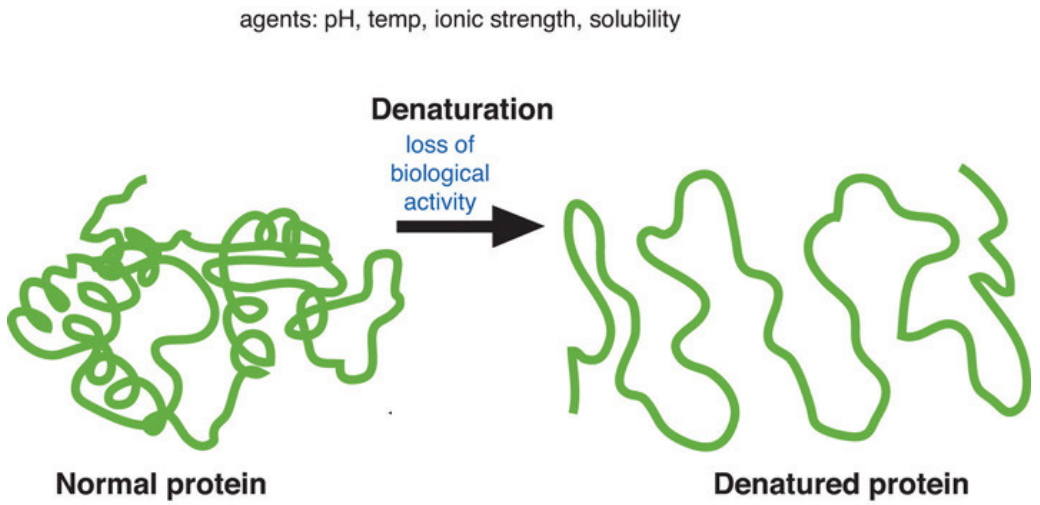

Formulation of Biopharmaceuticals
Microbiological consideration

Sterilization of Biopharmaceuticals

- Most proteins are administered **parenterally** and have to be **sterile**.
- In general, proteins are **sensitive to heat** (protein is denatured and by heat) and other regularly used sterilization treatments.
- They cannot withstand autoclaving, gas sterilization, or sterilization by ionizing radiation.
- Consequently, sterilization of the end product is <u>not possible</u>.



Sterilization of Biopharmaceuticals

- Protein pharmaceuticals have to be assembled under **aseptic** conditions, following rules in the pharmaceutical industry for aseptic manufacture.
- **Equipment and excipients** are treated separately and autoclaved or sterilized by dry heat (>160 ◦C), chemical treatment, or gamma radiation to minimize the bioburden.
- **Filtration techniques** are used for removal of micro bacterial contaminants.

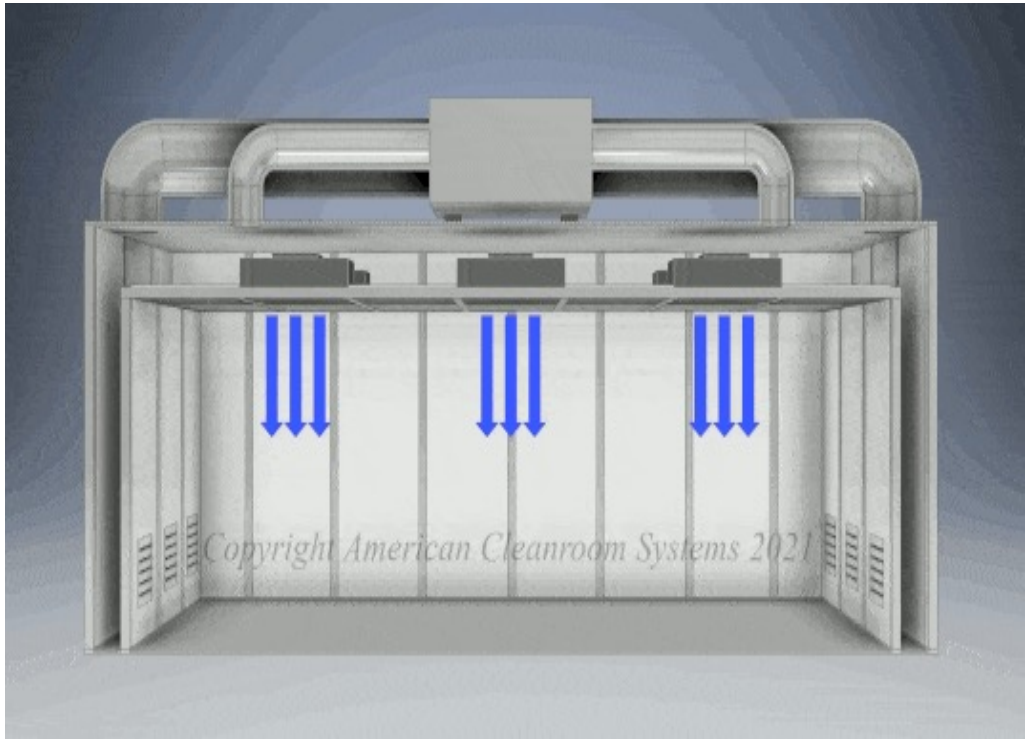
a. **Pre filters** remove the bulk of the bioburden and other particulate materials.

