu.eu/online-library/). Within HBM4EU, participation in ICI/EQUAS exercises is mandatory for laboratories that will analyse HBM4EU samples.

This report describes the 3rd ICI/EQUAS for BFRs in serum, which was conducted as EQUAS and was organised by UCT Prague (University of Chemistry and Technology, Prague; VŠCHT, Vysoká škola chemicko-technologická v Praze), Department of Food Analysis and Nutrition.

For this 3rd ICI/EQUAS, expert laboratories had to be selected according to the selection criteria described in HBM4EU-SOP-QA-001 and in agreement with the QAU.

The selection of the most relevant BFRs was previously done in WP9, and has been described in Deliverable report 9.2 v1.1. Based on this, a set of 10 target biomarkers was compiled to be included in the EQUAS for BFR analysis in serum.

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

2.1 Confidentiality

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

3 Control material

3.1 Preparation of control material

The bovine serum was obtained from Sigma Aldrich (USA). A total of three litres were purchased and delivered in a frozen state. Before the spiking procedure, the background concentrations of targeted BFRs were investigated. For this purpose accredited methods (ISO17025) using both, GC-MS and LC-MS instruments, used. In the testing material (referred to as “blank” in the study) all target biomarkers were < LOQ.

Before the spiking procedure, the serum was thawed at room temperature (20 °C). Then it was stirred for 30 min in a 3 L beaker using a magnetic stirrer. After that, three aliquots of 700 mL were transferred into the 1 L beaker (one aliquot for “blank” – non-spiked, one for serum level 1 and one for serum level 2). Each standard of target biomarkers was appropriately diluted into acetone and individually spiked into the serum at level 1 and serum at level 2 using a calibrated Eppendorf Multipette®. During the spiking procedure, the serum was mixed using a magnetic stirrer for the whole time, and when all compounds had been added, subsequent mixing for 30 min was performed. The aliquots of 10 mL from “blank” serum / level 1 serum / level 2 serum were placed into the tube and later analysed for homogeneity testing. For the participants´ analysis and stability testing, the aliquots of 5 mL were placed into the tube from each prepared material (“blank”, level 1, level 2). All tubes were placed into the freezer at -18 °C before analysis / dispatch.

3.2 Homogeneity of control material

The homogeneity of the control material was tested according to HBM4EU-SOP-QA-002. Ten tubes of the control material at both levels were randomly selected from the freezer and each sample was analysed in duplicates. In brief, two extraction procedures were used. For GC-MS amenable BFR (BDE-47, BDE-153, BDE-209, DP-anti, DP-syn and DBDPE) isolation based on three-step solvent extraction using a mixture of n-hexane:diethylether (9:1, *v/v*) followed by the purification using a solid phase extraction (SPE) on a Florisil® column was used (for details, please see Svarcova et al. 2019). For LC-MS amenable compounds (α-HBCD, γ-HBCD, 2,4,6-TBP and TBBPA) simple extraction by acetonitrile with formic acid was applied.

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

Table 2: Concentration of BFRs as obtained during the homogeneity testing (details see Appendix 1)

Biomarker	Level 1 (low)		Level 2 (high)	
	Mean (ng/mL)	RSDr (%)	Mean (ng/mL)	RSDr (%)
BDE-47	0.132	9	0.656	10
BDE-153	0.173	10	0.573	3
BDE-209	0.919	7	1.574	9
DP-syn	0.264	12	0.452	8
DP-anti	0.111	5	0.546	6
DBDPE	0.093	8	0.388	3
2,4,6-TBP	1.586	8	3.888	12
α-HBCD	0.596	9	4.593	7
γ-HBCD	0.284	10	5.413	5
TBBPA	2.527	7	9.537	4

3.3 Stability of control material

The stability of the control material was tested according to HBM4EU-QA-002. On the day of preparation of the control materials, randomly selected test serum samples of level 1 and level 2 were stored at -80 °C. After the deadline of submission of analysis results by the participants six test samples of both materials stored at -80 °C and six samples of both materials randomly selected from the -18 °C freezer, where the ICI samples were stored, were selected for analysis. For the analysis the previously described methods were used (see 3.2 Homogeneity of control material). The stability was evaluated using the Excel-sheet “HBM4EU ICI-EQUAS stability test CM v1”. The results are presented in **Appendix 2**. Generally no problem with stability was detected for tested compounds except for DP-syn at level 2, for which a statistic instability was detected.

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4 Organisational details

4.1 Participants

For the organisation of the 3rd ICI/EQUAS, the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine (IPASUM) at Friedrich-Alexander University of Erlangen-Nuremberg (IPASUM) conducted a survey to find expert laboratories for the analysis of BFRs in serum willing to participate in the project. Then, IPASUM evaluated their eligibility and selected expert laboratories in agreement with the QAU and according to HBM4EU-SOP-QA-001.

UCT Prague contacted the selected expert laboratories and sent them invitation letters by e-mail. It was indicated that participation would be free of charge, and that those who subscribed to this EQUAS would receive a kit containing the test materials needed for analysis. The final number of expert labs was five, three from Europe (HBM4EU consortium) and two from outside Europe (USA and Canada).

Participants of this 3rd ICI/EQUAS were laboratories from the HBM4EU consortium (including linked-third parties) that had been included as candidate laboratories for analyses in the frame of the HBM4EU project through WP9 (Task 9.2, Deliverable 9.3). Invitation letters (**Appendix 3**) and registration forms (**Appendix 4**) were sent by e-mail on 29/04/2019 to 28 laboratories. For registration, each participant was asked to provide which of 10 biomarkers were included in their scope. The participants were informed that participation will be free of charge. The deadline for registration was 23/05/2019. Out of 28 invited laboratories, 14 performed the assays and submitted results..

4.2 Dispatch and instructions

Test materials were dispatched to the participants under frozen conditions (on dry ice) on 04/06/2019. Each participant received one tube of burdened control materials of serum (level 1), one tube of burdened control materials of serum (level 2) and one tube of “blank” serum (non-spiked). Each sample consisted of approximately 5 mL serum.

Moreover, a letter with instructions on sample handling (**Appendix 5**), a sample receipt form to be sent back to UCT Prague upon receipt of the test material (**Appendix 6**) as well as a result submission form and a method information form (**Appendix 7**) were sent to the participants by e-mail. The latter form was used to extract relevant information related to the analytical method used for quantification.

Test materials were dispatched to the expert laboratories under frozen conditions (on dry ice) on the same date as for the participants, namely 04/06/2019. Each lab received six tubes of burdened control materials of serum (level 1), six tubes of burdened control materials of serum (level 2) and six tubes of “blank” serum (non-spiked). Each sample consisted of approximately 5 mL serum.

Participants and expert labs were asked to perform a single analysis of each sample using the same procedure as will be used for analysis of samples in the frame of HMB4EU and to report results following the instructions given. The deadline for submitting results was 15/07/2019.

4.3 Deviations from ICI/EQUAS SOPs

For this 3rd ICI/EQUAS, the HBM4EU-QA-SOPs (version 2) were followed. There were no deviations from these SOPs.

5 Data evaluation

5.1 False positives and <LOQ

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

A result was assigned as false positive when 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).

When a biomarker is reported as "<LOQ-value", AND an assigned value could be established for the biomarker in the control material, a further assessment was done to verify whether this result might be a false negative and to judge whether the LOQ is considered adequate (low enough) for analysis in the frame of HBM4EU. A result is a false negative when the LOQ of a biomarker is well below the assigned value, but the laboratory did not report a quantitative value. The LOQ is considered not adequate (too high) when:

1) the LOQ is substantially above the assigned value

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

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

In case no expert value could be obtained, the consensus value derived from the combined results from both participants and expert laboratories was used as an alternative. In this case the consensus value was calculated using robust statistics as described for ICI in HBM4EU-SOP-QA-003.

