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**DEPARTMENT OF BIOMEDICAL ENGINEERING**

**VII Semester**

**OBT357 BIOTECHNOLOGY IN HEALTH CARE**

**UNIT- 3 VACCINOLOGY**

## 3.6 Preservation and monitoring of microorganisms in seed lot systems

### Seed lot Systems:

- The **seed lot system** is a fundamental principle in vaccine production and quality control, designed to ensure the consistency, safety, and efficacy of vaccines from batch to batch.
- It is a hierarchical system that begins with a single, well-characterized master seed, from which all subsequent production lots are derived.

The system consists of two primary tiers:

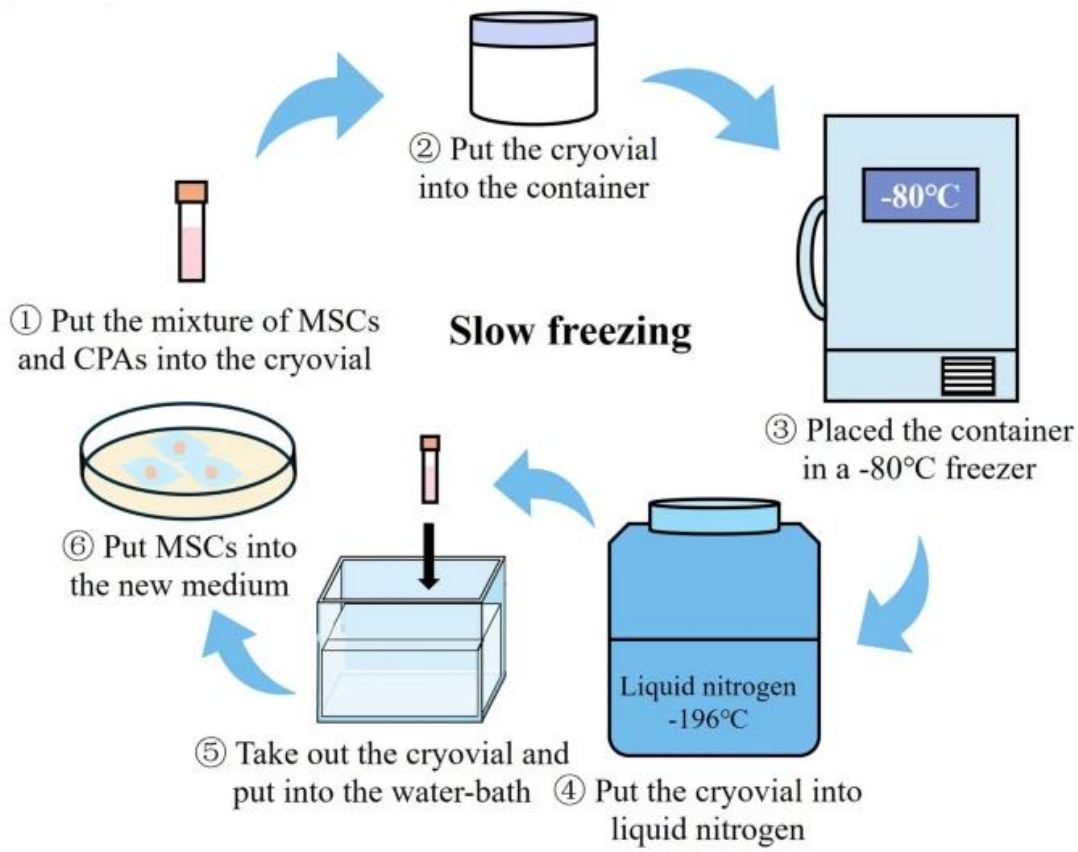
1. **Master Seed (or Master Cell Bank):** This is the original starting material for vaccine production. It's a single, small batch of the virus or bacteria that has been thoroughly tested and characterized. It is proven to be pure, genetically stable, and free from any contaminating agents. The master seed is stored under specific, controlled conditions (like in liquid nitrogen) to prevent any changes over time. It is only used to create the next tier of the system.
2. **Working Seed (or Working Cell Bank):** This is a larger quantity of the virus or bacteria derived directly from the master seed. It is used for the routine production of vaccine batches. The working seed is also rigorously tested to ensure it has the same characteristics as the master seed. By using the working seed for production, the master seed remains untouched, preserving its integrity and ensuring that all production runs are derived from the same, original, well-defined source.

