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# MIRAGE Guidelines for Reporting Mass Spectrometry Experiments for Glycan and Glycoproteomics Data

Version 1.0, October 10, 2025

Based on the MIRAGE MS Reporting Guidelines  
(Version 1.0, April 24, 2013, doi: [10.3762/mirage.2](https://doi.org/10.3762/mirage.2)).

## Published in

Lisacek F, Hackett WE, Thaysen-Andersen M, Karlsson NG, Klein J, Kolarich K, Maciej-Hulme ML, Okuda S, Packer NH, Struwe WB, Takahashi Y, Tiemeyer M, Zaia J, Aoki-Kinoshita KF, Kettner C (2025) Update and new implementation of the MIRAGE reporting guidelines for mass spectrometry experiments in glycoscience. *Molecular & Cellular Proteomics*. DOI: [10.1016/j.mcpro.2025.101473](https://doi.org/10.1016/j.mcpro.2025.101473).

These reporting guidelines replace the previous version from April 2013.

| Section  | Definition  | Type of expected information                          | Helpful tools  | Glycomics | Glyco-proteomics |
|--|---|---|--|-----------|------------------|
| <b>1. General Features</b>                         |   |   |  |           |                  |
| Data stamp   | The date on which the work described was completed  | Standard date 'YYYY-MM-DD' format                     |  | X         | X                |
| Responsible person or role and contact information | The primary contact person's name for this data set with affiliation and contact information  | Short free text                                       |  | X         | X                |
| Paper title and journal                            | The DOI of associated manuscript  | URL ( <a href="https://doi.org">https://doi.org</a> ) |  | X         | X                |
| <b>2. Experimental Design</b>                      |   |   |  |           |                  |
| No input required                                  | See MIRAGE Sample Preparation Guidelines  |   | <a href="https://doi.org/10.3762/mirage.1">https://doi.org/10.3762/mirage.1</a>  | X         | X                |
| <b>3. Data Processing &amp; Identification</b>     |   |   |  |           |                  |
| <b>3A. Data Pointers</b>                           |   |   |  |           |                  |
| Location of raw data                               | If made available in a public repository, the corresponding URL, or the information on <b>how to retrieve the raw data</b> . If not made available for public access, name the contact person or source and the internal coordinates of the data. | URL data set identifier/password or short free textx  | <a href="http://www.proteomexchange.org/">http://www.proteomexchange.org/</a><br><a href="https://glycopost.glycosmos.org/">https://glycopost.glycosmos.org/</a> | X         | X                |
| Location of processed data                         | If applicable, URL or the information on <b>how to retrieve processed data</b> .  | URL, dataset identifier/password or short free text   | <a href="http://www.proteomexchange.org/">http://www.proteomexchange.org/</a><br><a href="https://glycopost.glycosmos.org/">https://glycopost.glycosmos.org/</a> | X         | X                |

| Section   | Definition   | Type of expected information                                     | Helpful tools  | Glycomics | Glyco-proteomics |
|---|--|--|--|-----------|------------------|
| Location of any associated data                 | If <b>complementary experiments</b> were conducted in association with the present experiment, pointers to corresponding standard-compliant metadata unless otherwise accounted for in this report.  | URL, dataset identifier/password or short free text              | <a href="https://www.psidev.info/">https://www.psidev.info/</a>  | X         | X                |
| <b>3B. Peak List Generation</b>                 |  |  |  |           |                  |
| MS raw data processing software (if applicable) | The <b>name of the software</b> and the <b>version number</b> . Possibly specify commercial source or if open source. If applicable, specify whether change(s) were made to the original program code that may affect the results.         | PubMed ID, URL or URI, or short free text                        |  | X         | X                |
| MS raw data processing strategy                 | <b>Type of raw data processing</b> performed with the software (e.g. de-isotoping, charge deconvolution, peak picking). If applicable, parameters for the generation/selection/smoothing of peaks.   | Short text with possible drop down list or PubMed ID, URL or URI | Draw terms from:<br><a href="https://tinyurl.com/EBIOLS">https://tinyurl.com/EBIOLS</a><br><br>if an mzML file is uploaded, these can be extracted from it | X         | X                |
| Processed data file                             | Information about the processed MS data file. This includes the <b>file format and availability</b> ; if applicable the URI to access the file. Where available:<br>- the reference numbers of all the scans (as numbered in the raw file) | URI or short free text   |  | X         | X                |

