MCP MOLECULAR & CELLULAR PROTEOMICS

Important Information for MCP Authors

Guidelines to Authors for Publication of Manuscripts Describing Development and Application of Targeted Mass Spectrometry Measurements of Peptides and Proteins and Submission Checklist

The following Guidelines (Part 1, below) and Checklist (Part 2, below) are provided to aid authors in preparing their manuscripts and to inform them of the items that must be included in their manuscripts.

Authors will need to complete the Checklist during the submission process to help ensure that their manuscripts contain all the required information. Authors are to note the page numbers where the information can be found in the paper. Manuscripts will be checked to ensure they comply with the guidelines at the same time that the peer review is being performed. It is important to note that as with all other papers in MCP, the compliance check does not constitute a review of the manuscript as this ONLY determines if the article conforms to the guidelines, i.e., contains the requisite information. The compliance check does not judge the quality of the data or evaluate the scientific suitability of the manuscript.

Guidelines for Targeted MS Manuscripts

Reporting the Tier Level of the Analyses Used: Targeted proteomic analyses can be divided into three strata differing by the intended application and extent of analytical validation. This Tier structure was developed as a direct outcome of a meeting sponsored by the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) Program (see Carr et al. *Mol Cell Proteomics* 2014; PMID: 24443746). **Tier 1** measurements have the highest standards and generally correspond to clinical bioanalyses / diagnostic laboratory tests that measure a single analyte (or small numbers of analytes). **Tier 2** measurements are for research use assays, often highly multiplexed, for quantifying proteins, peptides and post-translationally-modified peptides. Tier 1 and 2 have two properties that together differentiate them from discovery experiments: **1)** the ability to measure repeatedly sets of analytes of interest within and across samples/experiments and **2)** they both employ internal standards for each analyte, for confident detection and precise quantification. **Tier 3** measurements, useful in early-stage biological studies, enable repeatable measurement of the same sets of analytes across experiments but **do not employ internal standards** for either accurate or precise measurement of the levels of each analyte. Tier 3 methods (including so-called Data Independent Analysis methods (DIA, including SWATH) do not constitute assays, but instead employ targeted strategies to bias towards detection of a predefined set of analytes. DIA methods are closer to data-dependent discovery approaches than to targeted MS methods like MRM and PRM, and will also need to comply with requirements for the former (see MS guidelines). The manuscript requirements are dependent on the Tier level of analysis performed. In the cover letter and in the manuscript, authors should state the Tier level of the analyses they have developed and applied.

For all Tiers, authors must provide the following written information:

- for human samples other than established cell lines, a Clinical Compliance check is required that specifically addresses how biofluids or tissue specimens were collected and handled, including IRB approval). Please see http://www.mcponline.org/site/misc/clinical.xhtml for details.
- name(s) of database(s) (e.g., PRIDE, MassIVE, etc.) where the raw data has been deposited and the access code(s) (see peptide/protein guidelines for information on raw data deposition).
- details of the experimental design and rationale, including statistical rationale for the design and/or regulatory guidance that was followed, as described in Section III of the Checklist, below.

- a subsection in the Experimental Methods with the header "Development and Analytical Validation Targeted MS Assays/Measurements" that includes the information described in section IV of the Checklist, below.

- the methods used for data collection and quantification for the samples and the values obtained including measurements of precision and/or accuracy.

For Tiers 1 and 2 analyses, authors are also required to provide all chromatograms used in quantification of analytes. This requirement (details for which are bulleted, below) is easily fulfilled using Skyline and Panorama (and possibly other tools in the future), and this tool combination also simplifies addressing the other requirements bulleted, below. If Skyline/Panorama or equivalent has not been used, authors may provide the above required chromatogram information in any format with a freely available viewer, such as PDF, as supplement at the time of first submission. Failure to provide either the necessary link to the Panorama files or equivalent, or to provide the chromatograms as pdf supplemental data may result in papers being returned without review.

As noted, this requirement pertains to Tiers 1 and 2 targeted analyses, only, and does not currently apply to label-free methods of quantification of the analytes (including cases where one or more labeled peptides have been used for normalization/quantification - but not for all analytes), as well as so-called Data Independent methods. These approaches fall under Tier III. However, for protein identifications based on a single peptide identification, or for post-translationally modified peptide identifications where DIA methods are employed, annotated spectra or ion chromatograms must be provided to present the evidence for the identifications (see MS guidelines).

- The chromatograms supplied must also include clearly marked integration boundaries or peak area shading used in the calculation of area under the curve (AUC), which was used as the basis for all reported quantities. Any smoothing or interpolation applied to the data must be reported and its relationship to the AUC calculation explained. The method for AUC calculation must be depicted visually using the graphed chromatograms and integration boundaries or peak area shading. If integration boundaries have been manually edited, this must be noted and details provided. A detailed explanation of the tool used to calculate the AUC from the graphed data must be provided. It is not required that the raw chromatogram point values graphed be made available. The actual (median) or estimated number of points across the peak should be reported for all the analytes in the assay (we recommend no fewer than 10-12 points across each peak). In the cases where actual value is not easily reported, an estimate may be provided from the cycle time or dwell time, retention time window used and number of concurrent transitions.

- If screen shots are provided (rather than the preferred mode of making data viewable with software such as Panorama), the chromatograms provided must include the full extent of the integrated peak, along with at least two peak widths of elution time (1 on either side) outside the peak itself to allow for some understanding of surrounding noise or potential interference. Chromatograms of both the analyte and isotopically-labeled standard (heavy peptide) must appear in a single view/page with matching retention time scales to allow reviewers and readers to ascertain coelution and assess shape matching.

