College of Pharmacy
Fifth Stage

Pharmaceutical Biotechnology

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Lectutre 3
Formulation of Biopharmaceuticals Microbiological Consideration

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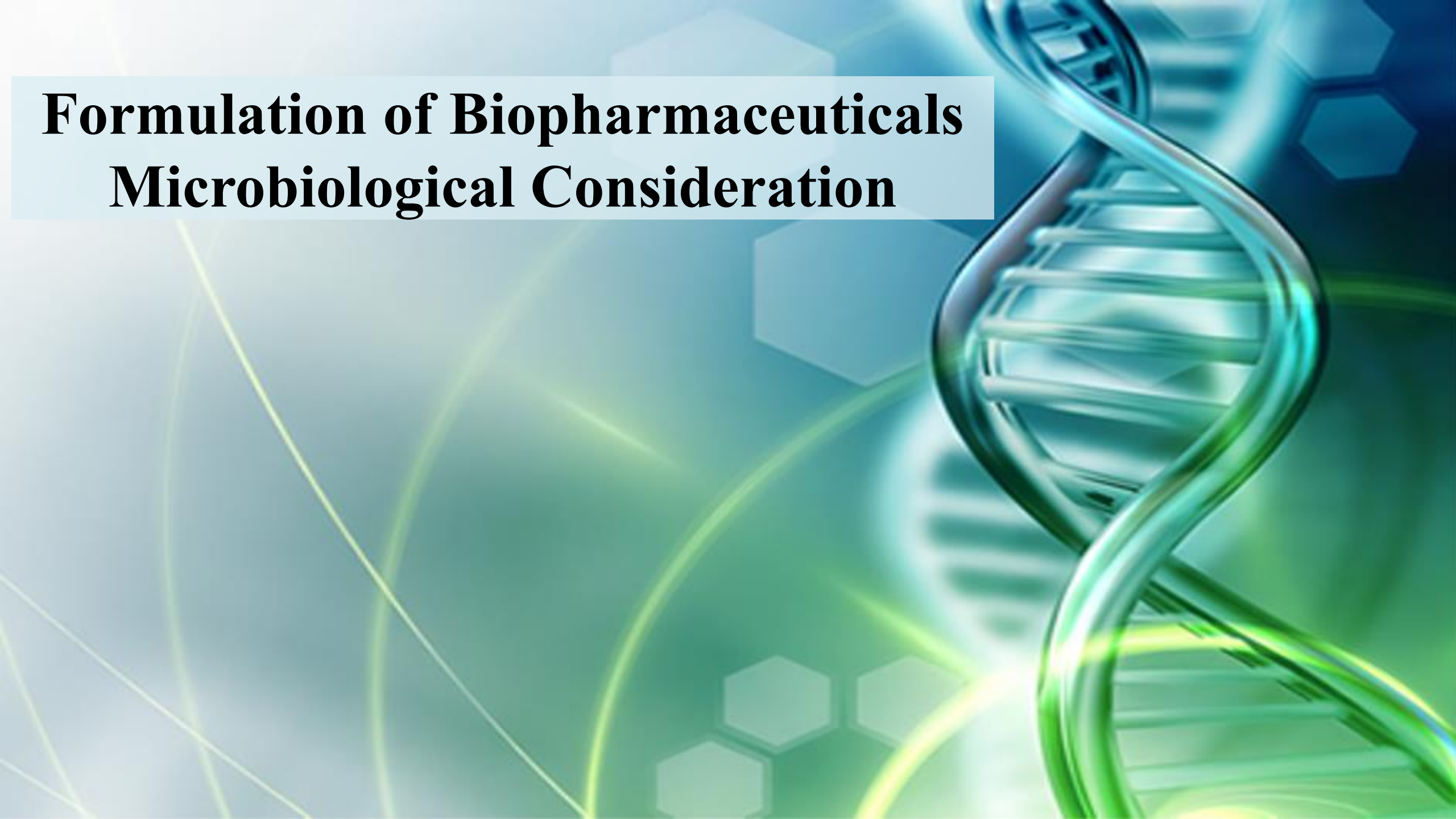
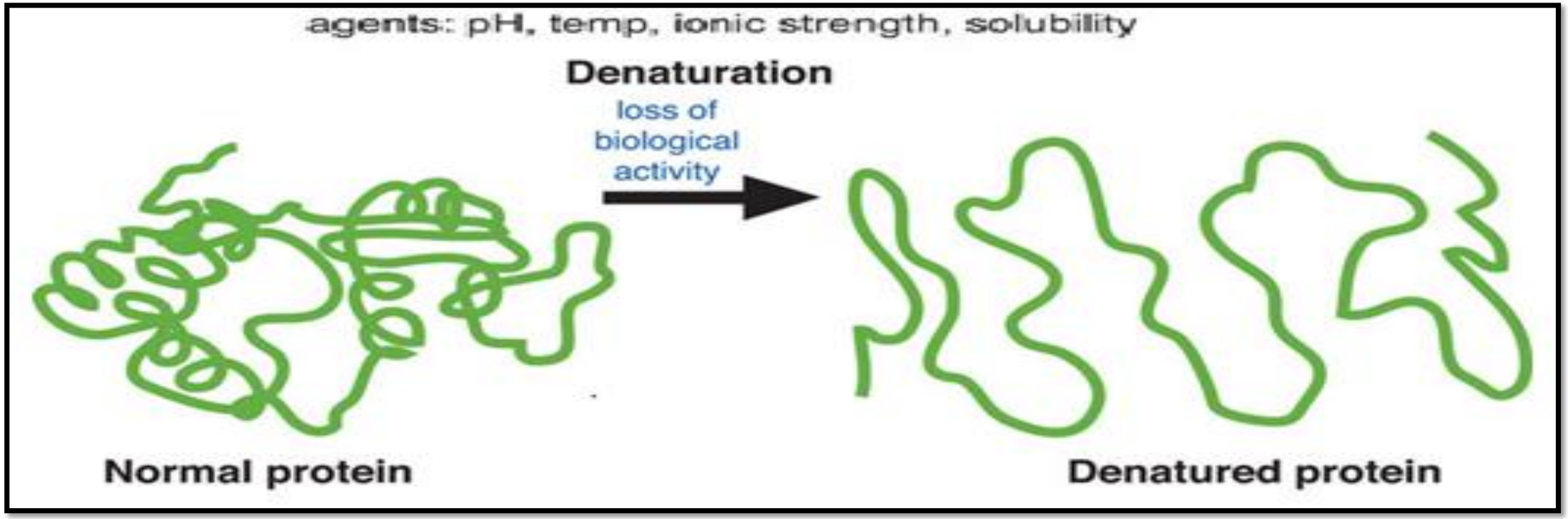


