Because it is necessary to correlate measures of drug-reactive antibodies with PK parameters, appropriately rigorous validation of the impact of ADA on the accuracy of the assay ("PK assay") used for quantitation of drug concentration should also be considered (e.g., see Sailstad et al., 2014). Although industry-driven "White Papers" can provide a useful source of technical options, it should be remembered that these have no official regulatory jurisdiction, and sponsors have flexibility to apply alternative approaches.

Finally, it is important to emphasize that the absence of standardized positive control antibody reagents precludes objective benchmarking of assay sensitivity: comparison to historical bioanalytical data is rarely possible because different assay conditions are used; and it is not feasible to define clinically impactful ADA levels prior to analyzing clinical samples with product/application-specific assays.

12.10 ADA ASSAY SPECIFICITY

The immunogenicity of therapeutic proteins is measured indirectly, using an *in vitro* assay that reflects binding of a mixed (in terms of binding specificity, amount, and avidity) antibody population in the test article with a fixed amount of antigen (typically labeled). Treatment-emergent immunogenicity is inferred by a difference in the signals for the pre- versus posttreatment samples.

Since versions of the same molecule produced by two independent manufacturing processes are to be compared, the author has found it helpful to include a demonstration of antigenic equivalence (or "bioanalytical similarity") of the respective product versions in the validation of assay specificity. Such demonstration (described below) then provides the rationale for applying a single assay format to measure clinical samples from subjects receiving either the biosimilar candidate or the reference product. This also demonstrates that chemical modification of the labeled antigen(s) used in the assay has not biased the relative binding in the solution phase of a positive control antibody to the different unlabeled versions.

A more important source of bias for detection of ADA induced by therapeutic monoclonal antibodies has been interference by residual circulating drug, which can lead to substantial underestimation of the true incidence of ADA formation. Sample pretreatment involving acid-dissociation, often in combination with affinity capture (Bourdage et al., 2007; Smith et al., 2007), has been widely used to achieve suitable assay sensitivity to detect ADA in the presence of clinically relevant levels of therapeutic monoclonal antibody.

Sponsors should also evaluate and minimize potential interference associated with nonspecific binding factors (e.g., Rheumatoid Factor, antihuman IgG Fc allelic antibodies, human antimouse antibody, as relevant) and target ligand present in samples from the different patient populations to be treated in clinical studies. Ideally, this aspect should be included in the initial assay development experiments to avoid having to redevelop and revalidate the ADA assay at a later stage. Inclusion of an excess of nonspecific human IgG in the assay buffer may be a useful strategy to overcome much of the nonspecific binding that can be observed in plasma or serum obtained from certain patient populations, for example, autoimmune subjects.