5.3 Target standard deviation (σ_T)

For calculation of the Z-scores, a fit-for-purpose relative target standard deviation (FFP-RSD_R) 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 1st and 2nd round.

5.4 ICI/EQUAS standard deviation (RSD_R)

To gain insight into the actual interlaboratory variability of each biomarker determination in this study, the robust relative standard deviation (RSD_R) 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.

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

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

In accordance with ISO 13528 and ISO 17043 and the deliverable D 9.4 "*The Quality Assurance/Quality Control Scheme in the HBM4EU project*, Z-scores are classified as presented in **Table 3**.

Table 3: Classification of Z-scores

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

5.6 Proxy-Z-scores

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

Proxy-Z-scores are classified as follows:

proxy-Z ≤ -3	false negative. Based on the LOQ provided, the laboratory should have been able to detect and quantify the biomarker. Performance is considered 'unsatisfactory'.
proxy-Z ≥3	the LOQ is considered too high to be fit-for-purpose in the frame of HBM4EU analysis. It also means that the LOQ is too high in comparison with other laboratories. (Note: proxy-Z can only be calculated when an assigned value could be established. If this is the case, this inherently means that reliable quantitative determination at a certain low level is feasible). Performance is considered 'unsatisfactory'.
-3 ≤ proxy-Z < -2	possible false negative. Performance is considered 'questionable'.
2 < proxy-Z ≤ 3	the LOQ is relatively high in relation to HBM4EU analysis and compared to other laboratories. Performance is considered 'questionable'.

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

6 Results and discussion

6.1 Results submitted by participants

In total, 14 candidate laboratories agreed to participate in this study and all of them submitted results.

The scope of BFR biomarkers measured by the laboratories varied substantially: from two to all ten target compounds (**Appendix 8**). BDE-47 and BDE-153 were measured by 13 labs, BDE-209 by 10 labs, DP-syn and DP-anti by eight labs, , α-HBCD and γ-HBCD by seven labs, DBDPE by five labs, TBBPA by four labs and 2,4,6-TBP was analysed by three labs.

It is worth mentioning that in several cases the scope of biomarkers in the registration form did not match the scope of submitted results. Two labs did not report the results for DBDPE (PT3BFR05 and PT3BFR11), one lab for TBBPA (PT3BFR11) and two for BDE-209 (PT3BFR11 and PT3BFR14). On the other hand, one lab (PT3BFR04) was able to report the results for TBBPA, which was not selected in their registration form.

Regarding submitted LOQs, a very high variability between participating laboratories was found (**Appendix 8**). Specifically, the LOQ for BDE-47 was in the range of 0.0003-0.2 ng/mL, for BDE-153 0.0003-1 ng/mL, for BDE-209 0.0001-0.1 ng/mL, for α-HBCD 0.005-2.24 ng/mL, for γ-HBCD 0.005-0.669 ng/mL, for TBBPA 0.0004-0.005 ng/mL, for DP-syn and DP-anti 0.0001-0.1 ng/mL, for DBDPE 0.025-0.8 ng/mL and for 2,4,6-TBP 0.010-0.049 ng/mL.

The individual analysis results of the laboratories are included in **Appendix 9** and **Appendix 11**.

6.2 Assigned values and (target) standard deviations

The assigned value was the expert-assigned value as derived from replicate analysis of the control materials by five expert laboratories as described in 5.2. Since not all expert labs covered all 10 biomarkers, the number of expert labs for BDE-47, BDE-153 and BDE 209 was five, and for DP-syn and DP-anti it was three.

The relative uncertainty was below the 18% (calculated as 0.7*σT) for BDE-47 (level 1 and level 2), BDE 153 (level 1 and level 2) and BDE 209 (level 2). The individual means of the expert labs were generally in good agreement with each other for these biomarkers. No outliers were identified.

In the case of BDE-209 at level 1 the relative uncertainty was 19%. Then the result from one expert lab was detected as an outlier (using Grubbs test). After exclusion of the outlier the relative uncertainty of results from the remaining four expert labs was 10% and met the requirement of minimum number of results for the establishment of the expert value.

For DP-syn and DP-anti at both levels the relative uncertainty of the expert-derived mean was in the range of 18.1 - 29%, which is too high to be used as assigned value. In the case of identification and removing of outliers, the requirement of the minimum number of experts will not be met (only two remaining labs). For this reason, the possibility of using the consensus value as an alternative to the expert-assigned value was investigated. The robust mean was determined as described for ICI in HBM4EU-QA-SOP-003, using the results of all laboratories (including expert labs). For DP-syn (level 1 and level 2) and for DP-anti (only level 1) this resulted in a sufficiently reliable assigned value suitable for determination of Z-scores. In the case of DP-anti (level 2), the uncertainty of the consensus value was not within the acceptable limits ($u \leq 0.7*\sigma T$) with respect to use for statistical evaluation of the data and calculation of Z-scores. The data set for this biomarker is unfit for the laboratory´s performance. Therefore, the consensus value was used as assigned value for DP-syn (level 1 and level 2) and DP-anti (level 1).

The similar approach was used for α-HBCD and γ-HBCD because the number of results from expert labs was below three and the consensus value was used as assigned value.

Finally, for TBBPA, 2,4,6-TBP and DBDPE the number of participating laboratories which tested these biomarkers in serum is too small to establish an assigned value. Moreover, in the case of DBDPE, the concentration in serum at both levels was too low, which resulted in most of the results being reported as < LOQ. For these three biomarkers no Z-scores or proxy-Z-scores could be determined.

In total, it was possible to establish assigned values for six biomarkers (BDE-47, BDE-153, BDE-209, α-HBCD, γ-HBCD and DP-syn) in both control materials and for one biomarker (DP-anti) at level 1. All assigned values and their uncertainties are included in **Appendix 9**.

The target standard deviation used for determination of the Z-scores was 25% (0.25*C) (see 5.3 and 5.5). To verify how this fixed target value compares to the actual interlaboratory variability of the results, the actual relative standard deviation (study RSD_R, robust statistics) derived from the participants' results (excluding the results from the expert labs) was calculated. The individual RSD_R's are included in **Appendix 9**. They ranged from 8% to 64% (median of all RSD_R's was 34%). In 9 out of 14 cases, the RSD_R exceeded the target standard deviation of 25%. The highest variability was observed for BDE-209, DP-syn and DP-anti at both levels.

From the data, it was also verified to what extent the robust mean of the participants deviated from the expert-value. Robust means with acceptable uncertainty could be derived in most cases except for TBBPA, 2,4,6-TBP and DBDPE. In general, the difference between the mean of the participants and the expert-assigned value was less than 20%. The only exception was BDE-209 at high concentration (difference of robust mean and expert value was 20.3%, 2.130 versus 1.771 ng/ml, respectively).

The calculated expert values and consensus values are summarized in **Table 4** and **Table 5**, respectively.