| Section   | Definition   | Type of expected information                  | Helpful tools | Glyco-omics | Glyco-proteomics |
|---|--|---|---------------|-------------|------------------|
|   | that were combined to produce a peak list,<br>- the total number of acquisitions combined to produce the peak list,<br>- whether the peak list was produced by summing or averaging the scans that are listed.   |   |               |             |                  |
| <b>3C. Glycomic &amp; Glycoproteomic Search</b> |  |   |               |             |                  |
| Identification software (if applicable)         | The <b>name of the software</b> and the <b>version number</b> . Possibly specify commercial source or if open source. If applicable, specify whether change(s) were made to the original program code that may affect the results.   | PubMed ID, URL or URI, or short free text     |               | X           | X                |
| Identification strategy                         | Search <b>strategy for precursor identification</b> , including if applicable, filtering of matches, use of database or spectral library, addition of manual pre- or post-search processing steps, inclusion of spiked synthetic glycans or glycopeptides, scoring methods for de-novo interpretation tools etc. | Short free text                               |               | X           | X                |
| Scoring algorithm                               | Method and software for <b>scoring</b> (for example, PSMs), along with the parameters supplied to the algorithm/method.  | Short free text, and PubMed ID, or URL or URI |               | X           | X                |

| Section  | Definition   | Type of expected information | Helpful tools  | Glyco-<br>mics | Glyco-<br>proteomics |
|--|--|------------------------------|--|----------------|----------------------|
| Taxonomy   | <b>Taxonomic details</b> as an NCBI TaxID. If not applicable, name of species as known by submitter.   | TaxID or short free text     |  | X              | X                    |
| Glycoconjugate search space                        | If database search, <b>type and version of the queried database(s) and glycan search space</b> .<br>If the database(s) is/are not available online, content details, including any information that will uniquely specify molecules and enable access. | URL or short free text       | <a href="https://www.uniprot.org/">https://www.uniprot.org/</a><br><a href="https://www.ncbi.nlm.nih.gov/refseq/">https://www.ncbi.nlm.nih.gov/refseq/</a>       |                | X                    |
| Glycan database                                    | List of <b>glycan compositions/structures</b> and/or the glycan masses considered in the search. Expected accession number in specified GlyTouCan version for each, unless none was assigned.  | List of IDs                  | <a href="https://glytoucan.org/">https://glytoucan.org/</a><br><a href="https://glycosmos.org/glycans/composition">https://glycosmos.org/glycans/composition</a> | X              |                      |
| Spectral library                                   | If spectral library search, type and size of spectral library (e.g., in-house or public)   | Short free text              |  | X              | X                    |
| Specified protease cleavage                        | In silico <b>cleavage pattern</b> as available on the search engine as well as cleavage rules if they have been defined by the user. Note if the software cleavage rules differ from the experimental procedure  | Short free text              |  |                | X                    |
| Number of missed protease cleavages                | Selected number of allowed <b>missed protease cleavage sites</b>   | Number                       |  |                | X                    |
| Additional parameters related to protease cleavage | If applicable, details of <b>semi-specific protease cleavage(s)</b>  | Short free text              |  |                | X                    |

| Section   | Definition  | Type of expected information            | Helpful tools | Glyco-<br>mics | Glyco-<br>proteomics |
|---|---|---|---------------|----------------|----------------------|
| Amino acid modifications  | <b>Amino acid modifications</b> (other than glycans) and their mode (fixed or variable) considered in the search. When applicable, specify custom modification and associated rules.  | Short text with possible drop down list |               |                | X                    |
| Glycan modifications  | <b>Natural or induced glycan modification</b> , e.g., permethylation, acetylation, reduction.   | Short text with possible drop down list |               | X              | X                    |
| Allowed adducts   | List of <b>adducts</b> that were allowed for the annotation.  | List of masses                          |               | X              | X                    |
| Loss and gain of small molecules  | Allowed <b>loss and gain of small molecules</b> , e.g. -CH <sub>2</sub> for undermethylation, loss of water or sialic acids   | Short text with possible drop down list |               | X              | X                    |
| Glycan fragmentation  | List of allowed <b>glycan fragmentation</b> for the annotation run (A,B,C,X,Y,Z). This includes cross-ring and glycosidic cleavages.  | Short text with possible drop down list |               | X              |                      |
| Precursor-ion and fragment-ion mass tolerance for tandem MS (when applicable) | For MS1 and MSn queries, the <b>mass tolerance</b> of precursor and fragment ions permitted by the search engine.   | Number                                  |               | X              | X                    |
| Thresholding and filtering  | Other settings to the software that filtered out certain sequences from the database (e.g. allow only certain glycan types (e.g., N-glycan) or restriction by composition). This also includes the usage of threshold for scoring values. | Short free text                         |               | X              | X                    |

