5.3.4.1 Endotoxins Endotoxins come from Gram-negative bacteria (e.g., *E. coli*) if they are used as the expressions system. The presence of endotoxins indicates bacterial contamination in raw materials, columns, water, and buffers. Endotoxins strongly bind to anion-exchange media even at high ionic strength and thus hydrophobic interaction chromatography and CIEC are used for their removal provided the target protein binds to the matrix. Binding of the target protein to CEX also allows effective endotoxin clearance. SEC can remove endotoxins provided the differences in molecular weight between the endotoxins and the protein are sufficient. However, SEC may be an unpredictable method since endotoxins range in size from subunits of 10–20 kDa in the presence of detergents to vesicles of 0.1 pm in diameter in the presence of divalent cations. In the absence of significant levels of divalent cations and surface-active agents, they dissociate into micelles of 300–1000 kDa.

Traditional inactivation methods (CIP) against endotoxins include acid hydrolysis, base hydrolysis, oxidation, alkylation, and heat and ionizing radiation. The use of a concentration-dependent matrix of sodium hydroxide is recommended for the removal endotoxins from chromatographic columns; endotoxins are destroyed by exposure to NaOH or peracetic acid but are not affected by ethanol.

5.3.4.1.1 Nucleic acids Nucleic acid contamination comes from host cell DNA/RNA or retroviral RNA. Molecules with more than 150–200 base pairs will behave as flexible coils in SEC, while molecules up to 18 base pairs behave as globular proteins. In between, the rigid rod structure should be expected. Their presence results in increased viscosity of the solution. Circular DNA is often supercoiled and will elute as a molecule of smaller size. Preventive action nucleic acid–free biopharmaceutical products are obtained by using nucleases (e.g., Benzonase) and/or by minimizing the release of nucleic acids from the host cell organism. AEIC has been shown to be effective in binding the highly charged nucleic acids at ionic strength at which most proteins elute. Due to the net negative charge and hydrophilic-binding character of the target protein to a CEX, hydrophobic interaction or affinity matrix may reduce the nucleic acid content. DNA binds to hydroxyapatite at low to moderate phosphate concentrations. Precipitation of nucleic acids with polyethyleneimine or magnesium chloride has been reported. The use of 1 M NaOH is recommended (make sure that equipment, filters, and chromatographic media are not affected by NaOH) for CIP. Nucleic acids are detected by monitoring absorption of light at 260 nm. The residual content of drug substance and/or drug product is usually measured by polymerase chain reaction (PCR) or amplification techniques. The maximum allowable content of nucleic acid per dose has been under continuous evaluation of the initially proposed content of 10 pg per dose suggested by CBER. WHO has stated that 100 pg per dose is acceptable. CBER now states that "lot-to-lot testing for DNA content in biological products produced in cell lines should be performed, and lot release