

The purpose of these guidelines is to provide sufficient information from which the reviewers/readers can evaluate, interpret, compare, and, if necessary, reproduce the reported study. They contain both mandatory and recommended information. The former are marked with asterisks.

Ethics Approvals

- *It is required to provide a statement of institutional Ethics/Animal Committee (IRB/ACUC) approval for use of human/animal biological materials for this research purpose including details of informed consent, if appropriate, for clinical or biomarker trial participation and/or primary cell line development.
- *It is also necessary to describe what mechanisms were employed to protect human subject confidentiality, such as procedures for identification/de-identification and coding of biospecimens.

Study Goals and Design

- A comprehensive description of the study design, including the overall goal(s) of the study, with specific attention to the following qualifications, should be included:
 - *Identify the stage/phase of the study, e.g. discovery, verification or validation, and state clearly the stage/phase that a candidate (biomarker, target, analyte) is at, e.g., exploratory/discovery; preclinical validation; etc.).
 - *Describe the flow of subjects/samples through the study, including the initial number of cases, the number included in each stage of the analysis (a diagram is recommended for more complex/larger studies) and reasons for subject dropout.
 - Justify any claims made regarding (candidate) biomarkers based on sound statistical and clinical assessment.
 - Describe the time period of the sample collection and/or study execution.

Subject Source and Description

- *It is essential to describe the source and classification of subjects (or materials obtained there from) with respect to as many of the parameters described below as are known:
 - Prospective or retrospective accrual
 - Stratification
 - Matching (gender, age, disease, etc)
 - Randomization of subject assignments, such as treatment, intervention, etc.
- * It is necessary to indicate the source of biospecimens (e.g. centralized biobank, internal biobank, clinical trial collection, surgery etc).

- *Describe inclusion/exclusion criteria for study and reference cohorts

- * For all patients from whom samples were derived, it is required to include a definition of disease or condition (including treatments if known) as indicated below:
 - disease description, such as subtype, stage, grade, histology and clinical score (if applicable).
 - disease type using standard medical terminology (ICD codes should be included where known), e.g. juvenile diabetes v. noninsulin-dependent diabetes.
 - any known potential confounders relative to the time of sampling, such as intra- or pre-operative status, administered drugs/anesthetics etc.

- *Describe demographic and clinical characteristics for study and reference (control) subjects, including age, gender, disease stage, and co-morbidities, etc.

- Describe trial treatment(s) or other intervention, if appropriate.

- Describe other exposures, interventions, and lifestyle factors that may affect results, such as, smoking history, etc.
- If appropriate, describe follow up and duration, include median and range.

Biospecimen Qualification

- Tissue
 - Indicate, if known/applicable:
 - Average time to tissue acquisition and processing (time to initial stabilization step), and range of times.
 - *Type of processing, e.g. formalin, ethanol, method of freezing, embedding medium.
 - Average storage temperature, and mean and range of duration of storage.
 - *Post-cutting fixation for frozen tissue.
 - *Methods of enrichment for relevant component(s) of biospecimen if applicable (e.g. micro dissection).
 - *Describe any histologic review of biospecimens used in experiments.

 - If immunohistochemical staining, or other testing, was done on tissue, indicate if pathology review was blinded and if agreement between reviewing pathologists was obtained.

 - If known, provide information regarding shipping of biospecimens to central repository, e.g., time, temperature.

- **Note:** Supporting histology (digitalized or original slides) may be requested by the reviewers and/or editors.
- For blood and biological fluid biospecimens, *reference published standard operating procedures if used, if not indicate:
 - *Method of collection.
 - *Tube type (and size if known) used for collection and storage.
 - *Use of additives such as anti-coagulants, preservatives, and protease inhibitors, if used.
 - Processing conditions including the time interval between collection and separation, *centrifugation conditions, temperature of processing, collection volume, time interval between processing and freezing.
 - Provide any information regarding shipping of biospecimens to central repository, e.g. time, temperature.
 - *Storage temperature and length of storage.
 - *Number of freeze thaw cycles.
 - Indicate if there were any variations in collection and processing across biospecimen set(s).
- *Primary cell lines, generation and use:
 - Provide clinical details regarding subject and biospecimen of origin
 - Provide conditions/protocols of cell line generation and characterization including passage number and number of clones analyzed.

Statistical Considerations

- * It is required to describe the statistical analysis strategy in detail, when applicable. This should include:
 - The central hypothesis that is being tested.
 - Model building and validation.
 - The rationale used to choose cut-off thresholds and other model parameters.
 - The independence of exploratory (training) and confirmative (test) analysis.
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- Provide the following details about the statistical analysis, if applicable:
 - Feature selection and multiple hypotheses testing strategy
 - the number of candidates (e.g., markers, targets, analytes) tested and number selected for more detailed analysis should be stated.
 - The details of any subgroup analysis performed should be provided.
 - It should be stated how missing values were handled, if applicable.
 - *Point estimates, p-values and/or confidence intervals should be given.
- The statistical presentation should also include a discussion of confounding factors and the methods employed to minimize their impact.

Technical Considerations

- Provide information, when applicable, about the performance characteristics (technical and process including fractionation, digestion etc.) of the analytical process/assay(s) used (e.g., mass spectrometry, protein, antibody, nucleic acid arrays, immunochemistry, 2D electrophoresis, or other measurement technology). Include for both the technical and processing steps (fractionation, digestion, etc.) noise assessment, reproducibility, normalization (e.g. array to array and protein to protein), measurement variation, specificity, limits of detection and quantitation. Authors should note that the guidelines for protein identification analyses for articles submitted to MCP are fully applicable to papers dealing with clinical proteomics and should also be consulted if this type of information is included.
- Describe quality control and quality assurance methods employed and how analysis of samples was conducted, e.g. replicate number, whether randomized etc.
- Describe any software packages and bioinformatics tools used for model building, pathway analysis or data visualization. Journal policy requires that all software employed is available to the general public.
- If applicable or known, list any other studies (preferably by literature reference) that have used the same or a subset of the samples employed regardless of the nature of the previous study.