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Evaluation of product antibody (mAb) heterogeneity in non-clonal cell pools for early pre-clinical development

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Abstract: During early stage of pre-clinical biologics development, grams of product antibody is needed for process development, formulation development, and analytical assay development. To accelerate the pre-clinical timeline, it's a common practice to use master wells (non-clonal stable cell pools) to generate development material. The advantage of using master wells is that the product antibody is generated from the same host cell line and expression vector in an earlier and shorter time. Thus, the purified product antibody can be representative to the final therapeutic product. However, the non-clonal nature of the cell pools can give rise to potential risk of heterogeneity in product quality exhibited in charge variants, glycan profile, etc. Herein, we present a case study on the evaluation of charge variant heterogeneity, its root cause, and impact. The increase fraction of acidic variants was first discovered in the in-process analytics by isoelectric focusing (iCIEF). Tryptic peptide mapping LC-MS analysis of purified drug substance further indicated an exchange of Lysine to Asparagine in the Fc region. Subsequent cDNA analysis of the single-cell clones from a master well that produced the purified product revealed a single substitution mutation that results in the amino acid substitution. Although the material used for development was a mixture of antibody product, process development, formulation development, and analytical development were not impacted. The risk of using mutated, potential non-representative variant was further mitigated by bridging studies, confirming product produced by single-cell clones. This case is a demonstration of a worst case scenario, in which a larger percentage (about 40%) heterogeneity was introduced via a point mutation at the DNA level. Nevertheless, overall time line for this program was not affected; thus the time saving benefits of this strategy outweigh the disadvantages and supports the use of non-clonal cell pool in the fast paced early stage development space.