Table 4: Expert-assigned values, associated uncertainty and standard deviations

	Expert-assigned values			Expert-assigned values		
	Level 1 - low			Level 2 - low		
	BDE-47	BDE-153	BDE-209	BDE-47	BDE-153	BDE-209
Expert value - mean of the mean (ng/ml)	0.151	0.184	1.197	0.644	0.549	1.771
No. of expert labs	5	5	4(+1*)	5	5	5
SD (ng/ml)	0.030	0.042	0.243	0.219	0.133	0.704
RSD (%)	20	23	20	34	24	40
u (%)	9	10	10	15	11	18

***** The results from one expert lab were identified as outlier and were removed for the calculation of expert-derived value

Table 5: Consensus values, associated uncertainty and standard deviations

	Level 1 - low				Level 2 - high			
	α-HBCD	γ-HBCD	Syn-DP	Anti-DP	α-HBCD	γ-HBCD	Syn-DP	Anti-DP*
Consensus value (ng/ml)	0.583	0.321	0.313	0.134	4.877	5.908	0.764	*0.769*
No. of participants (+ nb. of expert labs)	7(+1)	7(+1)	8(+1)	8(+1)	7(+1)	7(+1)	8(+1)	*8(+1)*
Robust SD (ng/ml)	0.115	0.029	0.045	0.032	0.941	0.594	0.310	*0.389*
u (ng/ml)	0.051	0.013	0.019	0.013	0.416	0.262	0.129	*0.162*
Target standard deviation 25%, σ_T (ng/ml)	0.146	0.080	0.078	0.034	1.219	1.477	0.191	*0.192*
u (%)	8.7	4.0	6.1	9.7	8.5	4.4	16.9	*21.1*

* u (0.162 ng/ml) of the consensus value for anti-DP was not within the acceptable limits u ≤ 0.7*σT (0.162 > 0.135 ng/ml)

6.3 Assessment of laboratory performance

Z-scores could be calculated for six biomarkers (BDE-47, BDE-153, BDE-209, α-HBCD, γ-HBCD and DP-syn) in both control materials and for one biomarker (DP-anti) at level 1 (**Appendix 9**). A graphical presentation of the Z-scores is provided in **Appendix 10**. A summary of a number of laboratories that reported results and the number of satisfactory/questionable/unsatisfactory scores are presented in **Table 6**.

Only one laboratory (PT3BFR03) reported '<LOQ-value' for BDE-153 at level 1 (low). In this case, a proxy-Z-score was calculated. This is indicated in **Appendix 9** as a Z-score between brackets.

The determination of assigned values was not possible for DP-anti at high level because of the high uncertainty of the consensus value.

The calculation of expert values or consensus values was not possible for DBDPE, TBBPA and 2,4,6-TBP as explained above. For this reason, no Z-score were provided (**Appendix 11**).

As a global overview, the proportion of satisfying results (-2 < Z-score < 2) was from 60% to 100%. The highest proportion of unsatisfactory scores was achieved for BDE-209 at both levels. In general, the highest number of satisfactory scores was determined for BDE-47 (100% at both levels), BDE-153 (92% and 85% at low and high level, respectively), α-HBCD and γ-HBCD (for both biomarkers 86% and 100% at low and high level, respectively), DP-syn (88% at both levels, respectively) and DP-anti (88% at low level).

Table 6: Summary of BFRs results assessment

Biomarker	Control material	No. of participants	No. of quantitative results	No. of satisfactory scores	No. of questionable scores	No. of unsatisfactory scores	% of satisfying z-scores	No. of successful labs
BDE 47	Level 1	13	13	13	0	0	100	13
	Level 2	13	13	13	0	0	100	13
BDE 153	Level 1	13	12	11	1	0	92	11
	Level 2	13	13	11	0	2	85	11
BDE 209	Level 1	10	10	6	1	3	60	6
	Level 2	10	10	6	1	3	60	6
Syn-DP	Level 1	8	8	7	1	0	88	7
	Level 2	8	8	7	1	0	88	7
Anti-DP	Level 1	8	8	7	0	1	88	7
	Level 2	8	8	no score	no score	no score	x	x
α-HBCD	Level 1	7	7	6	1	0	86	6
	Level 2	7	7	7	0	0	100	7
γ-HBCD	Level 1	7	7	6	0	1	86	6
	Level 2	7	7	7	0	0	100	7
TBBPA	Level 1	3	3	no score	no score	no score	x	x
	Level 2	3	3	no score	no score	no score	x	x
DBDPE	Level 1	4	1	no score	no score	no score	x	x
	Level 2	4	2	no score	no score	no score	x	x
2,4,6-TBP	Level 1	3	3	no score	no score	no score	x	x
	Level 2	3	3	no score	no score	no score	x	x

6.4 Conclusions and recommendations

In this HBM4EU 3rd ICI/EQUAS on BFR biomarkers in serum, 28 laboratories were invited, of which 14 submitted results. The overall participation rate was 50%. Three test materials were provided to each participant ("blank" material, spiked material at low level and spiked material at high level).

The scope of the laboratories varied substantially (from two to ten) and in most cases did not cover all target biomarkers. Only one laboratory was able to provide results for all BFR.

Evaluation of laboratories´ performance was realized for seven biomarkers – BDE-47, BDE-153, BDE-209, DP-syn, α-HBCD and γ-HBCD at both levels. For DP-anti the evaluation was possible only for level 1. The proportion of satisfying results (-2 < Z-score < 2) was from 60% to 100% and it is summarized in **Table 6**. In general, the highest number of satisfactory scores was determined for BDE-47 (100% at both levels), BDE-153 (92% and 85% at low and high level, respectively), α-HBCD and γ-HBCD (for both biomarkers 86% and 100% at low and high level, respectively), DP-syn (88% at both levels, respectively) and DP-anti (88% at low level).

The determination of assigned values was not possible for DP-anti at high level because of the high uncertainty of the consensus value.

Assigned values could not be determined for TBBPA, DBDPE and 2,4,6-TBP either due to a low number of reported results by expert laboratories and participants.

In this 3rd round, in contrast to 2nd round, it was not possible to assess performance for DP-anti at high level, because the uncertainty of the consensus value was not within the acceptable limits with respect to use for statistical evaluation of the data and calculation of Z-scores.

Obtained results confirm the reality of a quite significant core network of satisfactory laboratories for BDE-47, BDE-153, BDE-209, α-HBCD and γ-HBCD. However, compared to these biomarkers, there is a lower number of labs with satisfactory performance for DP-syn and DP-anti. Regarding TBBPA, DBDPE and 2,4,6-TBP globally, there is a low number of laboratories which analysed these compounds in serum.

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7 References

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

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Appendix 1: Homogeneity data

	BDE 47 - level 1		BDE 153 - level 1		BDE 209 - level 1		DP-syn - level 1		DP-anti - level 1		DBDPE - level 1	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.148	0.148	0.172	0.164	0.987	0.984	0.270	0.251	0.102	0.120	0.083	0.095
2	0.153	0.134	0.186	0.176	0.997	1.050	0.241	0.247	0.109	0.109	0.086	0.091
3	0.142	0.147	0.162	0.190	0.997	0.998	0.221	0.234	0.108	0.109	0.095	0.089
4	0.124	0.122	0.166	0.189	0.895	0.936	0.226	0.324	0.109	0.108	0.081	0.099
5	0.126	0.125	0.157	0.185	0.825	0.971	0.302	0.294	0.107	0.107	0.101	0.103
6	0.126	0.124	0.158	0.152	0.842	0.843	0.293	0.269	0.108	0.108	0.094	0.088
7	0.137	0.106	0.172	0.222	0.853	0.894	0.293	0.241	0.107	0.108	0.096	0.105
8	0.130	0.122	0.161	0.151	0.895	0.892	0.283	0.273	0.125	0.119	0.084	0.097
9	0.120	0.131	0.180	0.160	0.930	0.869	0.272	0.284	0.116	0.115	0.094	0.083
10	0.151	0.131	0.178	0.185	0.847	0.885	0.203	0.253	0.106	0.116	0.099	0.102
Grand mean	0.132		0.173		0.919		0.264		0.111		0.093	
Cochran´s test												
C	0.6043		0.4761		0.6489		0.589187918		0.6767		0.3414	
Ccrit	0.8674		0.8674		0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers		no outliers		no outliers		no outliers		no outliers		no outliers	
σ_T	0.0323		0.0433		0.2299		0.0659		0.0277		0.0233	
s_x	0.0087		0.0125		0.0647		0.0257		0.0052		0.0059	
s_w	0.0099		0.0164		0.0451		0.0319		0.0053		0.0077	
s_s	0.0052		0.0047		0.0563		0.0125		0.0037		0.0022	
Critical	0.0085		0.0114		0.0607		0.0174		0.0073		0.0062	
Ss < critical ?	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited		method suited		method suited	

