# MIRAGE Capillary Electrophoresis Guidelines

**Guidelines for reporting capillary electrophoresis experiments**

Version 1.0 from July 9, 2021

doi:10.3762/mirage.7

Authors: Guinevere Lageveen-Kammeijer, Erdmann Rapp, Deborah Chang, Pauline M Rudd, Carsten Kettner, Joe Zaia

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. General features</strong></td>
<td></td>
</tr>
<tr>
<td>Date stamp</td>
<td>The date on which the work described was initiated; given in the standard ‘YYYY-MM-DD’ format (with hyphens).</td>
</tr>
<tr>
<td>Responsible person/role</td>
<td>The (stable) primary contact person for this data set; this could be the experimentalist, lab head, line manager, principal investigator <em>etc.</em> Where responsibility rests with an institutional role (<em>e.g.</em> 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.</td>
</tr>
<tr>
<td>Experiment type</td>
<td>The CE mode <em>e.g.</em> CZE or CGE (or CEC, MEKC, CIEF, ITP, etc.), preconcentration (t-ITP), stacking.</td>
</tr>
<tr>
<td>Experiment aim</td>
<td>Glycofingerprinting (pattern comparison), identification (qualitative glycoprofiling), detailed glycan analysis, quantitation (quant. glycoprofiling), <em>etc.</em></td>
</tr>
<tr>
<td><strong>2. Sample</strong></td>
<td></td>
</tr>
<tr>
<td>Sample name(s) and descriptions</td>
<td>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, TeOF-marker and test samples. If calibrants, state concentrations of materials (see section 4).</td>
</tr>
<tr>
<td>Sample solution</td>
<td>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 background electrolyte. Manufacturer, order and lot numbers used.</td>
</tr>
<tr>
<td>Sample preparation</td>
<td>Any general and specific parameters, settings, conditions important for tracing the sample preparation history as outlined in the MIRAGE Sample Preparation Guidelines (doi:10.3762/mirage.1).</td>
</tr>
</tbody>
</table>
## Equipment

### 3.1 Instrumentation and other equipment

<table>
<thead>
<tr>
<th>Manufacturer, model, catalog number</th>
<th>The name of the manufacturer for a combined unit or component.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument details</td>
<td>Type of sample-capillary interface, type of cooling (if any), type of detector(s).</td>
</tr>
</tbody>
</table>

### 3.2 Control and data collection software

<table>
<thead>
<tr>
<th>Manufacturer, name, version</th>
<th>Name, version (or release date) and manufacturer of the control and data collection software. User modifications should be detailed.</th>
</tr>
</thead>
</table>

### 3.3 Capillary

<table>
<thead>
<tr>
<th>Capillary manufacture/sources</th>
<th>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, gels should be given as appropriate. Give reference or outline protocols.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary setup</td>
<td>Single capillary setup or capillary array setup (e.g. 4, 16, 48 or 96 in parallel).</td>
</tr>
<tr>
<td>Capillary dimensions</td>
<td>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 (µm).</td>
</tr>
<tr>
<td>Conditioning of a new /regeneration of an existing capillary</td>
<td>Flushing procedures prior to use. E.g. wash with 1M NaOH for 30 minutes followed by water for 10 minutes then BGE for 30 minutes 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.</td>
</tr>
</tbody>
</table>
## 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.

<table>
<thead>
<tr>
<th>Type of cal./norm.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No calibration/normalization at all.</td>
<td></td>
</tr>
<tr>
<td>Spiking for identification of single/individual peaks/structures.</td>
<td></td>
</tr>
</tbody>
</table>
| Internal or external separation time normalization:  
  - External: pre- and/or post-run process(es)  
  - Internal: in-run process |
| Peak height/area calibration for absolute quantification by spiking in of quant. standards (calibrants) identical to sample components/constituents (in-run process). |

Name/type of standards/calibrants, if not already given under 2.

