HORIZON2020 Programme
Contract No. 733032 HBM4EU

General guidance for new method development within HBM4EU and role of task 9.3 therein

Additional Deliverable

AD 9.1

WP 9 - Laboratory analysis and quality assurance

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1 Preface

This memo is primarily directed at all non-WP9 partners within HBM4EU in order to get guidance on how to submit requests for new method developments and on which information need to be provided together with the request. Only if procedures explained in this memo are followed, WP9 and WP9.3 can guarantee a well-structured, focussed, timely and substantiated support to these requests.

Because the possible bandwidth of requests for new method developments can both be highly diverse and highly specific, this memo cannot address all prerequisites for new method development requests in every detail, or applicable to all requests. Specific examples provided in this memo are not intended to favour or disfavour requests (in terms of specific biomarkers or specific matrices, the specific combination thereof, or specific analytical methodologies) but to better visualize the demands for requested information.

It also lies in the nature of new method developments and new method development requests that not all information demanded in this memo can be provided immediately or fully documented, or can be backed by already existing (published) data. Consequently, not all points in the provided check lists can or need to be answered with “yes”. Nevertheless, substantiated support can only be provided by WP9/WP9.3 if the current state of knowledge (biomarker, matrix, preanalytical and analytical methodology) is thoroughly described and presented, including all preliminary information and experiences already obtained at expert laboratories involved in the submission of new method development requests.

2 Preanalytical and analytical requirements for new method development within HBM4EU

2.1 Introduction

In HBM4EU, the focus is on generally applicable HBM methods (with specific biomarker/matrix combinations) and the generation of well interpretable, comparable, quality assured and EU-wide HBM data. Within WP9, including WP9.3 (new method development) and WP9.4 the activities are focused on analytics and Quality Assurance/Quality Control (QA/QC) of analytical procedures. WP9 will design and implement a QA/QC program for chemical analysis of HBM samples and support the aligned studies (new sampling and samples from biobanks) identified in WP8, with a view to improve and guarantee comparability and optimise data quality.

Before starting with the development of a new HBM4EU method as a new biomarker/matrix combination (different from those identified feasible in WP9.1, incl. D9.2) or the development of a new or improved analytical method, several general aspects must be considered. If the chemical is expected to cause harmful effects for humans (e.g. chemicals under regulation of REACH) and exposure to the general population or highly exposed subpopulations or occupationally exposed individuals is proven or can be expected and no alternative analytical method is available, the feasibility to develop a new method has to be investigated prior to the support by WP9.3.

Needs for new or improved method developments for the priority substances, identified in WP9.1 or as a result of an ICI/EQUAS in WP9.4 are under the umbrella of WP9. Nevertheless, external HBM4EU partners (outside WP9) may request the support of a new method for an exposure biomarker/matrix combination by experts in WP9. Therefore, this memo is intended to fit external WP9 requests (both from pillar 2 and 3) for new/improved method developments that will be channelled in WP9.

The sole ability to measure a biomarker in a certain matrix does not necessarily make this a useful and meaningful biomarker/matrix combination to be robustly used in HBM4EU. Task9.3 is involved in organising new methods development, selecting laboratories and preparing method development plans within WP9, and focuses on the feasibility of analytical methods to be included in the QA/QC assessment scheme in WP9.4 and to ensure the generation of interpretable and comparable HBM data for the general population (or occupationally exposed populations). Task9.3 experts will provide knowledge and support for the complete new analytical approach in order to generate robust and valid HBM data.

A new HBM method development will be supported only if the submitting party has checked sampling, preanalytical and analytical requirements of the new method or documents respective data determined by a third party and the whole approach for the specific matrix/biomarker combination appears to be scientifically and technically feasible and advisable for inclusion in EU-wide HBM studies by WP9/9.3. After discussion of all aspects the need for the new method to be developed or improved will be decided in WP9.3 together with all WP9 leaders. After finalization of all aspects of method development, the method needs to be included in the WP9.4 QA/QC assessment scheme, in order to be qualified to be used in HBM4EU and data produced thereof to be included in the pan-European HBM4EU dataset for comparable and interpretable EU HBM data. Important (new or refined) method development activities may be also justified for reaching the whole project's objectives in relation to research and scientific based support to policy within pillar 3 and related WPs under the responsibility of the concerned WP leaders there. In these cases WP9/9.3 can offer more tailor made or needs-adapted method development support in close collaboration with the respective WP leaders.

