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*Glycobiology*, 2022, **32**, 7, 580–587
https://doi.org/10.1093/glycob/cwac021
Advance access publication date 28 March 2022
**Glyco-Informatics**

# The minimum information required for a glycomics experiment (MIRAGE): reporting guidelines for capillary electrophoresis

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**The Minimum Information Required for a Glycomics Experiment (MIRAGE) is an initiative to standardize the reporting of glycoanalytical methods and to assess their reproducibility. To date, the MIRAGE Commission has published several reporting guidelines that describe what information should be provided for sample preparation methods, mass spectrometry methods, liquid chromatography analysis, exoglycosidase digestions, glycan microarray methods, and nuclear magnetic resonance methods. Here, we present the first version of reporting guidelines for glyco(proteo)mics analysis by capillary electrophoresis (CE) for standardized and high-quality reporting of experimental conditions in the scientific literature. The guidelines cover all aspects of a glyco(proteo)mics CE experiment including sample preparation, CE operation mode (CZE, CGE, CEC, MEKC, cIEF, cITP), instrument configuration, capillary separation conditions, detection, data analysis, and experimental descriptors. These guidelines are linked to other MIRAGE guidelines and are freely available through the project website https://www.beilstein-institut.de/en/projects/mirage/guidelines/#ce_analysis (doi:10.3762/mirage.7).**

*Key words*: data sharing; glycomics; glycoproteomics; guidelines; capillary electrophoresis.

## Introduction

Consistent reporting is essential for effective communication of experimental data both within and between different -omics domains. To achieve this, it is required that all data are acquired in an orderly and reproducible manner. The Minimum Information Required for Glycomics Experiments (MIRAGE) Commission is hosted by the Beilstein-Institut to develop reporting guidelines for glycomic experiments. These guidelines follow those in proteomics (MIAPE) (Taylor et al. 2007), enzymology (STRENDA) (Tipton et al. 2014), microarray (MIAME) (Brazma et al. 2001; Knudsen et al. 2005), metabolomics (MSI) (Members et al. 2007; Sumner et al. 2007; Spicer et al. 2017a, 2017b), and Lipidomics (LSI) (2020). Several reporting guidelines for glycoanalytics have already been developed by MIRAGE working groups, focusing on sample preparation (doi: 10.3762/mirage.1) (Struwe et al. 2016), mass spectrometry (MS; doi: 10.3762/mirage.2) (Kolarich et al. 2013), glycan microarrays (doi: 10.3762/mirage.3) (Liu et al. 2016), liquid chromatography (LC) (doi: 10.3762/mirage.4) (Campbell et al. 2019) and NMR (doi: 10.3762/mirage.5 and 10.376/mirage.6). These guidelines reflect the collective agreement by the MIRAGE Commission and Advisory Board established from representatives across the glycoscience community. Additional information concerning the guidelines and ongoing MIRAGE projects is available at http://www.beilstein-mirage.org.

Glycans require specific analytical and informatics tools as these chemical entities are biosynthesized in an adaptable non-template controlled manner. Separation modes such as LC, CE, and ion mobility spectrometry interfaced with MS now produce high-quality glycan profiling data that are made publicly available through glycomic data repositories that enable precise profiling of alterations in glycan expression during biological processes (Rojas-Macias et al. 2019). The ability to access and use such data depends on the availability of organized metadata that describe the glyco(proteo)mic samples and experimental conditions. Therefore, it is important to document the glycoanalytical sample preparation methods, which often involve enzymatic digestion, chemical derivatization, and/or separation steps to ensure reusability and reproducibility of experimental data and further data processing steps. The MIRAGE CE guidelines provide a checklist-like table that ensures that the researcher reported all relevant information in regard to a CE experiment. This information is intended to enable an improved interpretation, analysis and corroboration of data within both CE data collections and in a multi-attribute environment. Here, we present MIRAGE guidelines on how to report in a specific and reproducible manner all critical technical parameters in the metadata produced by CE. It should be noted that these guidelines are not intended as instructions to the glycoscience community on how experiments should be performed but rather to monitor that all relevant information is provided. For recommendations on how to achieve an efficient CE experiment, we recommend the following literature (Laroy et al. 2006; Mechref and Novotny 2009; Ruhaak et al. 2010;

**Received:** July 16, 2021. **Revised:** March 21, 2022. **Accepted:** March 22, 2022

Lu et al. 2018; Szigeti and Guttman 2019; Walsh et al. 2020; Cajic et al. 2021; Pralow et al. 2021).