<table>
<thead>
<tr>
<th>Analysis level</th>
<th>Description</th>
</tr>
</thead>
</table>
| Glycofingerprinting:  
  Only pattern comparison, no peak/structure assignment. |
| 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. |
| 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 (doi:10.3762/mirage.2). |
5. Run Processes

The protocol for a run 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; (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

<table>
<thead>
<tr>
<th>Temperature of capillary</th>
<th>Controlled temperature of capillary (if controllable).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auxiliary data channels</td>
<td>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.</td>
</tr>
<tr>
<td>Duration of data collection</td>
<td>Duration of data collection from detector (see Section 6) and auxiliary data channels (as listed above).</td>
</tr>
</tbody>
</table>

5.2 Step descriptors

<table>
<thead>
<tr>
<th>Step name</th>
<th>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).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step conditions</td>
<td>Description of the program used for the capillary separation; <em>e.g.</em> 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.</td>
</tr>
<tr>
<td>Pre-conditioning, flush and wash and background electrolyte/ampholytes solutions</td>
<td>Description of pre-conditioning, flush, wash and background electrolyte / ampholytes solutions in terms of components with concentrations. pH adjustments made should also be described including temperature.</td>
</tr>
</tbody>
</table>

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. Sample volume and concentration in the vial. |
### Temperature of sample storage
Controlled temperature of sample storage (if possible—instrument dependent).

### Injection type
State whether hydrodynamic (applying pressure) or electrokinetic (applying voltage) injection was performed. As for electrokinetic injection no injection volume can be given, but 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.

<table>
<thead>
<tr>
<th>Type of detection</th>
<th>Used detection method: UV-Vis, DAD, LIF, conductivity, MS, etc. (when using MS refer to the MIRAGE Mass Spectrometry Guidelines, doi:10.3762/mirage.2).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection specifics</td>
<td>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, doi:10.3762/mirage.2.</td>
</tr>
<tr>
<td>Detector calibration</td>
<td>Has a detector calibration step been carried out (yes/no), internal or external calibration. If external with which calibrant, what are acceptability criteria? For MS refer to MIRAGE Mass Spectrometry Guidelines, doi:10.3762/mirage.2.</td>
</tr>
</tbody>
</table>

### 7. Electropherogram/chromatogram, data processing.

<table>
<thead>
<tr>
<th>Software</th>
<th>Specify any software used to assist data interpretation (name, version). If open source software is used include website or download link to enable re-evaluation of data and results. When using MS refer to the MIRAGE Mass Spectrometry Guidelines, doi:10.3762/mirage.2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Database</td>
<td>Specify database used to assign structure based on standardized migration/retention time index (name, version).</td>
</tr>
<tr>
<td>Integration protocol</td>
<td>E.g. Gaussian, parabolic interpolation, etc.</td>
</tr>
<tr>
<td>Integration specifics</td>
<td>Minimum peak width, threshold (or height reject), shoulder sensitivity, minimum area shoulder sensitivity.</td>
</tr>
<tr>
<td>Migration/retention times</td>
<td>Dependent on whether a stationary phase/pseudo-stationary phase is used or not.</td>
</tr>
</tbody>
</table>
Note

For extended glycoprofiling via exoglycosidase sequencing the following should be included:

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Name of supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppliers Product Code</td>
<td>Suitable catalogue description including enzyme description in accordance with <a href="#">STRENGA</a></td>
</tr>
<tr>
<td>Exoglycosidase Preparation</td>
<td>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 &gt; 95%, activity of the enzyme, enzyme storage buffer, storage conditions</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>Total reaction time (mins) this may reflect expected behaviour (partial or complete digestion)</td>
</tr>
<tr>
<td>Control</td>
<td>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.</td>
</tr>
<tr>
<td>Protocol</td>
<td>Link to published/suitable exoglycosidase protocol</td>
</tr>
</tbody>
</table>