Cell Culture Systems

Course 5: Contamination

Assoc. Prof. Dr. Emrah Şefik Abamor

What is Contamination?

- Contamination of cell cultures is easily the most common problem encountered in cell culture laboratories, sometimes with very serious consequences. Cell culture contaminants can be divided into two main categories,
- **Chemical contamination**
- **Biological contamination**

Chemical Contamination

- Chemical contaminants such as impurities in media, sera, and water, including **endotoxins, plasticizers, and detergents**

Biological Contamination

- Biological contaminants such as **bacteria, molds, yeasts, viruses, mycoplasma**, as well as cross-contamination by other cell lines.

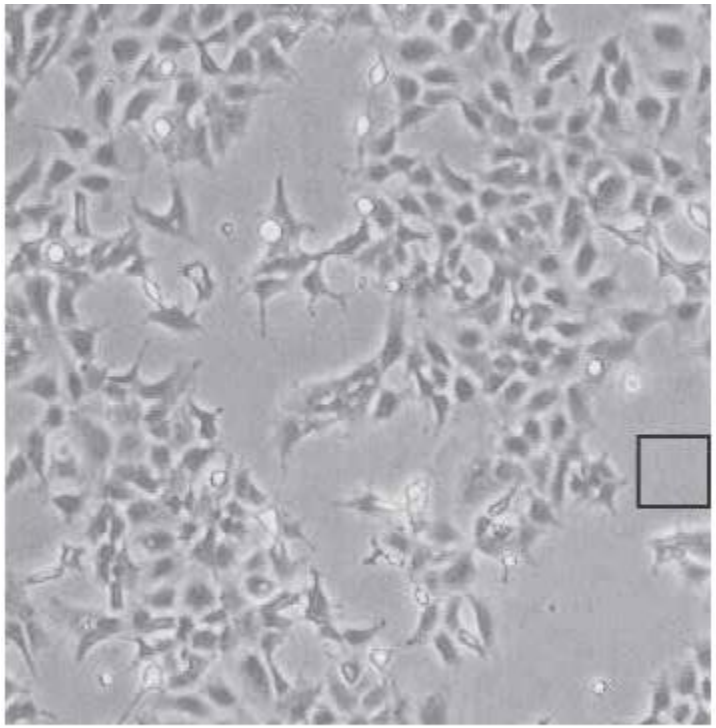
- While it is impossible to eliminate contamination entirely, it is possible to reduce its frequency and seriousness by gaining a thorough understanding of their sources and by following good aseptic technique.
- This section provides an overview of major types of biological contamination.

Bacteria

- Bacteria are a large and ubiquitous group of unicellular microorganisms.
- They are typically a few micrometers in diameter, and can have a variety of shapes, ranging from spheres to rods and spirals.
- Because of their ubiquity, size, and fast growth rates, bacteria, along with yeasts and molds, are the most commonly encountered biological contaminants in cell culture.

- Bacterial contamination is easily detected by visual inspection of the culture within a few days of it becoming infected.
- Infected cultures usually appear cloudy, sometimes with a thin film on the surface.
- Sudden drops in the pH of the culture medium are also frequently encountered.