| Section   | Definition   | Type of expected information  | Helpful tools   | Glyco-<br>mics | Glyco-<br>proteomics |
|---|--|---|---|----------------|----------------------|
| Any other relevant parameters                       | Any application-specific search parameters that may have an impact on the searched data and the interpretation of the results.   | Short free text   |   | X              | X                    |
| <b>3D. Glycoproteomic Search Output</b>             |  |   |   |                |                      |
| Data file   | Information about the software output data file. This includes the <b>file format and availability</b> ; if applicable the URI to access the file.   | URI or short free text  |   | X              | X                    |
| Identified proteins                                 | Protein accession number (e.g. UniProt) of all reported glycopeptides  | List of IDs   | <a href="https://www.uniprot.org/">https://www.uniprot.org/</a><br><a href="https://www.ncbi.nlm.nih.gov/refseq/">https://www.ncbi.nlm.nih.gov/refseq/</a>  |                | X                    |
| Peptide sequence                                    | Peptide sequence of all reported glycopeptides (notify any deviation from the expected protein cleavage specificity).  | List of peptide amino acid sequences  |   |                | X                    |
| Peptide sequence modifications (other than glycans) | List occurrence and position of amino acid modifications, whether artifactual (oxidized Met or Carbamidomethylated Cys) or natural (e.g. phosphorylation) or arising from amino acid variation(s). Refer to PTM-IDs when applicable. | List of positions of modified or variable amino acids   | <a href="http://www.unimod.org">http://www.unimod.org</a><br><a href="https://www.uniprot.org/docs/ptmlist">https://www.uniprot.org/docs/ptmlist</a><br><a href="https://bioportal.bioontology.org/ontologies/PSIMOD">https://bioportal.bioontology.org/ontologies/PSIMOD</a> |                | X                    |
| Glycosite position                                  | Glycosylated amino acid positions in the source protein and not peptide sequence. For N-sites acknowledge the N-X-S/T motif. FASTA format is recommended due to potential positional shifts.   | List of positions of glycosylated amino acids with corresponding reference to full protein sequence |   |                | X                    |

| Section  | Definition   | Type of expected information            | Helpful tools  | Glyco-<br>mics | Glyco-<br>proteomics |
|--|--|---|--|----------------|----------------------|
| <b>3E. Peak List &amp; Identification Annotation</b>   |  |   |  |                |                      |
| The following lines have to be repeated for each identified feature in the mass spectrum. This data could be provided in a combined annotation file (e.g., GlycoWorkbench file). | For glycomics, the features below can be recorded in Glyco-Workbench, spectra can be uploaded, annotated and additional information can be uploaded in "other" for each spectra, including observed mass, scoring and validation parameters. |   |  | X              |                      |
| MS level   | The <b>MS level</b> (e.g., MS2) at which spectra were acquired.  | Short text with possible drop down list |  | X              | X                    |
| Fragmentation type   | Type(s) of <b>fragmentation</b> used to identify molecules e.g., HCD, EThcD, CID, HCD and EThcD  | Short text with possible drop down list |  | X              | X                    |
| Ion mode   | The <b>ion mode</b> (positive or negative)   | Selection between positive/negative     |  | X              | X                    |
| Retention time   | The <b>retention time</b> of all reported detected glycomolecules (eg glycans, glycopeptides) (in minutes).  | List of numbers                         |  | X              | X                    |
| Precursor <i>m/z</i> and charge  | For MS2 spectra only, the <b>precursor <i>m/z</i></b> value and the <b>charge state</b> of the precursor ion.  | List of numbers                         |  | X              | X                    |
| Mass data  | The observed and expected masses   | List of numbers                         |  | X              | X                    |
| Confidence level   | The <b>confidence level</b> of all reported glycopeptides <sup>^</sup> (e.g., score or FDR).   | List of numbers                         | <a href="http://www.grits-toolbox.org/">http://www.grits-toolbox.org/</a><br><a href="https://glycoworkbench.sourceforge.informer.com/2.1/">https://glycoworkbench.sourceforge.informer.com/2.1/</a> |                | X                    |
| Glycans  | Details regarding the <b>glycan type</b> (e.g., N-, O-...) and <b>composition</b>  | List of numbers                         | <a href="https://glycosmos.org/glycans/composition">https://glycosmos.org/glycans/composition</a><br><a href="https://glytoucan.org/">https://glytoucan.org/</a>                                     | X              | X                    |