At present, the simplest way to provide the required chromatogram information is to have used Skyline for method development and data analysis. The Skyline files can then be uploaded into Panorama Public into a restricted area for confidential reviewer-only access. A tutorial on use of Panorama Public can be found at PanoramaWeb.org. Regardless of what tools are used, authors should state the versions of each software

tool that was used. MCP will update these guidelines as new tools become available that facilitate meeting the above requirements.

Targeted MS Manuscript Checklist
(Refer to Targeted MS Manuscript Guidelines for details)

Section I: General information (to be provided for all papers)

		Page	
Authors have stated the Tier level of the analyses they have developed and applied (i.e., Tier 1, Tier 2 or Tier 3 or a combination thereof).	Y		
Authors have supplied the names of the databases and the access codes for both the raw data and, if applicable, chromatogram files if the latter was not uploaded as supplementary material with the manuscript.	Y		
If human samples are used, authors have completed the Clinical Compliance check, including IRB approval and how biofluids or tissue specimens were collected and handled. See http://www.mcponline.org/site/misc/clinical.xhtml for more information.	Y		N/A

Section II: Chromatograms used in quantification of analytes (Tiers 1 and 2, only)

		Page	
If analyses reported are Tier 1 or Tier 2, authors have provided all chromatograms used in quantification of analytes and the names of the databases used and access codes, if applicable.	Y		N/A
If authors adjusted integration boundaries manually, was this noted and were the methods used described?	Y		N/A

Section III. Experimental design and rationale

		Page	
Authors provided a subsection in the Experimental Methods section with the header "Experimental Design and Rationale".	Y		
Authors described the criteria used to select the target peptides (e.g., uniqueness to proteoform, how PTM sites were avoided)	Y		
The sample size (n) (for each experiment), as a number (ranges are not acceptable)	Y		
The rationale for that (n) choice (e.g., statistical power of detection)	Y		
Numbers and types of controls employed	Y		
The number of replicates acquired, including a clear distinction between biological, process and/or technical replicates is provided	Y		
Authors described if samples were processed/run in a blinded fashion or not	Y		N/A
The criteria for the inclusion or exclusion of data points (if relevant)	Y		N/A
A description of the methods used for analysis, and the choice of the statistical cutoffs (p-value, FDR etc.) for both identification and quantification (e.g., t-test) are stated.	Y		

		Page
Justification for the statistical methods used for analysis is provided	Y	
Software used is described, including versions, and an explicit description of options and parameters used. Source code for 'in-house scripts' employed has been provided, if applicable.	Y	

Section IV. Information on development and analytical validation of targeted MS assays/measurements

		Page	
Type of Targeted MS Experiment Used (i.e., PRM, MRM/SRM)	Y		N/A
How the resulting precursor ions and the transitions were selected (e.g., in silico, from discovery experiments, from database/spectral library/literature) is described	Y		N/A
Methods used to optimize transition ion abundance are described	Y		N/A
Method(s) used to identify interferences are described	Y		
The transitions monitored for each analyte are provided (text of supplementary table)	Y		
Precision of measurements was determined and reported/listed	Y		N/A
The nature of the response-to-concentration response (e.g., linearity) was determined and reported, the curve fitting algorithm used is described and the response curves (if generated) are provided	Y		N/A
Chromatography and desalting methods (on-line as well as off-line, if employed) are fully described (including packing material, column dimensions, flow rates, mobile phases, etc.)	Y		N/A
Internal standards used (if any) are identified and how they were used is fully described	Y		N/A
The methods to characterize the internal standards, if used, are described (e.g., amino acid analysis; MALDI or ESI MS; HPLC-UV) • Note - for detailed information of characterization of internal standards, see Hoofnagle et al. Clinical Chemistry 62:1 48–69 (2016)	Y		N/A
How the concentration and stability of heavy peptide standards and/or labeled proteins were assessed initially and over the course of the study is described	Y		N/A
If limits of detection and quantification were determined, authors have provided the values determined including the calculations used	Y		N/A
If an evaluation of the system suitability for analysis (i.e., robustness, reproducibility, stability, carryover, etc.) was performed, authors have described the test and provided details or citation(s). • For example, see Abbatiello et al. Mol Cell Proteomics. 2013 12(9): 2623–2639; and Percy et al. J Proteomics. 2013 95: 66-76	Y		N/A
For studies carried out over weeks or months, authors have described the samples, methods and frequency of QC of the overall system performance and quantitative reproducibility over time • For example, see Simon Mol Cell Proteomics 2012	Y		N/A

Section V. Methods of data collection and data analysis

		Page	
Software used for peak picking and determination of peak area ratios is described	Y		N/A
Description of how transitions were selected and used to derive quantitative information is provided (e.g., were summed, peak area ratios used; if a single transition, how was it chosen (CV, intensity, interference free, other?). Any data filtering and processing used is described.	Y		N/A
Whether a single peptide or multiple peptides were used to generate a protein concentration is reported, and how the supporting/qualifying peptides were used is described	Y		N/A
If external calibration was used in the quantification, how this was done is clearly explained (including what calibrators were used, how equations were used to fit calibration curves, what QC samples were used to assess performance of the curves, what the acceptability criteria were for the QC samples, etc.)	Y		N/A
The precision (e.g., standard deviation, coefficient of variation, standard error) of the measurements for each peptide/protein is reported	Y		N/A