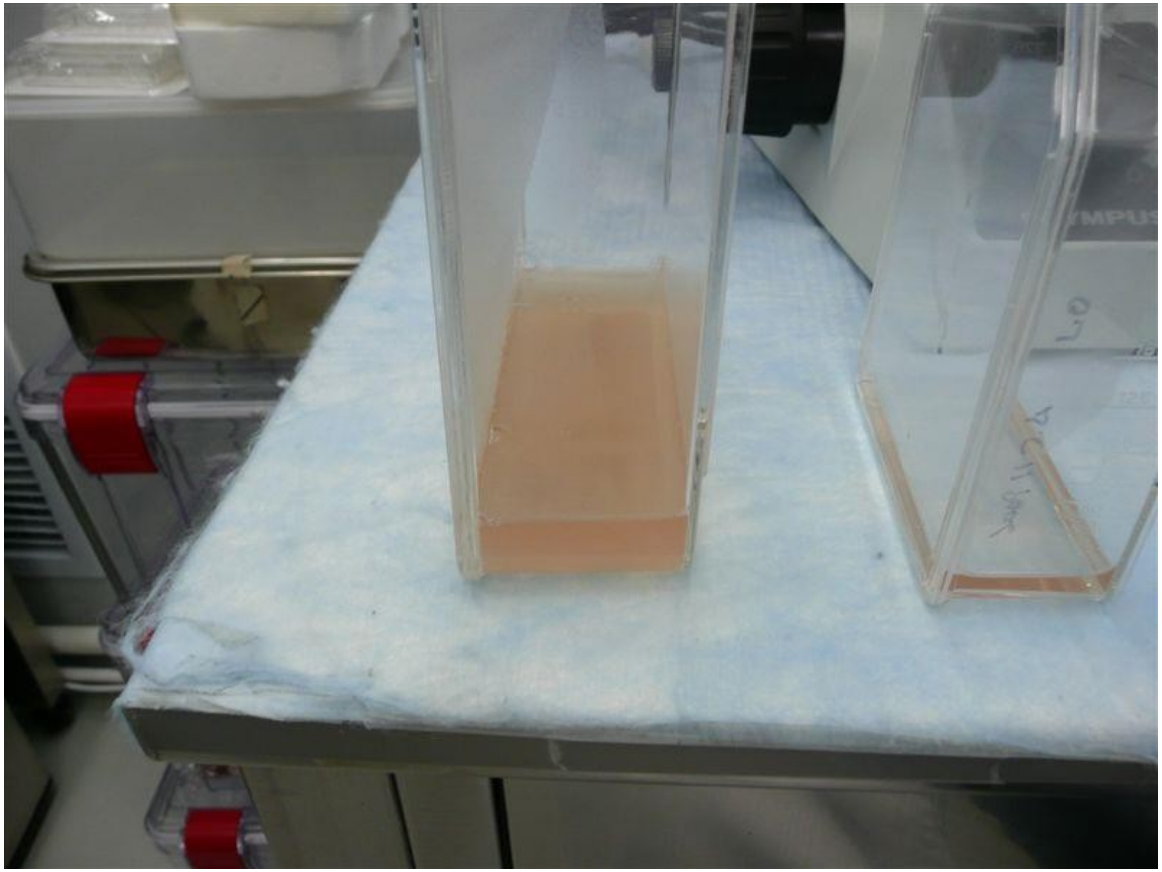
Bacterial Contamination in Culture

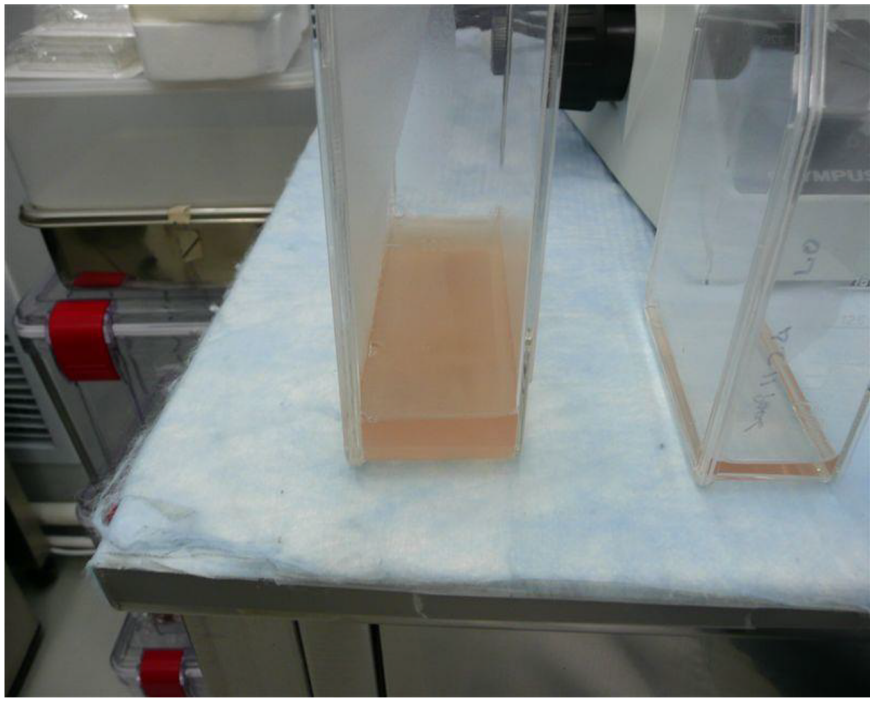


A

B

Bacterial Contamination in Culture

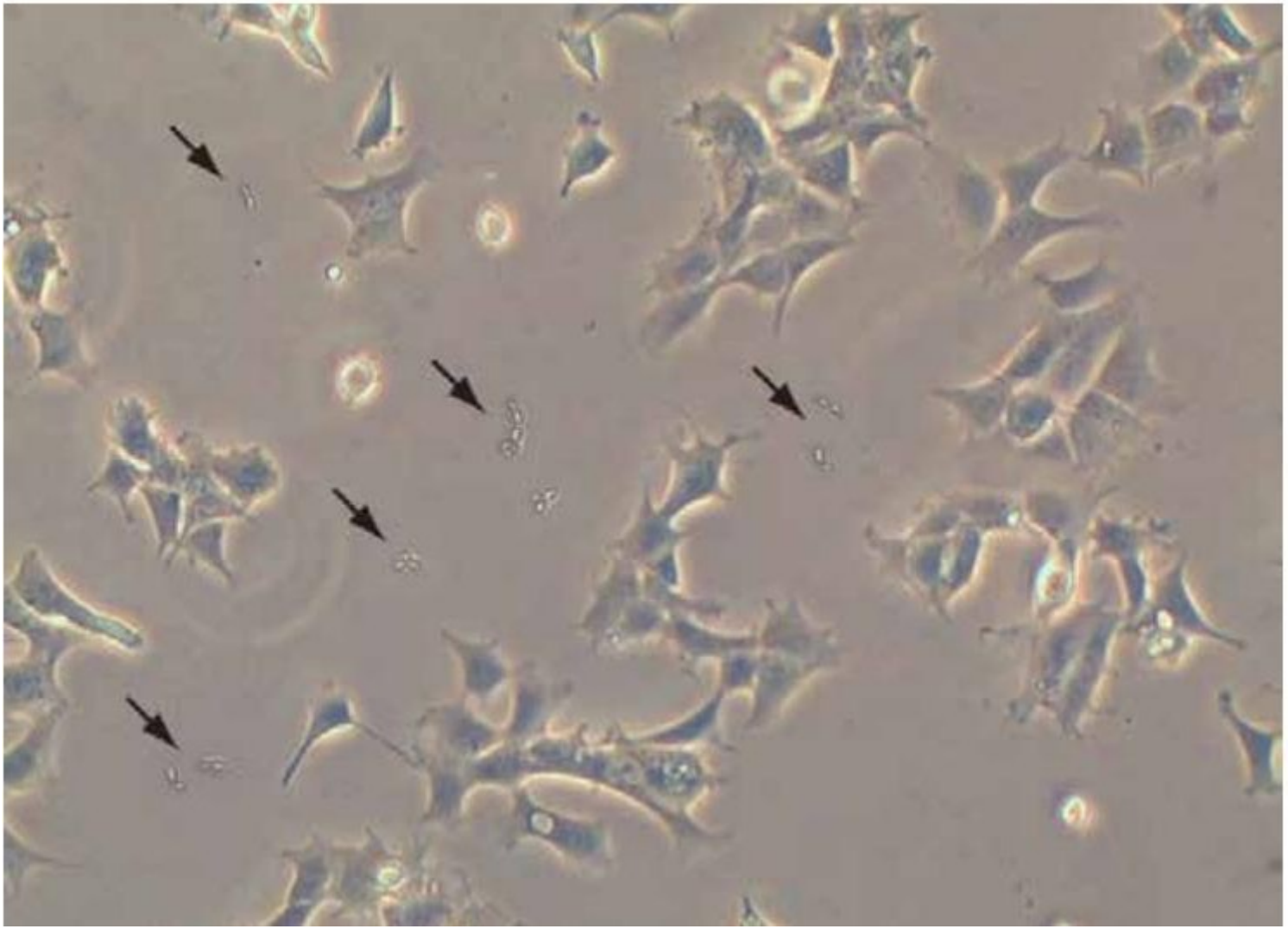




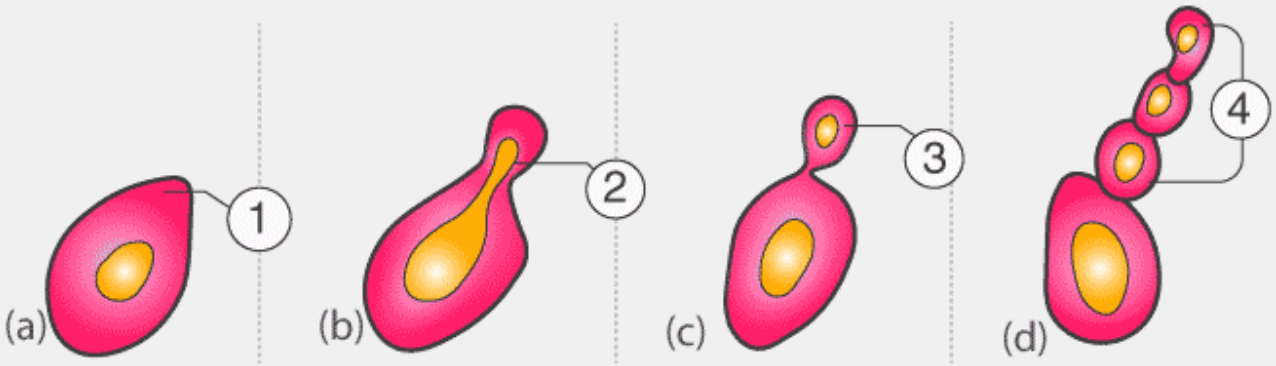
Culture media contaminated with bacteria (left) appear turbid and yellow whereas non-contaminated media (right) appear clear and red.

Yeasts

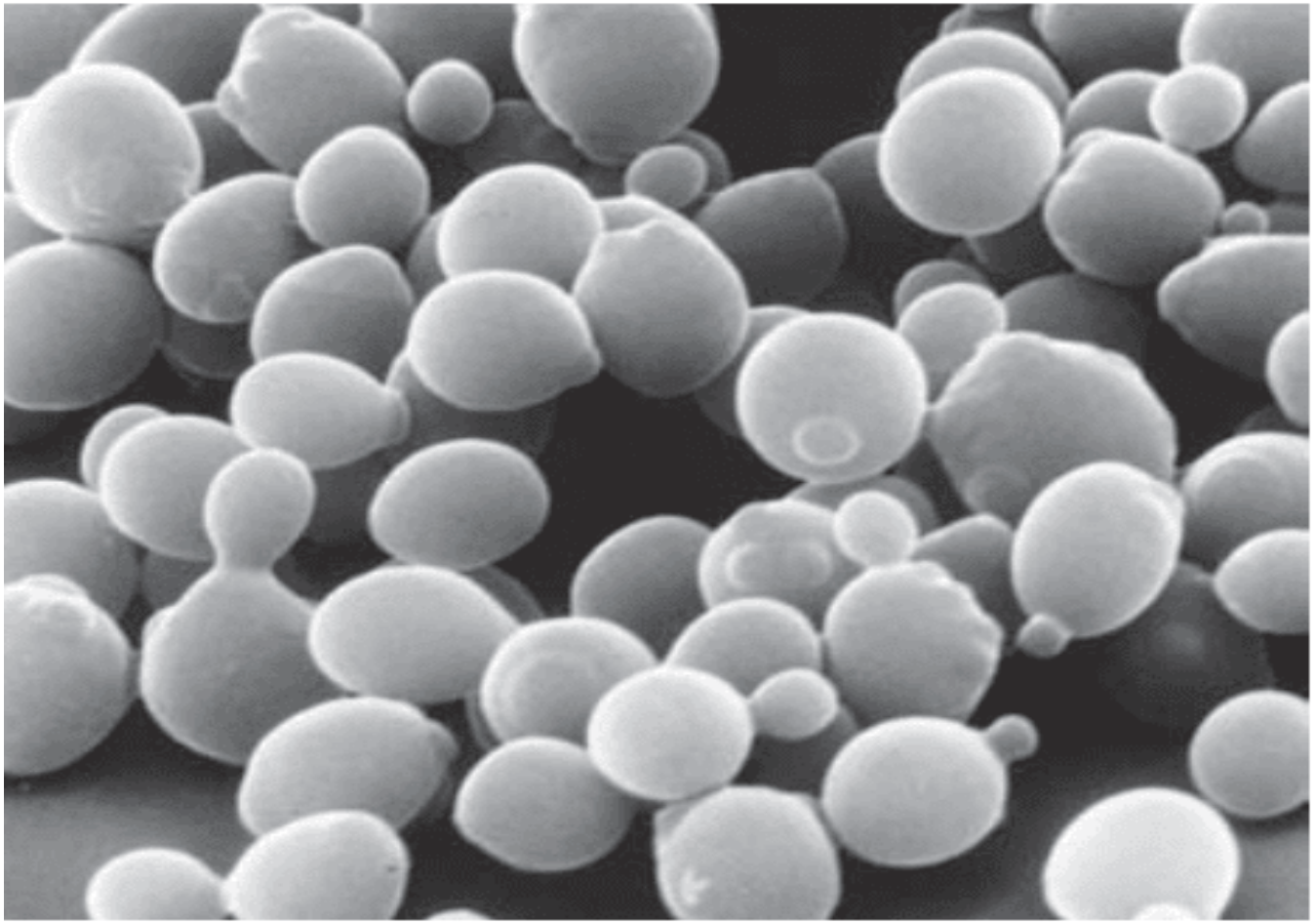
- Yeasts are unicellular eukaryotic microorganisms in the kingdom of Fungi, ranging in size from a few micrometers (typically) up to 40 micrometers (rarely).
- Like bacterial contamination, **cultures contaminated with yeast become turbid,** especially if the contamination is in an advanced stage.
- There is very little change in the pH of the culture contaminated by yeast until the contamination becomes heavy, **at which stage the pH usually increases.**



REPRODUCTION IN YEAST BY BUDDING



1 Yeast Cell | 2 Developing Bud | 3 New Bud | 4 Chain of buds


10 µm

Molds

- Molds are eukaryotic microorganisms in the kingdom of Fungi that grow as multicellular filaments called **hyphae.** A connected network of these multicellular filaments contain genetically identical nuclei, and are referred to as a colony or **mycelium.**