In specific situations, such as matrices being available only from certain subpopulations, being only available in very limited amounts, or being only accessible after complex or invasive procedures, or in case of other biomarker/matrix combinations than those identified as the most suitable ones by WP9, WP9.3 should be involved early in the process in order to assess the feasibility and scientific need to define a common procedure or if the proposal of the submitting party should be developed outside the umbrella of WP9. Nevertheless, whenever possible WP9 experts will try to assist on alternative approaches in order to provide solutions from the point of view of the QA/QC (e.g. alternatives to a complete ICIs/EQUAS scheme, validation or development of analytical methods, suggestions for expert labs etc.) in the understanding that this should not be at the expense of the core tasks, activities and objectives of WP9.

All available information on the envisaged new method development needs to be collected and provided by the submitting party to WP9.3, starting from the rationale of the respective biomarker/matrix selection with all information available on the respective biomarker in the respective matrix, including knowledge on the behaviour of the biomarker in this matrix (etc. kinetics, stability, homogeneity), sampling strategies of this matrix (with a thorough description of the sampled matrix), and all other aspects related to the preanalytical and analytical phase from analytical measurement technique, detection/quantification limits, quality assurance, etc. This substantiated information needs to be prepared by the submitting experts for the new matrix/biomarker combination as a feasibility paper, based upon this WP9.3 memo. With this document we provide demands for substantiated input and check-lists for the interested laboratories to gather the information in a comprehensive way. Before starting the support to develop a new method, the submitting experts for the specific biomarker/matrix combination or other analytical issues have to provide the information requested from the check-lists and to prepare a scientific review about the feasibility, applicability, and scientific justification for EU-wide HBM of the new method. Potentially problematic issues in the new method development should be highlighted by the interested parties, especially for non-classical HBM matrices (other than blood and urine) that have not been identified in WP9.1 (D9.1) in terms of harmonized, comparable collection of the respective matrix, influences of the collection procedure on the matrix (and the biomarkers therein), availability of control material for this matrix, and other potential issues in the preanalytical and analytical phase (such as e.g. external contamination, availability of analytical standards and (labelled) internal standards, limits of quantification needed to cover background exposures in the general population, etc.).

Thus, this document provides a general guidance on all information needed for new method development (biomarker/matrix-combinations) to be included in HBM4EU from WP9.3 and general WP9 perspective.

2.2 Choice and definition of biomarkers and matrices

It is very important to check the correct combination of biomarker(s) and matrices in order to safeguard that biomarker can and will provide reliable, comparable and interpretable results. Does the matrix correctly reflect enrichment or elimination pathways of the biomarker (e.g. placenta vs. blood samples of pregnant women)? Knowledge on the kinetics (accumulation, elimination) of the biomarker in the respective matrix needs to be provided. Is the matrix free of external interfering factors (for instance in nails)? Is the biomarker specific for the chemical (e.g. the untransformed chemical or a metabolite from the external compound)? The respective matrix itself must be able to be sampled in a robust and reproducible way. The matrix and biomarker must be stable during sampling, transportation and preparation procedures (see also “Criteria for prioritisation of biomarkers, matrices and analytical methods” in D9.1). For interpretability, the envisaged concentration measures need to be discussed and explained (e.g. mg biomarker / g of matrix, or

µg biomarker / ml of matrix) and if and how normalization/correction procedures can be applied (e.g. specific gravity or creatinine for urine, or lipid content for blood). Furthermore, the choice of the matrix should preferably follow a non-invasive sampling procedure (for instance urine, hair), but can also include blood samples for measures of persistent chemicals, to be widely applicable in HBM4EU. Exceptions to this general rule might apply if (new or refined) method development activities are justified for reaching objectives in relation to research and scientific based support to policy within pillar 3 and related WPs under the responsibility of the concerned WP leaders there.

For not well-established preparation procedures and HBM matrices, for example nails, hair, placenta or exhaled breath condensates (EBC), the rationale of the choice of the matrix must be proven by comparison of currently established analytical methods and their quality parameters (when available) and by considering whether the method is qualified to be used in HBM4EU.

