

## Hook Effect: What is “High Dose Hook Effect”?

For any ELISA to give accurate results there must be an excess of antibodies, both capture and enzyme conjugated, relative to the analyte being detected. It is only under the conditions of antibody excess that the dose response curve is positively sloped and the assay provides accurate quantitation. As the concentration of analyte begins to exceed the amount of antibody the dose response curve will flatten (plateau) and with further increase may paradoxically become negatively sloped in a phenomenon termed “High Dose Hook Effect”. Because the possibility exists that some samples may have analyte concentrations in excess of the antibody it is necessary to validate all sample types by dilutional linearity analysis to establish if they are on the valid, positively sloped region of the curve or on the negatively sloped hook region of the curve. **Failure to validate the potential for Hook Effect can result in severe under-estimation of true contaminant concentrations!**

The issue of hook effect in multiple antigen assays such as HCP ELISA can be more complex. The dose response curve for an HCP assay should be thought of as the cumulative dose responses of all HCPs individually with each HCP having its own hook region determined by the concentration of antibody to that particular HCP. We are practically and fundamentally limited in the amount of antibody that can be used in an HCP ELISA. It is not uncommon in HCP assays for some samples to have certain HCPs in concentrations exceeding the amount of antibody for that particular HCP. In such cases the absorbance of the undiluted sample may be lower than the highest standard in the kit however these samples will fail to show acceptable dilutional recovery/linearity as evidenced by an apparent and significant increase in HCP concentration with increasing dilution. This lack of dilutional linearity is actually the result of the hook effect for the subset of analytes in excess over their respective antibodies. Poor dilutional linearity (Hook Effect) is most likely to be encountered in samples early in the purification process. However if the purification process is selective for certain HCPs, it may also be seen in downstream and final product samples. Thus the establishment of dilutional linearity is a most critical experiment in the development and validation of HCP assays. Dilutional linearity studies are performed at a series of dilutions to establish what we term the “minimum required dilution” (MRD) for a given sample type. The MRD is the first dilution at which the dilution adjusted value for the sample in question and all subsequent dilutions remains essentially constant. The HCP value to be reported for such samples is the dilution corrected value at or greater than the established MRD. Once an MRD is established for a particular sample type, your SOP should reflect that this sample requires this dilution prior to assay.