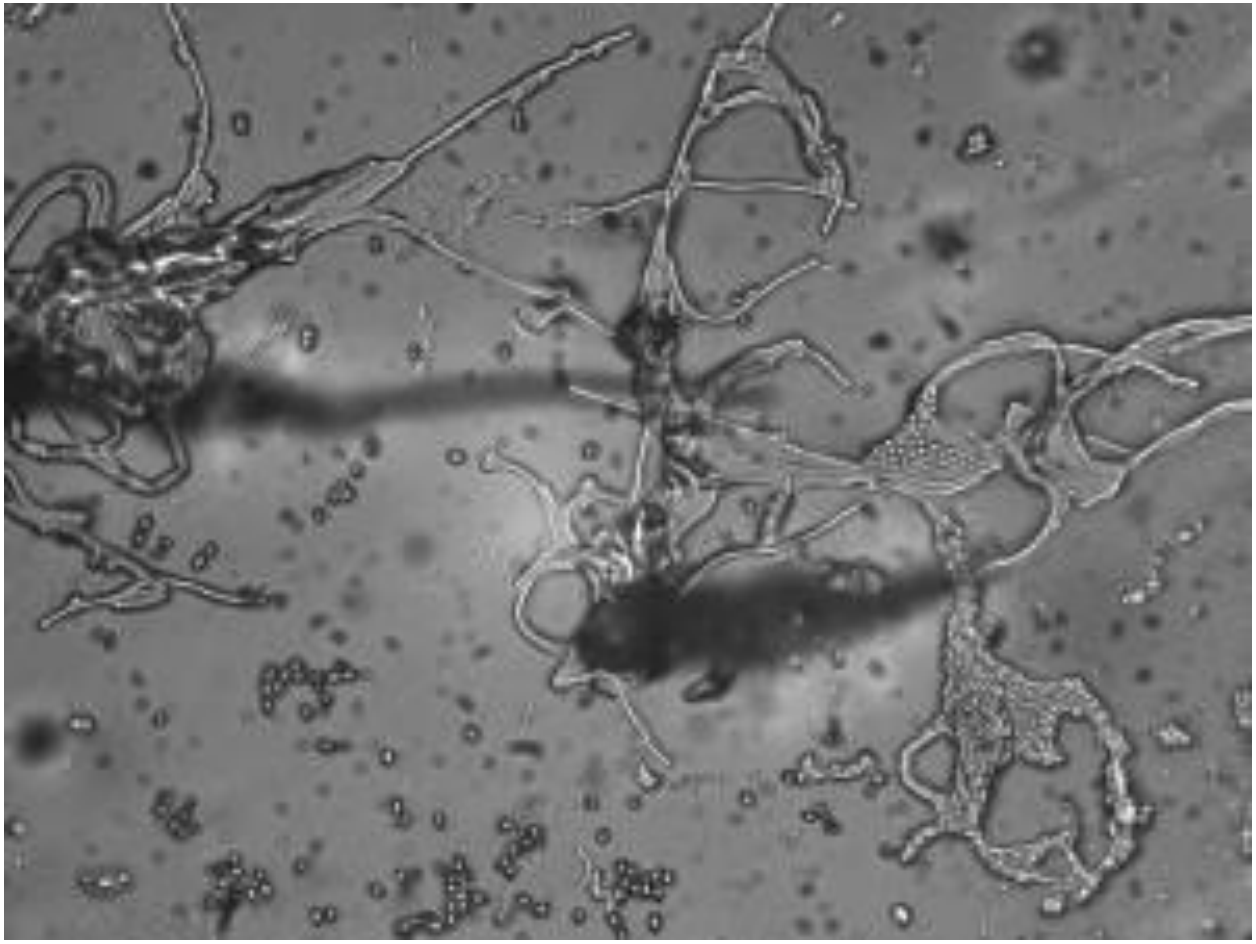
Molds

- Similar to yeast contamination, the pH of the culture remains stable in the initial stages of contamination, then **rapidly increases as the culture becomes more heavily infected and becomes turbid.**

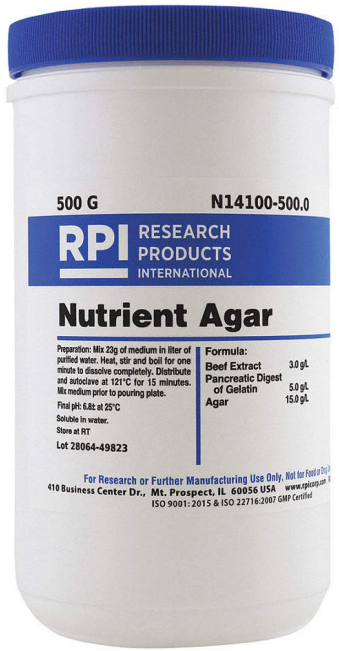
Molds

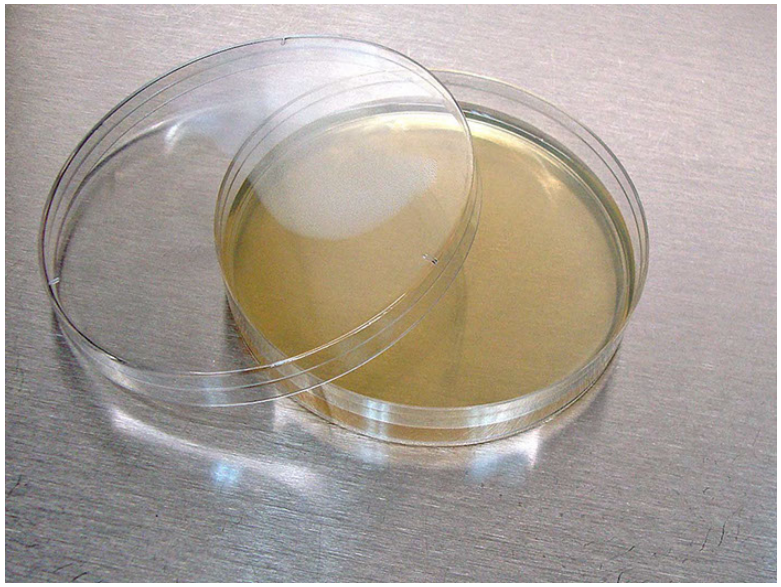
- Under microscopy, the mycelia usually appear as thin, wisp-like filaments, and sometimes as denser clumps of spores.
- Spores of many mold species can survive extremely harsh and inhospitable environments in their dormant stage, only to **become activated when they encounter suitable growth conditions.**