For instance, when preparation of nails or hair is considered, it must be guaranteed that the pre-analytical phase and the analytical phase can control for external contamination or interfering factors, providing reproducible sampling methods and homogeneous samples among the study population. This requires a detailed sampling protocol (type of nails; length and width of the sample; a certain age of the sample and others) and guidance prior to sampling (e.g. use of cosmetics and cleansing products, use of nail polish, use of gloves etc.), supported by a sampling questionnaire to collect relevant information prior to or by the time of the sampling. Another example is the choice of placenta tissue. Is the material homogeneous and representative among the study population? Does placenta sufficiently reflect exposure of pregnant women and/or their newborns? The sample has to represent the single individual and must be comparable with other samples within the population and in between populations in order to achieve a European wide comparable dataset.

Therefore, for each matrix/biomarker combination the submitting party has to check the feasibility to include the matrix/biomarker combination in an EU-wide HBM and the quality of the data (in terms of interpretability and analytical quality) that will be achieved.

The submitting party has to provide information on the reliability of the complete sampling, preanalytical and analytical procedures, especially for samples that are very susceptible to contamination, inhomogeneity or instability and to ensure sampling in accordance with standard operation procedures. Field workers and the laboratory staff must be extensively trained, particularly for novel sampling and analytical procedures. When available, a comparison between different matrices currently used for measuring the respective biomarker should be provided.

2.3 Preanalytical phase

Some biomarkers might be unstable, either due to their inherent physical/chemical properties, or due to the properties of/in the respective matrix (see criteria in D9.1). For those cases the submitting party must provide appropriate procedures to ensure stability of the biomarkers (in the respective matrix) and to establish specific actions for either enhancing the stability of the biomarker or developing a method that can prevent the instability. The submitting party has to consider interfering factors and how they can circumvent those matters (e.g. external contamination, temperature sensitivities, UV light sensitivities, transportation, storage and preparation conditions). The stability and homogeneity of the samples must be tested by using appropriate control material. The submitting party must describe all sampling and laboratory equipment requirements and whether similar equipment are needed for other interested parties to participate in an EU-wide HBM study according to the criteria for stability and homogeneity defined in D9.1.

2.4 Analytical phase

For the analytical phase the submitting party must describe all required pre-treatment steps (e.g. homogenisation, centrifugation, enzymatic treatment), extraction, derivatisation, separation, and/or clean-up steps. How much sample (volume or mass) is needed? Can the sampling, preparation and analytical procedure be standardised to an adequate internal standard? Are the results comparable with those from previous measures from the own laboratory or other laboratories? Are the quality parameters (e.g. precision, recovery, variability, and detection limits) within acceptable ranges? A pilot study with human material is strongly recommended. How many numbers of samples can be analysed by the laboratory (per day/week/month)? Is the new method feasible for conducting an EU-wide HBM study?

2.5 Conclusions

The feasibility to develop a new method has to be investigated prior to the support by WP9/WP9.3. Critical points in sampling and method development need to be identified and emphasized in the feasibility study by the submitting party.

The rationale and scientific and technical feasibility of the specific matrix/biomarker combination in EU-wide HBM must be described and proven.

It is very important to check the correct combination of biomarker(s) and matrices, if the biomarker should provide reliable, comparable and interpretable results for exposure and risk assessment. For each matrix-biomarker combination the submitting party has to control for the complete sampling, preanalytical and analytical procedure. For biomarkers with high instability the laboratory has to establish appropriate procedures to ensure stability and/or to establish specific actions for either enhancing the stability of the biomarker or developing a method that can circumvent the instability.

WP9.3 support can provide: checking the needs for the new method based on the feasibility paper and the check lists, identifying knowledge gaps, providing theoretical and practical knowledge transfer, involving laboratories with experience in developing new methods (D9.3), initiating discussions, telephone conferences etc. in close exchange with WP9.3 partners and the WP9 leaders.

A new method development appears to be feasible for inclusion in HBM4EU under WP9 if all pre- and analytical issues were sufficiently addressed and resolved, and if the new method (biomarker/matrix combination) can be included in the WP9.4 QA/QC measures (interlaboratory comparison investigations and external quality assessment scheme).

