HBM4EU science and policy for a healthy future

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Standard Operation Procedure

HBM4EU-SOP-QA-001

Organisation of Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS)

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1 Aim and application area

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.

Within HBM4EU, participation in ICI/EQUAS for a certain biomarker/matrix is mandatory for laboratories that will analyse HBM4EU samples. For this purpose, candidate laboratories need to participate in multiple proficiency rounds (ICI and/or EQUAS studies). In case of poor overall performance for a round, the QAU can decide to extend the programme and organise additional rounds. Each round will include two control materials containing different concentrations of the biomarker(s). Additional materials for informatory purposes may be included in a round. The rounds are divided in time in such a way that laboratories receive feedback on their performance well before the next round. This gives laboratories the opportunity to evaluate their results and perform corrective action if needed before participation in the next round for that biomarker/matrix.

This SOP describes the procedures and work flows for the organization of ICIs and EQUAS exercises as will be done in the frame of HBM4EU. For drafting this SOP, requirements as outlined in ISO/IEC 17043:2010, "Conformity assessment – General requirements for proficiency testing" have been taken into account.

The evaluation and scoring of the results of the participating laboratories will be done on an individual biomarker/matrix/concentration basis. Classification or decisions on eligibility of laboratories for HBM4EU analysis is not done by the ICI/EQUAS organiser and is beyond the scope of this SOP.

Details on preparation of control materials, evaluation of results, and reporting of results are described in separate SOPs (HBM4EU-SOP-QA-002/003/004).

2 Definitions

2.1 Interlaboratory comparability investigation (ICI)

ICI, elsewhere called proficiency test, assesses the comparability of analysis results for the same sample analysed by multiple laboratories in the same time frame. Each laboratory uses its own method. As measure of proficiency, Z-scores are calculated using the consensus value derived from the participants' results as assigned value, and a pre-set target standard deviation (e.g. fit-for-purpose standard deviation). Candidate laboratories are requested to apply the same procedure as they will use for analysis of samples in the frame of HBM4EU.

2.2 External Quality Assessment Scheme (EQUAS)

EQUAS is similar to ICI but instead of using the consensus value as assigned value, the mean concentration as established from data generated by designated expert laboratories is used. As in an ICI, Z-scores are calculated as a measure of proficiency. Candidate laboratories are requested to apply the same procedure as they will use for analysis of samples in the frame of HBM4EU.

2.3 Organiser

The laboratory responsible for organization of the ICI or EQUAS.

2.4 Coordinator

Person from the organiser responsible for the conduct of the ICI/EQUAS.

2.5 Expert Laboratory

Laboratory that is highly skilled and experienced and has a documented track record for determination of a certain biomarker/matrix combination. Expert laboratories are involved in EQUAS studies to establish expert-assigned values.

2.6 Biomarker

A biomarker is the target molecule of the HBM procedure in the biological material. In the case of conjugates, which are hydrolysed for the determination of the total concentration, the biomarker is defined as the target molecule after deconjugation.

2.7 Control material

Biological material used in an ICI / EQUAS, prepared and tested in a controlled process. Whenever available, the control materials (e.g. urine, blood/plasma/serum, milk, hair) are human materials (otherwise adequate surrogates).

2.7.1 Blank control material

Biological material with non-detectable or very low concentrations of the biomarker of interest. This material may be used for evaluation of procedural contamination and assessment of occurrence of false positives.

2.7.2 Burdened control material

Biological material containing the biomarker of interest (and/or its conjugates) through real-life exposure routes (e.g. diet, environmental, occupational) in a concentration range relevant for sample analysis in the frame of HBM4EU.

2.7.3 Spiked control material

Biological material artificially enriched with the biomarker of interest by addition of the biomarker to the control material. Spiked control materials are used in ICI/EQUAS when burdened materials are not available or do not contain the biomarker at sufficiently high concentrations. Where the biomarker is a conjugate, spiking is preferably done with the conjugate as far as available, otherwise the free form may be used.

2.8 Test sample

Coded aliquot of the control material, to be analysed by laboratories participating in an ICI / EQUAS.