Sterilization of Biopharmaceuticals

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



Solution

Protein pharmaceuticals assembled under aseptic conditions.

Sterilization of Biopharmaceuticals

- Protein pharmaceuticals have to be assembled under **aseptic conditions**, following rules in the pharmaceutical industry for **aseptic manufacture**.

1. Equipment and excipients are treated separately and **autoclaved or sterilized** by **dry heat (>160 °C), chemical treatment, or gamma radiation** to minimize the bioburden.

2. 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.

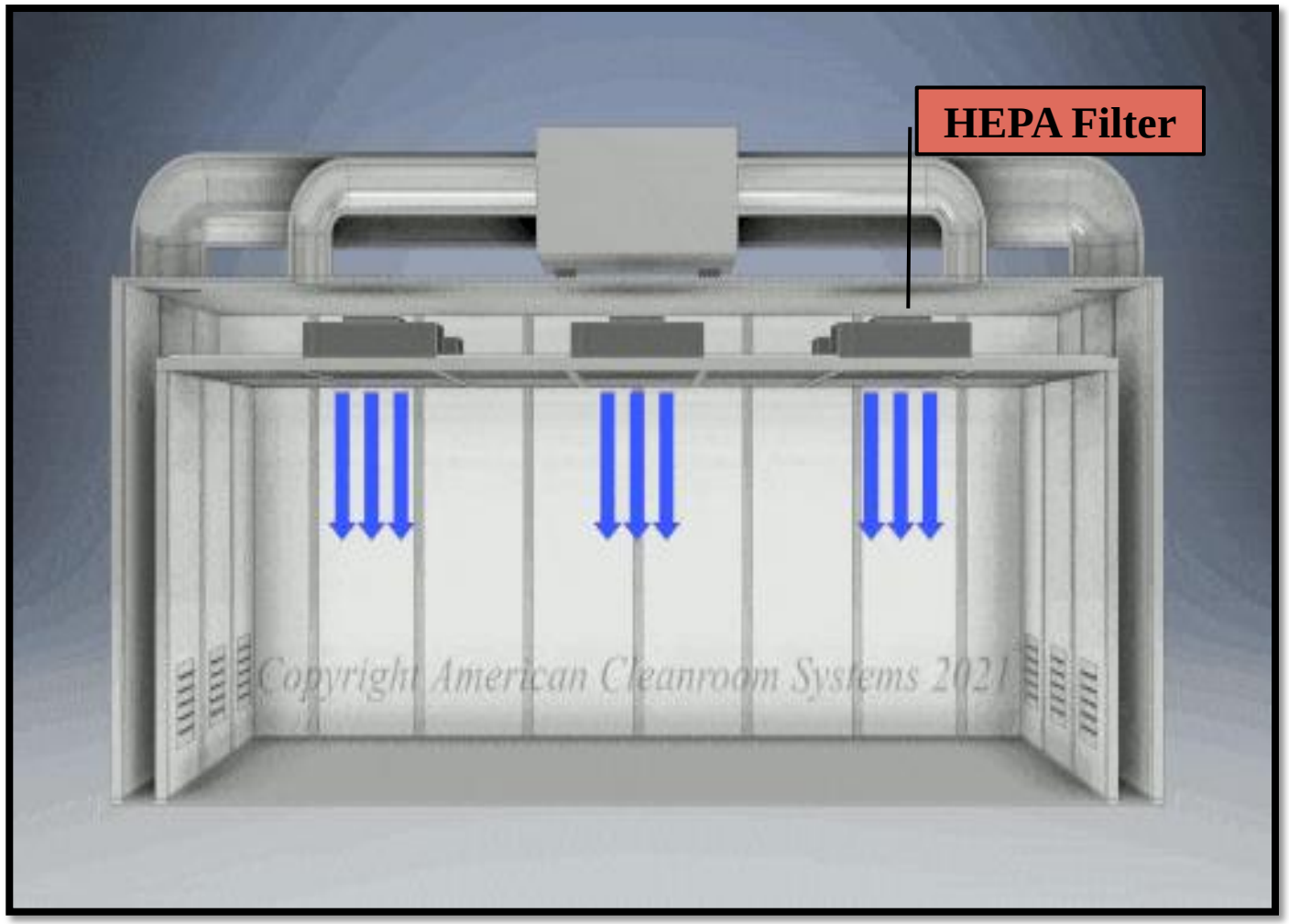

HATMAN

Work in Clean Room

- Assembly of the product is done in **class 100 clean room** (allow a maximum of only **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


凌威科技Class 100 Clean Room高潔淨無塵室實景


HEPA Filter
Copyright American Cleanroom Systems 2021

Use Proper Personal Protective Equipment (PPE)

- 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.
- Use Proper **PPE**, Regular **exchange** of filters, regular validation of **HEPA** equipment and thorough **cleaning** of the **room plus equipment** are **critical factors for success.**


Cap
(under hood)
Safety
glasses
Hood
Face mask
Gloves
Coveralls
Boots
Shoe
covers
(under boots)

