SMALL SCALE ASSAYS FOR PREDICITING ANTIBODY REDUCTION SUSCEPTIBILITY

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Antibody reduction is an active area of study in the cell culture process development field. Antibody reduction refers to the process by which the disulfide bonds in monoclonal antibodies are broken in a reducing environment, leading to the degradation of monomers into inactive byproducts such as half antibodies and individual heavy chains and/or light chains. This reduction can occur at multiple stages in a process, including the production bioreactor, clarification, harvest hold, and capture chromatography. While antibody reduction is a risk for all monoclonal antibody processes, it has been shown that different antibodies have vastly differing susceptibilities to reduction due to the number and structure of disulfide bonds. These susceptibilities can be broadly characterized by antibody subclass and by light chain subtype, although differences in reduction susceptibility are also present within these broad classes. In this study we demonstrate the development and use of a small-scale cell culture assay to predict the risk of reduction for different antibodies and the impacts of changing process parameters on reduction susceptibility.

Initially, a chemical reduction assay was used to assess susceptibility of different antibodies to reduction. This assay, while useful for predicting the risk level for different antibodies for reduction, cannot be used to assess different culture conditions as it only takes purified antibodies and subjects them to a chemical reducing reagent. This is significantly different to the complex environment present in a cell culture process, in which reduction is typically driven by enzymatic pathways. Further development was done to create a small-scale assay using cell culture fluid to confirm the findings of the chemical assay in a more relevant environment and to assess the effects of cell culture conditions on reduction susceptibility. An assay was developed in which cell culture supernatant was mixed with clarified cell lysate at different ratios in small, sealed vessels. In this way, the reducing environment of the bioreactor, clarification and harvest hold operations could easily be simulated at bench scale. This offers an improvement over chemical reduction assays, as it allows reduction susceptibility to be studied under different process conditions. Parameters such as percent lysis, temperature, and dissolved oxygen can be varied to assess the impact on antibody reduction. We demonstrate with this cell culture assay not just an ability to predict reduction risk based on the antibody but also an ability to assess the impacts of different process parameters on that risk. We also demonstrate the correlation of these parameters to changing oxidation reduction potential (ORP), which can act as a tool to predict the risk of reduction. This assay will be further used to study reduction mechanics and prevention techniques to assist process development and scale up.