nonsterile handling procedures. Working in closed systems and avoiding of raw materials of animal origin will help reduce the risk of infection. Strict control of equipment cleaning and sanitization procedures during processing will also help in reducing contamination risk. Removal of viruses may be inactivated by heat, radiation, chemical compound, or low pH, or removed by chromatography or filtration techniques. Due to molecular diversity, no specific chromatographic purification method can be recommended, and virus reduction factors must be determined for selected unit operations. Nanofiltration is a very efficient virus removal step often resulting in logarithmic reduction factors of 5–8. A commonly used reagent for cleaning chromatographic media is 0.1–1 M NaOH. Viruses can be successfully destroyed with peracetic acid (make sure that equipment, filters, and chromatographic media are not affected by NaOH or peracetic acid). A variety of purity analyses are available: monolayer cultures, test for pathogen viruses not able to grow in cell cultures in both animals and eggs, test for retroviruses, endogenous viruses, or viral nucleic acid, and test for selected viruses using mouse, rat, and hamster antibody production tests. It is necessary to document the utilization of adequate virus removal and inactivation strategies to ensure the exclusion of contaminating viruses. Different MoAs should ensure overlapping and complementary levels of protection. The purification process is validated with respect to virus removal and inactivation. The final product is rarely tested if continuous mammalian cell lines have been used as an expression system.

5.3.4.4 Prions Prions come from transmissible spongiform encephalopathies including scrapie in sheep and goats, chronic wasting disease in mule deer and elk, bovine spongiform encephalopathy (BSE) in cattle, Kuru, and Creutzfeldt-Jakob disease in humans. The disease-causing agents (prions) generally replicate in infected individuals without evidence of infection detectable by available diagnostic tests applicable in vivo. The primary source of contamination of a recombinant product is the use of animal-derived raw materials, which could harbor bovine prions (BSE agent). Currently, there are no assays that are sensitive or specific enough to test raw materials or sources and the only reliable prevention is to include barriers, such as avoidance of animal or human raw materials (e.g., trypsin, serum, transferrin, bovine/human serum albumin, protein supplements, peptones). However, this is not always possible (e.g., in the propagation of cells for the establishment of cell banks), and inactivation and removal procedures during downstream processing become of interest. Milk is unlikely to present any risk of prion contamination. Filtration has to be proven efficient in the removal of prion particles. Thus, size exclusion partitioning of abnormal prion particles using normal flow filtration or tangential flow filtration resulted in significant reduction of the infectious agent. The most effective inactivation methods include chloride dioxide, glutaraldehyde, 4 M guanidium thiocyanate, sodium dichloroisocyanurate, sodium metaperiodate, 6 M