**Table 6: Beyond the mAb Series – Fusion Proteins, Bispecifics, and Peptides Applications**

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**Scope:**

During the past 2 decades, our industry invested heavily in developing and engineering novel and safe molecules such as fusion, bispecific and peptide biotherapeutics. However, with every new modality, the complexity of these molecules may pose a considerable challenge from an analytical standpoint. This roundtable aims to discuss the application challenges and identify potential solutions for improved characterization. In some cases, this may require developing new instrumentation/software or a new chemistry solution to allow scientists to confidently characterize existing and new molecule types in the ever-growing pipeline diversity.

**Questions for Discussion:**

1. How do you tackle the analytical characterization of a new modality?
2. What gaps exist around new modality analysis (vaccines, Gene Therapy, CAR-T, etc)? Can we apply existing CE solutions, or is new development required?
3. What gaps exist around non-therapeutic material encountered during development (polysorbate, cyclodextrins, etc)? Can we apply existing CE solutions or is new development required?
4. If you encounter a roadblock, for example, no adequate solution from a vendor (instrument, chemistry kit, software). How do you solve the problem?

**Discussion Notes:**

With new modalities development and characterization come different types of challenges. Below are the general thoughts around the table.

Challenges related to new molecule characterization:

- New kinds of fragmentation and stability
- Early molecule assessment groups will perform initial assessments using platform methods before hand-off. This helps understand method suitability and new CQAs that may be of concern.
- Standard methods generally work well and should be tried first. If a problem arises it is helpful to work closely with other groups (LC/MS teams) for comprehensive characterization work.

- Being diligent during developability is critical to determine whether platform methods are OK or need adjustments, i.e. drug substance stability
- CQA upstream transfer to downstream to characterize in parallel with the early stage to be ahead specifically if considering multi-specific antibodies

Challenges related to size and HMW aggregates:

- CQA characterization of aggregates (homodimers vs safety); hetero-aggregates vs safety
- /efficacy
- Size exclusion or other analytical techniques needed for orthogonality to CE
- In bispecific mAbs, the LC's are similar in MW. How to resolve these LC's to baseline? Options to address this issue would be different gel formulations that allow for the separation between these two closely related species.
- If SDS-Page has different formulations that can do S=S separations. Why can't CE address this with new gel chemistry?

Charge Heterogeneity:

- Denaturation of sample is relevant if interaction with antigen is native. The concern is if
- denaturing the sample changes the charge profile compared to non-denaturing conditions.
- It is important to understand what is changing. For example: if an aggregate species runs with the main peak, then a different mechanism of separation is needed to understand and quantify.
- Bispecific LOD/LOQ of the LC's and HC's must be corrected to the Ɛ coefficient as it may have 1 – 2 % difference
- To simplify charge het analysis of complex modalities would be to de-glycosylate, treat with sialidase and remove phosphorus species. However, this approach is not adequate to QC due to complex sample preparation.
- Charge het and sub-unit analysis are methods that everyone in development and QC can benefit from. Critical to characterize the charge profile of the Fc region as it has Important biological implications. However, it is not a holistic approach but is still relevant.
- Analytical control feedback to engineering designs to construct something that can be developed and manufacturable for selection. Bispecific molecules may be easier because
- engineering design groups can make the standard so the analytical group can characterize and improve the method.
- Fusion proteins may need to undergo reduction to simplify their notorious complex profiles.
- Two-dimensional CE/MS is very attractive for this challenge, where 1D would be CZE and 2D would be peptide mapping.