2.9 Assigned value

Concentration of a biomarker considered to be the best estimate of the true value for a control material. In an ICI, the consensus value is used as assigned value. In an EQUAS, a target value as established by expert laboratories is used as assigned value.

2.10 Consensus value

Concentration of a biomarker in a test material derived from the results of the participants in an ICI.

2.11 Expert-assigned value

Concentration of a biomarker in a test material as derived from the results of expert laboratories.

2.12 Target standard deviation (σ_T)

The standard deviation for proficiency (target standard deviation) determines the performance boundaries in an ICI/EQUAS. It is used for calculation of Z-scores.

2.13 ICI / EQUAS standard deviation (RSD_R)

Standard deviation calculated for a biomarker in a control material from the results submitted by the participants.

2.14 Outliers

Analysis result characterized as deviation from the other results in a study.

2.15 Robust statistics

Statistical method to minimize the influence of outliers on the calculated mean and standard deviation [Analytical Methods Committee, 1989a&b].

2.16 False positive

Reported presence or a concentration for a biomarker by a participant in an ICI / EQUAS that has been demonstrated during the evaluation of the ICI/EQUAS not to be present in the control material.

2.17 False negative

Situation where a biomarker is present in the test material but no numerical value is reported by the participant in an ICI / EQUAS study.

2.18 Z-score

The Z-score is a value used to classify the performance of a laboratory within an ICI or EQUAS.

3 Procedures

3.1 General

3.1.1 Tasks, responsibilities and requirement of the organiser

For each ICI / EQUAS a team is established with the following tasks and responsibilities:

- Coordinator, experienced in organization of ICI / EQUAS studies, responsible for:
    - coordination of the study (planning, progress)
    - contact person for the participating laboratories
    - reporting
- Scientific expert with sufficient knowledge and experience of the parameter studied: responsible for scientific relevance and quality of the study
- Technical staff, experienced with and responsible for:
    - logistics (invitation of candidate participants, sample shipment, collection of results and questionnaires)
    - preparation of control material and test samples
    - interpretation of analysis data of control material (homogeneity/stability)
- Optionally: statistician to assist in data interpretation

Notes:

- ICI/EQUAS organisers are institutions from the HBM4EU consortium (including linked-third parties). When not available, organisations outside the consortium may be considered as an alternative option.

- EQUAS involves expert laboratories which can be any competent organisation, either from the HBM4EU consortium or external.

- In principle the organiser of an ICI performs all tasks mentioned above. If not possible, the organiser may outsource the preparation of the test material and/or the associated analyses (i.e. analysis of test samples for homogeneity and stability testing). In that case, the laboratories preparing the control material and performing the test sample analysis will report the analysis results to the organiser (template see HBM4EU-SOP-QA-002) after which the organiser will perform the statistical analysis to assess homogeneity and stability of the control material.

3.1.2 Communication with the participating laboratories

Communication with the laboratories will be done by the organizer. Participating laboratories will remain anonymous in emails sent to multiple addresses unless agreed otherwise. Disclosure of expected or assigned concentrations will only be done after the deadline of result submission.

3.1.3 Conflict of interest

A laboratory involved in the organisation of an ICI/EQUAS for a certain biomarker/matrix, and/or the final preparation of the control material, and/or unblinded analysis of test samples for

homogeneity and stability, cannot participate in the ICI/EQUAS studies for that particular biomarker/matrix combination. Consequently, that laboratory cannot demonstrate its proficiency and not apply as candidate for analysis of HBM4EU samples for that parameter.

A laboratory performing the homogeneity/stability testing without *a priori* knowledge of the concentrations (e.g. analysis data from elsewhere, known spiking levels) is still eligible as candidate for analysis of HBM4EU samples.

In case of an EQUAS study, an expert laboratory can be involved in preparation of the test material and/or homogeneity/stability testing, and its results can be used for establishment of the expert-assigned value. Regarding qualification as candidate for analysis of HBM4EU samples for a certain biomarker/matrix, an expert laboratory from the HBM4EU consortium is exempted from the obligation to participate in EQUAS studies for that biomarker (but wherever possible needs to participate in ICI studies).

Exceptions to these rules are only possible upon agreement of the QAU and must exclude any conflict of interest.