	BDE 47 - level 2		BDE 153 - level 2		BDE 209 - level 2		DP-syn - level 2		DP-anti - level 2		DBDPE - level 2	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	0.663	0.708	0.598	0.571	1.522	1.665	0.488	0.518	0.567	0.547	0.370	0.375
2	0.613	0.766	0.562	0.582	1.829	1.742	0.426	0.448	0.522	0.541	0.389	0.384
3	0.717	0.695	0.574	0.607	1.861	1.547	0.446	0.450	0.511	0.455	0.397	0.371
4	0.635	0.552	0.581	0.594	1.459	1.537	0.429	0.450	0.522	0.541	0.378	0.386
5	0.626	0.622	0.574	0.563	1.476	1.586	0.405	0.404	0.557	0.511	0.399	0.392
6	0.643	0.604	0.553	0.564	1.463	1.474	0.500	0.429	0.582	0.541	0.378	0.387
7	0.663	0.708	0.560	0.543	1.644	1.393	0.460	0.406	0.590	0.597	0.402	0.407
8	0.613	0.766	0.558	0.610	1.696	1.711	0.488	0.518	0.582	0.545	0.394	0.405
9	0.717	0.695	0.580	0.558	1.562	1.465	0.426	0.448	0.555	0.590	0.400	0.380
10	0.565	0.558	0.578	0.550	1.501	1.347	0.446	0.450	0.522	0.547	0.374	0.398
Grand mean	0.656		0.573		1.574		0.452		0.546		0.388	
Cochran´s test												
C	0.3883		0.3955		0.4090		0.4491		0.2784		0.3310	
Ccrit	0.8674		0.8674		0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers		no outliers		no outliers		no outliers		no outliers		no outliers	
σ_T	0.1444		0.1261		0.3463		0.0994		0.1202		0.0854	
s_x	0.0515		0.0131		0.1258		0.0309		0.0261		0.0082	
s_w	0.0550		0.0185		0.1156		0.0234		0.0237		0.0107	
s_s	0.0337		0.0009		0.0956		0.0260		0.0200		0.0033	
Critical	0.0433		0.0378		0.1039		0.0298		0.0361		0.0256	
Ss < critical ?	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited		method suited		method suited	

Appendix 1: Homogeneity data (continued)

	2,4,6-TBP - level 1		α-HBCD - level 1		γ-HBCD - level 1		TBBPA - level 1	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	1.492	1.442	0.568	0.576	0.313	0.338	2.539	2.503
2	1.458	1.477	0.598	0.494	0.331	0.237	2.472	2.638
3	1.580	1.613	0.580	0.567	0.288	0.264	2.462	2.547
4	1.673	1.620	0.582	0.686	0.271	0.281	2.577	2.458
5	1.489	1.603	0.570	0.527	0.274	0.278	2.653	2.629
6	1.683	1.518	0.591	0.568	0.292	0.287	2.637	2.382
7	1.652	1.692	0.621	0.694	0.320	0.301	2.452	2.308
8	1.445	1.460	0.639	0.551	0.281	0.279	2.122	2.547
9	1.492	1.742	0.625	0.649	0.296	0.260	2.398	2.631
10	1.700	1.887	0.564	0.668	0.249	0.246	2.564	3.020
Grand mean	1.586		0.596		0.284		2.527	
Cochran´s test								
C	0.4294		0.2239		0.7490		0.3588	
Ccrit	0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers		no outliers		no outliers		no outliers	
σ_T	0.3965		0.1311		0.0625		0.5560	
s_x	0.1070		0.0386		0.0211		0.1264	
s_w	0.0855		0.0493		0.0243		0.1703	
s_s	0.0883		0.0166		0.0122		0.0386	
Critical	0.1047		0.0393		0.0188		0.1668	
Ss < critical?	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited	
	2,4,6-TBP - level 2		α-HBCD - level 2		γ-HBCD - level 2		TBBPA - level 2	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	4.046	3.901	4.838	4.647	5.572	5.587	10.193	9.115
2	3.971	4.262	5.021	4.179	4.995	5.623	9.975	9.989
3	3.402	4.112	4.858	4.212	5.371	5.192	9.738	9.863
4	3.192	3.260	4.003	4.216	5.651	5.168	9.091	9.366
5	3.055	4.121	4.239	4.152	4.896	4.838	9.486	9.917
6	3.604	3.588	4.686	4.784	5.693	5.305	9.324	9.286
7	4.071	4.165	4.909	4.967	5.252	5.667	9.412	9.170
8	3.544	4.253	4.273	4.929	5.450	5.910	9.553	9.452
9	4.104	4.329	4.526	5.037	5.436	5.410	9.640	9.574
10	4.893	3.894	4.699	4.678	5.704	5.539	9.797	8.797
Grand mean	3.888		4.593		5.413		9.537	
Cochran´s test								
C	0.3433		0.3691		0.3220		0.4621	
Ccrit	0.8674		0.8674		0.8674		0.8674	
C < Ccrit ?	no outliers		no outliers		no outliers		no outliers	
σ_T	0.9721		1.1482		1.3532		2.3842	
s_x	0.3506		0.2586		0.2306		0.2541	
s_w	0.4068		0.3100		0.2476		0.3546	
s_s	0.2004		0.1371		0.1500		0.0410	
Critical	0.2566		0.3031		0.3573		0.6294	
Ss < critical?	homogeneity adequate		homogeneity adequate		homogeneity adequate		homogeneity adequate	
sw < 0.5 σT ?	method suited		method suited		method suited		method suited	

Appendix 2: Stability data

Biomarker	BDE 47 level 1		BDE 47 level 2		BDE 153 level 1		BDE 153 level 2		BDE 209 level 1		BDE 209 level 2	
time (days)	0	40	0	40	0	40	0	40	0	40	0	40
	0.177	0.148	0.582	0.635	0.190	0.187	0.553	0.562	0.984	0.895	1.665	1.797
	0.163	0.148	0.581	0.552	0.189	0.189	0.563	0.607	0.997	0.936	1.861	1.810
	0.133	0.153	0.574	0.626	0.185	0.176	0.495	0.581	0.936	0.825	1.459	1.664
	0.175	0.134	0.564	0.622	0.222	0.196	0.578	0.563	0.971	0.971	1.829	1.917
	0.111	0.142	0.610	0.643	0.180	0.163	0.515	0.564	0.843	0.842	1.696	1.834
	0.108	0.147	0.578	0.604	0.185	0.173	0.613	0.578	0.895	0.843	1.711	1.827
Average	0.145	0.145	0.582	0.614	0.192	0.181	0.553	0.576	0.937	0.885	1.704	1.808
Std dev	0.031	0.006	0.015	0.033	0.015	0.012	0.043	0.017	0.059	0.059	0.143	0.082
x0-xa (difference)		-0.001		-0.032		0.011		-0.023		0.052		-0.105
Test 'consequential instability':												
σH		0.032		0.128		0.042		0.122		0.206		0.375
0,3*σH		0.010		0.038		0.013		0.036		0.062		0.112
x0-xa<0,3*σH? (consequential instability)		NO		NO		NO		NO		NO		NO
Test 'significant difference':												
t		0.071		2.162		1.404		1.217		1.534		1.557
t-crit		2.228		2.228		2.228		2.228		2.228		2.228
Significant difference		NO		NO		NO		NO		NO		NO

