HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Criteria for prioritisation of biomarkers, matrices and analytical methods

Deliverable Report

D 9.1

WP 9: Laboratory analysis and quality assurance

Deadline: April, 2017

Upload by Coordinator: 27th April 2017

Entity	Name of person responsible	Short name of institution	Received [Date]
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All partners in task 9.1, task leaders in WP9 as well as CGLs were given the opportunity to comment on the evolving drafts of the criteria and were invited to take part in the regular phone and web meetings where the content of the criteria was discussed in detail. Thus, this deliverable has passed a thorough peer-review process by experts within the consortium. According to the Annual Work Plan for year 1, the criteria for prioritisation of biomarkers, matrices and analytical methods should be circulated to the National Hubs (NH) via the National Hub Coordinator and National Hub Contact Points. However, after consultation with the NHC, WP leader and Pillar 2 Leaders, circulation of the draft criteria through the NHs was deemed unnecessary, since partners involved in the elaboration were experts in the field and the workload to the NHs should be limited.

1 Criteria for prioritising exposure biomarkers and matrices

1.1 Elaboration process

Establishing a list of criteria to evaluate exposure biomarkers (EB) and matrices (M) is the first step in the procedure to identify and select the best exposure biomarkers and matrices for the whole HBM4EU project.

In order to develop the list of criteria, the work was done in a consensual manner between partners of WP9 and other experts within the consortium. First, Agence Nationale de Santé Publique (ANSP) and Karolinska Institute (KI) drew up a draft list of criteria from the scientific literature and their experience in human biomonitoring (HBM).

During this work, a difficulty arose to distinctly separate criteria to apply to exposure biomarkers or criteria to apply to matrices. Therefore, we established a list of criteria to prioritise exposure biomarker/matrix (EB/M) as a "couple". This approach offers the opportunity to do the prioritisation in a single step, which is simpler. It also offers the possibility to make a comparison between the same exposure biomarker that may be present in different matrices (for example cadmium in blood or in urine).

An easy ranking procedure was used in order to ask experts (see Contributors above) to score each criterion from 0 to 10, for not relevant to very relevant criteria, respectively. Experts were invited to justify their scores and add comments. It was also possible for them to add new criteria if considered relevant. At the end of this first consultation, scores were returned from experts to ANSP and KI, who made a synthesis.

The comments and scores lead to identification of two main groups of criteria. The first one, with criteria that were judged as important by a large majority of the contributors and the second one, with criteria that were more debated. Consequently, we decided to split the criteria into two levels of importance without further ranking. We propose that if the selection of exposure biomarker/matrix is not accomplished after the evaluation using the set of criteria at the first level, the second level of criteria can be used, but their evaluation should be considered more supportive than mandatory.

Different criteria will apply depending on the categories of substances of interest (A, B and C). Descriptions (taken from the "Scoping document/Overview of prioritized substances") of the substance categories are as follows:

A) Sufficient data are already available,

B) Only insufficient data are available, and

C) No data are available, published and/or no biomarkers have been established.

1.2 List of criteria

The following list of criteria is intended to help in the selection of the best couple of exposure biomarkers/matrices in future HBM studies planned within HBM4EU and HBM surveys across Europe.