3.2 Work flow ICI

The table below describes the steps and indicative timeline for the conduct of an ICI. Time t=0 corresponds to shipment of the test samples.

Time (d)	Description	details see
t<-49	Drafting ICI plan	3.2.1
-42<t<-35	Invitation and registration of candidate participants	3.2.2
t=-28	Closure of registration of participants	3.3.3
	Go/no go decision ICI:	
	(postponement or cancellation when number of participants <7)	
-42<t<-35	Preparation of list of participants details (contacts, address, lab code, etc.)	
	Production/preparation of control material	3.2.4
-35<t<-7	Homogeneity and stability testing of control material	3.2.4
	Aliquotation and coding of test samples	
-7<t<0	Preparation of test sample sets for shipment	3.2.5
-28<t<0	Preparation of documentation/shipment arrangements	3.2.6-3.2.8
	(instructions, receipt form, method questionnaire, courier service, dry ice)	
t=0	**Shipment of test samples to participants**	**3.2.9**
	Communicate shipment to participants	
0<t<7	Collect sample receipt forms	
	Re-send if necessary	
21<t<28	Send out reminder of deadline approaching	
t=28	Deadline submission of results	3.2.10

	Registration and verification of results	
	Analysis for stability verification of test samples	
28<t<35	Statistical evaluation of results	3.2.11
	If necessary: inquire additional information from laboratories	
0<t<48	Writing draft ICI report	3.2.12
48<t<56	Discussion/approval of draft report by/with HBM4EU QAU	
56<t<63	Distribution of final report to participants and HBM4EU QAU	
t<70	Archiving of ICI documentation/correspondence	3.2.13

3.2.1 ICI plan

An ICI is initiated by drafting an ICI plan. The plan should include:

- Title of the ICI, date of issue,
- Organizer details (organization, staff involved and tasks)
- Other parties involved (if any)
- Information of material, biomarkers to be analysed, and indication of concentration range
- Number of control materials and test samples to be sent to each laboratory
- Origin of control material and clearance/approval of use for ICI purposes
- Procedure of preparation and analysis of the test material by the organizer
- Procedure of transport of test samples
- WP9 helpdesk contact (https://www.hbm4eu.eu/private/wp9-help-desk/)

As a brief summary of the ICI plan, the form in Appendix 1 must be completed and sent to the HBM4EU QAU.

3.2.2 ICI invitation and registration

After an optional pre-announcement, an invitation to participate in an ICI with a registration form will be sent (template see Appendix 2) to candidate laboratories by email. The invitation letter will include:

- Subject of the ICI (matrix/biomarker)
- Aim of the ICI
- The number of test samples to be analysed, and the amount of control material of each sample that will be provided
- Calendar with key-dates: deadline registration, sample shipment, deadline submission analysis results, expected date preliminary and final report
- Any special requirements for participation in the ICI if applicable (e.g. minimum required LOQ, in/exclusion of certain methods)

- Statement that 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.

- Statement participation is free of charge.

Note: costs for organisation, preparation of control material, test samples and courier cost are covered by the organizer through HBM4EU, but only for participants that are partners or linked-third parties of the HBM4EU consortium. In case of any participants from outside the HBM4EU consortium, a fee will apply.

- Statement that participating laboratories themselves are responsible for custom clearance and associated costs if applicable.

The registration form will include:

- name/address of the laboratory

- name of contact person, telephone number and email address

- address for delivery of the test samples

- statement to be signed by the contact person to agree with the conditions mentioned in the invitation letter, and that the laboratory will analyse the ICI samples and submit results before the indicated deadline

- organizer details (institute, coordinator, contact details) and information how to submit the registration form

Upon registration, the participant will receive a confirmation and a random lab code. The organizer keeps a confidential record linking the participants to the lab codes. This record will be submitted to the QAU who will keep a database of all ICI/EQUAS participants and results.

3.2.3 Closure of registration

Registration closes after the deadline indicated in the invitation letter. However, the organizer can allow additional laboratories to participate until shipment of the samples in specific cases, when a sufficient number of test samples is available.

