Table 13: System Suitability Strategies for Mass Spectrometers and LC/MS Methods

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**Scope:**
System suitability testing is an integral part of many analytical procedures including mass spectrometry analysis. Lack of evaluating LC/MS systems prior to analyzing samples may generate data with poor quality, which could potentially mislead data interpretation and possibly cause extensive investigations and delays in the development timelines for biotherapeutic products. This roundtable aims to discuss different aspects of system suitability in LC/MS methods as applied to in-depth characterization of biotherapeutic products. Topics include approaches and strategies for determining system suitability samples for different LC/MS methods, confirming instrument performance, and ensuring reproducible and accurate results for regulatory submission. System suitability for MS in QC (i.e., multi-attribute method) will also be discussed.

**Questions for Discussion:**

1. Do teams/organizations perform LC and MS system check prior to experiments (e.g., tune/calibrate MS for specific assays)?
2. What are the choices of system suitability samples for different LC-MS characterization assays? Should the system suitability samples reflect sample preparation process, instrument performance for particular molecules, or both?
   a. External standards (e.g., NIST mAb)
   b. Prepared standards (protein digest standards)
   c. Internal standards (reference material)
   d. System suitability samples for LC vs. LC-MS
3. What are considerations for specific methods?
   a. Intact and subunit analysis
   b. Peptide mapping
      1) Characterization peptide mapping
      2) Host cell protein analysis
      3) Sequence variant analysis
   c. Released glycan characterization
4. System suitability samples for MS in QC (i.e., Multi-attribute method, MAM)
5. Acceptance criteria for system suitability samples for different assays?
Discussion Notes:

1. Do teams/organizations perform LC and MS system checks prior to experiments (e.g. tune/calibrate MS for specific assays)?
   a. About 50% of participants indicated that they perform system checks prior to experiments
   b. How much system suitability (SS) do you need to do for early development studies vs characterization (e.g. for the BLA)?
      i. Most companies seemed to agree that some form of SS needs to be done even at early stages of development. How much SS remained unanswered.
   c. How do you check whether the instrument, digestion solutions, etc. are working?
      i. See examples under peptide mapping (response to Question 3)

2. What are the choices of system suitability samples for different LC-MS characterization assays? Should the system suitability samples reflect sample preparation process, instrument performance for particular molecules or both?
   a. External standards, prepared standards, internal standards (reference standards) are all used. Examples of external standards include NISTMab and Pierce 15-mer peptide retention time mix
   b. SS samples should reflect the sample preparation process and instrument performance for particular molecules. Examples:
      i. For one company, SS samples for peptide mapping include a frozen Mab digest, Mab from the current process, and a reference standard
      ii. For one company, the SS samples for oligo analysis include a blank and an oligo mixture of different length oligos with known masses bracketing the test sample. Oligo standards used in non-GMP studies are made in house. For GMP studies, Oligos from a CMO that conform to GMPs are used
   c. Participants noted that it’s hard to tell whether the problem is from the instrument or the SS sample used. Therefore, qualification of CMO/Vendors for external standards is important.

3. What are considerations for specific methods?
   a. Intact and subunit analysis
      i. SS is least restricted for Intact mass analysis
      ii. Predigested sample are used both as a SS and calibrant for subunit analysis
   b. Peptide mapping
      i. Examples of SS samples for peptide mapping
         1. Combination of Frozen Mab digest, Mab from the current process, and reference standard
         2. Pierce 15-mer peptide retention time mix
      ii. Many participants agreed on the importance of running a blank between SS samples to reduce carryover. One company has seen ~1% carry over for hydrophobic peptides. Number of blanks between samples depends on the samples one is looking at e.g. really small quantities of HCP
      iii. Sample stability:
1. Frozen sample degradation over time is an issue to consider.
2. Sample stability during runs is another issue to consider. For Example, autosampler stability when you have too many samples.
   a. Some companies run SS at the beginning and end of each campaign and sometimes between test samples to check stability during runs
   b. Unexpected deamidation or oxidation from the instrument: One option is by comparing to a standard you know
   c. How about oxidation & deamidation in the standard? Based on data collected previously to make sure it can be compared. Another option is to run the same reference material in the beginning and end of the queue
iv. How do you evaluate whether the digest solution is working?
   1. Look at missed cleavages in the sample
   2. Look at the profile of a known protein digest
v. How do you evaluate whether the instrument is working?
   1. Spike test sample with a peptide standard and check intensity of instrument and ability to see low peaks and missed cleavages
   2. Some use a digested BSA standard (1%)
   3. Some use a digested Internal QC sample
   4. All is done by data analysis e.g. using mascot search
vi. SS for MS in QC (i.e., MAM)
   1. Unfortunately, not a lot of companies have MS in QC at this point. Although there is a consensus that SS is extremely important for applications like MAM. Both external and internal standards are necessary to ensure LC-MS application
   c. Released glycan characterization
      i. See example above of SS samples used for oligo characterization
4. Acceptance criteria for system suitability samples for different assays
   a. Retention time, Intensity, Resolution are important criteria to assess SS for both LC and MS
   b. Important to distinguish what SS means for each part of the assay e.g. Resolution for MS is to confirm accuracy of masses and not coelution of the peptides
   c. SS criteria are typically the same for both internal and external standards and require finding a defined number of peptides. Easy for commercial standards. For internal standards, look for select peptides grouped into easy, medium and hard to find peptides. Confirm by data analysis e.g. mascot search
   d. One company uses historical comparison for intensity and mass accuracy for glycan analysis.
   e. Is Mass Spec linear? EIC and peak area might be better for addressing linearity issues compared to peak intensity, depending on the nature of the target (peptides vs. oligos)