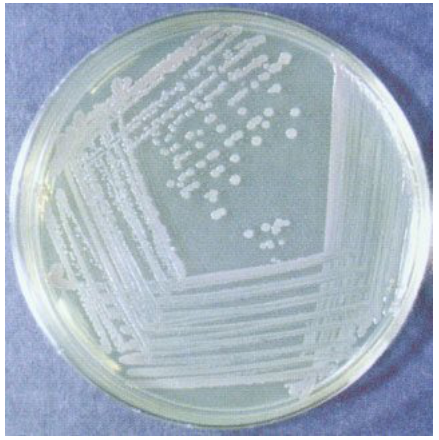
Mold Contamination



Contamination Control







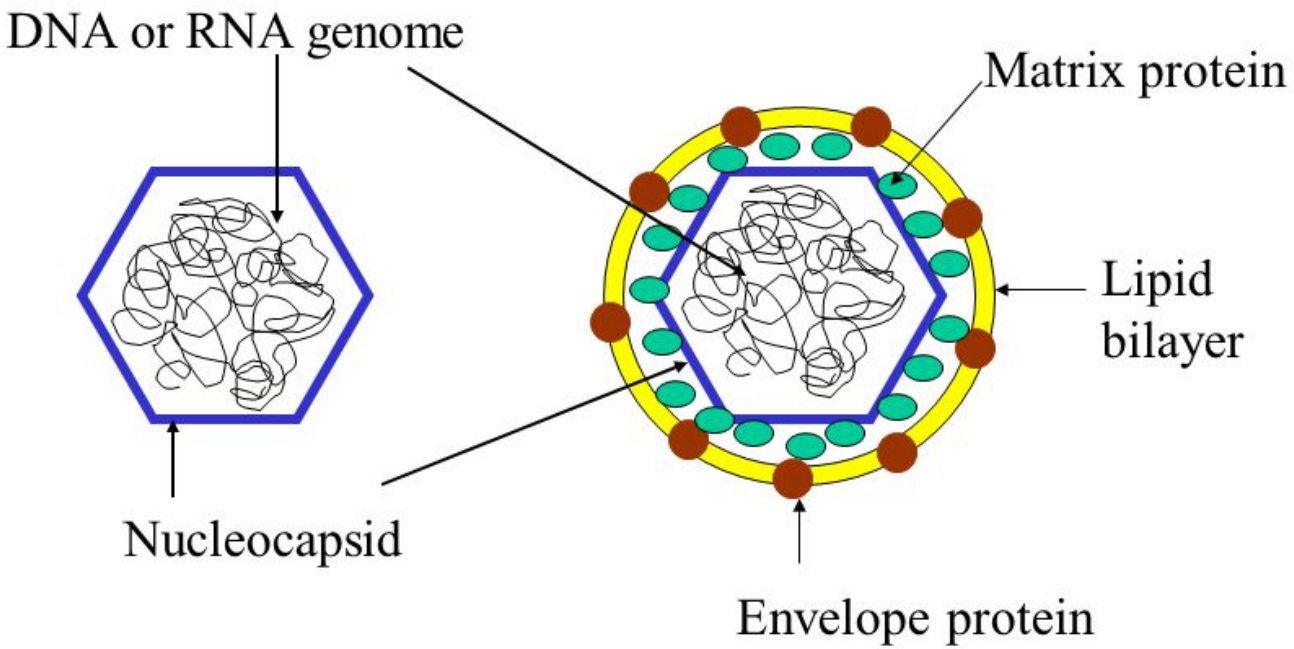
Contamination Control

Viruses

- Viruses are microscopic infectious agents that take over the host cell's machinery to reproduce.
- Their extremely small size makes them very difficult to detect in culture, and to remove them from reagents used in cell culture laboratories.
- Because most viruses have very stringent requirements for their host, they usually do not adversely affect cell cultures from species other than their host.