The ICI can proceed only when at least seven laboratories have registered. A lower number will not enable proper statistical evaluation of the data (i.e. calculation of Z-scores) and results in cancelation or postponement of the ICI. If needed, the registration deadline can be extended and the invitation of candidate laboratories can be expanded to laboratories outside HBM4EU or outside the EU after approval by the Pillar leaders.

In case an ICI cannot proceed due to an insufficient number of participants, it may be replaced by an extra EQUAS round.

3.2.4 Preparation of control material and test samples

The procedures of preparation and coding of control materials, testing of homogeneity and stability are described in HBM4EU-SOP-QA-002.

3.2.5 Preparation of sample sets for shipment

Sample sets will be packed in a bag or box by random selection of the individual test samples. Each sample set will be labelled with a (lab) code. The organizer will record which test samples are in which sample set. The total number of sample sets prepared should exceed the number required for the participating laboratories by 20%, with a minimum of 5 sample sets.

3.2.6 Instruction letter

Simultaneously with shipment of the samples, the participants will receive an instruction letter (template see Appendix 3), a sample receipt form (3.2.7) and a result submission form (3.2.8) by email.

The instruction letter will include:

- the number of test samples in the box

- the matrix and biomarkers to be analysed for

- way of storage of the samples upon receipt, until analysis

- any pre-treatment before analysis (thawing/equilibration to room temperature, re-homogenisation)

- deadline for submission of results and instructions for submission

- statement that content has to be checked upon receipt and sample receipt form has to be completed and returned

- statement that the samples need to be analysed in the same way as is done for analysis of samples in the frame of HBM4EU (i.e. no replicate analysis when the laboratory's SOP specifies single analysis; same method, way of quantification, etc.)

- any specific reporting requirements (e.g. number of significant figures, correction/or not for recovery)

3.2.7 Sample receipt form

Upon receipt of the samples by the laboratory, the content needs to be checked and a receipt form (template see Appendix 4) needs to be returned by the participant to the organizer. The sample receipt form will include the following items to be filled in by the participant:

- the codes of the test samples received

- the condition of the sample upon arrival (e.g. dry ice present; frozen, thawed, any damage/leakage etc.)

- date of receipt

The form needs to be completed and signed by the participant and sent by email to the organizer (details to be provided on the form). The organizer checks whether the codes of the test samples matches with their records. In case samples did not arrive in good condition, an alternative set may be sent.

3.2.8 Result submission form

A result submission form (paper, template see Appendix 5, or web application) is provided by the organizer and needs to be used by the participants to submit the results and method information to the organizer.

The result submission form will include*:

- code of the test sample
- list of biomarkers to be analysed, unit (e.g. ng/ml), space to fill in the analysis result
- the limit of quantification (LOQ) of the method used

Method information may include*

- amount of sample used for analysis
- deconjugation method (if applicable)
- extraction/digestion (solvent, conditions [time, temperature, agitation])
- cleanup (LLE, SPE, ...)
- derivatisation
- instrumental analysis method

GC (injector, injection volume, column)

LC (injection volume, column, eluent)

Detection: MS (single quadrupole, MS/MS triple quadrupole, MS^n ion trap, TOF-HRMS, Orbitrap-HRMS); Ionisation mode

Other:

- internal standard used and moment of addition (e.g. to sample, to final extract, ...)
- method of quantification (isotopic dilution, standard addition, multi-level, matrix-matched standards)
- method/criteria for identification
- specification whether or not method is validated and ISO17025 accredited
- information on repeatability, intermediate precision, measurement uncertainty
- space for remarks/further details by the participant

* the items may depend on the method of analysis

3.2.9 Shipment of samples

Samples are preferably shipped on Monday-Tuesday to ensure delivery the same week and avoid storage in a courier distribution centre over the weekend. For the date of shipment, also take national holidays into account to ensure the laboratory is able to receive the samples. Depending on sample and biomarker stability, samples are shipped at ambient conditions, with ice packs, or with dry ice.

3.2.10 Sample analysis

Laboratories get 4 weeks for sample analysis and reporting the results. Ten days before the deadline, a deadline reminder will be sent to those laboratories that did not yet submit their results.

