For human therapeutic monoclonal antibody products, fractionation of a polyclonal antiserum raised in animals may be necessary to remove antispecies reactive antibodies that are not representative of the immune response of humans to the same product. Alternatively, animals could be immunized with the $F(ab)_2$ domain of the therapeutic monoclonal antibody, or an anti-idiotypic monoclonal antibody reagent could be used for products in which the human immune response is known to be directed primarily to the complementarity-determining regions (CDRs) (e.g., adalimumab, van Schouwenburg et al., 2013; or infliximab, van Schie et al., 2015). In the case of fusion proteins or conjugates, assay validation should include positive control antibodies reactive with the different moieties of the molecule.

12.12.1 Validation of Antigenic Equivalence ("Bioanalytical Similarity")

Although the positive control antibody reagent can only represent a surrogate index of the ADA response to be measured in the assay, the author has found it useful to demonstrate that the positive control antibody reacts in an equivalent manner with the biosimilar and reference products. Antigenic equivalence can be demonstrated by using a competitive binding format of the screening assay in which a wide (e.g., 100-fold) concentration-range of unlabeled versions of the biosimilar and reference products are added as competing antigens to inhibit the signal for binding of the positive control antibody to the labeled biosimilar antigen in the assay. Visual overlap of the inhibition curves (or, if considered necessary, a statistical test of equivalence) may then be used as evidence that there are no detectable differences—at least when using a surrogate antibody—in the antigenicity of the biosimilar and reference products. In the case that the curves do not overlap, or fail a statistical test of equivalence, biosimilarity might be questioned.

12.13 CONFIRMING SPECIFICITY OF POSITIVE SIGNALS

Since clinical samples are required to be tested in an operator-blinded manner, preferably in a single assay format that uses the biosimilar product as the antigen, it becomes relevant to confirm specificity of screened positive samples by competitive inhibition with unlabeled biosimilar and reference product tested in parallel. In any case, the screening assay cut point is set to yield a 5% false positive rate, so the confirmatory assay step is essential to correctly distinguish true from false positives. The confirmatory assay cut point should be established in a statistically rigorous manner to enable reliable classification of sample results as positive or negative. Different regulatory agencies may recommend different levels of stringency according to the risks associated with the product, such that sponsors will need to justify the approach actually applied.

Typically, a high molar excess of competing antigen is used in the confirmatory step of ADA assays, potentially undermining the value of the test to discriminate differential reactivity of the ADA with the biosimilar and reference antigens. An alternative approach using two concentrations of competing antigen—one representing submaximal inhibition of the positive control signal in the assay and the other representing a just maximal inhibitory level—might provide a more instructive