Criteria for Exposure Biomarkers/Matrices			
First level of importance*			
	Substance Categories		
	A: Sufficient data available	*B: Insufficient data available*	*C: No data available*
Specificity	EB/M concentration reflects exogenous exposure. The EB/M concentration is an exclusive consequence of environmental/occupational exposure.		EB/M concentration might not reflect exclusively exogenous exposure to the substance, but is a correct indication of exposure.
Biological sensitivity	The measured concentration of the EB/M correlates strongly with the substance intake dose. Variations of EB/M concentration reflect precisely the variation of exposure to the substance of interest.		The measured concentration of the EB/M is an acceptable indication of the substance intake dose.
Limit of quantification (LOQ)	The LOQ of a validated analytical method is low compared to commonly measured concentrations in the general population. The EB/M with the highest frequency of quantified data available is preferable. Typically more than 60% of the data could be quantified in the target population, but this may depend on study design and population.		Only a few studies are available and the quantification has been done using non-validated analytical methods.
Measurement validity	The EB/M concentration in the sample is not likely to be altered by contamination with a ubiquitous parent substance from the environment preceding and during the analysis. Variations in matrix composition can be easily corrected for (e.g. creatinine in urine, lipids in serum).		Sample contamination by a ubiquitous parent substance might occur, but the level of contamination is low compared to expected levels and special precautions can be applied to minimize the amount of contamination.
Matrix availability and sample collection	The sample collection of the relevant matrix is not considered too invasive. Easy collection and transportation of the required amount of sample with a validated sampling protocol is beneficial. It is advantageous if it is possible to determine more than one EB in the same matrix.		It is relatively easy to obtain a sufficient sample volume for a required number of samples at a reasonable cost.
Stability after sample collection	The EB/M is stable in the sample for many hours during transportation to the laboratory or before storage in a biobank. Optimal transportation conditions to ensure the stability are relatively easy to achieve.		If stability data is missing, stability should be assessed. For compounds with low stability, sample degradation can be prevented by an adaptation of transportation conditions or implementation of particular sampling operating procedures.
Stability during storage	The cryo-preservability of EB/M is sufficient to guarantee a high stability during storage in the biobank.		If stability of the EB/M is not guaranteed during storage in biobanks, it is recommended to analyse the sample as soon as possible.
Half-life	The EB/M should preferably have a half-life sufficiently long to avoid an excessive intra-individual variability in EB/M concentration measurement.		

***not in ranked order**

Criteria for Exposure Biomarkers/Matrices			
Second level of importance*			
	Substance categories		
	A: Sufficient data available	*B: Insufficient data available*	*C: No data available*
Analytical method availability	At least one validated, and publicly available, analytical method exists to measure EB/M concentration.		An analytical method exists, or is likely to be validated in the near future and could be used to produce new data.
Individual susceptibility	The formation of the EB/M in the human body is not prone to individual susceptibility (e.g. enzyme polymorphism).		Not relevant
Background of data	The EB/M has been used in many European HBM surveys to study the substance of interest. The EB/M allows comparison with historical data to characterize the temporal variation of exposure across Europe. Data on the toxicological profile of EB/M or health guidance values are available. This criterion should not be used to exclude selection of a better EB/M regarding the other criteria.		Not relevant

***not in ranked order**

2 Criteria for prioritising analytical methods

2.1 Elaboration process

The criteria for prioritisation of analytical methods will be used in the evaluation of analytical methods applied for determination of the prioritised substances (1st prioritisation round). Furthermore, the criteria will be used beyond the currently prioritised compounds, i.e. in future evaluations of analytical methods following forthcoming prioritisation rounds. It was therefore deemed important, in deliberations within WP 9, to identify general rather than compound-specific criteria. Based on this premise, Aarhus University (AU), University of Latvia (LU) and the Norwegian Institute of Public Health (NIPH) prepared draft versions of the criteria for the analytical methods that were circulated to all contributors. The comments received were compiled and considered for the following draft. Specific questions were discussed in regular conference calls.

The final criteria should be applicable to analytical methods of different maturity, as could be expected for substances of the categories A, B and C (see above for definitions). In the long term, this work can contribute to the identification of or development towards analytical reference methods at EU level.

2.2 List of criteria

These criteria are intended to facilitate the selection of the most appropriate analytical method for conducting HBM of substances of the three different categories A, B and C. The table below consists of method-specific criteria and general considerations.