3.2.11 Evaluation of results

The (statistical) evaluation of results is described in HBM4EU-SOP-QA-003.

3.2.12 ICI report

Reporting will be done by the organizer according to HBM4EU-SOP-QA-004. The draft report will be sent to the HBM4EU QAU and after approval to the participants. The report will also be published on the HBM4EU website. In case significant errors are discovered in the final report, a revision will be drafted (details see HBM4EU-SOP-QA-004).

3.2.13 Remarks and complaints

Participants have the possibility to send in their remarks or complaints to the organizer and the HBM4EU QAU. These will be examined and feedback will be provided. If appropriate, corrective actions will be taken.

3.2.14 Archiving

All documentation, i.e. ICI plan, invitations, instructions, reports, data files on test materials, email communications, etc. will be archived by the organiser either electronically (with at least one backup) or as paper files for a period of at least 5 years.

3.3 Work flow EQUAS

The work flow for organization of an EQUAS as done within the frame of HBM4EU is very similar to that of organization of an ICI. The main difference is that the test samples used are characterized by at least three designated expert laboratories. From this exercise a mean concentration is obtained that will be used as the expert-assigned value.

As the work flow of EQUAS is similar to ICI, the procedures as described in 3.2 also apply for EQUAS, with the following differences:

- for EQUAS, expert laboratories to characterize a control material need to be identified and recruited (3.3.1). The procedure for establishment of the expert assigned values is described in 3.3.2.

- for EQUAS there is no requirement with respect to the number of participants because the assigned value is not based on the participants' results but on a concentration determined by expert laboratories.

3.3.1 Selection of expert laboratories

The HBM4EU QAU will select expert laboratories for a specific biomarker/matrix combination. Candidates are typically laboratories with routine experience in biomarker analysis, that published their analytical procedure in peer-reviewed publications or have been acting as collaborators in an application study (population and occupational study, respectively), which is published peer-reviewed. Expert laboratories are recruited through literature, HBM4EU network (e.g. through chemical substance group leaders), and can be laboratories outside HBM4EU or EU.

Selection criteria include:

- number of years of experience with the biomarker/matrix combination of interest

- application of highly sensitive and selective analytical techniques for the analysis (sufficiently low LOD, LOQ)

- application of isotopically labelled standards for quantification

- availability of in-house validation reports, data on on-going intra-laboratory performance (e.g. control charts), ISO17025 accreditation for the biomarker of interest.

- success rate in inter-laboratory comparisons, external quality assessment schemes or at least comparative results in application studies

The minimum number of expert laboratories required for establishment of an expert-assigned value is three. Exceptions need to be approved by the QAU.

3.3.2 Establishment of expert-assigned value

Preparation of the control material and test samples, and homogeneity and stability testing are done as described in HBM4EU-SOP-QA-002.

Each expert laboratory will analyse the control material in at least 6-fold. The results obtained by the expert laboratories will be submitted to the EQUAS organiser who will calculate the means and (relative) standard deviation for each expert laboratory. Using the individual means of the expert laboratories, the mean of the means will be calculated, its relative standard deviation, and the relative uncertainty of the mean of the means which is given by:

$u_e = RSD_e / sqrt(N)$

with u_e = relative uncertainty of the mean of the mean concentrations from the expert labs

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

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

The mean concentration derived from the expert laboratories is considered suitable for use as assigned value in EQUAS studies when $u \leq 0.7*\sigma_T$.

When $u > 0.7*\sigma_T$, the individual means are checked for outliers. For this the Grubbs' outlier test is used. When an individual expert mean is identified as Grubbs' outlier, it is rejected from the data set[1] and the relatively uncertainty is calculated again. If the condition $u \leq 0.7*\sigma_T$ is still not met, then the uncertainty of the expert-derived mean is too high to be used as assigned value. In this case no assessment of participants' performance is possible for the applicable biomarker. This is also the case when the number of (remaining) individual expert means is less than three.

