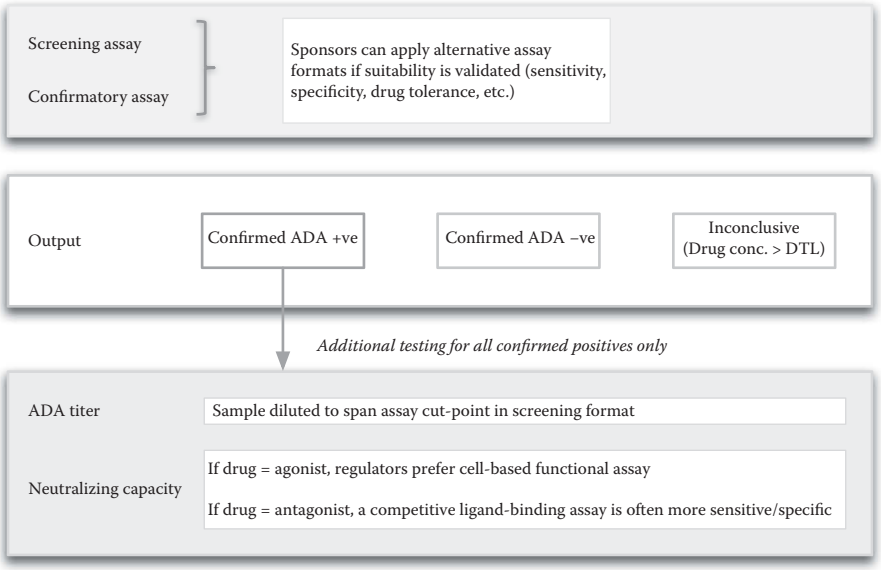
comparison that human ADA responses to either product were reacting in a comparable manner to the respective antigens. Although this alternative approach does not represent a regulatory expectation, it could provide supporting evidence that the measurement of antigenicity, used to infer immunogenicity, is not biased by the choice of antigen for the ADA assay; and that the individual human polyclonal immune responses to the treatment appear to react in solution phase in an equivalent manner with the respective product versions.

For some biosimilar products (e.g., insulin glargine), it is relevant to test the cross-reactivity of confirmed positive signals with related products (other insulins to which pretreated subjects may have been exposed to) and the endogenous molecule (native human insulin). Indeed, this was part of the bioanalytical evidence presented to support the EU approval of Abasaglar (EPAR for Abasaglar).

12.14 ADA TESTING STRATEGY

The multitier testing scheme that is recommended for bioanalysis of any biological medicinal product is equally applicable to the development of biosimilars. This multitier assay strategy is illustrated in Figure 12.1.



ADA, antidrug antibody; DTL, drug tolerance limit.

FIGURE 12.1 Multitier test scheme for detection of ADA. All clinical samples are tested in the screening assay to detect the presence of ADA; samples with signals above the cut-point are then tested to confirm reactivity with solution-phase competing antigen; only the confirmed positive samples are further tested to estimate ADA titer (reciprocal of minimum dilution yielding a signal above the assay cut-point) and capacity to neutralize a relevant biological function of the drug. In the case that the drug level in the sample exceeds the validated drug tolerance limit, samples are classified as "inconclusive" for ADA.