Calibration of HCP Assays

The absolute quantitation of HCP assays is exceedingly difficult for a number of reasons. While ELISA is inherently a quantitative method when applied to a single analyte, ELISAs that attempt to measure simultaneously all of the hundreds of potential HCP contaminants in the same well using a single reporter/detector system will at best be semi-quantitative. Many arbitrary choices and assumptions go into the making of anti-HCP antibodies and later the choice of material to use as "standards."

How to obtain the HCP material for immunogen is the first choice that must be made. Do we use a null cell line or a mock transfected cell line? Or one with the actual product plasmid and what differences in HCP might we see from each? At what point in the purification process do we obtain the HCPs for immunogen or standards? What if the array and relative concentrations of HCPs in final product are different from the HCP in the ELISA standards? How does one assign a total HCP concentration to a mixture of many proteins with different molecular weights? What effect will the different affinities of antibodies to various HCPs as well as the different concentrations of those antibodies have on the ability of the assay to be quantitative? With only Western blot as a relatively insensitive method to detect individual proteins how do we know that we have antibody to all the HCPs present in a given sample type?

These questions are in essence the limitations of ELISA for HCP analysis. But before we "throw out the baby with the bath water" we should recognize that despite those many arbitrary choices and limitations, ELISA remains a very valuable and the most sensitive method at our disposal to detect HCP contamination. As long as we understand the assay's limitations and acknowledge the arbitrary decisions made in its development it will provide us with very valuable information for process development, process control, and final product lot to lot quality control.

Due to the impracticality of obtaining real final product HCPs that co-purify with product down to the final step, most HCP assays will utilize a source of HCPs from upstream in the purification process. Those HCPs typically will not come from the actual product cell line but rather a null cell or mock transfected cell line. Our initial approach to calibrate this material is to simply perform a BCA total protein assay using bovine serum albumin (BSA) as an arbitrary standard. When standards made with the HCP material calibrated by BCA give reasonable "stoichiometric agreement" with the amounts of antibody used in the ELISA then we feel the calibration by BCA is good enough. What is meant by "stoichiometric agreement" is that we know how much antibody is used in the ELISA. It is the quantity of antibody that actually dictates the analytical range and dose response curve of the ELISA. If we assume an average molecular weight for the total HCPs then we can reasonably estimate HCP concentrations across the valid

analytical range of the assay. When the BCA assay concentrations do not reasonably approximate the ELISA stoichiometry, we process the HCP material further. Such processing involves various purification steps to remove components registering in the BCA assay but that are in fact not HCP or at least not immunoreactive HCPs. This processing might involve simple diafiltration or ultimately affinity purification against the anti-HCP antibody. The purification stops as soon as the BCA concentration gives a realistic stoichiometric agreement in the ELISA.