Biomarker	DP anti level 1		DP anti level 2		DP syn level 1		DP syn level 2		DBDPE level 1		DBDPE level 2	
time (days)	0	40	0	40	0	40	0	40	0	40	0	40
	0.120	0.125	0.522	0.557	0.284	0.324	0.518	0.655	0.083	0.095	0.389	0.375
	0.109	0.119	0.511	0.547	0.283	0.302	0.518	0.638	0.095	0.089	0.397	0.371
	0.107	0.116	0.522	0.522	0.294	0.294	0.450	0.605	0.101	0.103	0.399	0.386
	0.108	0.115	0.511	0.541	0.293	0.293	0.500	0.631	0.094	0.105	0.378	0.387
	0.119	0.106	0.541	0.511	0.272	0.269	0.460	0.593	0.084	0.102	0.394	0.405
	0.115	0.116	0.555	0.455	0.253	0.293	0.426	0.536	0.099	0.091	0.400	0.380
Average	0.113	0.116	0.527	0.522	0.280	0.296	0.479	0.610	0.093	0.098	0.393	0.384
Std dev	0.006	0.006	0.018	0.037	0.016	0.017	0.038	0.043	0.008	0.007	0.008	0.012
x0-xa (difference)		-0.003		0.005		-0.016		-0.131		-0.005		0.009
Test 'consequential instability':												
σH		0.025		0.116		0.062		0.105		0.020		0.086
0,3*σH		0.007		0.035		0.018		0.032		0.006		0.026
x0-xa<0,3*σH? (consequential instability)		NO		NO		NO		YES		NO		NO
Test 'significant difference':												
t		0.926		0.286		1.666		5.576		1.169		1.484
t-crit		2.228		2.228		2.228		2.228		2.228		2.228
Significant difference		NO		NO		NO		YES		NO		NO

Biomarker	2,4,6-TBP level 1		2,4,6-TBP level 2		alpha HBCD level 1		alpha HBCD level 2		gama HBCD level 1		gama HBCD level 2	
time (days)	0	40	0	40	0	40	0	40	0	40	0	40
	1.673	1.650	5.021	5.014	0.568	0.619	4.647	4.839	0.288	0.289	5.572	5.542
	1.683	1.849	4.858	4.403	0.576	0.633	4.212	4.785	0.281	0.283	5.587	5.174
	1.692	1.803	4.909	5.082	0.582	0.602	4.838	4.643	0.274	0.294	4.995	5.108
	1.742	1.811	4.967	5.349	0.580	0.567	4.858	5.091	0.292	0.312	5.623	5.293
	1.700	1.861	4.929	5.415	0.570	0.586	4.784	5.201	0.281	0.319	5.371	5.140
	1.887	1.710	5.037	5.405	0.527	0.571	4.967	4.940	0.296	0.296	5.192	5.348
Average	1.729	1.781	4.954	5.111	0.567	0.596	4.718	4.916	0.285	0.299	5.390	5.267
Std dev	0.081	0.083	0.068	0.386	0.020	0.026	0.269	0.205	0.008	0.014	0.254	0.163
x0-xa (difference)		-0.051		-0.158		-0.029		-0.199		-0.014		0.123
Test 'consequential instability':												
σH		0.380		1.090		0.125		1.038		0.063		1.186
0,3*σH		0.114		0.327		0.037		0.311		0.019		0.356
x0-xa<0,3*σH? (consequential instability)		NO		NO		NO		NO		NO		NO
Test 'significant difference':												
t		1.079		0.984		2.136		1.438		2.114		0.995
t-crit		2.228		2.228		2.228		2.228		2.228		2.228
Significant difference		NO		NO		NO		NO		NO		NO

Biomarker	TBBPA level 1		TBBPA level 2	
time (days)	0	40	0	40
	2.539	3.167	10.193	9.484
	2.462	2.450	9.975	12.218
	2.653	2.771	9.738	10.256
	2.637	3.040	9.797	9.817
	3.020	2.360	9.486	10.120
	2.398	2.727	9.989	10.483
Average	2.618	2.752	9.863	10.397
Std dev	0.220	0.317	0.245	0.958
x0-xa (difference)		-0.134		-0.534
Test 'consequential instability':				
σH		0.576		2.170
0,3*σH		0.173		0.651
x0-xa<0,3*σH? (consequential instability)		NO		NO
Test 'significant difference':				
t		0.851		1.322
t-crit		2.228		2.228
Significant difference		NO		NO

Appendix 3: Copy of letter of invitation

HBM4EU science and policy for a healthy future

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study
BFR/Round 3

Title of ICI/EQUAS: BFR in serum

Dear Colleague,

within the frame of HBM4EU,

Prof. Dr. Jana Hajslova & Dr. Darina Lankova

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

announce the 3rd round of ICI/EQUAS for the determination of BFR in serum. 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.

Test samples
The matrix will be serum. The participants will receive:
- 3 different materials of serum (5 ml each) for determination of BFR in serum

Target biomarkers
Please see registration form for BFR/Round 3 for the biomarkers potentially present in the test samples. We would be pleased if your laboratory could participate with the analysis of as most as possible BFR.
LOQs should allow the analysis of BFR in samples of the general population.

Calendar:

Deadline for registration	23-05-2019
Distribution of test samples (projected)	28-05-2019
Deadline for reporting the results (projected)	08-07-2019

HBM4EU science and policy for a healthy future

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

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

Confidentiality:
All laboratory specific information will be treated confidentially, and will never be disclosed to third parties (government, accreditation bodies) except the HBM4EU QAU, without permission of the laboratory.

Contact information organiser:

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

Email of organiser: darina.lankova@vscht.cz
Email for registration: VSCHT-hbm4EU@vscht.cz
Phone: 00420 22044 4312

Appendix 4: Copy of registration form for participation

HBM4EU science and policy for a healthy future

HBM4EU: Registration form for participation in ICI / EQUAS study BFR/Round 3.

Title of ICI/EQUAS: BFR in serum

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

Parameter	Participation	
	Yes	No
BDE-47		
BDE-153		
BDE-209		
α-HBCD		
γ-HBCD		
TBBPA		
Syn-DP		
Anti-DP		
DBDPE		
2,4,6-Tribromophenol		

Participating laboratory:
name of institution

address of the laboratory

name of 1st contact person, telephone number and email address

name of 2nd contact person, telephone number and email address

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Address for delivery of the test samples
name of institution

address of the laboratory

The above laboratory will participate in the ICI/EQUAS study **BFR/Round 3**.
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:

VSCHT-hbm4EU@vscht.cz

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

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

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

HBM4EU science and policy for a healthy future

HBM4EU: Result submission form ICI/EQUAS study BFR/ Round 3.

Title of ICI/EQUAS: BFR in serum

Laboratory name & code:

Contact person:

Institute:

Address:

Country:

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

Date:

Signature:

HBM4EU science and policy for a healthy future

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

VSCHT-hbm4EU@vscht.cz

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

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

Jana Hajslova
Email: jana.hajslova@vscht.cz
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Prof. Ing. Jana Hajslova, CSc.
(for the ICI/EQUAS organisers)

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

HBM4EU science and policy for a healthy future

HBM4EU: Acknowledgement of receipt form ICI/EQUAS study BFR/Round 3.

Title of ICI/EQUAS: BFR in serum

Laboratory name:

Contact person:

Contents of parcel:
- 3 tubes with 5 ml serum

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

Date of receipt (dd-mm-yyyy):

Code on tube	Damage/leakage	Remarks

Name: Signature:

Date:

After signing this form, please scan and send the pdf. to: VSCHT-hbm4EU@vscht.cz

HBM4EU science and policy for a healthy future

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

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(for the ICI/EQUAS organisers)

Appendix 7: Copy of method information form for participation in 3rd ICI/EQUAS

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Method information form for participation in ICI/EQUAS BFR in serum BFR/Round 3.