b. The **final "sterilizing"** step before filling the vials is filtration through 0.2 or 0.22 µm membrane filters.

Sterilization of Biopharmaceuticals
Clean Room

- Assembly of the product is done in class 100 clean room.
- This class means: maximum 100 particles > 0.5 µm per cubic foot).
- This rooms contains a laminar airflow that is filtered through HEPA (high efficiency particulate air) filters.
- **Cleanrooms** are used in practically every industry where small particle can adversely affect the manufacturing process.

Clean Room

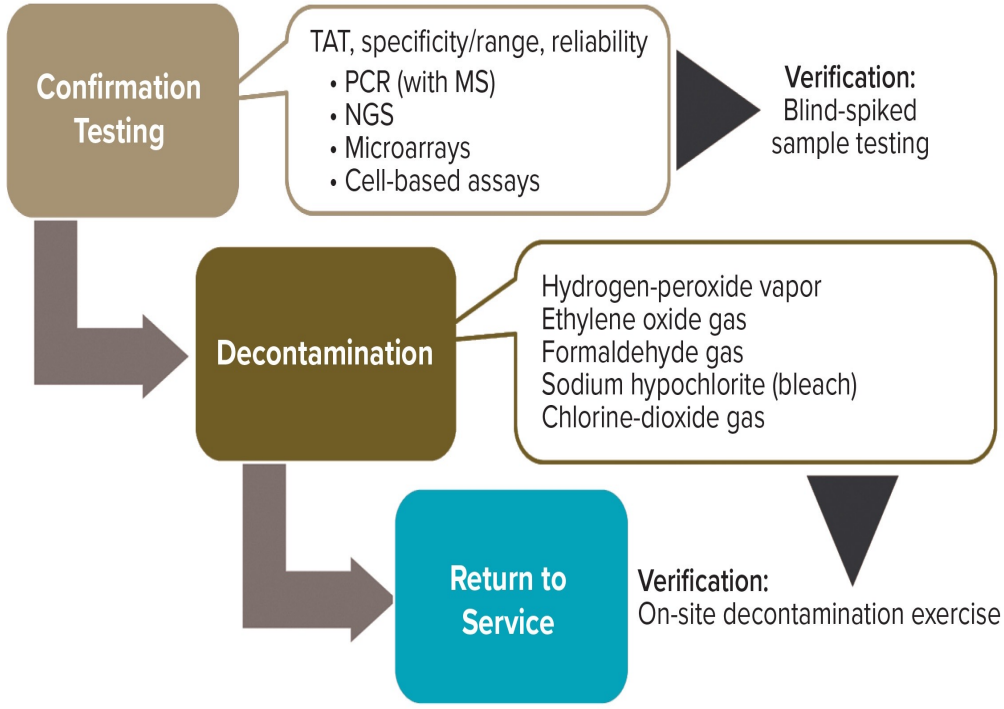


Clean Room

- The “human factor” is a major **source of contamination**.
- Well-trained operators wearing **protective cloths** (face masks, hats, gowns, gloves, or head-to-toe overall garments) should operate the facility.
- Regular exchange of filters.
- Regular validation of HEPA equipment.
- Thorough cleaning of the room plus equipment are critical factors for success.

