None of these assays can assess the ability of the antibodies to neutralize the biological activity of the therapeutic proteins that is an important element in the assessment of immunogenicity. For the evaluation of the neutralizing ability, the use of an appropriate noncell-based competitive ligand-binding assay or a cell-based neutralization assay is required.

Testing for immunogenicity is performed during the preclinical and clinical phases. The U.S. FDA *Guidance for Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins* states that an immunogenicity assay should, in addition to being sensitive, also be able to detect all isotypes, in particular, IgM and all IgG isotypes. The recommended sensitivity is 250 to 500 ng/mL. Studies are performed in three steps: screening, confirmation, and characterization of positives. Initial screening can result in false positives, and, therefore, the initial screening assay is usually followed by a confirmatory assay. After identification and confirmation of positive samples, a full characterization of ADAs in terms of assessment of isotype (class or subclass), binding stability, epitope specificity, and neutralizing capacity gives valuable information of the nature of the studied immune response. The IgG4 is second to IgG1 as the major isotype in ADAs developed for therapeutic mAbs. IgG4 has been associated with immune responses generated under conditions of high doses and prolonged exposure to therapeutic proteins. IgG4 ADAs can be difficult to detect in traditional bridging or homogenous ELISA and ECL™ (enhanced chemiluminescent) assays due to their bispecific nature.

7.3.6 Cell-based neutralization assays

The development of improved assays, particularly cell-based assays for the detection of neutralizing antibodies that allow immunogenicity to be determined with precision and the comparison of immunogenicity data between biopharmaceuticals, are critical for the development of less immunogenic and safer biopharmaceuticals.

The biological activity of a therapeutic is often evaluated using an in vitro cell-based assay based on a functional aspect of the protein or the MOA. These assays can be categorized into those that detect signaling responses soon after the protein-receptor interaction has occurred (early stage) or those that provide a measurable readout after the culmination of a cellular response (late stage). Since these assays assess the cellular response in vitro to a protein, they constitute an ideal and appropriate logical approach for the development of a cell-based neutralization assay. It should be realized that different types of bioassay procedures can be used as the basis of a neutralization assay for a biological.

A cell-based neutralization assay can be defined as an in vitro assay utilizing cells that interact with or respond to the therapeutic either directly or indirectly in a measurable manner in the presence of a test sample for the detection of antiproduct neutralizing antibodies. The detection