Enveloped and non-enveloped viruses

- Non-enveloped virus
- Enveloped virus



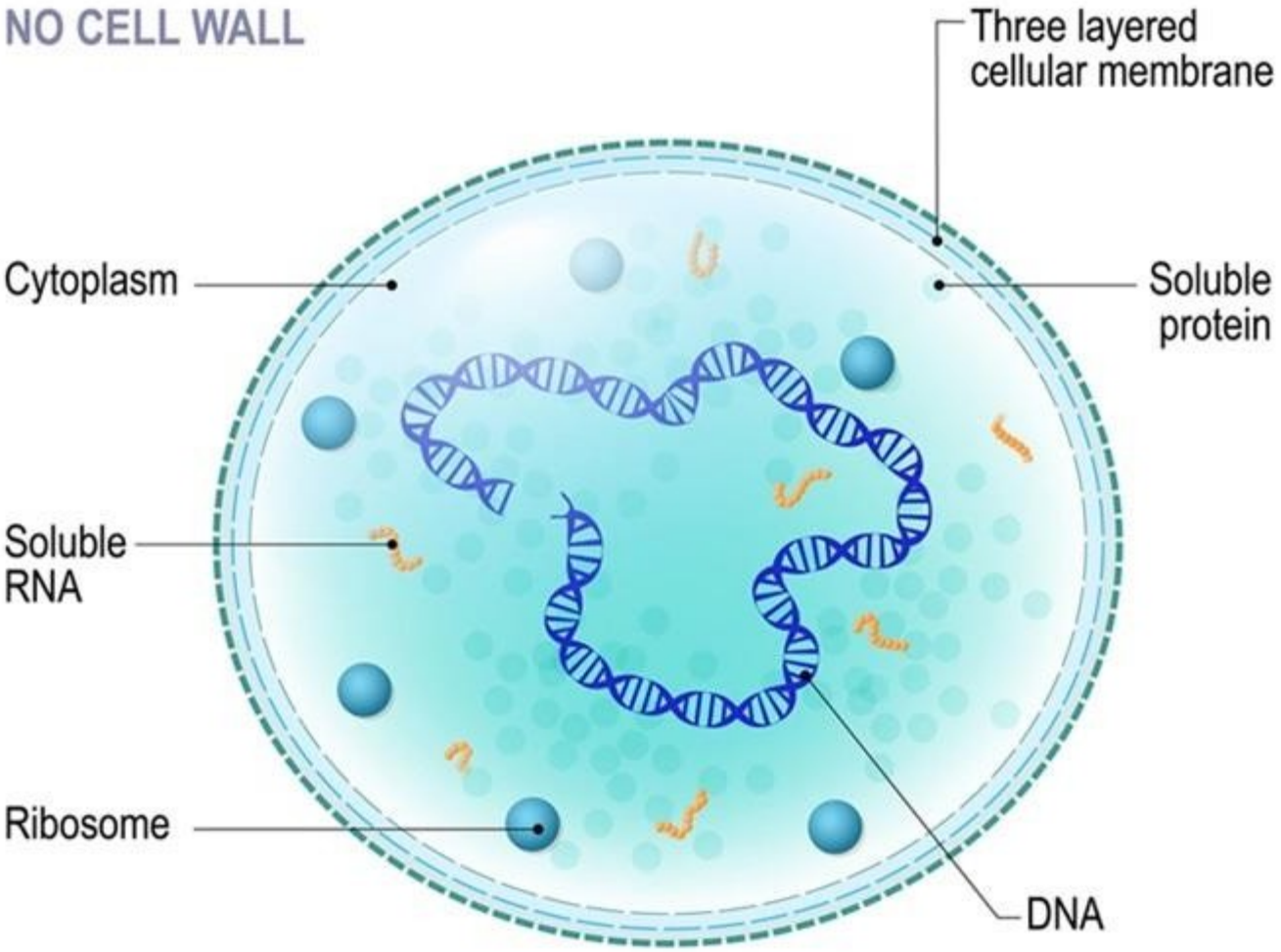
Viruses

- However, using virally infected cell cultures can present a serious health hazard to the laboratory personnel, especially if human or primate cells are cultured in the laboratory.
- Viral infection of cell cultures can be detected by **electron microscopy, immunostaining with a panel of antibodies, ELISA assays, or PCR with appropriate viral primers.**

Mycoplasma

- **Mycoplasma are simple bacteria that lack a cell wall, and are considered the smallest self-replicating organism.**
- Because of their extremely small size (typically less than one micrometer), mycoplasma are very difficult to detect until they achieve extremely high densities and cause the cell culture to deteriorate; until then, there are **often no visible signs of infection.**

Mycoplasma


NO CELL WALL
Three layered cellular membrane
Cytoplasm
Soluble protein
Soluble RNA
Ribosome
DNA

Mycoplasma

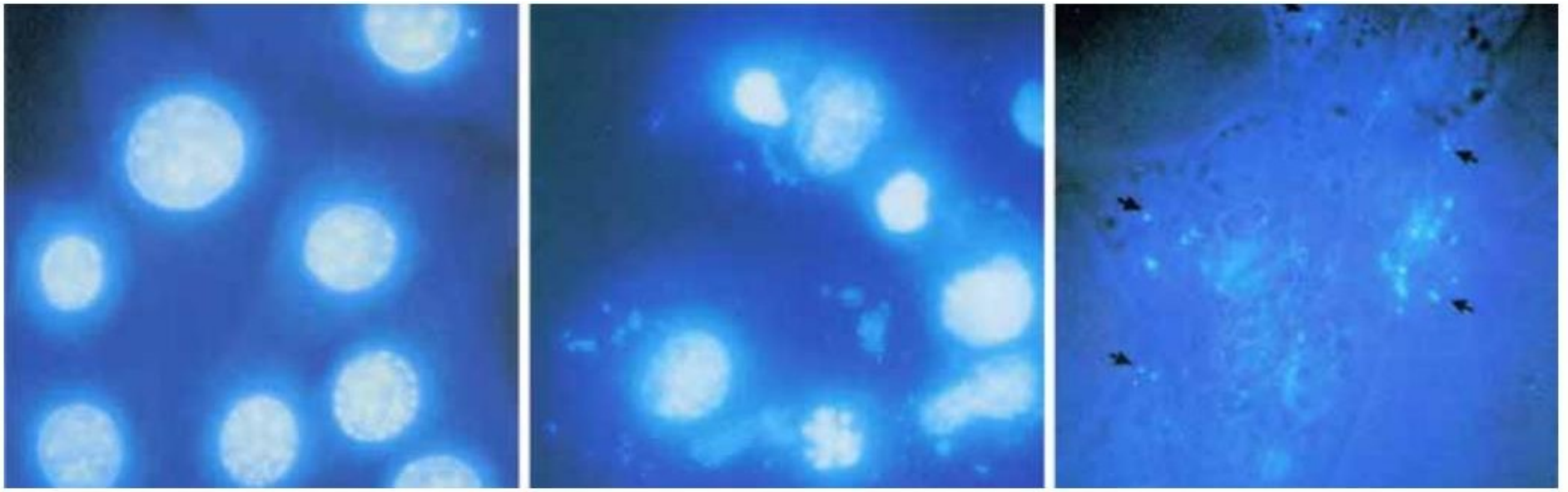
- The effects of mycoplasma infection are more insidious than those of bacteria and fungi, inducing several long term effects in cell cultures. These include:
- **Altered growth rate**
- **Morphological changes**
- **Chromosome aberrations**
- **Alterations in amino acid and nucleic acid metabolism**

Mycoplasma

- Some slow-growing mycoplasma may persist in culture without causing cell death, but they can alter the behavior and metabolism of the host cells in the culture.
- Chronic mycoplasma infections might manifest themselves with decreased rates of cell proliferation, reduced saturation density, and agglutination in suspension cultures

Mycoplasma

- However, the only assured way of detecting mycoplasma contamination is by testing the cultures periodically using fluorescent staining, ELISA, PCR, immunostaining, autoradiography, or microbiological assays.



A B C

Cross Contamination

- While not as common as microbial contamination, extensive cross-contamination of many cell lines with HeLa and other fast-growing cell lines is a clearly established problem with serious consequences.

- Obtaining cell lines from reputable cell banks, periodically checking the characteristics of the cell lines, and practicing good aseptic technique are practices that will help you avoid cross-contamination.
- DNA fingerprinting, karyotype analysis, and isotype analysis can confirm the presence or absence of cross-contamination in your cell cultures.

Antibiotic Usage

- Antibiotics should never be used routinely in cell culture, because their continuous use encourages the development of **antibiotic-resistant strains**
- allows low-level contamination to persist, which can develop into full-scale contamination once the antibiotic is removed from media, and
- may hide mycoplasma infections and other cryptic contaminants.
- Further, some antibiotics might cross-react with the cells and interfere with the cellular processes under investigation.

Aseptic Technique

- Aseptic Technique
  - ✓Refers to a procedure that is performed under sterile conditions.
  - ✓This includes medical and laboratory techniques, such as with microbiological cultures.

How can microorganisms be killed?

1. Denaturation of proteins (e.g. wet heat, ethylene oxide)
2. Oxidation (e.g. dry heat, hydrogen peroxide)
3. Filtration
4. Interruption of DNA synthesis/repair (e.g. radiation)
5. Interference with protein synthesis (e.g. bleach)
6. Disruption of cell membranes (e.g. phenols)

Factors that influence efficacy of disinfection/sterilization

1. Contact time
2. Physico-chemical environment (e.g. pH)
3. Presence of organic material
4. Temperature
5. Type of microorganism
6. Number of microorganisms
7. Material composition

Some terms

- **Sterilization:**

  It is the process by which article, surface or medium is made free from all microorganisms either in the vegetative or spore state.

- **Disinfection:**

  It is the process by which an article, surface or medium is made free from all pathogenic microorganisms (that is organisms that are capable of giving rise to infection).

Antiseptics :

Antiseptics :

- Chemical disinfectants which can safely applied to living tissues and are used to prevent infection by inhibiting the growth of microorganisms.

Asepsis :

- Technique by which the occurrence of infection into an uninfected tissue is prevented.

Classification

There are two types of sterilization: physical and chemical.

1. Physical sterilization includes:

- heat
- radiation
- filtration

2. Chemical sterilization includes:

- Alcohols
- Aldehydes
- Phenolics
- Oxidizing agents
- Quaternary ammonium compounds
- ethylene oxide gas
- Others

METHOD OF STERILIZATION

* THREE METHOD :-

1. Physical method
   a)Dry heat sterilization
   b)Moist heat sterilization
   c)Sterilization by radiation (gamma radiation)

2. Chemical method
   a) Gaseous sterilization
   b) Sterilization by disinfectant

3. Mechanical method
   Pass through bacteria-proof filter

DRY HEAT STERILIZATION

- Instrument- 'OVEN'

OVEN : -

specially designed instrument - electrically heated and thermostatically controlled.

Expose at 160 ºC for 1 hour.

Advantage-

it is suitable method for sterilization of substances destroyed by moisture.

Disadvantage-

long heating time, high temperature.

oven

Hot air oven:

- Most widely used method
- Electrically heated and fitted with a fan to even distribution of air in the chamber.
- Fitted with a thermostat that maintains the chamber air at a chosen temperature.
- Temperature and time:
  - » 160 C for 2 hours.
  - » 170 C for 1 hour
  - » 180 C for 30 minutes.

Uses of Hot Air Oven

- **Sterilisation of**

1. Glassware like glass syringes, petri dishes, pipettes and test tubes.
2. Surgical instruments like scalpels, scissors, forceps etc.
3. Chemicals like liquid paraffin, fats etc.

– Precautions :

1. Should not be overloaded
2. Arranged in a manner which allows free circulation of air
3. Material to be sterilized should be perfectly dry.
4. Test tubes, flasks etc. should be fitted with cotton plugs.
5. petridishes and pipetts should be wrapped in paper.
6. Rubber materials and inflammable materials should not be kept inside.
7. The oven must be allowed to cool for two hours before opening, since glass ware may crack by sudden cooling.

MOIST HEAT STERLIZATION

- Instrument- 'AUTOCLAVE'

Heating process in autoclave - saturated steam under pressure is allowed to penetrated through materials for 15 minutes and temperature 121° c.

Advantage-

micro organism are killed most efficiency in lesser time due to high pressured saturated steam

Disadvantage-

unsuitable for materials not withstanding temperature of 115°C or more during heating

AUTOCLAVE

- Steam sterilisation is usually carried out in a metal vessel known as autoclave, which can be filled with steam at a pressure greater than atmospheric pressure. Sterilisation can thus be achieved at temperatures considerably above the boiling point of water; laboratory autoclaves are commonly operated at steam pressure of 15 lb/in^2, above atmospheric pressure, which corresponds to a temperature of 120°C.