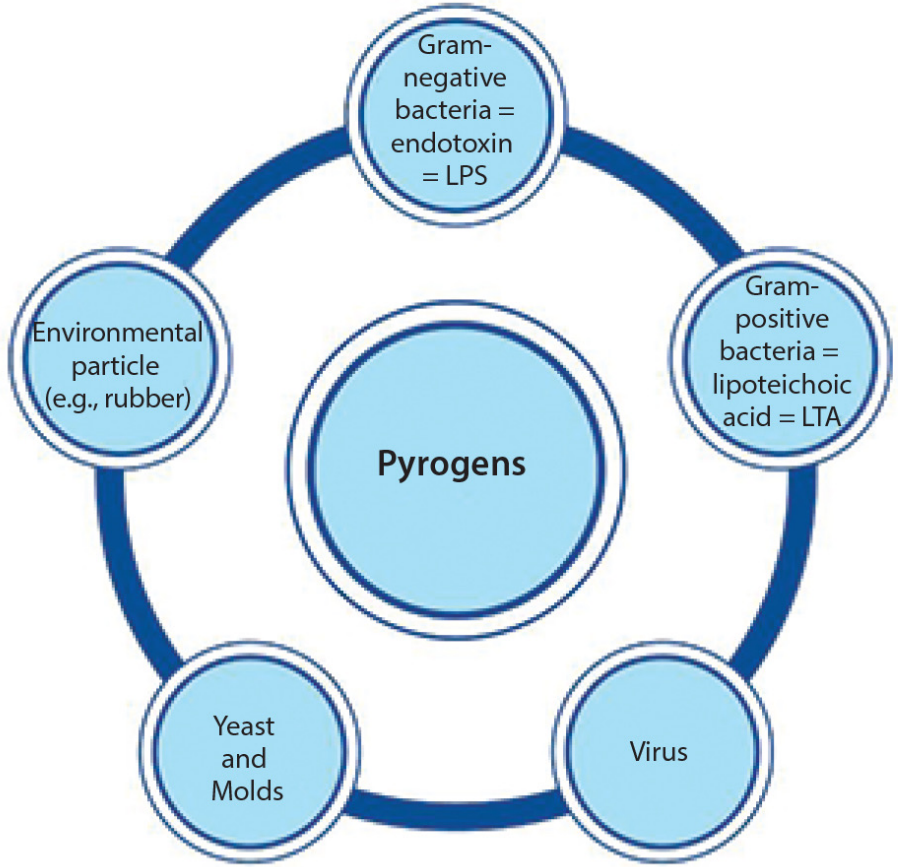
Viral Decontamination

- As recombinant DNA products are **grown in microorganisms**, these organisms should be **tested for viral contaminants**.
- Appropriate measures should be taken if viral contamination occurs.
- In the rest of the manufacturing process, no (unwanted) viral material should be introduced.
- Excipients with a certain risk factor such as blood-derived human serum albumin should be **carefully tested before use**, and their presence in the formulation process should be **minimized.**



Pyrogen Removal

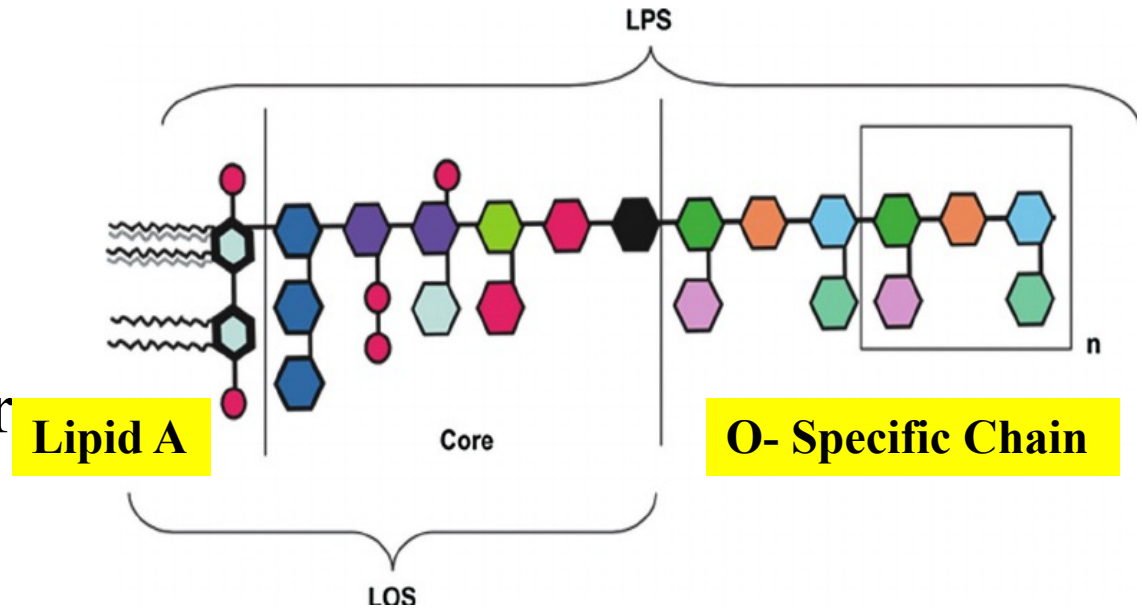
- Pyrogens are compounds that induce fever (act by preventing of calcium and sodium balance in the anterior hypothalamus).
- Exogenous pyrogens (pyrogens **introduced** into the body, **not generated** by the body itself) can be derived from bacterial, viral, or fungal sources.
- Bacterial pyrogens are mainly endotoxins produced by gram-negative bacteria. (They are lipopolysaccharides)