[1] a maximum of one Grubbs' outlier is allowed to be removed per biomarker and per control material

4 References

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

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

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

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

Göen T, Schaller KH, Drexler H: External quality assessment of human biomonitoring in the range of environmental exposure levels. International Journal of Hygiene and Environmental Health 215 (2012), 229-232

Schindler BK, Esteban M, Koch HM et al.: The European COPHES/DEMOCOPHES project: Towards transnational comparability and reliability of human biomonitoring results. International Journal of Hygiene and Environmental Health 217 (2014), 653-661

Appendix 1a. ICI plan

ICI/EQUAS: <code/round> Date of issue: dd-mm-yyyy

Title of the ICI: <substance group> in <matrix>

Organiser: <name institute>

<address institute>

Coordinator: <name>

<email, telephone>

Sub-contracting

Preparation of control material	Homogeneity/stability testing
No / Yes to:	No / Yes to:
<name institute>	<name institute>
<address institute>	<address institute>
<contact person + email>	<contact person + email>

Control materials

<describe type and number of control materials to be prepared>

<describe origin of control materials, any pretreatment before use (make reference to approval for use for ICI purposes)>

<burdened material or blank material to be spiked?>

Analytes

Biomarkers included: <provide list of individual analytes and the anticipated concentration>

Storage conditions: <container (volume+material) + temperature + max. time (if known)>

ICI schedule:

Announcement / Invitation of laboratories	dd-mm-yyyy
Deadline registration	dd-mm-yyyy
Distribution of test samples	dd-mm-yyyy
Deadline submission of results	dd-mm-yyyy
Preliminary report (Table with results and Z-scores)	dd-mm-yyyy
Final report	dd-mm-yyyy

Appendix 1b. EQUAS plan

ICI/EQUAS: <code/round> Date of issue: dd-mm-yyyy

Title of EQUAS: <substance group> in <matrix>

Organiser: <name institute>

<address institute>

Coordinator: <name>

<email, telephone>

Sub-contracting

Preparation of control material	Homogeneity/stability testing
No / Yes to:	No / Yes to:
<name institute>	<name institute>
<address institute>	<address institute>
<contact person + email>	<contact person + email>

Control materials

<describe type and number of control materials to be prepared>

<describe origin of control materials, any pretreatment before use (make reference to approval for use for ICI purposes)>

<burdened material or blank material to be spiked?>

Analytes

Biomarkers included: <provide list of individual analytes and the anticipated concentration>

Storage conditions: <container (volume+material) + temperature + max. time (if known)>

Expert laboratories (at least 3):

Laboratory-1: <name institute>

<address institute>

Contact person: <name>

<email, telephone>

Appendix 1b. EQUAS plan (continued)

Laboratory-2: <name institute>
<address institute>
Contact person: <name>
<email, telephone>

Laboratory-3: <name institute>
<address institute>
Contact person: <name>
<email, telephone>

EQUAS schedule:

Announcement / Invitation of laboratories	dd-mm-yyyy
Deadline registration	dd-mm-yyyy
Distribution of test samples	dd-mm-yyyy
Deadline submission of results	dd-mm-yyyy
Preliminary report (Table with results and Z-scores)	dd-mm-yyyy
Final report	dd-mm-yyyy

Appendix 2. Invitation and registration form

HBM4EU: Announcement / invitation to participate in ICI / EQUAS study <code/round>.

Title of ICI/EQUAS: <substance group> in <matrix>

Within the frame of HBM4EU, <name organiser> announces the <n> round of ICI/EQUAS for the determination of <substance group> in <matrix>.
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 <matrix>. The participants will receive <n> samples, <x g or ml> each.

Target biomarkers
The biomarkers potentially present in the test samples are: <provide list>
Optionally: for each of the biomarkers the required/recommended LOQ is specified

Calendar:

Deadline registration	dd-mm-yyyy
Distribution of test samples	dd-mm-yyyy
Deadline submission of results	dd-mm-yyyy
Preliminary report	dd-mm-yyyy
Final report	dd-mm-yyyy

Registration
For registration <provide details how to register, refer to registration form>
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:
<name coordinator> <email> <telephone>
<name institute> <address institute>

Appendix 2. Invitation and registration form (continued)

HBM4EU: Registration form for participation in ICI / EQUAS study <code/round>.