## General principles for CE guidelines

Since the early 1990s, CE has proven to be a powerful technique for the separation of carbohydrates (Liu et al. 1991). In a liquid-filled capillary, the presence of an electric field will ensure a migration of charged molecules towards the electrode of the opposite charge. Based upon this principle, separation is obtained by differences in the electrophoretic mobility of each analyte. Therefore, in CE, it is relatively easy to separate acidic glycans from neutral glycans as the separation will be driven by the acidic monosaccharides. These monosaccharides (e.g. *N*-acetylneuraminic acid and glucuronic acid) as well as the glycan-linked sulphate or phosphate groups, will be negatively charged over a wide pH range. Next to charge, the hydrodynamic volume of analytes plays an important role in separation, allowing the differentiation of glycan isomers by CE (Mittermayr and Guttman 2012).

In the field of glyco(proteo)mics, several different operation modes of CE (Table 1) have been applied for the analysis and characterization of glycan structures, including conventional capillary zone electrophoresis (CZE) (Stefansson and Novotny 1994; Feng et al. 2017) and micellar electrokinetic capillary chromatography (MEKC) (Camilleri et al. 1995; Hutterer et al. 2000; Feng et al. 2017), which separates the analytes based upon differences in zone velocity. In the case of capillary electrochromatography (CEC) (Guryca et al. 2007), the separation is achieved by combining 2 principles; namely those of LC and CE. Here, the capillary is embedded with a chromatographic bed and the mobile phase is driven by an electric field. Next to these modes, capillary gel electrophoresis (CGE) (Feng et al. 2017) is a commonly used mode which separates the analytes based upon size, charge and shape as the analytes will move through a stationary phase or immobile phase (gel). Moreover, this platform can be multiplexed up to 96 capillaries (e.g. by using a DNA analyzer), making it an attractive approach for screening applications due to its high-throughput (Laroy et al. 2006; Schwarzer et al. 2008; Ruhaak et al. 2010). Separation can also be achieved based upon differences in isoelectric point (pI) and is known as capillary isoelectric focusing (cIEF) in which migration occurs as long as the analyte is charged. This mode is mainly applied to the characterization and development of biopharmaceuticals (Suba et al. 2015). The last operation mode being used is capillary isotachophoresis (cITP), where analytes separate in distinct zones by using two buffers with different compositions. Here, the first buffer has the highest mobility (leading electrolyte) and the second buffer contains an analyte with the lowest mobility (terminating electrolyte). A subform of this operation mode, called transient-ITP (tITP), is often used as a preconcentration step commonly used prior to CZE (Gahoual et al. 2014).

CE is known for its high resolving power and limited sample consumption (loading capacity of 1–50 nL), but becomes an even more compelling platform when combined with sensitive detectors such as laser-induced fluorescence (LIF), UV absorbance, or mass spectrometry. To enable optical detection of released glycans, an introduction of a chromophore or fluorophore is required. Commonly known labels for this purpose are 2-aminoacridone (2-AMAC) (Camilleri et al. 1995; Hutterer et al. 2000), 2-aminobenzamide (2-AB) (Guryca et al. 2007), 2-aminobenzoic acid (2-AA) (Kamoda et al. 2006), 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) (Stefansson and Novotny 1994), and 8-aminopyrene-1,3,6-trisulfonic acid (APTS) (Evangelista et al. 1996; Guttman et al. 1996; Chen and Evangelista 1998; Schwarzer et al. 2008; Mechref and Novotny 2009; Thiesler et al. 2016; Szigeti and Guttman 2019). From these, APTS is the most frequently applied label in the CE field, as it contains three negatively charged functional groups, introducing an overall negative charge to the analyte and guaranteeing a rapid separation. Moreover, an overall high sensitivity and specificity is obtained using an excitation at 488 nm, which prevents background interferences of other biomolecules. However, the identification of new analytes remains a time-consuming task, as it requires various experiments to enzymatically release specific monosaccharides of the carbohydrates for sequencing purposes. In addition, no easy distinction can be made between glycan species when co-migration occurs, but additional glycan sequencing or hyphenation of CE to MS can overcome these challenges. Glycan sequencing allows not only the identification of unknown glycan structures, but also the identification and quantification of different glycans beyond multi-structure peaks (Thiesler et al. 2016; Cajic et al. 2021). By using the high separation power of CE in combination with (tandem) MS, a high resolving power is achieved, structural information can be obtained and co-migrating analytes can be identified based upon differences in their mass (Lageveen-Kammeijer et al. 2019).

These guidelines are established to standardize the reporting of glyco(proteo)mic CE experiments for publication. To ensure that the results are reproducible, it is important to report specific CE parameters including capillary properties, coatings, background electrolyte (BGE), injection parameters, sample matrix, detector settings, as well as the handling and manipulation of data, including the software version, consulted libraries, and statistical methods being applied. These guidelines are applicable for CE platforms coupled to an optical detector (LIF/UV) and those hyphenated with MS. Furthermore, the use of standards should be described for calibration as well as alignment in the separation domain (e.g. using Glucose Unit values) (Guttman et al. 1996; Jarvas et al. 2016; Cajic et al. 2021). For the in-depth characterization of complex carbohydrates, the guidelines describe the use of excoglycosidases (Guttman and Ulfelder 1997); especially applicable for CE platforms equipped with an optical detector. The guidelines are subdivided into the following sections; (i) general features, (ii) description of the sample, (iii) equipment, (iv) type of analysis, (v) run processes, (vi) detection, and (vii) data processing of the electropherogram/chromatogram (Fig. 1). The full specification is provided in Table 2 and the most recent version can be obtained from the MIRAGE website (https://www.beilstein-institut.de/en/projects/mirage/guidelines/#ce_analysis).

Three example data sets are included to illustrate how to report on a CE experiment, which is in compliance with the guidelines (Supplementary Information). The first dataset describes the analysis of fingerprinting the *N*-glycome of human plasma using xCGE-LIF (Hennig et al. 2016). For this purpose, the released *N*-glycans were labeled with APTS. Supplementary Fig. S1 illustrates an example of an aligned

**Table 1.** Different modes of operation in the field of CE.

| Capillary mode | Description |
|---|---|
| cITP | Analytes separate in distinct zones. For this purpose, the sample is injected between two different buffers. The first buffer has the highest mobility of all analytes present during the analysis, also known as the leading electrolyte. The second buffer, following after the sample (or positioned at the outlet), has the slowest mobility, also known as the terminating electrolyte.<br>Key parameters: Composition and pH of the leading and terminating electrolyte |
| cIEF | Separation based upon differences in isoelectric point (pI). The analyte will migrate as long if it is charged.<br>Key parameters: Concentration of anolyte (acidic) and catholyte (basic), presence of ampholytes in the sample and the generation of the pH gradient. Addition of pI markers to determine the pI of the anolyte |
| MEKC | Separation based upon hydrophobicity, analytes separated due to differential partitioning between micelles (pseudostationary phase) and the aqueous buffer solution. With this technique, neutral analytes can be separated.<br>Key parameters: pH, surfactant concentration, additives, polymer coating, robust, and controllable EOF |
| CZE | Most commonly used technique for CE–MS. Separation is based upon the ratio of the charge of the analyte to its mass. Analytes will separate in distinct zones.<br>Key parameters: Constant field strength, pH of the BGE as well as homogeneity. |
| CEC | Separation determined by the migration and adsorption effects of the stationary phase and electroosmotic flow (EOF) of the mobile phase.<br>Key parameters: Stationary phase and mobile phase. |
| CGE | Separation based upon size, charge and shape of the analyte by moving through a stationary phase gel. The EOF is suppressed by the presence of a capillary wall coating.<br>Key parameters: Constant field strength, pH of the BGE, medium, and gel concentration. Only electrokinetic injection can be utilized. |

Disclaimer: Only the key parameters are provided for each CE separation mechanism.