Criteria for analytical methods			
Method-specific criteria*			
	Substance Categories		
	A: Sufficient data available	*B: Insufficient data available*	*C: No data available*
Sample preparation	Sample preparation procedures are well established and applied routinely for relevant biological matrices.	Appropriate sample preparation procedures may be available to some extent, but have not necessarily been established for all EB/M combinations. A certain level of development (mainly adaptation) could be necessary.	Sample preparation procedures are typically not yet available and have to be developed. The effort needed for this development will depend on the possible adaptation of an existing protocol (e.g. for adding new substances from an already known family).
Standards	The use of standards of target EBs and internal standards (among these isotopically labelled standards where relevant), is mandatory and they are commercially available at reasonable costs.	The use of standards of target EBs and internal standards (among these isotopically labelled standards where relevant), is advisable. Standards are not necessarily commercially available or might be offered by only one or few suppliers, Longer times of delivery or higher costs may occur.	Determination of this substance might be the first tentative identification of the EB/M. Standards might not be commercially available at all.
Validation	The method is well-established in multiple laboratories. Comprehensive validations have been performed (e.g., parameters such as accuracy and precision have been tested at concentrations close to limits of detection (LOD) and/or limits of	The method is established and full within laboratory validations have been carried out in some research laboratories, based on common guidelines (e.g. ICH guidelines and GLP). Concentrations might still be reported using methods subjected to	No method validation is expected. Assessment of critical parameters might be in progress.

	quantification (LOQ)) according to common guidelines (e.g. ICH guidelines[1] and GLP[2]). Participation in inter-laboratory comparisons and/or use of certified materials is in place (if these options are available for the EB/M).	less rigorous validation procedures (e.g., in-house controls, lack of assessment of some parameters like matrix effects, precision, accuracy, etc.). Large-scale studies and interlaboratory comparisons are expected in the near future.	
Selectivity	A low extent of interferences has been demonstrated. The measured concentrations are that of the EB/M.	Potential interferences might not be fully controlled for some EB/M.	Selectivity has not necessarily been assessed.
Sensitivity			
- ***Determination of limits of detection (LOD) and limits of quantification (LOQ)***	LODs and LOQs have been determined for each EB/M and have usually been reported in comprehensive validations.	LODs and LOQs are available for some individual EB/M, but not necessarily for all EB and all matrices of interest for HBM.	Highly dependent on availability of standards. When available, the LOD and LOQ might have been determined for individual studies, but not as part of a validation procedure.
- ***Quantifiable compounds***	In general, LOQs have been proven to be sufficiently below the concentrations in a high proportion of the samples of a population.	LOQs may appear in some cases higher than the expected exposure, but enable quantification of most biomarkers in a reasonable number of samples of the population. Risk of low detection rates, and subsequent biased upper bound risk assessment.	Quantification is reliable only when the standard is available. For some compounds, only semi-quantitative determinations are possible.
- ***Robustness***	Limited variation in the LODs and LOQs. Controlled environment-	Some variation in LODs and LOQs can occur (e.g. variable blanks	High variation in LODs and LOQs or absent/insufficient

[1] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
[2] Good Laboratory Practice

	laboratory conditions (e.g. low background levels).	and/or instrument performance).	information to properly quantify this parameter. Interferences cannot be ruled out.
- ***Comparability***	Similar LODs and LOQs have been obtained by most laboratories.	Variability in LODs and LOQs exists, for example due to different analytical approaches for the determination of the EB/M.	Low comparability of LODs and LOQs or absence of information. Subject to method development and validation.
Uncertainty and Accuracy			
- ***Uncertainty (at lower concentrations)***	The uncertainty has been assessed according to common guidelines and is sufficiently low for the purpose of the project. Concentrations close to LOQs have been evaluated in the validation.	The uncertainty has been assessed, but might exceed guidelines for validation of analytical methods for certain EB/M combinations.	The uncertainty has not been assessed, or if assessed, it might exceed commonly accepted values.
- ***Uncertainty (at higher concentrations)***	The uncertainty is sufficiently low for the purpose of the project.	The uncertainty is sufficiently low for the purpose of the project.	The uncertainty has not been assessed, or if assessed, might exceed commonly accepted values.
- ***Accuracy – availability of QC measures***	The accuracy has usually been assessed using external QC measures such as certified reference materials or relevant interlaboratory comparisons.	The accuracy has mainly been assessed using internal QC measures, recovery tests or comparisons with an independent analytical method, although some external QC measures might be available for some EB/M.	The accuracy has usually not been evaluated yet.
- ***Accuracy - assessment***	The accuracy is within the limits given by guidelines for validation of analytical methods (e.g. ≤20% deviation, depending on the	The accuracy might be compromised by several factors (e.g. not optimal internal standards, blank contamination at low concentrations). A wider range of accuracies is	The accuracy has usually not been assessed, but indications from similar EB might be available. High