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

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

Further remarks/observations:

Date:

Signature:

Appendix 8: Scope and LOQs (ng/ml) as provided in the method information submitted by the laboratories

Lab code	BDE-47	BDE-153	BDE-209	α-HBCD	γ-HBCD	TBBPA	Syn-DP	Anti-DP	DBDPE	2,4,6-TBP	Total
PT3BFR01	0.005	0.005	0.050	0.005	0.005	0.005	0.020	0.020	0.800	0.010	**10**
PT3BFR02	0.004	0.004	0.035	NA	NA	0.004	0.010	0.010	0.100	0.049	**8**
PT3BFR03	0.2	1	NA	NA	NA	NA	NA	NA	NA	NA	**2**
PT3BFR04	0.00074-0.002963	0.0053-0.12649	0.007975-0.028301	0.002	0.002	0.001	0.002114-0.006311	0.001868-0.005602	2.315	NA	**9**
PT3BFR05	0.0114	0.0116	0.0515	0.0123	0.0127	NA	0.0114	0.0114	NA	NA	**7**
PT3BFR06	0.0001	0.0001	0.0001	0.010	0.010	0.005	0.0001	0.0001	NA	0.001	**9**
PT3BFR07	NA	NA	NA	0.03	0.03	NA	NA	NA	NA	NA	**2**
PT3BFR08	0.001	0.001	0.15	NA	NA	NA	0.001	0.001	NA	NA	**5**
PT3BFR09	0.005	0.005	0.1	NA	NA	NA	NA	NA	0.2	NA	**4**
PT3BFR10	0.01	0.005	0.009	NA	NA	NA	0.005	0.005	0.025	NA	**6**
PT3BFR11	0.001	0.001	NA	0.25	0.25	NA	0.1	0.1	NA	NA	**6**
PT3BFR12	0.00152-0.00163	0.00368-0.00392	0.0354-0.0382	0.176-2.24	0.134-0.669	NA	NA	NA	NA	NA	**5**
PT3BFR13	0.00003-0.00005	0.00003-0.00031	0.001-0.0060	NA	NA	NA	NA	NA	NA	NA	**3**
PT3BFR14	0.050000	0.05000	NA	NA	NA	NA	NA	NA	NA	NA	**2**
Total	**13**	**13**	**10**	**7**	**7**	**4**	**8**	**8**	**5**	**3**	

NA – not analysed

Appendix 9: Assigned values and participant's performance

	BDE 47: Level 1			BDE 47: Level 2			BDE 153: Level 1			BDE 153: Level 2		
Z-score based on	**expert value ($n_{expert\ labs}$=5)**			**expert value ($n_{expert\ labs}$=5)**			**expert value ($n_{expert\ labs}$=5)**			**expert value ($n_{expert\ labs}$=5)**		
Number of participants	**13**			**13**			**13**			**13**		
Number of quantitative results	**13**			**13**			**12(+1)**			**13**		
Expert / Consensus value (ng/ml)	**0.151**			**0.644**			**0.184**			**0.549**		
Uncertainty of assigned value (ng/ml)	**0.013**			**0.098**			**0.019**			**0.059**		
Relative uncertainty (%)	**8.6**			**15.2**			**10.3**			**10.7**		
Relative FFP-target standard deviation (%)	**25**			**25**			**25**			**25**		
Study RSDr (%)	**24**			**24**			**25**			**33**		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	0.144	-0.2	satisfactory	0.590	-0.3	satisfactory	0.215	0.7	satisfactory	0.655	0.8	satisfactory
PT3BFR02	0.123	-0.7	satisfactory	0.460	-1.1	satisfactory	0.202	0.4	satisfactory	0.547	0.0	satisfactory
PT3BFR03	0.201	1.3	satisfactory	0.743	0.6	satisfactory	< 1.000	(17.7)	unsatisfactory	1.062	3.7	unsatisfactory
PT3BFR04	0.097	-1.4	satisfactory	0.395	-1.5	satisfactory	0.106	-1.7	satisfactory	0.324	-1.6	satisfactory
PT3BFR05	0.132	-0.5	satisfactory	0.518	-0.8	satisfactory	0.193	0.2	satisfactory	0.594	0.3	satisfactory
PT3BFR06	0.128	-0.6	satisfactory	0.485	-1.0	satisfactory	0.201	0.4	satisfactory	0.594	0.3	satisfactory
PT3BFR07	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR08	0.160	0.2	satisfactory	0.657	0.1	satisfactory	0.215	0.7	satisfactory	0.685	1.0	satisfactory
PT3BFR09	0.119	-0.8	satisfactory	0.514	-0.8	satisfactory	0.188	0.1	satisfactory	0.636	0.6	satisfactory
PT3BFR10	0.223	1.9	satisfactory	0.892	1.5	satisfactory	0.304	2.6	questionable	1.097	4.0	unsatisfactory
PT3BFR11	0.114	-1.0	satisfactory	0.463	-1.1	satisfactory	0.128	-1.2	satisfactory	0.430	-0.9	satisfactory
PT3BFR12	0.143	-0.2	satisfactory	0.560	-0.5	satisfactory	0.193	0.2	satisfactory	0.599	0.4	satisfactory
PT3BFR13	0.129	-0.6	satisfactory	0.523	-0.8	satisfactory	0.193	0.2	satisfactory	0.623	0.5	satisfactory
PT3BFR14	0.167	0.4	satisfactory	0.513	-0.8	satisfactory	0.220	0.8	satisfactory	0.534	-0.1	satisfactory

	BDE 209: Level 1			BDE 209: Level 2			Syn-DP: Level 1			Syn-DP: Level 2		
Z-score based on	**expert value ($n_{expert\ labs}$=4*)**			**expert value ($n_{expert\ labs}$=5)**			**consensus value**			**consensus value**		
Number of participants	**10**			**10**			**8**			**8**		
Number of quantitative results	**10**			**10**			**8**			**8**		
Expert / Consensus value (ng/ml)	**1.197**			**1.771**			**0.313**			**0.764**		
Uncertainty of assigned value (ng/ml)	**0.121**			**0.315**			**0.045**			**0.310**		
Relative uncertainty (%)	**10.1**			**17.8**			**14.4**			**40.6**		
Relative FFP-target standard deviation (%)	**25**			**25**			**25**			**25**		
Study RSDr (%)	**64**			**57**			**35**			**45**		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	1.820	2.1	questionable	1.960	0.4	satisfactory	0.295	-0.2	satisfactory	0.784	0.1	satisfactory
PT3BFR02	1.753	1.9	satisfactory	3.177	3.2	unsatisfactory	0.368	0.7	satisfactory	1.128	1.4	satisfactory
PT3BFR03	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR04	0.269	-3.1	unsatisfactory	0.452	-3.0	unsatisfactory	0.163	-1.9	satisfactory	0.367	-1.5	satisfactory
PT3BFR05	1.060	-0.5	satisfactory	1.650	-0.3	satisfactory	0.313	0.0	satisfactory	0.804	0.2	satisfactory
PT3BFR06	0.853	-1.1	satisfactory	1.650	-0.3	satisfactory	0.324	0.1	satisfactory	0.711	-0.2	satisfactory
PT3BFR07	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR08	1.418	0.7	satisfactory	2.535	1.7	satisfactory	0.373	0.8	satisfactory	0.895	0.5	satisfactory
PT3BFR09	1.170	-0.1	satisfactory	2.260	1.1	satisfactory	NA	x	x	NA	x	x
PT3BFR10	0.224	-3.3	unsatisfactory	0.794	-2.2	questionable	0.337	0.3	satisfactory	1.151	1.5	satisfactory
PT3BFR11	NA	x	x	NA	x	x	0.100	-2.7	questionable	0.184	-2.2	questionable
PT3BFR12	0.978	-0.7	satisfactory	1.740	-0.1	satisfactory	NA	x	x	NA	x	x
PT3BFR13	0.107	-3.6	unsatisfactory	0.194	-3.6	unsatisfactory	NA	x	x	NA	x	x
PT3BFR14	NA	x	x	NA	x	x	NA	x	x	NA	x	x

Appendix 9: Assigned values and participant's performance (continued)