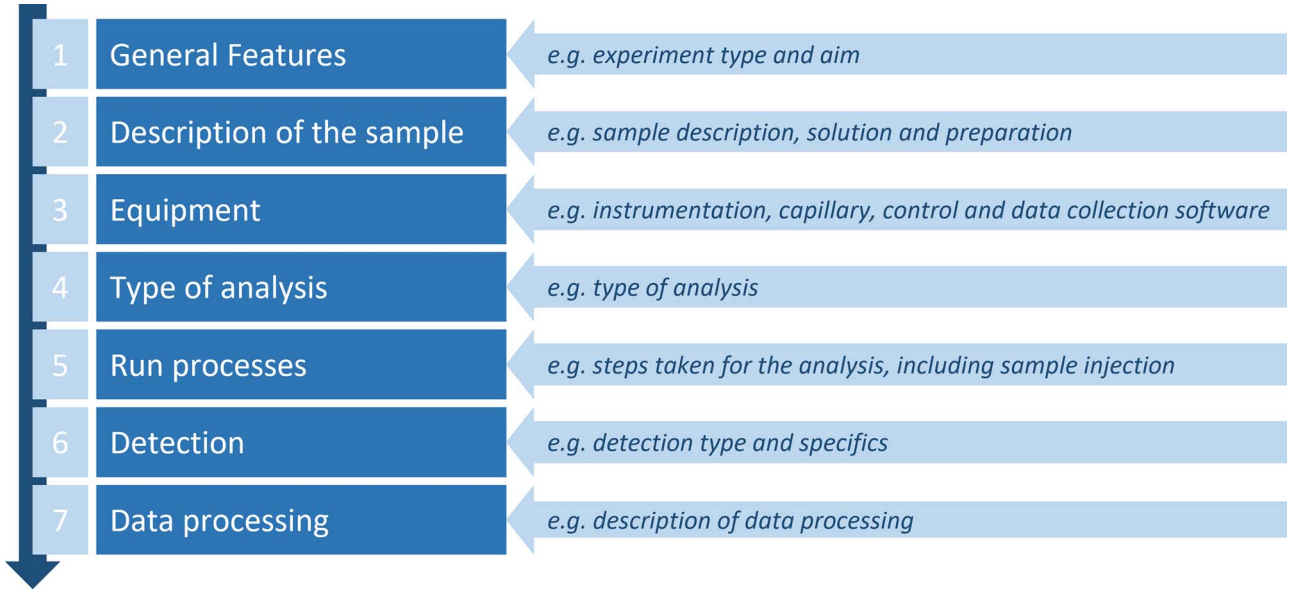


**Fig. 1.** The main sections of the MIRAGE CE guidelines. Section 1 is a general description of the experiment whereas Section 2 provides details regarding the sample that is being analyzed. Sections 3–5 provide insights into the hardware and settings used for the analysis. Sections 6 and 7 focus on the data generation and processing.

(normalized) electropherogram and Supplementary Table S1 provides information about the 31 most abundant peaks and the underlying *N*-glycans. The second example, describes the analysis of PNGaseF released *N*-glycans from pooled human plasma, followed by a linkage-specific derivatization of sialic acids and labeling procedure with Girard's reagent P by sheathless CE-ESI-MS (Lageveen-Kammeijer et al. 2019) and is accompanied by a summary of identified *N*-glycans. Extracted ion electropherograms and a summed MS spectrum are provided alongside with a supporting MS2 peak list (Supplementary Fig. S2 and Supplementary Table S2, respectively). The third example illustrates the assignment of

**Table 2.** MIRAGE guidelines with the CE guideline: MIRAGE sample preparation guidelines (Struwe et al. 2016) and MIRAGE mass spectrometry guidelines (Kolarich et al. 2013).

