Table 4: New Developments in Analytical Methods for Novel Modalities and PAT

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Scope and Questions for Discussion:

Recent years the interest for developments of analytical methods for novel modalities and as PAT have increased. Analytical methods that are capable of measuring physical and chemical process parameters and characterize novel antibody modalities or products are in focus. We will discuss how to develop new techniques and some of the challenges one can encounter during method development.

Discussion Notes:

Challenge: Protein aggregation (is not totally assessed/understand yet)

- Is right not addressed with native SEC-MS (Aggregates may not elute from column)
- ZipChip might be an option to overcome this (elution problem), also a very fast technology (magic technology)
- MS is not the easiest technology – depended on type of MS/interface chosen

Challenge: HCP analysis

- HCP analysis needs also orthogonal method next to ELISA, to make sure if the amounts are correct
- Nowadays mass spectrometry is also as fast as ELISA, so totally comparable, however sometimes HCPs are missed with the ELISA assay
- Validation of LC-MS of HCP quantification was already performed
- Providers for reagents/kits need to provide consistent materials over a long range

Challenge: Method transfer

- Training of lab employees (need to be demonstrated to authorities)
- Trending as a short term indicator to see if something goes wrong (automated trending strategies

Challenge: Novel modalities

- Antisense oligonucleotides – not clear to which extend they need to be characterized
- Which combination of methods should be chosen
- Molecular biology is necessary to be involved in analytics more then compared to antibody characterization
- Antisense oligonucleotides seem to be much more heterogeneity compared mAbs

Challenge: AAV

- Viral protein purity as well as DNA purity\identity
- Transfection\transduction efficiency
- Characterization and especially purification of AAVs (90% during production are empty) – so far solved with ultracentrifugation (high amount of materials are necessary
- Empty vs full: new automated western blot platform – high throughput, very hands-off – very good for quantification
- Transmission electron microscopy hard to implement in industry/GMP environment (manually counting) – new software necessary (but problems with partially fille
- CE-SDS for viral protein purity/content - 3 proteins 10:1:1 ratio (batch depended), also some ribosome skipping of VP3
- DNA methylation is also of importance, as well as truncations of DNA (ITRs) – truncations are detected in CGE as a broad peak

Challenge: Fusion-proteins

- Need for QC friendly methods
- Peptide-FC fusion proteins is hard to characterize with MS orthogonal methods (because they are in between mAbs and peptide characterization)
- Possibility to cleave peptide from Fc part and analyze it apart (smaller changes can be detected on peptide level)
- IdeS for cleavage of mAb fusion proteins (depending on the sequence, sometimes not possible to cleave for the IdeS enzyme), however sometimes miss cleavages
- Differences of IdeS between different vendors, sometimes only possible to use for research
- Alternatives to IdeS for cleavage show a higher level of miss-cleavages

Challenge: capturing of Fab portions

- Protein A is not working due to missing Fc portion
- Cappa select is a possibility to overcome this