	Anti-DP: Level 1			α-HBCD: Level 1			α-HBCD: Level 2		
Z-score based on	**consensus value**			**consensus value**			**consensus value**		
Number of participants	**8**			**7**			**7**		
Number of quantitative results	**8**			**7**			**7**		
Expert / Consensus value (ng/ml)	**0.134**			**0.583**			**4.887**		
Uncertainty of assigned value (ng/ml)	**0.032**			**0.051**			**0.416**		
Relative uncertainty (%)	**23.9**			**8.7**			**8.5**		
Relative FFP-target standard deviation (%)	**25**			**25**			**25**		
Study RSDr (%)	**35**			**31**			**18**		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	0.133	-0.02	satisfactory	0.500	-0.54	satisfactory	4.525	-0.27	satisfactory
PT3BFR02	0.136	0.06	satisfactory	NA	x	x	NA	x	x
PT3BFR03	NA	x	x	NA	x	x	NA	x	x
PT3BFR04	0.087	-1.31	satisfactory	0.490	-0.60	satisfactory	4.298	-0.45	satisfactory
PT3BFR05	0.138	0.12	satisfactory	0.532	-0.33	satisfactory	4.610	-0.21	satisfactory
PT3BFR06	0.156	0.62	satisfactory	0.470	-0.73	satisfactory	3.450	-1.11	satisfactory
PT3BFR07	NA	x	x	0.639	0.36	satisfactory	5.740	0.67	satisfactory
PT3BFR08	0.254	3.36	unsatisfactory	NA	x	x	NA	x	x
PT3BFR09	NA	x	x	NA	x	x	NA	x	x
PT3BFR10	0.152	0.51	satisfactory	NA	x	x	NA	x	x
PT3BFR11	0.100	-0.94	satisfactory	0.710	0.82	satisfactory	5.640	0.59	satisfactory
PT3BFR12	NA	x	x	1.020	2.83	questionable	5.840	0.75	satisfactory
PT3BFR13	NA	x	x	NA	x	x	NA	x	x
PT3BFR14	NA	x	x	NA	x	x	NA	x	x

	γ-HBCD: Level 1			γ-HBCD: Level 2		
Z-score based on	**consensus value**			**consensus value**		
Number of participants	**7**			**7**		
Number of quantitative results	**7**			**7**		
Expert / Consensus value (ng/ml)	**0.321**			**5.908**		
Uncertainty of assigned value (ng/ml)	**0.013**			**0.262**		
Relative uncertainty (%)	**4.0**			**4.4**		
Relative FFP-target standard deviation (%)	**25**			**25**		
Study RSDr (%)	**61**			**8**		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	0.296	-0.32	satisfactory	5.777	-0.09	satisfactory
PT3BFR02	NA	x	x	NA	x	x
PT3BFR03	NA	x	x	NA	x	x
PT3BFR04	0.297	-0.31	satisfactory	6.150	0.16	satisfactory
PT3BFR05	0.317	-0.05	satisfactory	5.800	-0.07	satisfactory
PT3BFR06	0.310	-0.14	satisfactory	5.180	-0.49	satisfactory
PT3BFR07	0.349	0.34	satisfactory	6.770	0.58	satisfactory
PT3BFR08	NA	x	x	NA	x	x
PT3BFR09	NA	x	x	NA	x	x
PT3BFR10	NA	x	x	NA	x	x
PT3BFR11	0.430	1.35	satisfactory	6.060	0.10	satisfactory
PT3BFR12	1.010	8.57	unsatisfactory	6.260	0.24	satisfactory
PT3BFR13	NA	x	x	NA	x	x
PT3BFR14	NA	x	x	NA	x	x

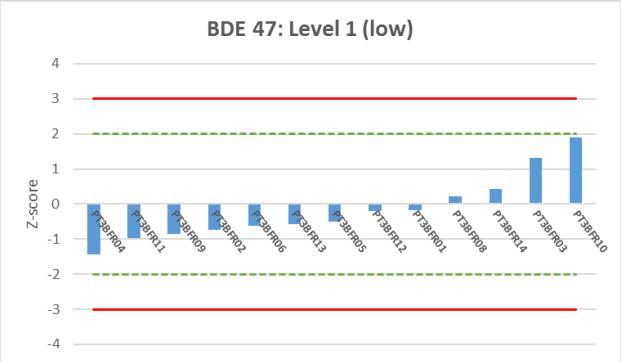
Legend to tables in Appendix 9:

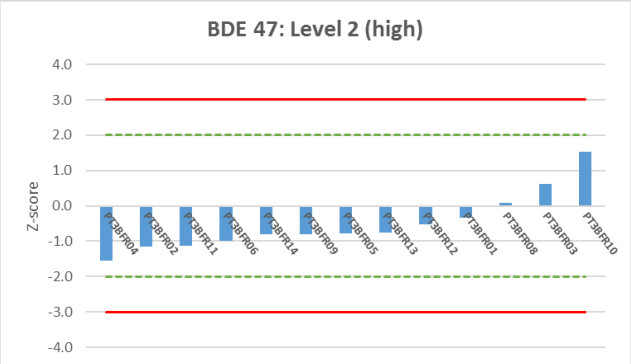
Z-score based on expert value = calculation of assigned value was based on the results obtained from expert laboratories.

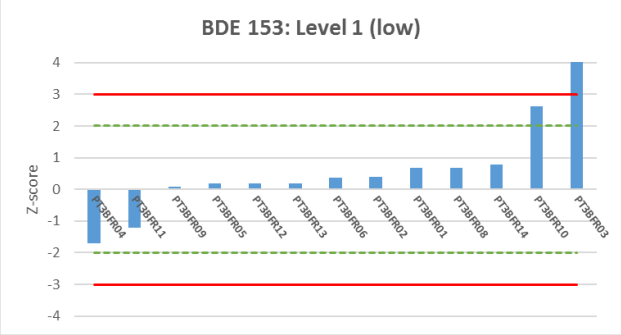
Z-score based on consensus value = no expert value was calculated because the number of valid results from expert laboratories was below 3. Instead, the consensus value based on the combined results of participants and expert laboratories was used as assigned value.

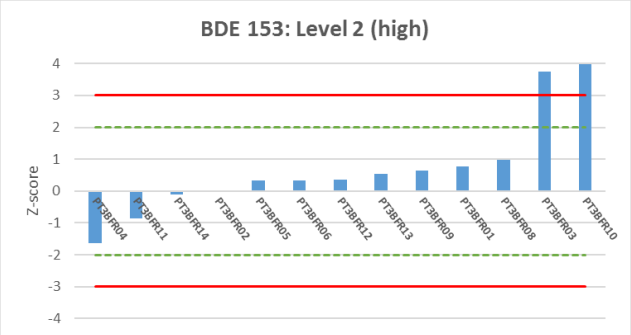
Z-score between brackets (x.x): laboratory reported result as '<LOQ-value'. A proxy-Z-score was calculated using the LOQ as result (see paragraph 5.6).

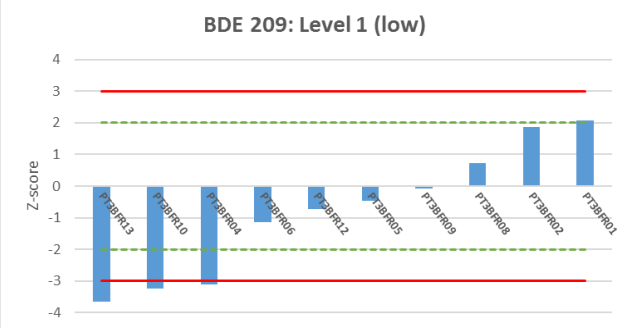
Appendix 10: Graphical representation of the Z-scores