Viral Decontamination

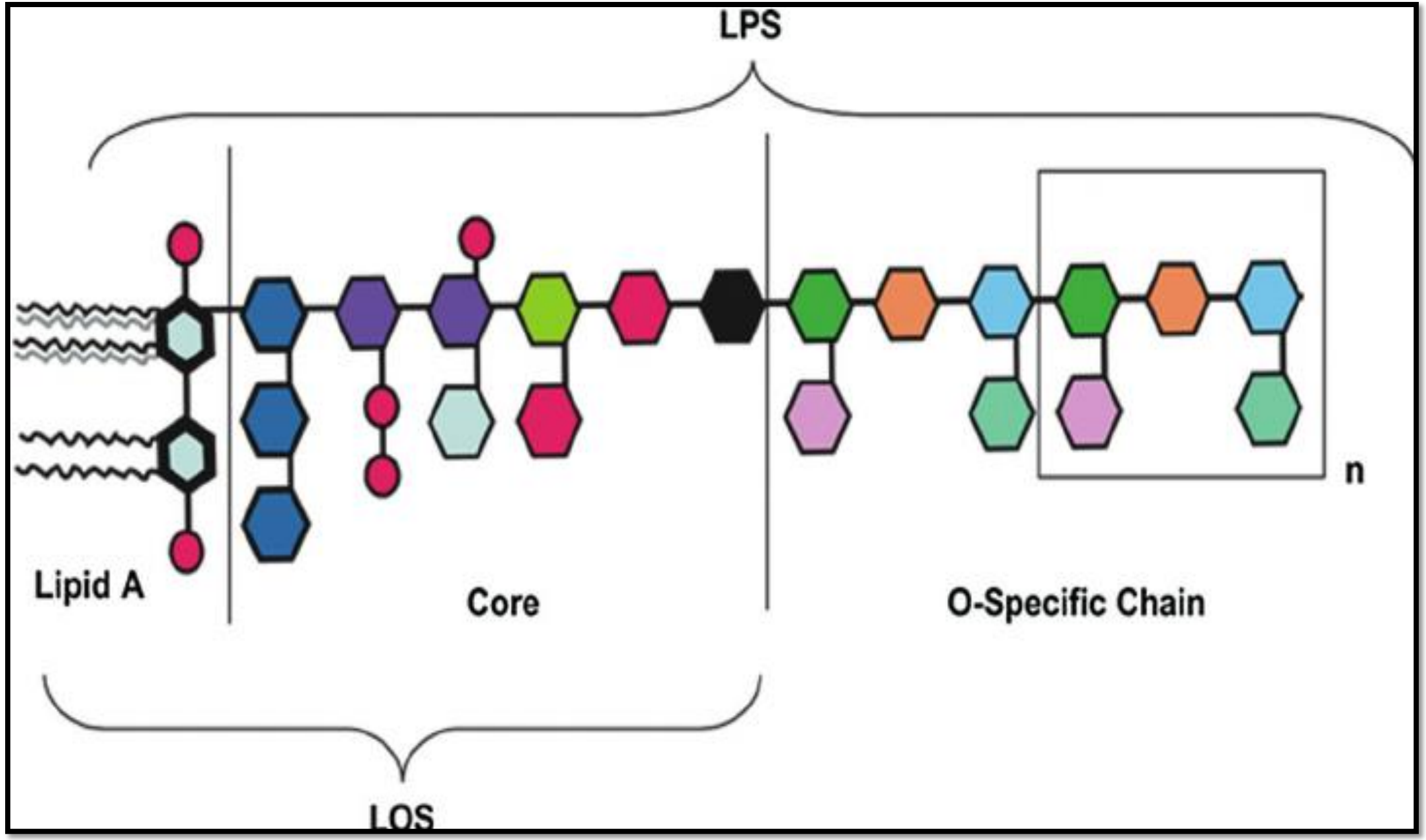
- As recombinant DNA products are **grown in microorganisms**, these organisms should be **tested for viral contaminants**.
- Appropriate measures should be taken if viral contamination occurs.
- In the rest of the manufacturing process, **no (unwanted) viral material** should be **introduced**.
- **Excipients**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

Pyrogen Removal

- **Pyrogens** are compounds that induce **fever**.
- **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 (LPS))

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.**


LPS
n
Lipid A
Core
O-Specific Chain
LOS

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 adsorb to surfaces** indicate that these compounds are **amphipathic** in nature (possessing both **hydrophilic** (water-loving, polar) and **lipophilic** (fat-loving) properties).