	concentration level and EB/M).	expected (e.g. ≤40% deviation).	uncertainty must be expected.
Recovery	The EB recoveries are usually in the range of 80-120%. If outside this range, the use of proper internal standards compensates the deviations.	Variable recoveries might be expected (e.g. 50-150%). There is a stronger need to compensate the deviations with a proper internal standard.	Recoveries have rarely been assessed.
Range/Linearity	The method provides acceptable precision and accuracy for the relevant concentration range. The linear range has been evaluated for the determination of the relevant EB/M.	The method mainly provides acceptable precision and accuracy for higher concentrations. Awareness of potential issues exists at low concentrations. The linear range has usually been evaluated for the relevant EB/M, although less attention might have been paid to keeping all determined concentrations within the linear range.	Optimal working range has rarely been evaluated.
Robustness			
- ***Response to small changes in the analytical process***	The robustness has been assessed, and only small variations within acceptable limits have been identified due to minor changes in the analytical procedure/conditions.	The robustness has been assessed, and variations can occur due to several factors (e.g. EB stability, instrument performance, environment and/or operating conditions, etc.).	The robustness has likely not been assessed. Any significant variations, which could affect the analytical result, should be reported.
- ***Method precision/ repeatability***	The repeatability/intermediate precision has been evaluated according to common guidelines. It is within an acceptable range. Control charts are usually used for the assessment of precision.	The repeatability/intermediate precision has often been assessed, but the standard deviations can be higher than the recommendations given in the guidelines for validation of analytical methods.	The repeatability/ intermediate precision has likely not been assessed.

***not in ranked order**

General considerations*			
	Substance Categories		
	A: Sufficient data available	*B: Insufficient data available*	*C: No data available*
Geographical coverage	Method available in several National Hubs (NHs).	Method available in some NHs, but laboratories in most NHs are in a position to expand existing methods (e.g. experience with similar methodology).	Method available in one or few NHs, geographical coverage uncertain.
Research topic	The method development is generally not a research topic, except for refinements/improvements or extensions.	The method development might still be a research topic, and is usually based on the adaptation of existing methods for related compounds.	The method development is exclusively a research topic.
Costs			
- ***Costs per analysis***	A reliable price per analysis can be given, reflecting labour use and use of consumables.	A price per analysis can be given, but might have to include method adjustments (e.g. new matrix) or establishment of QA/QC measures.	Prices are likely to be rough estimates. Highly dependent upon findings during method development.
- ***Unexpected costs***	Low risk of unexpected costs due to technical or logistical difficulties associated with the analysis.	Risk of unexpected costs due to technical or logistical difficulties associated with the analysis (e.g. need for sample treatment, re-analyses, dilutions, etc.).	High risk of unexpected costs due to low predictability of potential technical or logistical difficulties associated with the analysis.
Time lines			
- ***Predictability***	Time required for the analysis can be predicted with some certainty.	Time required for the analysis might be subject to method development and prediction is thus uncertain.	Time required for the analysis will depend on progress with method development and validation.

- ***Risk of delays***	Low risk of delays due to technical or logistical difficulties associated with the analysis.	Moderate risk of delays due to technical or logistical difficulties associated with the analysis.	Potentially a high risk of delays due to technical and/or logistical difficulties associated with the analysis.

***not in ranked order**