The most common methods for preserving microorganisms in a seed lot system are:

**1. Cryopreservation (Freezing)**

This is the most widely used and preferred method for long-term storage of both viruses and bacteria. The microorganisms are frozen at extremely low temperatures, which halts all metabolic activity.

- **Process:** The microorganisms are suspended in a cryoprotectant solution (like glycerol or dimethyl sulfoxide, DMSO) which protects them from damage caused by ice crystal formation. The mixture is then slowly frozen, often in a controlled-rate freezer, and stored at very low temperatures, typically in **liquid nitrogen** (-196°C) or in an ultra-low-temperature freezer (-80°C).
- **Advantage:** This method ensures the highest level of genetic stability over decades.




The Figure illustrates a step-by-step process for cryopreserving mesenchymal stem cells (MSCs) using a slow freezing technique

- **Put the mixture of MSCs and CPAs into the cryovial**: A mixture of MSCs (mesenchymal stem cells) and CPAs (cryoprotective agents, e.g., glycerol or DMSO) is prepared to protect the cells from damage during freezing. This mixture is transferred into a cryovial, a small, sterile tube designed for low-temperature storage.
- **Put the cryovial into the container**: The cryovial is placed into a specialized container, likely a controlled-rate freezing device, to ensure slow freezing and minimize ice crystal formation that could harm the cells.
- **Placed the container in a -80°C freezer**: The container with the cryovial is moved to a -80°C freezer for initial freezing. This slow freezing step helps reduce cellular stress and maintain viability before transferring to even lower temperatures.
- **Put the cryovial into liquid nitrogen**: After initial freezing, the cryovial is transferred into liquid nitrogen at -196°C. This ultra-low temperature provides long-term storage, halting biological activity and preserving the cells indefinitely when stored properly.
- **Take out the cryovial and put into the water-bath**: To retrieve and revive the cells, the cryovial is removed from liquid nitrogen and thawed quickly in a water bath (typically at 37°C) to restore the cells to a viable state.
- **Put MSCs into the new medium**: Once thawed, the MSCs are transferred into a fresh culture medium to recover and resume growth under controlled conditions.

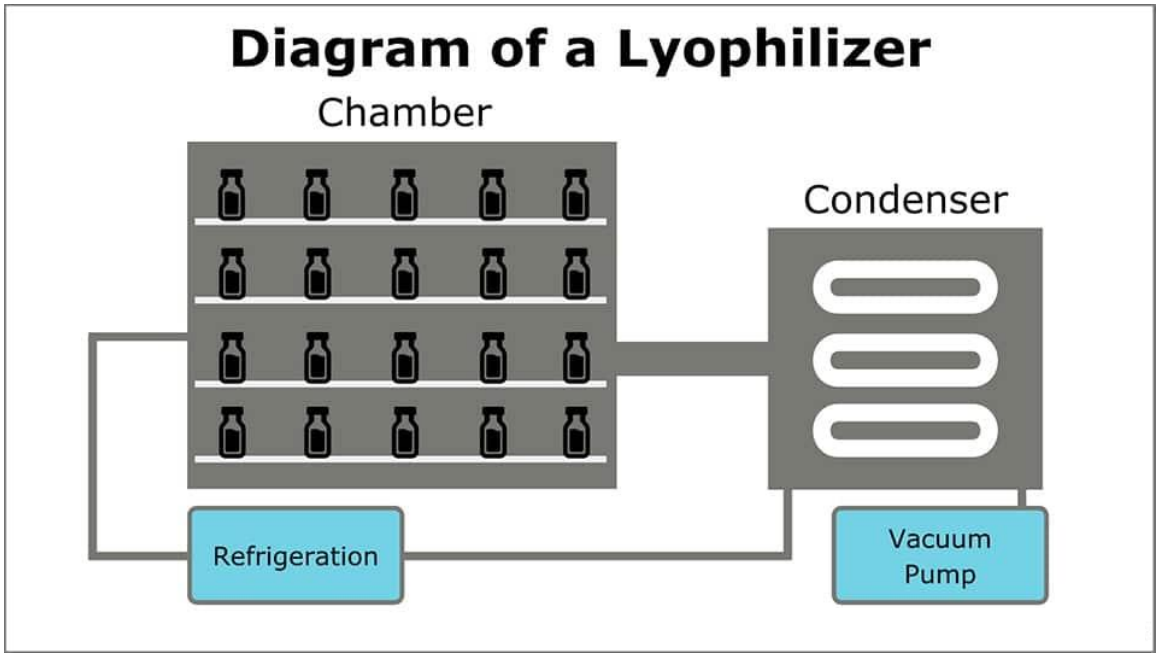
## 2. Lyophilization (Freeze-Drying)