3 Submission process

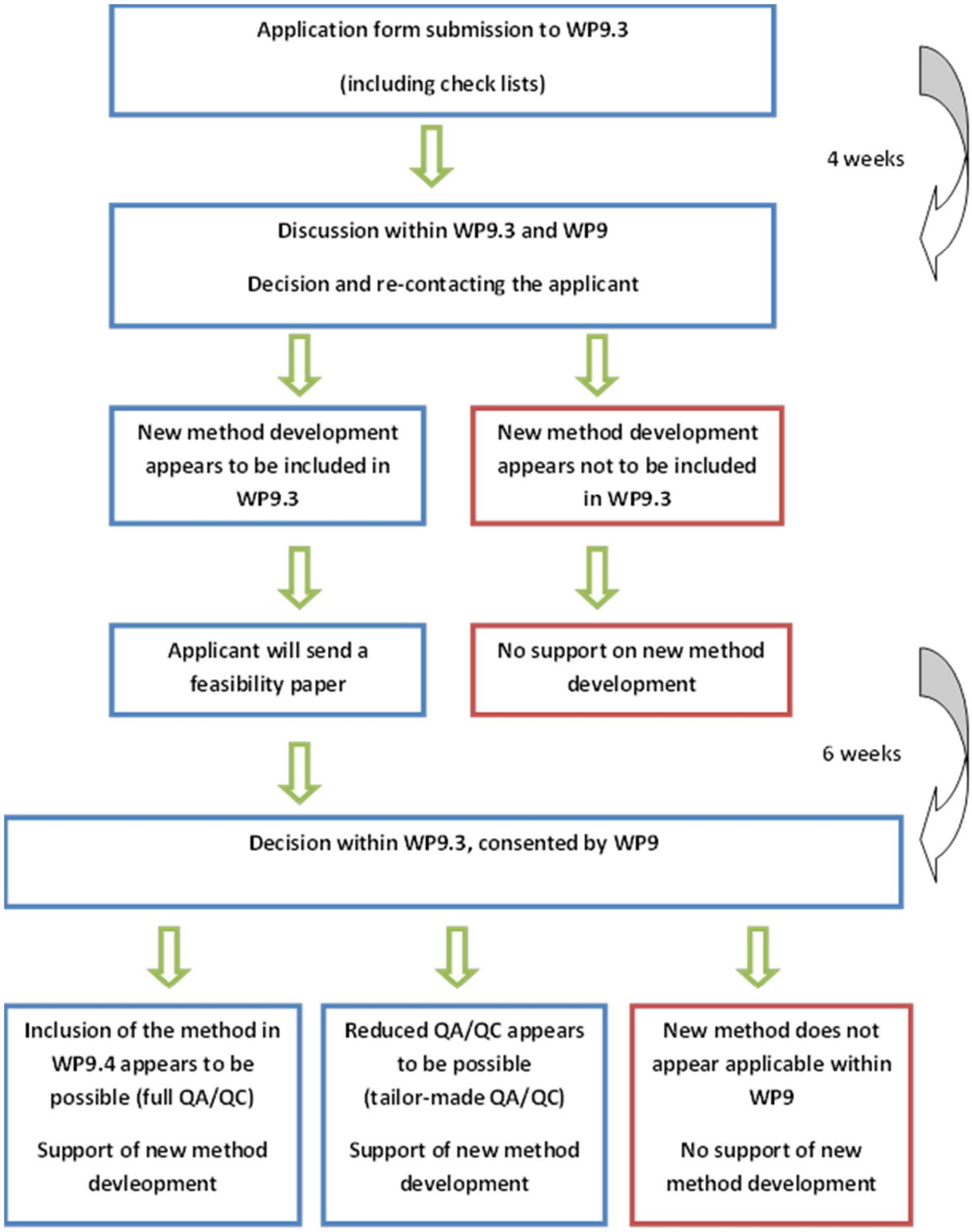
The process of applying for a new biomarker/matrix combination to be considered for new method development within WP9.3 is initiated by sending a short application form (see below, Appendix A) to WP9.3 (WP9@ipa-dguv.de). In this short application form the submitting party has to present key information on the submitting party itself, its role within HBM4EU, involved WPs (and tasks) proposing or concluding the demand for this new method, general aspects on the biomarker/matrix combination, the intended population for investigation (general population, occupational, etc.) and general aspects on the new analytical method itself. If available, discussion results from all previously involved WPs (specifically mentioning the involved WPs and tasks) concluding the demand for this new method should be shortly summarized. This short application form should be accompanied by the filled out check lists (see below, Appendix B).

