HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

The Quality Assurance/Quality Control Scheme in the HBM4EU project

Deliverable Report

D 9.4

WP 9 - Laboratory analysis and quality assurance

Deadline: September 2017

Upload by Coordinator: 3 November 2017

Entity	Name of person responsible	Short name of institution	Received [Date]
Coordinator	Marike KOLOSA-GEHRING	UBA	11/10/2017
Grant Signatory	Marike KOLOSA-GEHRING	UBA	11/10/2017
Pillar Leader	Argelia CASTAÑO	ISCIII	11/10/2017
Work Package Leader (WP9)	Argelia CASTAÑO Marta ESTEBAN	ISCIII ISCIII	11/10/2017
Task leader (Task 9.4)	Thomas GÖEN	IPASUM	11/10/2017

Responsible author	Thomas GÖEN (IPASUM)	E-mail	thomas.goeen@fau.de
Short name of institution		Phone	+49 9131 8526121
Co-authors	Holger Koch, Jean Philippe Antignac, Adrian Covaci, Jana Klanova, Marta Esteban, Argelia Castaño.		

Table of contents

1 Authors and Acknowledgements

Lead authors
Thomas Göen, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg (IPASUM)

Contributors
Holger Koch, Institute for Prevention and Occupational medicine of the German Social Accident Insurance (IPA)

Argelia Castaño and Marta Esteban, Institute of Health Carlos III (ISCIII)

Jean Philippe Antignac, French National Institute of Agronomic Research (INRA)

Adrian Covaci, University of Antwerp (UAntwerp)

Jana Klánová, Masaryk University (MU)

2 Introduction

One of the tasks of the European Human Biomonitoring Initiative (HBM4EU) is closing the data gaps for the exposure to prioritised substances through the analysis of human samples, either using bio-banked samples, samples from ongoing studies or by implementing surveys at EU level in a harmonised way.

These chemical analyses will be done by laboratories selected according to defined criteria (Work Package 9). A network of European laboratories will be created to assure that analytical methods are state of the art that quality is guaranteed so as to increase capacities for the analysis of exposure biomarkers in all sample matrices. A specific selection process for involved laboratories will ensure a high rate of participation, since all countries will be able to propose the participation of national laboratories via their National Hubs, in coordination with the National Hub Coordinator (Tasks 9.2 and 9.4).

A crucial issue within the selection process of candidate laboratories for the analyses of HBM4EU samples is the compliance with the Quality Assurance/Quality Control (QA/AC) Scheme in the HBM4EU project (Task 9.4).

The objective of the QA/QC scheme is the assessment and securing of comparability of the analyses of HBM4EU samples on selected biomonitoring parameters between the HBM4EU candidate laboratories as well as to the data of other international established laboratories and studies.

3 Definitions

The Quality Assurance Unit (QAU) will serve as a central unit for all questions related to chemical analysis and quality assurance during the whole period of the project. Members of the QAU developed a number of tools and approved them for their suitability in former projects (DEMOCOPHES, COPHES).

The QAU is composed of experts from the following HBM4EU partner institutions:

- Institute of Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg (IPASUM), Erlangen, Germany;
- The National Centre for Environmental Health of the Instituto de Salud Carlos III (ISCIII), Madrid, Spain;
- The Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Bochum, Germany;
- The Department of Food Analysis and Nutrition of the University of Prague (VSHT), Prague, Czech Republic and,
- The French Institute of Agronomic Research (INRA), Nantes, France.

It is supported by associated experts from the Research Centre for Toxic Compounds in the Environment of the Masaryk University (MU), Brno, Czech Republic; University Antwerp (UAntwerp), Belgium and the Institute of Food Safety of the Wageningen University (RIKILT), Wageningen, the Netherlands.

Inter-laboratory Comparison Investigations (ICI) are based on the direct comparison between candidate laboratories selected for the analytical analysis of a special parameter within the HBM4EU project (see Deliverable 9.3 - Database of candidate laboratories for the 1st prioritisation round of substances). Inter-laboratory comparison investigations are particularly needed, if analytical know-how on a parameter is not far spread, if only few labs can (or state to be able to) perform certain analyses, if a sufficient number of expert laboratories for EQUAS investigations cannot be identified, or if the parameter itself needs closer investigation (e.g. in terms of conjugation status, enzyme used, isomeric composition, selection of native or spiked control material etc.).