| Classification | Definition |
|---|---|
| **1. General features** | |
| Date stamp | The date on which the work described was initiated; given in the standard "YYYY-MM-DD" format (with hyphens). |
| Responsible person/role | The (stable) primary contact person for this data set; this could be the experimentalist, lab head, line manager, principal investigator, etc. Where responsibility rests with an institutional role (e.g. one of a number of duty officers) rather than a single person, give the official name of the role rather than any one person. In all cases give affiliation and stable contact information, which consists of (i) Name, (ii) Postal address and (iii) Email address, (iv) ORCID. |
| Experiment type | The CE mode (e.g. CZE, CGE, CEC, MEKC, CIEF, ITP, etc.), preconcentration (t-ITP), stacking. |
| Experiment aim | Glycofingerprinting (pattern comparison), identification (qualitative glycoprofiling), detailed glycan analysis, quantitation (quant. glycoprofiling), etc. |
| **2. Sample** | |
| Sample name(s) and descriptions | Name and concentration of sample(s) (if known) including any label, marker, or tag applied that will be used for detection, such as fluorescent labels (by name only). Identify and give source to possible controls, system suitability standard, sample-related standards/calibrants, $t_{EOF}$-marker, and test samples. If calibrants, state concentrations of materials (see Section 4). |
| Sample solution | The components with concentrations and pH (excluding the sample itself) of the sample solution that is to be injected into the capillary including leading electrolyte (if used) and BGE. Manufacturer, order and lot numbers used. |
| Sample preparation | Any general and specific parameters, settings, conditions important for tracing the sample preparation history as outlined in the MIRAGE sample preparation guidelines (Struwe et al. 2016) (doi: https://www.beilstein-institut.de/en/projects/mirage/guidelines/#sample_preparations). |
| **3. Equipment** | |
| 3.1 Instrumentation and other equipment | |
| Manufacturer, model, catalog number | The name of the manufacturer for a combined unit or component. |
| Instrument details | Type of sample-capillary interface, type of cooling (if any), type of detector(s). |
| 3.2 Control and data collection software | |
| Manufacturer, name, version | Name, version (or release date) and manufacturer of the control and data collection software. User modifications should be detailed. |
| 3.3 Capillary | |
| Capillary manufacture/sources | If the capillary was purchased pre-made (e.g. coated, with window, or pre-cut lengths) then include the model name, catalogue number, manufacturer, and lot number. If the capillary has been manufactured "in house" then supplier of silica capillary, catalogue number and lot number should be given. If using a coated, gel filled, packed, or monolithic capillary, the manufacturer, catalogue number, lot number, type, surface modifications, particle and pore size, and gels should be given as appropriate. Give reference or outline protocols. |
| Capillary setup | Single capillary setup or capillary array setup (e.g. 4, 16, 48 or 96 in parallel). |
| Capillary dimensions | The exact dimensions of the capillary employed: from inlet to detection window (effective length, cm); from inlet to outlet (total length, cm); and the inner and outer diameters of the capillary ($\mu$m). |
| Conditioning of a new/regeneration of an existing capillary | Flushing procedures prior to use; e.g. wash with 1 M NaOH for 30 min followed by water for 10 min then BGE for 30 min at room temperature for conditioning uncoated fused silica capillary or coating procedures, including frequency of refreshing coating for dynamic coatings. If an existing capillary is being regenerated, the capillary history should be considered. |

(*Continued*)