Pyrogen Removal

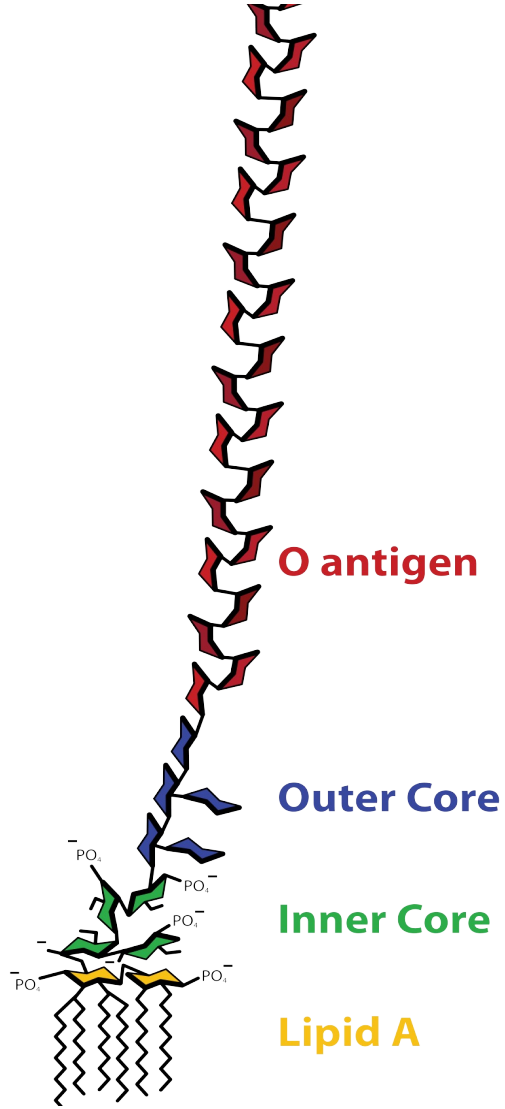
- Most properties of endotoxins are accounted for by the active, insoluble "**lipid A" fraction** being solubilized by the various sugar moieties (circles *with different colors)*.

- Although the general structure is similar, individual endotoxins **vary** according to their **source** and are characterized by the **O-specific antigenic chain.**



Pyrogen Removal

- Lipid-A structure is **similar** in thousands of different endotoxins.
- Another general property shared by endotoxins is their **high, negative electrical charge**.
- Their tendency to aggregate and to form large units with M_W of over 10^6 in water and their tendency to adsorb to surfaces indicate that these compounds are amphipathic in nature (possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties).



Pyrogen Removal

Pyrogen must be removed from different component of the biopharmaceutical product as follow:

A. Glass or vials:

- Pyrogens are **stable** under standard autoclaving conditions but break down when heated in the dry state.
- For this reason, equipment and container are treated at temperatures **above 160 °C (autoclave temperature)** for prolonged periods (e.g., 45 min dry heat at 250 °C, or 650 °C for 1 min).

B. The product:

- Pyrogen removal of recombinant products derived from bacterial sources should be an integral part of the preparation process.
- Ion exchange chromatographic procedures (**utilizing its negative charge**) can effectively reduce endotoxin levels in solution.

Pyrogen Removal

C. Excipients:

- Excipients used in the protein formulation should be essentially endotoxin-free.
- Also For solutions, "**water for injection**" should be (freshly) distilled or produced by reverse osmosis.
- Endotoxin molecules tend to form micelles or vesicles in aqueous solution and can be removed by filtration

D. Physical Component such as stoppers and tubing:

- Rinsing or dilution with **pyrogen-free water systems** is most common.

Summary Of microbial consideration

- Biopharmaceuticals must be **sterile** → this can be done by filtration.
- Biopharmaceuticals must be processes in **clean room** to prevent contamination.
- Biopharmaceuticals must be **tested for viral** contamination and treated using appropriate measures.
- Biopharmaceuticals drug, containers, equipment and everything related to it must be pyrogen free → can be don using various measures.