Title of ICI/EQUAS: <substance group> in <matrix>

Laboratory:
<name institution>
<address of the laboratory>
<name of 1st contact person, telephone number and email address>
<name of 2nd contact person, telephone number and email address>

Address for delivery of the test samples
<name institution>
<address of the laboratory>

The above laboratory will participate in the ICI/EQUAS study **<code/round>.**
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: <provide details>

Contact information organiser:
<name coordinator> <email> <telephone>
<name institute> <address institute>

Appendix 3. Instruction letter

Dear participant,

Thank you for participation in HBM4EU ICI/EQUAS study <code/round> for the determination of <substance group> in <matrix>.

You will receive a parcel containing <x> test samples. <specify date of shipment> Each sample consists of approximately <y g or ml> <matrix>. The parcel will be shipped under <ambient/frozen/dry-ice> 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 <ambient/frozen/dry-ice> conditions until analysis.
- Analyse the samples for the biomarkers indicated in the invitation letter <ref/dd-mm-yyyy>
- Thaw the samples and re-homogenise them according to your own procedure.
- Analyse the samples using the same procedure as will be used for analysis of samples in the frame of HBM4EU.
- Carry out a single analysis for each sample.
- For submission of results and method information use the forms provided.
- The deadline for submission of analysis results and method details is dd-mm-yyyy.

If you have any questions or need any assistance, please contact:
<name> <email>

Contact information organiser:
<name coordinator> <email> <telephone>
<name institute> <address institute>

For questions not related to logistics of the ICI/EQUAS, you can contact the WP9 helpdesk:
https://www.hbm4eu.eu/private/wp9-help-desk/

Appendix 4. Sample receipt form

HBM4EU: Sample receipt form ICI / EQUAS study <code/round>.

Title of ICI/EQUAS: <substance group> in <matrix>

Laboratory code:

Contact person:

Institute:

Address:

Country:

Contents of parcel:
- <n> containers/tubes with <matrix>
- Sample receipt form

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

Date of receipt (dd-mm-yyyy):

Conditions: ambient / partly thawed / frozen / dry-ice still present

Code on Container	Damaged/leakage	Remarks
	Yes / No	
	Yes / No	

Name: Signature

Date:

After signing this form, please scan and send the pdf to: <provide details>

Contact information organiser:
<name coordinator> <email> <telephone>
<name institute> <address institute>

Appendix 5. Result submission form <alternatively an excel file can be provided>

HBM4EU: Sample receipt form ICI / EQUAS study <code/round>.

Title of ICI/EQUAS: <substance group> in <matrix>

Laboratory code:

Contact person:

Institute:

Address:

Country:

For each of the analytes from this ICI/EQUAS round:
- provide the method number (state a number that corresponds to the method number used in the method information form)
- Indicate number of years of experience with the determination of the biomarker (<1 year, n years, >5 years)
- provide the LOQ
- report NA for not analysed/not included in the scope of the method used
- report <LOQ when the result is below the limit of quantification
- report a numerical value when found above LOQ, express to three significant figures (e.g. 0.543), in the units indicated by the organiser

Example result table:

				Result	
	Method	Experience		sample code =	sample code =
Biomarkers	#	# years	LOQ (ng/ml)	ng/ml	ng/ml
name-1					
name-2					
name-3					
name-4					
name-5					
name-6					
name-7					
name-8					
name-9					
name-10					

Please mention here any specific observations/remarks (if any):

Appendix 6. Method information form (example for LC/GC-based methods) <alternatively an excel file can be provided>

Method number	**1**	
ISO17025 accredited	yes/no	
SAMPLE PREPARATION		
amount sample extracted		g or ml
Deconjugation	No / yes	
- chemical	Reagent / pH/ temp / time	
- enzymatic	Enzyme / pH / temp / time	
Extraction		
- pH adjustment		
- LLE;	solvent(s) / time / shaking	
- SPE; material	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		
Quantification		
Use of internal standard (IS)	yes/no	
- isotopic label	yes/no (optionally, specify label used for each biomarker)	

- 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	
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	
Precision data (from initial /on-going validation		
Repeatability		
Intermediate precision		
Expanded measurement uncertainty		

Further remarks/observations: