Table 9: What is the State of the Art Regarding Methods for Analysis of Glycans and Glycoproteins?

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Scope:
Even subtle changes in bioprocessing conditions can alter the cellular synthesis of glycans and as a consequence, the physicochemical properties, safety, efficacy, and immunogenicity of the glycoprotein product. For the demonstration of glycosylation consistency, robust, information-rich, and reproducible methods for glycan analysis are required for regulatory filings of glycoprotein-based biotherapeutics. However, there are several challenges associated with the analysis of glycans and glycoproteins. For example, the complex nature of glycans and associated methodologies makes it not always possible to directly compare results between laboratories. This roundtable aims to discuss the applications, challenges and recent advances in glycan/glycoprotein analysis.

Questions for Discussion:
1. What are the traditional and novel applications commonly used for the analysis of glycans and glycoproteins?
2. What are the challenges of glycan and glycoprotein analysis and how to overcome those?
3. What are the recent advances in glycan and glycoprotein analysis in terms of separation techniques, instrumentation and data analysis?
4. What do we need to improve performance of glycan and glycoprotein analysis with respect to reproducibility, accuracy, and sensitivity?

Discussion Notes:

- Traditional ways of analysing glycans are based on released and fluorescently labelled glycans. This approach, however, has some limitations in terms of unknown occupancy and location of glycans, which is important in more complex glycoproteins. One of disadvantages pointed out was that contaminants of glycans released from low abundant proteins, such as HCPs can interfere with the assay results.
- Despite the released and fluorescently labelled glycans analysis is known to be a standard and widely performed approach it was thought that analysis of glycol-peptides using Mass Spectrometry can provide more insightful information, including location and structure of glycans
- APTS or hydrazine derivatisation was used instead of 2AB to enhance analysis by Mass Spec and support more detailed structural characterisation
- Analysis of intact proteins is also popular, especially in proteins with simpler glycosylation pattern
- It was concluded that for high throughput or early stage projects detection sensitivity is not an issue and typically intact or reduced mass analysis of proteins should suffice, however, for late stage projects more sensitive and quantitative approaches are recommended.
- Although LC separations seem to be standard, CE separations are recommended for high throughput and increased sensitivity
- The biggest challenge identified in glycan analysis was related to poor software perfomance, inconsistent annotation and need for manual checks which slows down the analysis.
- New data libraries would be highly welcomed to help the end user with data analysis