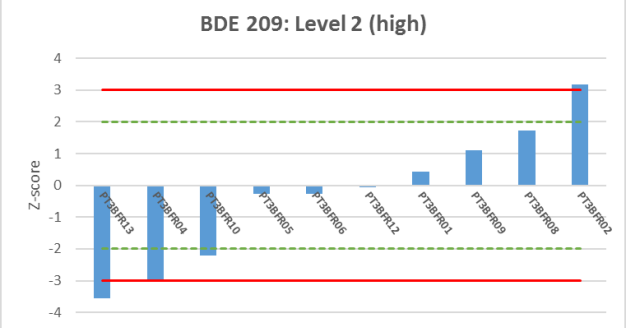




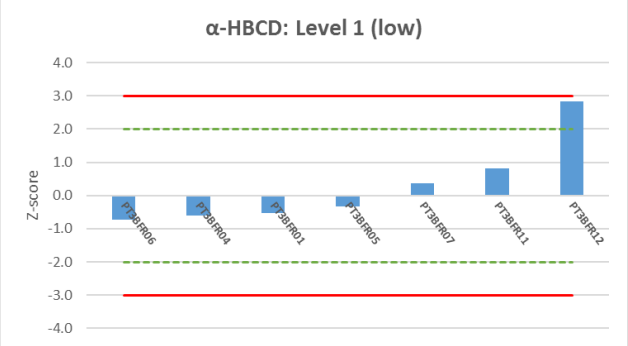


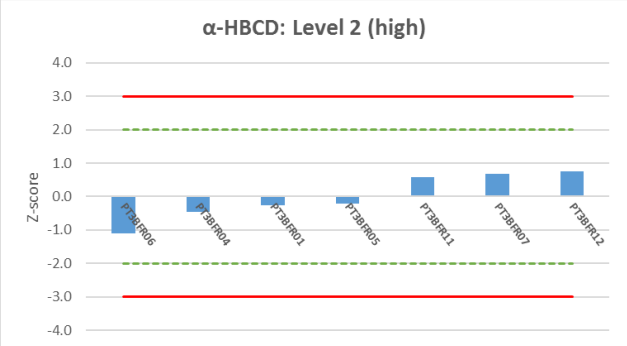


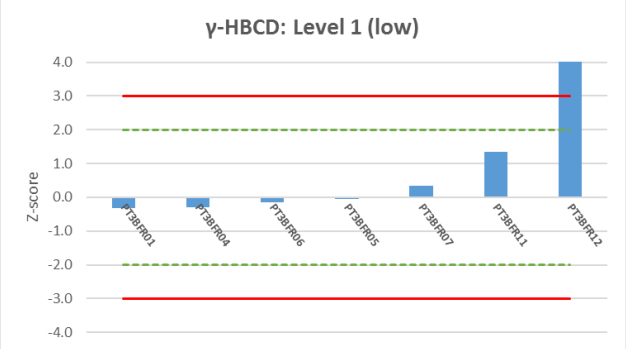


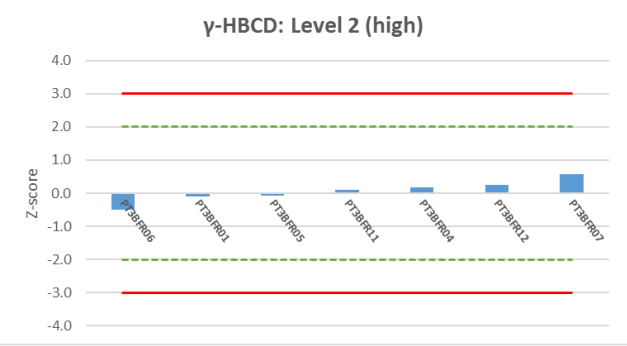


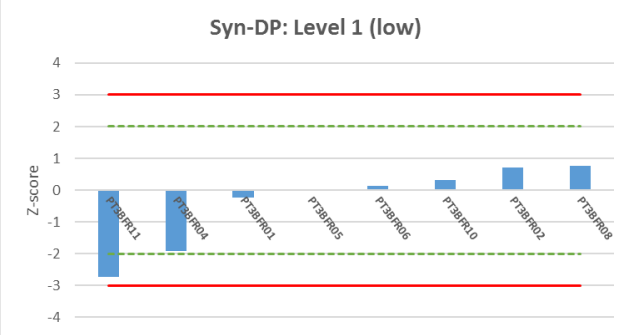
Appendix 10: Graphical representation of the Z-scores (continued)

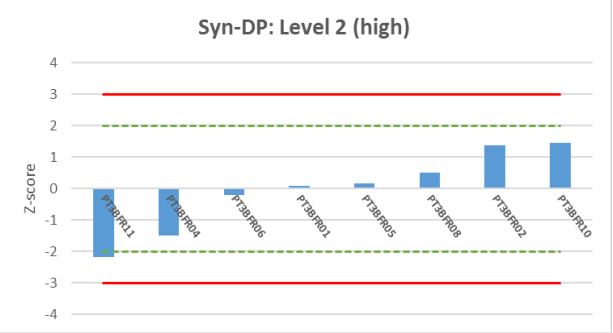












Appendix 11: Reported results for which no assigned value and Z-scores were calculated

	TBBPA: Level 1			TBBPA: Level 2			DBDPE: Level 1			DBDPE: Level 2		
Z-score based on	x			x			x			x		
Number of participants	4			4			5			5		
Number of quantitative results	4			4			1(+4)			2(+3)		
Expert / Consensus value (ng/ml)	NC			NC			NC			NC		
Uncertainty of assigned value (ng/ml)	NC			NC			NC			NC		
Relative uncertainty (%)	NC			NC			NC			NC		
Relative FFP-target standard deviation (%)	NC			NC			NC			NC		
Study RSDr (%)	NC			NC			NC			NC		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	2.278	x	x	7.519	x	x	< 0.800	x	x	< 0.800	x	x
PT3BFR02	1.737	x	x	14.102	x	x	< 0.100	x	x	0.531	x	x
PT3BFR03	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR04	0.787	x	x	10.427	x	x	< 2.315	x	x	< 2.315	x	x
PT3BFR05	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR06	2.900	x	x	9.870	x	x	NA	x	x	NA	x	x
PT3BFR07	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR08	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR09	NA	x	x	NA	x	x	< 0.200	x	x	< 0.200	x	x
PT3BFR10	NA	x	x	NA	x	x	0.071	x	x	0.418	x	x
PT3BFR11	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR12	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR13	NA	x	x	NA	x	x	NA	x	x	NA	x	x
PT3BFR14	NA	x	x	NA	x	x	NA	x	x	NA	x	x

	2,4,6-TBP: Level 1			2,4,6-TBP: Level 2			Anti-DP: Level 2		
Z-score based on	x			x			consensus value		
Number of participants	3			3			8		
Number of quantitative results	3			3			8		
Expert / Consensus value (ng/ml)	NC			NC			0.769		
Uncertainty of assigned value (ng/ml)	NC			NC			0.389 (unfit)		
Relative uncertainty (%)	NC			NC			50.6		
Relative FFP-target standard deviation (%)	NC			NC			25		
Study RSDr (%)	NC			NC			44		
	Value	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**	**Value**	**Z-score**	**Classification**
PT3BFR01	2.128	x	x	5.795	x	x	0.830	x	x
PT3BFR02	0.73	x	x	6.41	x	x	1.051	x	x
PT3BFR03	NA	x	x	NA	x	x	NA	x	x
PT3BFR04	NA	x	x	NA	x	x	0.402	x	x
PT3BFR05	NA	x	x	NA	x	x	0.779	x	x
PT3BFR06	1.610	x	x	4.730	x	x	0.747	x	x
PT3BFR07	NA	x	x	NA	x	x	NA	x	x
PT3BFR08	NA	x	x	NA	x	x	1.216	x	x
PT3BFR09	NA	x	x	NA	x	x	NA	x	x
PT3BFR10	NA	x	x	NA	x	x	1.159	x	x
PT3BFR11	NA	x	x	NA	x	x	0.213	x	x
PT3BFR12	NA	x	x	NA	x	x	NA	x	x
PT3BFR13	NA	x	x	NA	x	x	NA	x	x
PT3BFR14	NA	x	x	NA	x	x	NA	x	x

Legend to the table in Appendix 11

Z-score based on x = no assigned value was calculated because number of valid results from expert laboratories was below 3, and no meaningful robust mean (consensus value) and study RSD_R could be calculated because the number of values from the participants was below 7.