Lyophilization involves removing water from the microorganism preparation, leaving a dry, powdery residue. This process is also highly effective for long-term preservation.

- **Process:** The microorganism culture is first frozen and then placed under a vacuum. The vacuum causes the frozen water to sublimate (turn directly from a solid to a gas), leaving the dry material behind. This process is often done in the presence of a cryoprotectant or a stabilizing agent, like a sugar.

- **Advantage:** The resulting material is a stable powder that can be stored at lower but less extreme temperatures (like 4°C or -20°C) and can be easily transported without the need for liquid nitrogen.

A **lyophilizer** is a machine used for **freeze-drying** microorganisms, vaccines, enzymes, and other biological materials. It removes water by sublimation (ice → vapor, without becoming liquid), preserving the material for long-term storage.




- **Freezing** – Product is cooled to very low temperature in the chamber.
- **Primary Drying (Sublimation)** – Under vacuum, ice in the product turns directly into vapor.
- **Secondary Drying (Desorption)** – Remaining bound water molecules are removed by slightly warming the sample.

3. **Deep Freezing (−20°C to −80°C):**

- Less stable than liquid nitrogen but still useful for medium-term storage.

4. **Periodic Transfer (subculturing at intervals):**

- Rarely used today because it increases risk of mutations and contamination.

### 3.6.2 Preparation Steps for Preservation:

1. **Harvesting**: Collect microorganisms from late log or early stationary phase cultures for optimal resistance to freezing.
2. **Cryoprotectants**: Add agents like glycerol (5-10%) or dimethyl sulfoxide (DMSO) to protect cell membranes. Glycerol is less toxic for most microbes, while DMSO penetrates larger cells better.
3. **Equilibration**: Allow 15-60 minutes at ambient temperature for the cryoprotectant to penetrate cells, avoiding prolonged exposure to prevent toxicity.
4. **Aliquoting and Storage**: Distribute into sterile vials or ampoules, label with codes (e.g., strain, passage number, batch), and store in monitored freezers with stabilizers.

Storage conditions are strictly controlled: e.g., -86°C freezers with daily temperature monitoring (range: -75°C to -85°C), humidity control, and uninterruptible power supplies. Backup storage and off-site reserves are recommended for disaster recovery.

### 3.6.3 Monitoring of Microorganisms in Seed Lots:

Monitoring ensures seed lots remain safe, potent, and free from contaminants throughout their lifecycle, from establishment to use in production. This involves regular testing for identity, purity, viability, and stability, as per WHO GMP and FDA guidelines. No other infectious materials should be handled in the same area during seed lot preparation to avoid cross-contamination.

Monitoring activities include:

1. **Identity Testing:**
   - ❖ Confirm strain identity using phenotypic tests, biochemical assays, PCR, or sequencing.
2. **Purity Testing:**
   - ❖ Check for contamination (bacterial, fungal, mycoplasma, viral).
   - ❖ Culture-based methods + molecular methods.

3. **Viability Testing:**
    - ❖ Assess recovery and growth after thawing or rehydration.
4. **Genetic Stability Testing:**
    - ❖ Verify that no mutations, plasmid loss, or antigenic drift has occurred.
    - ❖ Techniques: restriction analysis, sequencing, protein profiling.
5. **Potency/Functionality Testing:**
    - ❖ Confirm that the microorganism retains its intended biological activity (e.g., antigen expression, virulence attenuation, metabolic function).
6. **Environmental Monitoring (supportive):**
    - ❖ Ensure storage facilities (freezers, liquid nitrogen tanks) are properly maintained with alarms, temperature logging, and backup systems.

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