Based upon the application form and accompanying documents the proposal for a new method development (specific biomarker/matrix combination) will be discussed within WP9.3 and within WP9. In due time, WP9.3 will re-contact the applicant providing comments on this application.

These comments then need to be specifically addressed in the more detailed feasibility paper on the proposed new method development. The feasibility paper should cover all aspects of new method development, such as the rationale for the new method to be considered in WP9.3, for selecting the specific biomarker/matrix combination, for the proposed analytical method and their advantage(s) compared to already existing and validated HBM methods etc. All available information on the biomarker and matrix regarding specificity, stability, homogeneity etc. must be provided (together with the available relevant literature) and also a description of the difficulties that must be solved. The check lists serve as a guide to this feasibility paper (but might be freely amended according to the special demands or circumstances related to the new method).

Based on the full feasibility paper, WP9.3, consented with all WP9 tasks, will communicate the decision whether to support new method development for the new biomarker/matrix combination, or not. Only given a high likelihood that the proposed new method can be transferred into the HBM4EU wide QA/QC program of WP9 (WP 9.4), a positive decision can be rendered. For specific biomarkers/matrix combinations (e.g. when HBM is planned for a smaller subpopulation or specific groups) a reduced QA/QC plan could be provided. Likewise, support of new method development activities may be also justified for reaching the whole project's objectives in relation to research and scientific based support to policy within pillar 3 and related WPs under the responsibility of the concerned WP leaders there.

Flow chart of the submission process



4 References

D9.1: WP 9: Laboratory analysis and quality assurance. Criteria for prioritisation of biomarkers, matrices and analytical methods.

D9.2: WP9 - Laboratory analysis and quality assurance. Prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances.

D9.3: WP 9 - Laboratory analysis and quality assurance. Candidate laboratories to develop new analytical methods in HBM4EU WP9.

5 Appendix

5.1 Appendix A: Application form

The application form can be requested as word-file per email: WP9@ipa-dguv.de

1. GENERAL INFORMATION

SUBMITTER	
Submission date:	
Organisation/Company:	
Department/Faculty/Institute:	
Address:	
Postcode:	
Town:	
Country:	
Role within HBM4U (provide work packages/tasks):	
Involved partners for demanding new/improved method development within HBM4EU (provide work packages/tasks):	
Responsible contact:	
Title	
Surname	
First name	
Function	
Phone	
Email	
ADDITIONAL CONTACT PERSON (OPTIONAL)	
Title	
Surname	
First name	
Function	
Phone	
Email	

2. INFORMATION ON THE METHOD

Name of the method:
Abbreviations used in the submission:
Summary of the method:
Target population (e.g. general population, specific subgroups, occupational):
Briefly describe the intended purpose:
Describe whether the method represents an improvement compared to an existing method *(e.g. better information, lower LOQ, increase the high-throughput, etc.)*:
Describe the limitations of the method *(technical limitations, applicability to restricted populations, etc.)*:
Stated if any component of the method is patented, copyright protected, trade-marked, registered ,etc.:

5.2 Appendix B: Check-lists

The check-lists below are intended to give guidance for the feasibility study/paper to be prepared by the submitting party for new method development. Depending on the new method and depending on the biomarker/matrix combination proposed, additional aspects might be added by the submitting party for completeness, and due to their own experience with the respective method and matrix/biomarker combination. Important references or ground laying literature need to be provided together with the feasibility study/paper to WP9.3.