External Quality Assessment Schemes (EQUAS) compare the analytical proficiency of candidate laboratories selected for the analysis of a special parameter within the HBM4EU project with the proficiency of expert laboratories.

Expert laboratories must feature particular experience in the analysis of the specific parameter, which is the topic of an EQUAS. Evidences for particular experience are the use of analytical benchmark techniques for the special parameter, the frequent application of the special parameter in population studies, basic research on the metabolism and kinetics of the special parameter, and successful results for the special parameter in external quality assessment schemes.

4 Quality assurance scheme

4.1 ICI/EQUAS organiser

The proficiency tests within HBM4EU should be organised by an institution with adequate experience in the execution and evaluation of inter-laboratory exercises of chemicals and metabolites in biological material, preferably in human material as well as for the special biomonitoring parameter.

The ICI/EQUAS organiser should have a sufficient reliable procedure for the homogeneity and stability testing of the prepared ICI/EQUAS material available that will be developed in detail in a separate procedure.

The ICI/EQUAS organiser may not be part of the candidate laboratories competing for the analyses of HBM parameters in HBM4EU samples. In case of a laboratory competing for the analysis also compete for ICI/EQUAS organisation for the same parameter, special measure will be applied to guarantee the fair participation of this laboratory.

These restrictions do not apply to tailor-made comparison tests as a preparatory platform for ICI/EQUAS exercises (see 4.2).

4.2 Proficiency test sequences

The quality assurance programme for each selected biomonitoring parameter of the prioritised substances of HBM4EU should include at least three proficiency tests. According to the experience of the candidate laboratories and the results of the first run, the sequence of the programme may be completed by

- One ICI run and two EQUAS runs or,
- Two ICI runs and one EQUAS run.

Nevertheless, the sequence can be expanded by additional runs if a poor or unsatisfactory comparability is found in the runs executed. The QAU will decide which sequence is appropriate for the selected biomonitoring parameter and whether an extent of the scheme is necessary.

ICIs can be performed in various stages and should include close exchange between laboratories and the QAU, in order to communicate and exchange problems and offer solutions, e.g. by web conferences.

In the case of an insufficient number of candidate laboratories for a regular ICI/EQUAS scheme the QAU can mandate tailor-made comparison tests, e.g. by the exchange of samples between the laboratories.

4.3 ICI/EQUAS materials

ICI/EQUAS materials are produced by the ICI/EQUAS organisers or have to be purchased by them.

To ensure evident information on the quality assurance of HBM analyses, the materials for the ICI/EQUAS runs should to be prepared based on human materials or adequate surrogates. However, the use of native biological materials implies unknown or unpredictable native background levels of the HBM parameter. Thus, each ICI/EQUAS should contain three materials of the following composition/origin:

- Material A (biological material without spiking),
- Material B (biological material spiked with the HBM parameter at low level),
- Material C (biological material spiked with the HBM parameter at high level).

All samples of the special ICI/EQUAS run have to be prepared at the same time using the same lot of the biological material. This approach enables additional cross-check evaluation, e.g. by comparison of the differential between Material B result and Material A result and the spiking amount of Material B. The control material will be extensively tested for stability and homogeneity of the materials that has to be tested before distribution to the laboratories that participated ICI/EQUAS application. Each ICI/EQUAS material shall be sent in triplicate with hidden attribution to the exercise participants.

Spiking of ICI/EQUAS materials should be performed, if possible, using the chemical conformation really occurring in the human material, e.g. a metabolite occurs mostly or completely as conjugate. If the real conformation (e.g. conjugate) is not available, the application of real human samples of different exposure levels should be included in the ICI/EQUAS programme. ICI/EQUAS of this approach should contain following composition:

- Material A (human biological material originally contain the HBM parameter at a low level),
- Material B (human biological material originally contain the HBM parameter at a high level).

The approach can also be supplemented by materials which contain originally exposed material spiked with additional amounts of the HBM parameter. Also in this approach, each ICI/EQUAS material shall be sent in triplicate with hidden attribution to the exercise participants.

Dependent on the parameter and the analytical challenges it can be necessary to include reagent blanks and calibration standard samples, respectively, in ICI/EQUAS runs too.

4.4 ICI/EQUAS workflow

The ICI/EQUAS workflow covers tasks which have to be prepared preliminary before the ICI/EQUAS runs and tasks of exercising and evaluation the runs.