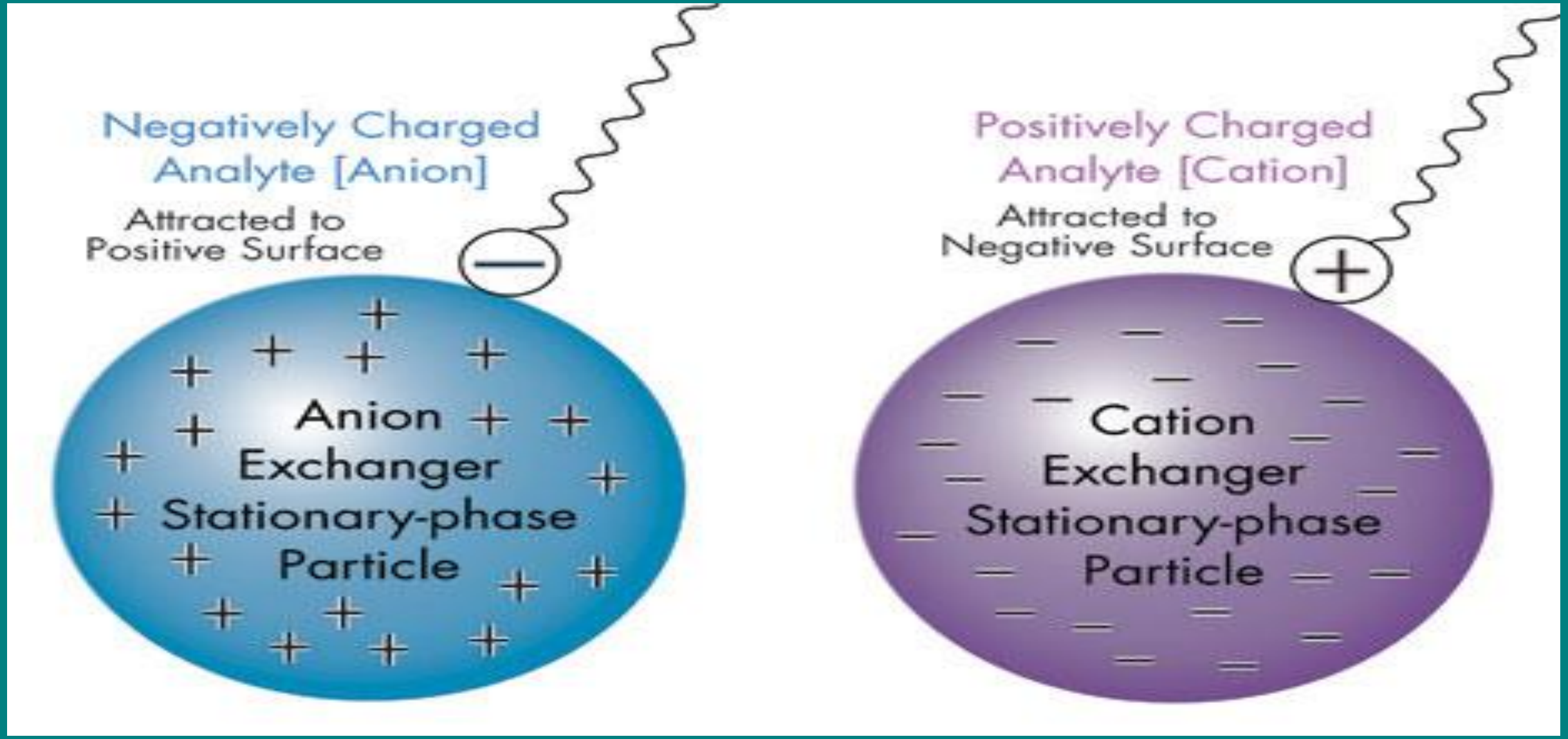
Pyrogen Removal

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

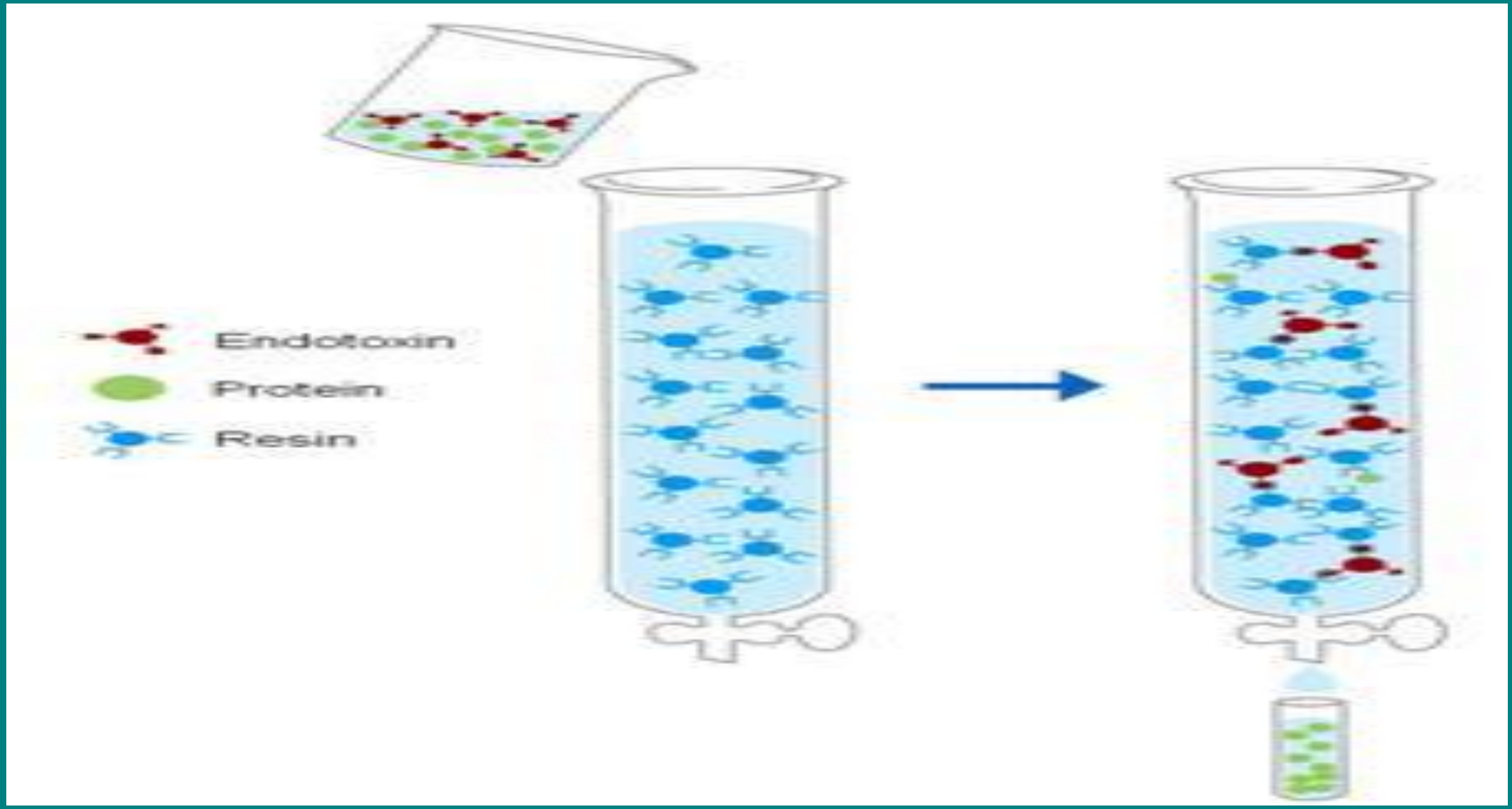
1. Glass or vials: Pyrogens are stable under standard autoclaving conditions but break down when heated in the dry state. For this reason, equipment and container are treated at **temperatures above 160 ˚C (autoclave temperature)** for **prolonged periods** (e.g., **45 min dry heat at 250 ˚C, or 650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

2. 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**.