The check-lists can be requested per email: WP@ipa-dguv.de

5.2.1 Check-list No. 1: Preanalytical terms and conditions.

Exposure biomarker/matrix combination (EB/M):	_____ / _____	
Preanalytical terms and conditions		
1. Matrix	The choice and applicability of the exposure biomarker/matrix (EB/M) combination should be described in a short review with appropriate references.	Please provide description and references.
	If validated EB/M combinations and/or analytical methods are available the different methods should be compared.	
	The matrix has to be defined precisely (e.g. type, weight, length, age, homogeneity, required precautionary actions before sampling etc.).	Please provide a detailed description of the matrix.
2. Specifity	The EB/M concentration is an exclusive consequence of environmental/occupational exposure.	….. yes ….. no
	EB/M concentration might not reflect exclusively exogenous exposure to the substance, but is a correct indication of exposure.	….. yes ….. no
3. Biological sensitivity	The behaviour of the biomarker in the matrix is known (kinetics, stability, homogeneity).	….. yes ….. no
	The measured concentration of the EB/M correlates with the substance intake dose.	….. yes ….. no
	Variations of EB/M concentration reflect precisely the variation of exposure to the substance of interest.	….. yes ….. no
	The measured concentration of the EB/M is an acceptable indication of the substance intake dose.	….. yes ….. no
4. 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.	….. yes ….. no
	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.	….. yes ….. no
	Variations in matrix composition can be easily corrected for (e.g. creatinine in urine, lipids in serum). Possible correction/normalization procedures need to be presented.	….. yes ….. no
	The sample collection of the relevant matrix is considered not too invasive.	….. yes ….. no

5. Matrix availability/sample collection	Easy collection (not sophisticated equipment or highly trained staff needed) and transportation of the required amount of sample with a validated sampling protocol is developed.	….. yes ….. no
	It is relatively easy to obtain a sufficient sample volume for a required number of samples.	….. yes ….. no
	Use of not interfering sampling material is possible.	….. yes ….. no
	It is possible to determine more than one EB in the same matrix.	….. yes ….. no
6. Stability after sample collection / during storage	The EB/M is stable in the sample for many hours during transportation to the laboratory or before storage in a biobank.	….. yes ….. no
	Optimal transportation conditions to ensure the stability are relatively easy to achieve.	….. yes ….. no
	Sample degradation can be prevented by an adaptation of transportation conditions or implementation of particular sampling operating procedures.	….. yes ….. no
	The cryo-preservability of EB/M is sufficient to guarantee a high stability during storage in the biobank.	….. yes ….. no
	Stability of the EB/M is not guaranteed.	….. yes ….. no
7. Half-life	The EB/M has a half-life sufficiently long to avoid an excessive intra-individual variability in EB/M concentration measurement.	….. yes ….. no
	The half-life of the EB/M sample is short, excessive intra-individual variability in EB/M concentration measurement must be considered.	….. yes ….. no
	Provide necessary information on kinetics/distribution/elimination in order to interpret the biomarker concentration in the respective matrix.	
8. Individual susceptibility	The formation of the EB/M in the human body is prone to individual susceptibility (e.g. enzyme polymorphism).	….. yes ….. no
9. Background of data	The EB/M has been used in HBM surveys to study the substance of interest.	….. yes ….. no
	The EB/M allows comparison with existing HBM data.	….. yes ….. no
	Data on the toxicological profile of EB/M or health guidance values are available.	….. yes ….. no
10. Laboratory equipment	The laboratory sufficiently provides the required equipment.	….. yes ….. no
	Participating laboratories must have the same or similar equipment.	….. yes ….. no

5.2.2 Check-list No. 2: Analytical terms and conditions.

Exposure biomarker/matrix combination (EB/M):	_____ / _____	
Choice of analytical method	_____	
Analytical terms and conditions		
1. Analytical method availability	At least one validated and publicly available, analytical method exists to measure the EB/M concentration in humans (please provide references).	….. yes ….. no
	An analytical method exists, or is likely to be validated in the near future and could be used to produce new data (please provide more information).	….. yes ….. no
2. Sample preparation	Sample preparation procedure is well established and applied routinely for relevant biological matrices.	….. yes ….. no
	Appropriate sample preparation procedures are available to some extent, but have not necessarily been established for the EB/M combination. A certain level of development (mainly adaptation) could be necessary.	….. yes ….. no
	Sample preparation procedures are not yet available and have to be developed.	….. yes ….. no
	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).	….. yes ….. no
3. Control material	Certified control material is available.	….. yes ….. no
	Control material in the same matrix is available. Range of concentration:_____	….. yes ….. no
	Control material has to be prepared and tested. Please define in more detail the choice and appropriateness of the control material: _____	….. yes ….. no
4. Standards	Standards of target EBs and internal standards (among these isotopically labelled standards when relevant) are available.	….. yes ….. no
	Standards are not commercially available or might be offered by only one or few suppliers, longer times of delivery may occur.	….. yes ….. no
	Determination of this substance is the first tentative identification of the EB/M. Standards might not be commercially available at all.	….. yes ….. no
5. Validation	The method is established and full within laboratory validations have been carried out, based on common guidelines (e.g. ICH guidelines and GLP).	….. yes ….. no
	Concentrations will be reported using methods subjected to less rigorous validation procedures (e.g., in-house controls, lack of assessment of some parameters like matrix effects, precision, accuracy, etc.).	….. yes ….. no
	Large-scale studies and interlaboratory comparisons are expected in the near future.	….. yes ….. no