**Table 2.** Continued

| Classification | Definition |
|---|---|
| **4. Type of analysis** | |
| Describe the type and aim of the experiment and the type of glycoanalysis performed, including as applicable separation time and/or peak height/area normalization/calibration, which standards/calibrants were used. | |
| Type of cal./norm. | • No calibration/normalization at all.<br>• Spiking for identification of single/individual peaks/structures.<br>• Internal or external separation time normalization: External: pre- and/or postrun process(es)<br><br>Internal: in-run process<br><br>• Peak height/area calibration for absolute quantification by spiking in of quant. Standards (calibrants) identical to sample components/constituents (in-run process). |
| Analysis level | Name/type of standards/calibrants, if not already given under section 2.<br><br>• Glycofingerprinting: Only pattern comparison, no peak/structure assignment.<br>• Glycoprofiling: Peak/structure assignment by database matching of normalized separation times without supporting exoglycosidase digestions (or complementary MS analysis). Reporting of all (potential) structures co-migrating within a specified separation time range is recommended.<br>• Detailed glycoanalysis: Validation of database matching via exoglycosidase sequencing or by MS; i.e. confirmation of assigned structures by sequential/parallel exoglycosidase digestion and or by complementary mass spectrometry analysis). If using MS refer to the MIRAGE mass spectrometry guidelines (Kolarich et al. 2013) (doi:10.3762/mirage.2). |
| **5. Run Processes** | |
| The protocol for a CE analysis normally follows the order (i) preconditioning prior to the first use of a capillary (various flush steps, designed to clean/activate/coat the inner walls of the capillary), (ii) preconditioning (carried on each analysis); (iii) injection; (iv) separation; and (v) post-conditioning (again, various flush steps). Each of these steps needs to be defined as specified (Sections 5.2, 5.3, and 6, as applicable). There are also parameters that should be specified across the whole run (Section 5.1). Voltages and pressures should be described in terms of polarity (+ or −) and direction, respectively. | |
| 5.1 Run descriptors | |
| Temperature of capillary | Controlled temperature of capillary (if controllable). |
| Auxiliary data channels | Descriptions of the auxiliary channels set-up to monitor current, power, voltage, polarity, and pressure applied and values obtained for all steps. State if this is to be used as indication of system suitability. |
| Duration of data collection | Duration of data collection from detector (see Section 6) and auxiliary data channels (as listed above). |
| 5.2 Step descriptors | |
| Step name | Descriptor for an individual step in the run. This includes: pre-conditioning, flush, wash, injection, stacking, focusing, mobilization and separation steps. (Guidelines for each type of step are not described in separate sections of this document, due to this being method specific). |
| Step conditions | Description of the program used for the capillary separation; e.g. pressure or voltages all given relative to time and vial locations and contents. This information should include voltage mode (positive/negative, step and hold, or gradient) if applicable. The frequency of vial and/or mobile phase/buffer/gel exchange replenishment intervals should also be detailed. |
| Pre-conditioning, flush and wash and BGE/ampholytes solutions | Description of pre-conditioning, flush, wash and BGE / ampholytes solutions in terms of components with concentrations. Any pH adjustments that are made should also be described including the capillary temperature. |
| 5.3 Sample injection | |
| To describe sample injection, provide a complete description in line with section 5.2 (named appropriately), plus the following additional information. N.B. If a sample stacking, electrofocusing experiment has been carried out this must be specified in Section 5.2; Step Name. | |
| Sample name(s) | Reference one of the descriptions given under section 2.<br>Sample volume and concentration in the vial. |
| Temperature of sample storage | Controlled temperature of sample storage (if possible—instrument dependent). |

(*Continued*)

