Table 2: Charge Variants Analysis (cIEF/icIEF, IEC, CZE)

SESSION 1:
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SCOPE:
Within pharmaceutical industry, the charge variant analysis of biomolecules is very important for the characterization of new molecules, process control and for release test. The common techniques currently used for charge heterogeneity testing are ionic exchange chromatography (IEC), capillary isoelectric focusing (cIEF), image capillary electrophoresis (icIEF) and more recently CZE.
This table will discuss about the technologies available on the shelf and of the main challenges for implementing chare variants analysis.

QUESTIONS FOR DISCUSSION:
1. What is the Table's experience with development of these methods?
2. Which kinds of samples are analyzed?
3. Is there a versatile/polyvalent method compared to the others? What are their assets/drawbacks?
4. Are there some tricky points to check before implementing these analyses?

DISCUSSION NOTES:
Ionic exchange chromatography (IEC) or cIEF: There are no "rules" to say which technology is better than the other for my analysis. Both can be used. But sometimes it could be difficult to compare the patterns as some peak shifting can be observed. The main advantage of IEC (and traditional IEF) is the possibility to collect each peak after analysis. Then each one can be injected in mass spectrometry for identification. This methodology could be preferred to a direct coupling cIEF-MS; this coupling being not straightforward.

CZE (method of He and al.) can be used for mAbs with pI $\geq$ 7, for proteins at lower pIs, some method development is needed. CZE seems to be more flexible compared to the other technologies: no need of cartridge "dedicated" like for ICE analysis and the sample preparation is easier than for cIEF and imaging capillary electrophoresis. However, some issues related to batch to batch quality of EACA were raised. CZE method is used preferably for hydrophobic molecules comparing to ion exchange chromatography methods.

The importance of screening charge variants at discovery level in order to establish a corridor for monitoring was discussed. Biologics are inherently heterogeneous and complex profiles are expected, hence it is important that the charge fingerprint is consistent from batch to batch.

Imagery capillary electrophoresis is faster than the other methods, although CZE can be fast as well. Actually the sample preparation for the CZE is quite easy compared to the cIEF or imaging capillary electrophoresis.
IEC on ADC sample is very complex; some very broad peaks are obtained.

Some tips to implement these technologies:

- These methods are running with buffers that contain high concentrations of salts, urea and/or gel, so you have to pay attention to clean the devices.
- For cIEF or imagery capillary electrophoresis you need to desalt your sample before the analysis and "mix" it (strongly) before the injection due the viscosity of the mixture (ampholytes…). Due to the viscosity you have to take care to "pipette" some accurate volumes.
- The salt concentration of sample has low impact in CZE and even less in IEC.
- The quality of ampholytes is crucial and some variability can be observed lot to lot. Same observation for the columns used in IEC.
- For IEC, several people preferred pH gradients over salt gradients.
- Some variability in the quality of aminocaproic acid (for CZE) could be observed lot to lot (see also CEPharm 2017 troubleshooting session).