Ion exchange chromatography



Ion exchange chromatography



Pyrogen Removal

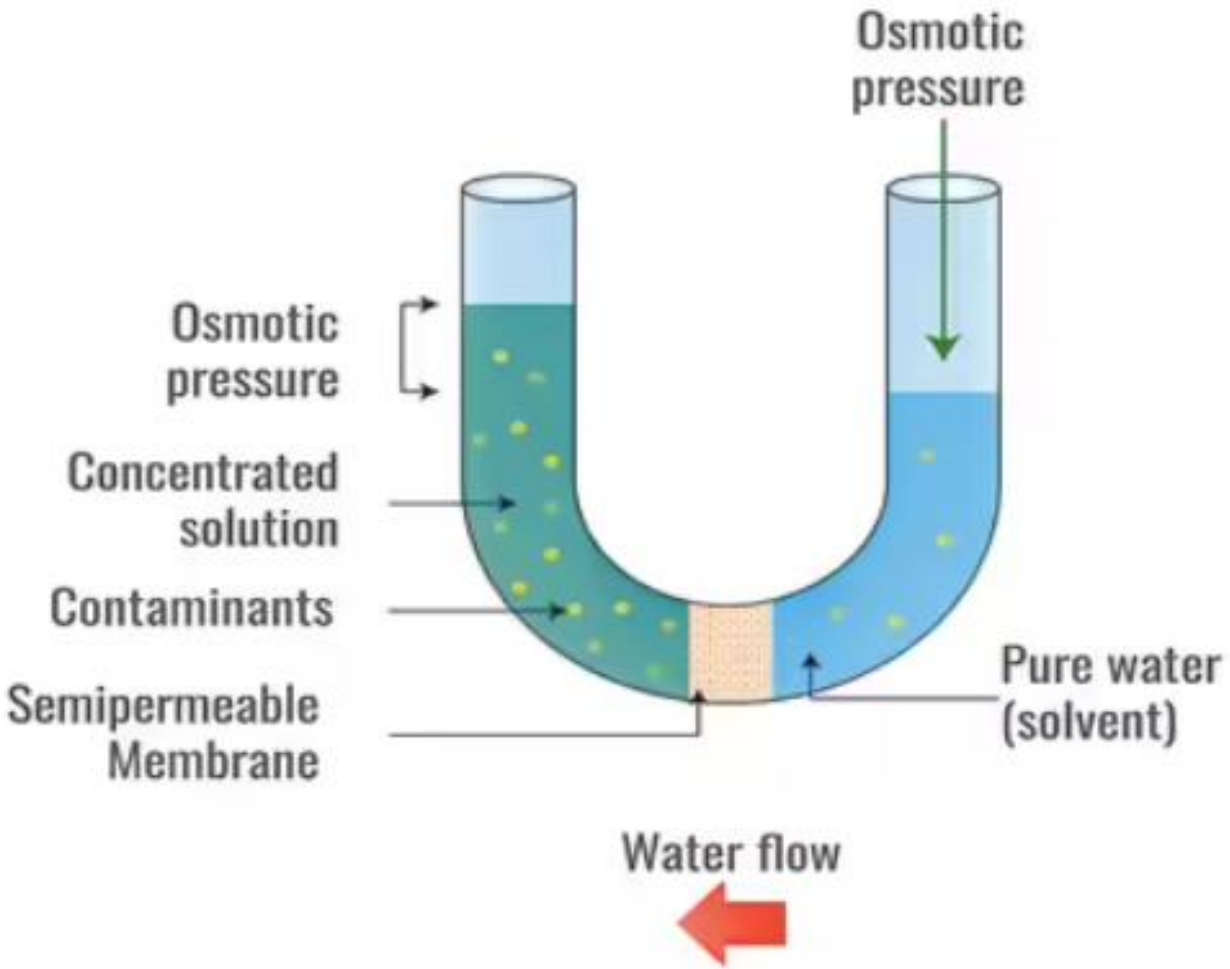
3. Excipients:

- **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**

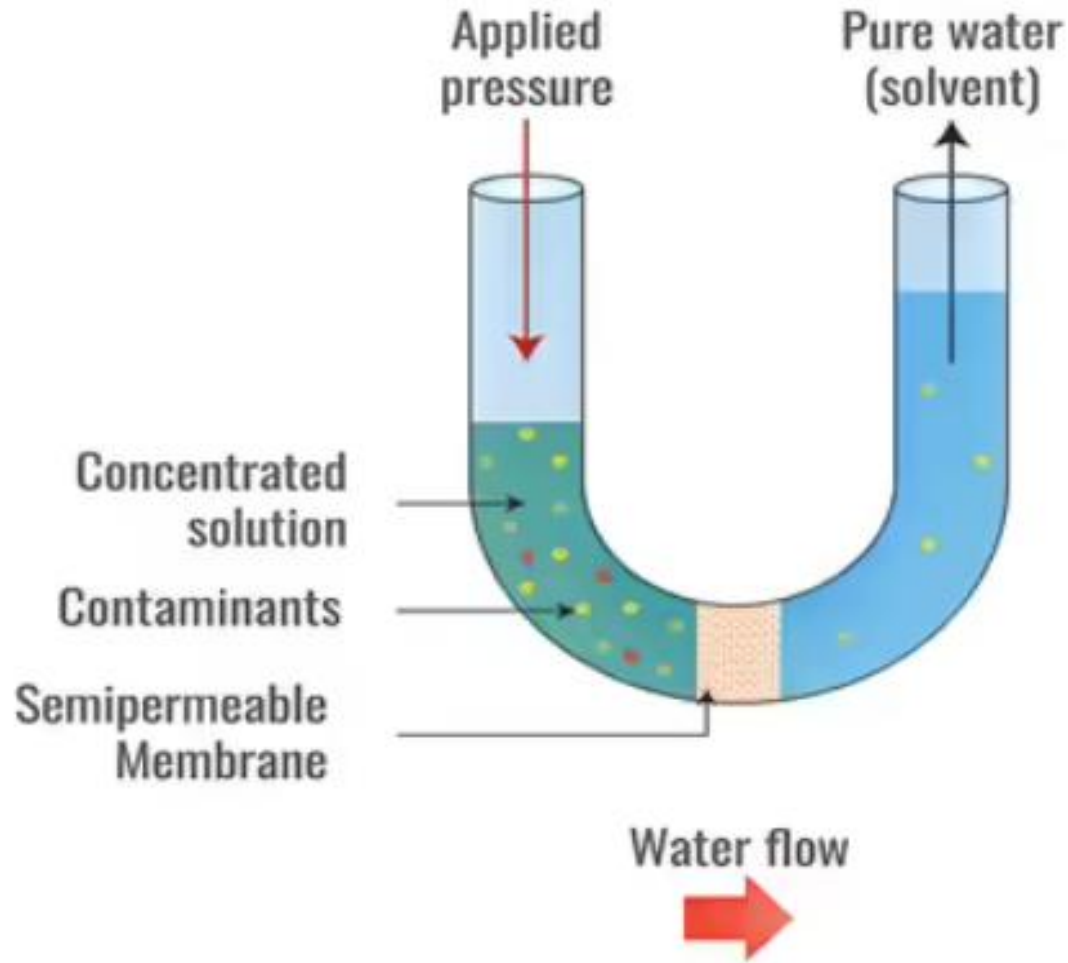
4. Physical Component such as **stoppers and tubing**:

- **Endotoxins** can also be inactivated on utensil surfaces by **oxidation** (e.g. **peroxide**) or **dry heating** (e.g. **30 minutes dry heat at 250°C**).

Osmosis and Reverse Osmosis



Osmosis



Reverse Osmosis


Thank You