same as the target, it is possible that neutralizing antibodies could cause interference. Possible ADA interference can be verified by using an ADA positive control (PC) and fortifying it in PK validation samples. The selection of this positive control should be carefully considered so that it reflects, as closely as possible, the potential antibody population in test samples.

During the sample analysis, the PK results can be correlated with the ADA results. A drop in PK with a corresponding positive response in ADA analysis can indicate possible interference. Another possible approach could be to look at the subjects that were positive for neutralizing antibodies. Individual results (e.g., positive PK result for predose samples, failure of incurred sample reproducibility, and nonparallelism of PK samples) should be carefully evaluated and could indicate ADA interference.

*4.13.4.1 Immunogenicity assay challenges* Immunogenicity testing is a critical component of the safety and the efficacy assessment of biosimilars. Biosimilar guidance suggests that immunogenicity is monitored by tracking the rate of incidence, the time for antibodies to form, the persistence of antibodies, the magnitude of the response, and the type of response.

## 4.13.5 Assay development

The immunogenicity of biosimilar products is also tested side by side, just like the PK profile, with the originator product, requiring a common point of comparison; however, unlike small molecules, the biological samples used for immunogenicity testing is a mixture of ADAs against the drug. The drug is often used as the capture reagent in the ADA assay. If the biosimilar product is used as a capture reagent, it may not bind and detect ADAs unique to the originator product and vice versa. This lack of cross-reactivity creates a risk of observing false negative results, making a product appear less immunogenic. As a consequence, two assays are validated, one for each of the product very early in the development stage. However, if cross-reactivity is demonstrated with the originator product, then the biosimilar product alone can be used with one assay method, substantially reducing the cost. Single assays are also accepted where the incidence of immunogenicity is low such as filgrastim. Generally, a single assay is more robust since comparing the responses that include the incidence of positive results, isotype distribution, and titer when using two methods can be difficult to validate and conduct. Two assays are more likely to product false negative outcomes. While the ADA studies provide a comparison of immunogenicity, these observations need testing for being clinically meaningful.

## 4.13.6 Assay controls

A major challenge in developing ADA assay is the availability of PC that establishes sensitivity, specificity, drug tolerance, and assay precision;