organic solvents (make sure that equipment, filters, and chromatographic media are not affected by NaOH or organic solvents).

5.3.4.7 Microbial agents Microbial agents and fungi come from infection of the bioreactor during cell culture. Other sources are contaminated water, buffers, raw materials, chromatographic columns, and equipment. Fermentation and cell culture bioreactors are prone to microbial infections. As the use of antibiotics in large-scale operations should be avoided, strict demands on the design of bioreactors and handling procedures are the key measures to avoid microbial infections. Test for microorganisms at the end of production assures that no infections have taken place during culture. The nature of samples and buffers used during downstream processing make these excellent growth substrates for microorganisms. For that reason, water quality control, sterile filtration of buffers prior to use, sterile filtration of intermediary products, and effective cleaning and sanitization procedures are key elements of the downstream operations. Filtration through 0.22 μm filters. Bacterial spores are typically removed by means of 0.1 μm filters. Cleaning with 60%–70% v/v ethanol is a commonly used disinfectant against microbial agents; often 20% v/v ethanol is used as a storage solution for chromatographic resins, but the solution has no sporicidal effect, while 0.1–1.0 M NaOH is widely used to kill microorganisms. Peracetic acid has both bacterial and sporicidal effects. Viable cells can be identified by spread out of the cell suspension or sample solution on agar plates.

5.3.4.8 Mycoplasma Mycoplasmas have long been recognized as a contaminant of continuous cell cultures caused by an infection of the cell line or the bioreactor. Working in closed systems under good manufacturing practices (GMPs) will reduce the risk of infection. The end-of-production test includes screening for mycoplasma. Mycoplasmas are extremely sensitive to osmotic shock and pH extremes and should not constitute a problem in downstream processing provided sanitization and cleaning in place procedures are carried out according to GMPs. Mycoplasmas are resistant to most antibiotics. Frequent testing (at every passage) is recommended. The cell culture is discarded upon infection. Cleaning with 0.1–1 M NaOH will inactivate the mycoplasma. Mycoplasmas are difficult to detect; the only reliable way of demonstrating infection is by agar plating, fluorescent dyeing of DNA, or by PCR. Recently, a selective biochemical test that exploits the activity of certain mycoplasma enzymes has been made commercially available.

5.3.4.9 Des-amido forms Des-amido forms are target protein derivatives in which one or several of the glutaminyl or asparaginyl amino acid residues are converted to the corresponding acids (glutamyl and aspargyl). The deamidation reaction is slow at pH 3–5, at low temperatures, and at low conductivity. Deamidated forms are removed by HP-IEC and HP-RPC. Des-amido forms are detected by analytical HP-IEC,