	No method validation is expected. Assessment of critical parameters will be in progress.	….. yes ….. no
6. Selectivity	A low extent of interferences has been demonstrated. The measured concentrations are that of the EB/M.	….. yes ….. no
	Potential interferences are not fully controlled for the EB/M.	….. yes ….. no
	Selectivity has not been assessed.	….. yes ….. no
7. 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.	….. yes ….. no
	LODs and LOQs are available for some individual EB/M, but not necessarily for all EB and all matrices of interest for HBM.	….. yes ….. no
	When standards are available, the LOD and LOQ will be determined for individual studies, but not as part of a validation procedure.	….. yes ….. no
	The LOQ of a validated analytical method is low compared to commonly measured concentrations in the general population.	….. yes ….. no
	Only a few studies are available and the quantification has been or will be done using non-validated analytical methods.	….. yes ….. no
- **Quantifiable compounds**	In general, LOQs have been proven to be sufficiently below the concentrations in a high proportion of the samples of a population.	….. yes ….. no
	LOQs may appear in some cases higher than the expected exposure, but enable quantification of the biomarker in a reasonable number of samples of the population.	….. yes ….. no
	No standard is available; only semi-quantitative determinations are possible.	….. yes ….. no
- **Robustness**	Limited variation in the LODs and LOQs. The environment-laboratory conditions are well controlled (e.g. low background levels).	….. yes ….. no
	Some variation in LODs and LOQs can occur (e.g. variable blanks and/or instrument performance).	….. yes ….. no
	High variation in LODs and LOQs or absent/insufficient information to properly quantify this parameter. Interferences cannot be ruled out.	….. yes ….. no
- **Comparability**	Similar LODs and LOQs have been obtained by most laboratories.	….. yes ….. no
	Variability in LODs and LOQs exists, for example due to different analytical approaches for the determination of the EB/M.	….. yes ….. no
	Low comparability of LODs and LOQs or absence of information.	….. yes ….. no
8. Uncertainty and Accuracy		
	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.	….. yes ….. no

- **Uncertainty (at lower and higher concentrations)**	The uncertainty has been assessed, but might exceed guidelines for validation of analytical methods for the EB/M combination.	….. yes ….. no
	The uncertainty was assessed but it exceeded commonly accepted values.	….. yes ….. no
	The uncertainty has not been assessed.	….. yes ….. no
- **Accuracy - assessment**	The accuracy is within the limits given by guidelines for validation of analytical methods (e.g. ≤20% deviation).	….. yes ….. no
	The accuracy has mainly been assessed using internal QC measures, recovery tests or comparisons with an independent analytical method.	….. yes ….. no
	The accuracy has usually not been evaluated yet.	….. yes ….. no
	The accuracy has usually not been assessed, but indications from similar EB are available.	….. yes ….. no
- **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.	….. yes ….. no
	Variable recoveries are detected (e.g. 50-150%). There is a stronger need to compensate the deviations with a proper internal standard.	….. yes ….. no
	Recoveries have rarely been assessed.	….. yes ….. no
- **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 EB/M.	….. yes ….. no
	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.	….. yes ….. no
	Optimal working range has rarely been evaluated.	….. yes ….. no
9. 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.	….. yes ….. no
	The robustness has been assessed, and variations occurred due to several factors (e.g. EB stability, instrument performance, environment and/or operating conditions, etc.).	….. yes ….. no
	The robustness has likely not been assessed. Any significant variations, which could affect the analytical result, should be reported.	….. yes ….. no
- **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.	….. yes ….. no
	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.	….. yes ….. no

	The repeatability/ intermediate precision has not been assessed.	….. yes ….. no
- **Method reliability**	The within-laboratory reproducibility of the test method has been evaluated.	….. yes ….. no
	The transferability of the method to other laboratories has been evaluated.	….. yes ….. no