A general preliminary step before starting the ICI/EQUAS programme is the preparation of a questionnaire for participating laboratories, with the aim to extract data which may support cause analysis of deviating results of an ICI/EQUAS run. The items of this questionnaire depend on the properties of the parameter and the analytical techniques applied. This implies a special questionnaire for each HBM parameter.

Special steps which have to be performed preliminary before starting the EQUAS runs are:

- Survey for expert laboratories based on deliverable 9.2,
- Definition of criteria for selecting expert laboratories based on deliverable 9.2,
- Invitation of expert laboratories.

Steps which have to be executed periodically for each ICI and EQUAS run are

- Production of ICI/EQUAS materials (1 week),
- Homogeneity and stability testing of ICI/EQUAS materials (4 weeks),
- Shipment of the ICI/EQUAS material (1 week),
- Receipt of results and questionnaires (4 weeks),
- Evaluation of the results (1 week),
- Communication of the results,
- Discussion and conclusions on the results with respect to error detection and correction, e.g. by web or telephone conferences (1 week).

Each ICI/EQUAS run should be performed within an interval of 3 – 4 months.

4.5 Evaluation of ICI/EQUAS results

The main goal of Work Package 9 (WP9) is to guarantee the quality of the analytical results in HBM4EU. Therefore, it is necessary to define strict criteria even if this implies that not all countries can analyse their own samples. Otherwise the results derived from the activities within the European HBM Platform will not be reliable and comparable.

Taking this into account, the Quality Assurance Unit of WP9 have defined a minimum of two ICIs and one EQUAS exercises to be performed for each biomarker with different concentrations covering the range of levels found in the general population as described before. In principle, an expert judgment and discussion of the results has to be performed, particularly in the case of divergent clusters of results. For quantitative evaluation, the results from the participating laboratories will be rated using the classical z-scores indicators, which is an international accepted criteria for evaluation of results (see below). A valid quantitative evaluation approach needs a minimum of three participating laboratories (candidate laboratories or expert laboratories). Additionally, to the quantitative evaluation of the comparability of the laboratories, results of replicates will be used for the calculation and evaluation of laboratory repeatability.

Participating laboratories should obtain satisfactory results in the three exercises (for all the samples per round).

4.5.1 Evaluation of ICI results

The results of the ICI runs will be quantitative evaluated regarding comparability using the approach of consensus value from participants according ISO 13528. With this approach, the assigned value X for the test material is the robust average of the results reported by all participants in the round, calculated using Algorithm A in Annex C of ISO 13528 (2005).

The algorithm yields robust values of the average and standard deviation of the data to which it is applied.

Denote the p items of data, sorted into increased order, by:

$x_1, x_2, \ldots, x_i, \ldots, x_p$

Denote the robust average and robust standard deviation of these data by x* and s*.

Calculate initial values for x* and s* as:

$x^* =$ median of x_i $\quad (i = 1,3, \ldots, p)$

$s^* = 1.483$ median of $|x_i - x^*|$ $\quad (i = 1,3, \ldots, p)$

Update the values x* and s* as follows. Calculate:

$\delta = 1.5\ s^*$

For each x_i (i = 1,2, …,p), calculate:

$x_i^* = \{ x^* - \delta$, if $x_i < x^* - \delta$; $x^* + \delta$, if $x_i > x^* + \delta$, x_i, for all other data $\}$

Calculate the new values x* and s* from:

$x^* = \sum x_i^*/p$

$s^* = 1.134 \sqrt{\sum (x_i^* - x^*)^2/(p-1)}$

where the summation is over i.

The robust estimation x* and s* may be derived by an iterative calculation, i.e. by updating the values x* and s* several times using the modified data, until the process converges. Convergence may be assumed when there is no change from one iteration to the next in the third significant figure of the robust standard deviation and of the equivalent figure in the robust average.

The evaluation of the participant's performance results by calculation of the z-score:

$$z = \left| x_i - x^* \right| / \delta$$

Thus results will be evaluated according to:

Value	**Performance**
z-score ≤ 2	Satisfactory
2 > z-score < 3	Questionable*
z-score ≥ 3	Unsatisfactory

*) Margin for improvement after expert evaluation, discussions and advise.

Thus, the result of the participant will be directly approved if the z-score is ≤ 2. The participant will pass the ICI run successfully if her/his results of all samples are approved by the z-score evaluation.