| Section                        | Definition   | Type of expected information                 | Helpful tools   | Glyco-<br>mics | Glyco-<br>proteomics |
|--------------------------------|--|--|---|----------------|----------------------|
|                                | (e.g. Hex5HexNAc2), GlyTouCan accession number.  |  | <a href="https://glyconnect.expasy.org/compozitor/">https://glyconnect.expasy.org/compozitor/</a> |                |                      |
| Glycan structure features      | The <b>glycan structural features</b> inferred from observed data (previous row.   | Types: Composition, topology, fully defined  |   | X              | X                    |
| Orthogonal approaches          | Additional information used for evaluation of confidence. This may include the exoglycosidase treatment, lectin recognition, reference/internal database/standard etc. (e.g., unique fragmentation or PGC-specific retention time patterns,...). | Short text with possible drop down list      |   | X              |                      |
| Any other relevant information | Any additional information that has an impact on identification.   | Short free text                              |   | X              | X                    |
| <b>3F. Validation</b>          |  |  |   |                |                      |
| Manual curation and validation | If applicable, <b>validation</b> of the output data and identifications (e.g., manually curated/filtered).   | Short free text with possible drop down list |   | X              | X                    |
| Structural ambiguity           | Can the data explain the type of monosaccharides, sequence, linkage position and configuration and site position of the reported glycan on the peptide carrier?  | Short free text with possible drop down list |   | X              | X                    |

| Section                                      | Definition  | Type of expected information                         | Helpful tools | Glyco-<br>mics | Glyco-<br>proteomics |
|--|---|--|---------------|----------------|----------------------|
| <b>4. Quantification &amp; Statistics</b>    |   |  |               |                |                      |
| <b>4A. Quantification Method Description</b> |   |  |               |                |                      |
| Quantification method                        | Methodology used for quantification (e.g., MS1 precursor intensity, MS2 fragment intensity, duplex stable isotope labeling, multiplex isobaric tag labeling, label-free method based on spectral count, etc.); if a methods paper is cited, any significant deviations should be noted. | PubMed ID or short text with possible drop down list |               | X              | X                    |
| Quantification software                      | The <b>name of the software</b> and the <b>version number</b> . Possibly specify commercial source or if open source. If applicable, specify whether change(s) were made to the original program code that may affect the results.  | PubMed ID, URL or URI, or short free text            |               | X              | X                    |
| Replicate information                        | The number of sample or biological replicates and the number of technical replicates.   | Numbers  |               | X              | X                    |
| Reference points                             | The quantitation and <b>standards used</b> . If the quantitation was not label-free, the labels used and any randomization system used with the labeling.   | Short text with possible drop down list              |               | X              | X                    |
| <b>4B. Quantification Processing</b>         |   |  |               |                |                      |
| Data normalization                           | Performed <b>data normalization</b> (e.g. logarithmized or square rooted  | Short text with possible drop down list              |               | X              | X                    |

| Section                           | Definition  | Type of expected information            | Helpful tools | Glyco-omics | Glyco-proteomics |
|-----------------------------------|---|---|---------------|-------------|------------------|
|                                   | abundance). Description of, if any, the TIC proportionate normalization of abundance values. Describe TMT normalisation if applicable.                              |   |               |             |                  |
| Relative abundance transformation | Possible data transformation into <b>relative abundance</b> (e.g. at each site or for each protein), if at all.   | Short text with possible drop down list |               | X           | X                |
| Other transformation technique    | If any transformation was applied, details of the filtered and/or processed input intensity values.   | Short free text                         |               | X           | X                |
| Replicate aggregation             | The calculation method <b>aggregating / combining the values</b> from experimental replicates and/or groups (e.g., average, geometric average, weighting, etc. ...) | Short text with possible drop down list |               | X           | X                |
| Evaluation method                 | <b>Evaluation method</b> applied to the quantification software (or manual calculation) result.   | Short text with possible drop down list |               | X           | X                |
| Acceptance criteria               | The <b>acceptance criteria</b> and quantitative measures of variability (e.g. standard error).  | Short text with possible drop down list |               | X           | X                |
| Data variance                     | If applicable, <b>observed variance</b> between technical replicates, within sample groups, and across the dataset.   | List of numbers                         |               | X           | X                |
| Outliers                          | Definition of the range of observations and details of any outliers or exceptions in either samples or individual molecules.  | Numbers and short free text             |               | X           | X                |

| Section                   | Definition   | Type of expected information              | Helpful tools | Glyco-<br>mics | Glyco-<br>proteomics |
|---------------------------|--|---|---------------|----------------|----------------------|
| <b>4C. Assessments</b>    |  |   |               |                |                      |
| Data analysis software    | The <b>name of the software</b> and the <b>version number</b> . Possibly specify commercial source or if open source. If applicable, specify whether change(s) were made to the original program code that may affect the results. | PubMed ID, URL or URI, or short free text |               | X              | X                    |
| Statistical test overview | <b>Statistical tests</b> performed and their parameters and thresholds of significance as applicable. If applicable, multiple testing corrections performed and reasoning behind the use of the applied tests.                     | Short text with possible drop down list   |               | X              | X                    |
| Clustering overview       | Possible <b>clustering tests</b> performed, their parameters and thresholds of significance. If applicable, multiple testing corrections performed and reasoning behind the use of the applied tests.                              | Short free text                           |               | X              | X                    |