9.11.1 Process-Related Impurities and Contaminants

These are derived from the manufacturing process and are classified into three major categories: cell substrate-derived, cell culture-derived, and downstream-derived.

1. Cell substrate-derived impurities include, but are not limited to, proteins derived from the host organism and nucleic acid (host cell genomic, vector, or total DNA). For host cell proteins, a sensitive assay, for example, immunoassay, capable of detecting a wide range of protein impurities is generally utilized. In the case of an immunoassay, a polyclonal antibody used in the test is generated by immunization with a preparation of a production cell minus the product-coding gene, fusion partners, or other appropriate cell lines. The level of DNA from the host cells can be detected by the direct analysis of the product (such as by hybridization techniques). Clearance studies, which could include spiking experiments at the laboratory scale, to demonstrate the removal of cell substrate-derived impurities, such as nucleic acids and host cell proteins, may sometimes be used to eliminate the need for establishing the acceptance criteria for these impurities.
2. Cell culture-derived impurities include, but are not limited to, inducers, antibiotics, serum, and other media components.
3. Downstream-derived impurities include, but are not limited to, enzymes, chemical and biochemical processing reagents (e.g., cyanogen bromide, guanidine, and oxidizing and reducing agents), inorganic salts (e.g., heavy metals, arsenic, and nonmetallic ion), solvents, carriers, ligands (e.g., MAbs), and other leachables.

For intentionally introduced, endogenous, and adventitious viruses, the ability of the manufacturing process to remove and/or inactivate viruses should be demonstrated, as described in the ICH guidance *Q5A Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*.

9.11.2 Product-Related Impurities, Including Degradation Products

The following represents the most frequently encountered molecular variants of the desired product and lists relevant technology for their assessment. Such variants may need considerable effort in isolation and characterization, in order to identify the type of modification(s). Degradation products arising in significant amounts during the manufacture and/or storage should be tested for and monitored against appropriately established acceptance criteria.

9.11.2.1 Truncated Forms

Hydrolytic enzymes or chemicals may catalyze the cleavage of peptide bonds. These may be detected by HPLC or SDS-PAGE. Peptide mapping may be useful, depending on the property of the variant.