**Table 2.** Continued

| Classification | Definition |
|---|---|
| Injection type | State whether hydrodynamic (applying pressure) or electrokinetic (applying voltage) injection was performed. For electrokinetic injection, no injection volumes can be provided; also for hydrodynamic injections (which are in the low nL range), it is often not possible to state absolute injection volumes, at least injection voltage/pressure and time/duration should be given. |
| Injection geometry | State whether short or long end injection. |
| **6. Detection** | |
| This section documents the process and the methods employed both to allow analytes to be detected. | |
| Type of detection | Used detection method: UV–Vis, DAD, LIF, conductivity, MS, etc. When using MS refer to the MIRAGE mass spectrometry guidelines (Kolarich et al. 2013) (doi:10.3762/mirage.2). |
| Detection specifics | Details of detection wavelengths; reference wavelengths, bandwidth, emission wavelength, and bandwidths of laser if used; data collection rate. When using MS refer to the MIRAGE mass spectrometry guidelines (Kolarich et al. 2013) (doi:10.3762/mirage.2). |
| Detector calibration | Has a detector calibration step been carried out (yes/no), internal or external calibration. If external with which calibrant, what are acceptability criteria? When using MS refer to the MIRAGE mass spectrometry guidelines (Kolarich et al. 2013) (doi:10.3762/mirage.2). |
| **7. Electropherogram/−chromatogram, data processing** | |
| Software | Specify any software used to assist data interpretation (name, version). If open source software is used include web site or download link to enable re-evaluation of data and results. When using MS refer to the MIRAGE mass spectrometry guidelines (Kolarich et al. 2013) (doi:10.3762/mirage.2). |
| Database | Specify database used to assign structure based on standardized migration/retention time index (name, version). |
| Integration protocol | E.g. Gaussian, parabolic interpolation, etc. |
| Integration specifics | Minimum peak width, threshold (or height reject), shoulder sensitivity, minimum area shoulder sensitivity. |
| Migration/retention times | Dependent on whether a stationary phase/pseudo-stationary phase is used or not. |
| Note: For extended glycoprofiling via exoglycosidase sequencing the following should be included. | |
| Supplier | Name of supplier |
| Suppliers Product Code | Suitable catalogue description including enzyme description in accordance with STRENDA (doi:10.3762/strenda.18) |
| Exoglycosidase Preparation | Volume and concentration (also of sample), (any deviation from manufactures recommendations). Expressed in units where one unit is defined as the amount of enzyme required to cleave >95%, activity of the enzyme, enzyme storage buffer, storage conditions |
| Reaction Time | Total reaction time (min) this may reflect expected behavior (partial or complete digestion) |
| Control | Describe any external controls used to monitor enzyme activity, include the data of these controls, provide details if digestion protocols were adjusted on the basis of the controls. |
| Protocol | Link to published/suitable exoglycosidase protocol |

glycopeptides from a tryptic digest of $\alpha$1-acid glycoprotein using CE-MS data (Khatri et al. 2017). Extracted ion electropherograms for the assigned glycopeptides are shown in Supplementary Fig. S3. It should be noted that both CE-MS examples do not include an internal standard such as a neutral marker and is rather common for most CE-MS experiments. This is unfortunate as CE-MS is known for its low robustness in terms of migration time. In order to improve the repeatability of a study as well as to compare results from different laboratories or experiments, it would be recommended to include an internal standard, which will help to determine the electroosmotic mobility and normalize/align the migration times. This guideline, on how to report the minimal information required for glycomic experiments by CE, will be evaluated over time and the community is encouraged to contact the MIRAGE consortium to make the guidelines more comprehensive if it is found that specific information is missing.

## Conclusions

Biomedical scientists who use glycomic, proteomic and/or glycoproteomic measurements will benefit from the usage of these guidelines that define the minimum information

required for reporting a CE-based glycomics experiment. This will facilitate sharing and re-use of data and assigning appropriate credit. It will assist software and tool developers as they provide workflows for processing CE-glycomics data. Eventually, it will enable long-term data stewardship to enable effective data mining.

The MIRAGE-CE guidelines are designed to support biomedical research community efforts to report experimental data with transparency to maximize their impact. These guidelines include metadata identifiers and descriptors, the dissemination and adopting of which will allow the reuse of reported data. These descriptors, including sample preparation, experimental settings, and data analysis workflows, establish the provenance of experimental datasets as essential information.

The availability of these recommended reporting guidelines are supported by journals including *Glycobiology*, *Molecular and Cellular Proteomics*, *Glycoconjugate Journal* to ensure complete and transparent use of glycomics data. We expect additional journals to adopt the guidelines going forward. This will assure more rigorous the management and stewardship of published data collections required for discovery and innovation that exploits glycoscience experiments.

## Supplementary material

Supplementary material is available at Glycobiology Journal online.

## Data availability

The most recent version of the MIRAGE-CE guidelines as well as all other MIRAGE guidelines, can be found on the project website: https://www.beilstein-institut.de/en/projects/mirage/guidelines/#ce_analysis doi:10.3762/mirage.7, registered with FAIRSharing: https://fairsharing.org/bsg-s001623/.

## Acknowledgements

This work was reviewed and edited by the MIRAGE Advisory Board members. The MIRAGE project is funded by the Beilstein-Institut and is acknowledged by all members of the commission.

## Funding

The Beilstein-Institut, Frankfurt am Main, Germany, is the funding body of the MIRAGE initiative.

## Conflict of interest statement

None declared.

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