Table 6: Identifying New Attributes of Drugs Using Capillary Electrophoresis

Session 1:

Facilitator: Richard Rustandi, *Merck & Co., Inc.*

Scribe: Colleen Reed, *Pfizer, Inc.*

Session 2:

Facilitator: Eoin Cosgrave

Scribe: Emmanuel Kizekai, *Amgen, Inc.*

Scope:

The purpose of this roundtable is to explore how CE technology can be applied to identifying attributes of protein therapeutics. The discoveries of new modalities such as bispecifics, BiTEs, ADCs, masked antibodies and others have been rapidly advancing due to better understanding of biological systems and diseases progressions as well as innovations in protein and bioconjugate engineering.

Understanding these new modalities' critical quality attributes (CQAs) such as charge heterogeneity, clippings, etc., and their relationship to process parameters are an expectation in regulatory filings for product characterization. Many therapies exhibit high potency and are subsequently formulated at low protein concentration, which in turn introduces challenges for analysis. Other therapies with novel formats (masked antibodies, bispecifics, fusion proteins, etc) present challenges due to the absence of experience with methods that can be used to monitor their CQAs. CE presents unique advantage to analyze these molecules as the methods require small sample volume and protein load and deliver high resolution separation between species. However, there still exist challenges with some of these molecules that impede CE from being used as an orthogonal method for attributes identity.

Questions for Discussion:

1. What are some of the new modalities that are being work on at your company?
2. What are the types of attributes organizations are experiencing that are causing analytical headaches?
3. Are CE methods used for charged heterogeneity?
4. Are there examples of CE-based methods that have been used to identify new attributes?
5. Do low protein concentration and small volume present an issue for analyzing fractionated species?
6. What other CE based technologies are you looking into?
7. Is CE-MS a reliable technology and can it be applied to the newer modalities?

Discussion Notes:

Session 1:

What are challenges with bi-specific molecules?

- Overcome low concentrations (0.5 – 1 mg/mL)
  - Sensitivity issue
- Goal – develop methods to assess CQA's
  - CE-SDS with minimal optimization
    - Suggest: Make antibody against bispecific, and run on CE and Western Blott
    - Should be able to make antibody in ~3 months
    - Label the antibody for separation

- AAV
  - SDS-PAGE to CE-SDS

        - Goal - Assess Impurity level and ratios of VP1/VP2/VP3 (process consistency)
        - Challenge protein level, low titer
        - UV or LIF depending on titer (challenges with labeling)
    - Methods for Empty/Full ratio
        - Currently in industry some Ion Exchange- LC
        - iCIEF
            - pI difference really small
            - Can we use reducing, urea?
        - CZE
            - Density difference
            - Use stacking
            - Not really explored
        - Intermediates need to be taken into consideration
    - Method for total content
            - Adeno Virus quant by CZE with 1 or 2 point reference
    - HCP
        - Current ELISA
        - New going to Simple Western
    - Post-translational modifications
        - Structure function studies needed
        - Unsure which are CQA's
    - MicroChip used for quick screening cell lines
    - Alkaline Gel on CE
        - DNA integrity
        - Move by transgene configuration
        - Replace gel ID method for QCR
    - Capsid ID?
    - Glycosylation?
    - Aggregation – may be separated by CZE
    - Consider that a sample centration step may be required and another method must be used to check for impurity loss
- Oncolytic Virus
    - Very low concentrating, 0.2 ug/mL
    - Using Silver stain gel currently