4.5.2 Evaluation of EQUAS results

The results of the EQUAS runs will be evaluated using the approach of consensus value from expert laboratories according ISO 13528. With this approach, the assigned value X for the test material is the robust average of the results reported by the expert laboratories in the round, calculated using Algorithm A in Annex C of ISO 13528 (see 4.4.1 Evaluation of ICI results).

The evaluation of the participant results by calculation of the z-score:

$$z = \left| x_i - x^* \right| / \delta$$

The result of the participant will be approved if the z-score is ≤ 2. The participant will pass the EQUAS run successfully if her/his results of all samples are approved by the z-score evaluation.

4.5.3 Permission criteria

A participant fulfils the quality criteria of the HBM4EU call if she/he passes 3 of ICI/EQUAS runs successfully. Exceptions from this rule have to be approved and justified by the QAU.

5 External quality assurance measures beyond the selection process

Quality assurance measures should be maintained after approval of the candidate laboratories concomitant with the analysis of HBM4EU samples. Such sustainable continuation of QA measures beyond the ICI/EQUAS runs can be realised by

- the use of Certified Reference Materials (CRM) and,
- the participation at public accessible international proficiency tests.

Certified Reference Materials are provided for a few HBM parameters by the National Institute of Standards and Technology of the US Department of Commerce (NIST) and European Commission's Joint Research Centre (formerly Institute for Reference Materials and Measurements (IRMM)). The spectrum covers mainly the determination of some toxic elements in blood and urine. The materials are available at the institutions itself but also by several commercial distributors.

Several national proficiency schemes of laboratory medicine include also the determination of some toxic elements in blood and urine. International established external quality assessment programmes which cover both inorganic and organic HBM parameters are the proficiency schemes of the Centre de Toxicologie du Québec at the Institut National de Santé Publique du Quebec (CTQ-INSPQ) and German External Quality Assessment Scheme (GEQUAS). The schemes offer the quality assurance of HBM parameters of well-established but also new emerging chemical substances and provide two runs per year at least.

6 Internal quality control measures

Each laboratory selected for the analysis of HBM4EU samples has to perform internal quality control measures during the HBM4EU application. These measures include at least the purchase or in-house preparation of appropriate quality control (QC) materials, the analysis of at least one sample of each material in each analytical series and the evaluation of the QC results using quality control charts. The QC material should consist of (human) biological material at two different levels at least. The levels should be selected in respect of the prospected distribution of results from the population studies.

7 Ethical issues of QA/QC scheme

Human biomonitoring studies are essential tools to understand the association between environmental exposure and health. They should finally lead to a better understanding and prevention of environmentally induced adverse health effects. Nevertheless, the studies should assure the right balance between the protection of the study participant and the progress of research with the impact on public health.

An important component of beneficial studies is the generation of accurate data. Quality assurance is an essential tool to guarantee the generation of accurate data and thus benefits the profit scale of the ethical balance of HBM studies.

On the other hand ethical issues have to be considered during quality assurance processes. This belongs to the data protection of laboratories participating in ICI and EQUAS runs as well as the adequate supply and careful use of biological material for quality assurance measures.

The organiser of ICI and EQUAS runs as well as the QAU members has to guarantee the confidential handling of all data and the communication of ICI and EQUAS results considering the anonymity of the participants.

Decisions on the acceptance of candidate laboratories should only refer on aggregated data and must not include data of unsuccessful applicants.

8 References

Centre de Toxicologie du Québec at the Institut National de Santé Publique (CTQ-INSPQ). External quality assessment schemes, Québec, Canada. Access by the link https://www.inspq.qc.ca/en/ctq/eqas

European Commission Joint Research Centre (JRC) Certified Reference Material Cataloque. Geel, Belgium. Access by the link https://crm.jrc.ec.europa.eu/

German External Quality Assessment Scheme (GEQUAS) External quality assessment scheme for analyses in biological materials. Erlangen, Germany. Access by the link http://www.g-equas.de/

ISO 13528 (2005) Statistical methods for use in proficiency testing by interlaboratory comparisons. International Organization for Standardization, Geneva

National Institute of Standards and Technology of the US Department of Commerce (NIST) Standard Reference Materials (SRM). Gaithersburg, USA. Access by the link https://www.nist.gov/