Temperatures above 100°C

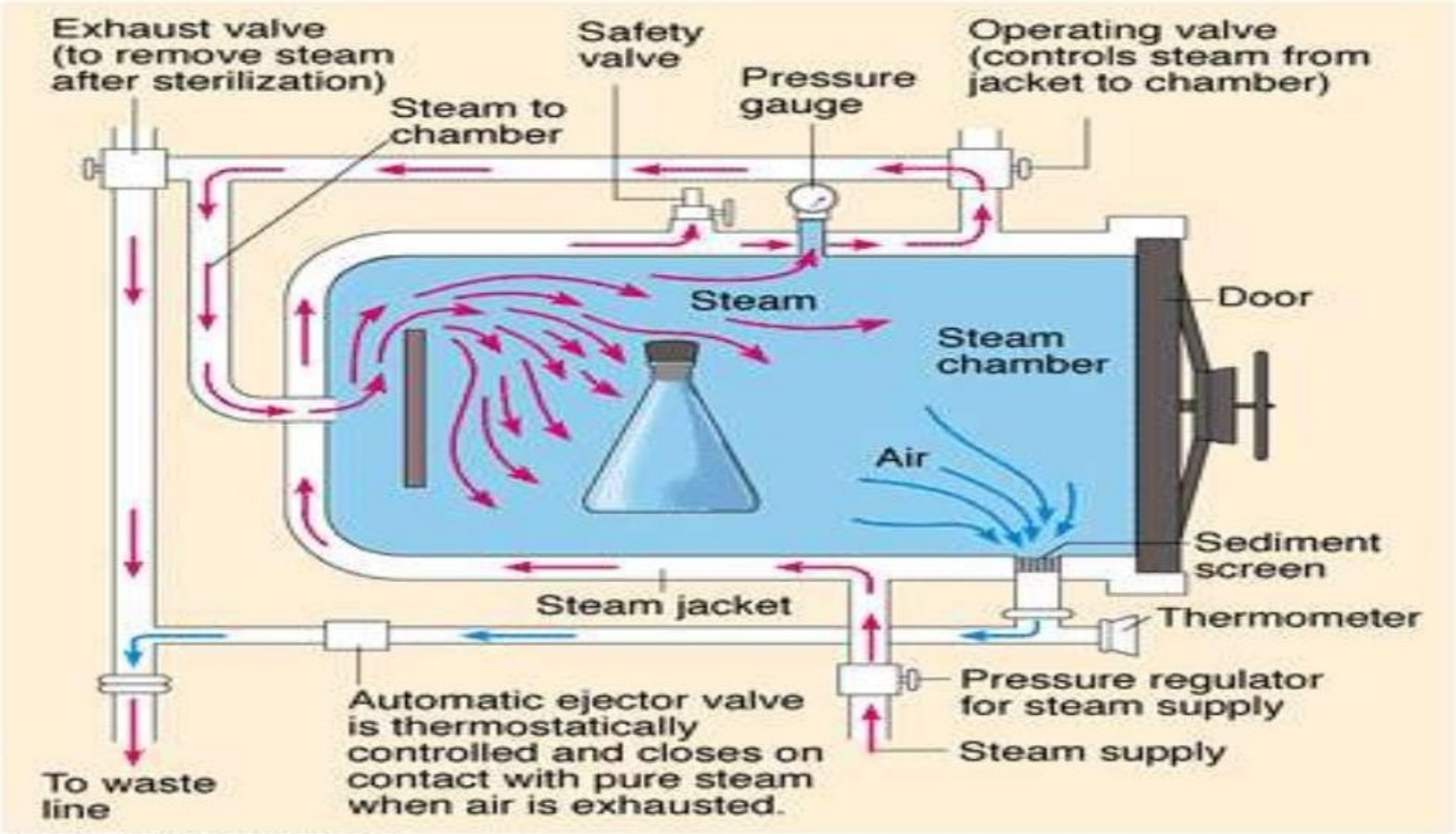
III. A temperature above 100°C

Autoclave :

-Steam above 100°C has a better killing power than dry heat.

-Bacteria are more susceptible to moist heat.

Autoclave: Closed Chamber with High Temperature and Pressure



Uses of Autoclaves:

- **Uses :**
  1. Useful for materials which can not withstand high temp.
  2. To sterilize culture media, rubber material, gowns, dressings, gloves etc.

Radiations :

- **Radiations :**
  - Ionizing radiations
  - Non - Ionizing radiations

I- Non-ionizing rays :

- Non-ionizing ultra violet rays are low energy rays with poor penetrative power.
- **Mechanism of action:**

1- UV rays inhibits DNA replication.

- *UV rays have **bactericidal** effect but don't kill spores hence they are not efficient for complete sterilization.*
- *They can be used in surface disinfection.*

I- Non-ionizing rays :

- Non-ionizing ultra violet rays are low energy rays with poor penetrative power.
- **Mechanism of action:**

1- UV rays inhibits DNA replication.

- *UV rays have **bactericidal** effect but don't kill spores hence they are not efficient for complete sterilization.*
- *They can be used in surface disinfection.*

- **Radiation**
  - Nonionizing radiation
    - Wavelengths greater than 1 nm
    - Excites electrons, causing them to make new covalent bonds
      - Affects 3-D structure of proteins and nucleic acids
    - UV light causes pyrimidine dimers in DNA
    - UV light does not penetrate well
    - Suitable for disinfecting air, transparent fluids, and surfaces of objects

II- Ionizing rays:

- Ionizing rays are high-energy rays with good penetrative power .
- These high-energy radiations damage the **nucleic acid** of the microorganism which kill all bacteria, fungi, viruses and spores.
- Gamma rays are mainly used for sterilization of prepacked disposable plastic items that can't withstand heat e.g. plastic syringes, catheters and gloves, and for sterilization of antibiotics, vitamins, hormones, glasswares and fabrics.
- **Examples** : X- rays, Gamma rays.

Filtration:

- **. Filtration:**
  - Useful for substances which get damaged by heat.
  - To sterilize sera, sugars and antibiotic solutions.
  - To obtain bacteria free filtrates of clinical samples.
  - Purification of water.

Filtration Sterilization :

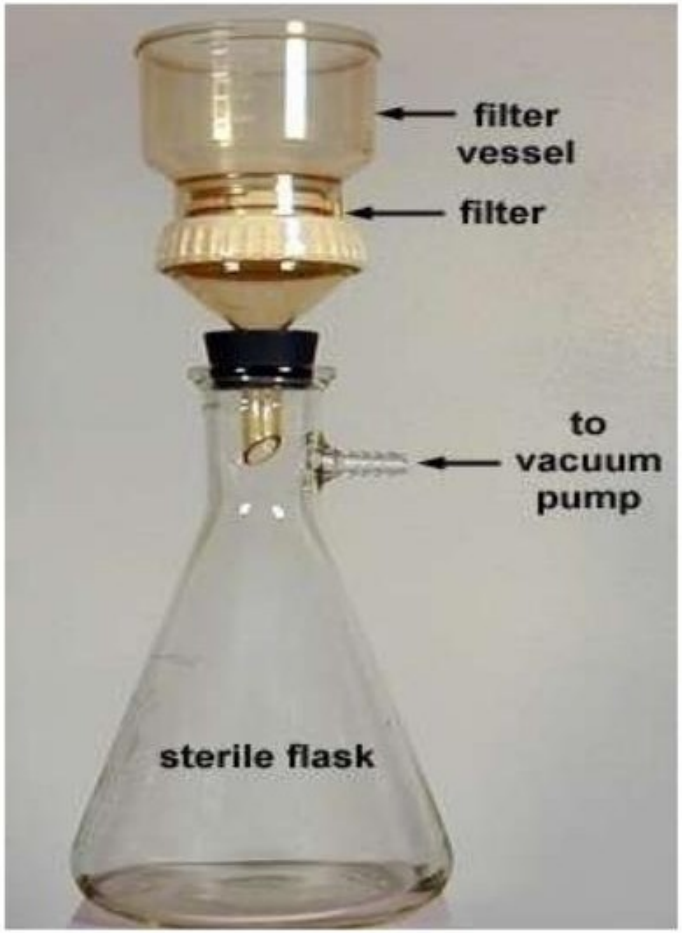
- Filtration process does not destroy but removes the microorganisms.
- Used in the treatment of heat sensitive injections and ophthalmic solutions, biological products, air and other gases for supply to aseptic areas.
- Application of filtration for sterilization of gases: **HEPA (High efficiency particulate air)** used in Laminar air flow cabinets.

Several Types of Filters

- Types of filters:
  1. Candle filters
  2. Asbestos disc filters
  3. Sintered glass filters
  4. Membrane filters
  5. Air filters
  6. Syringe filters

Filtration

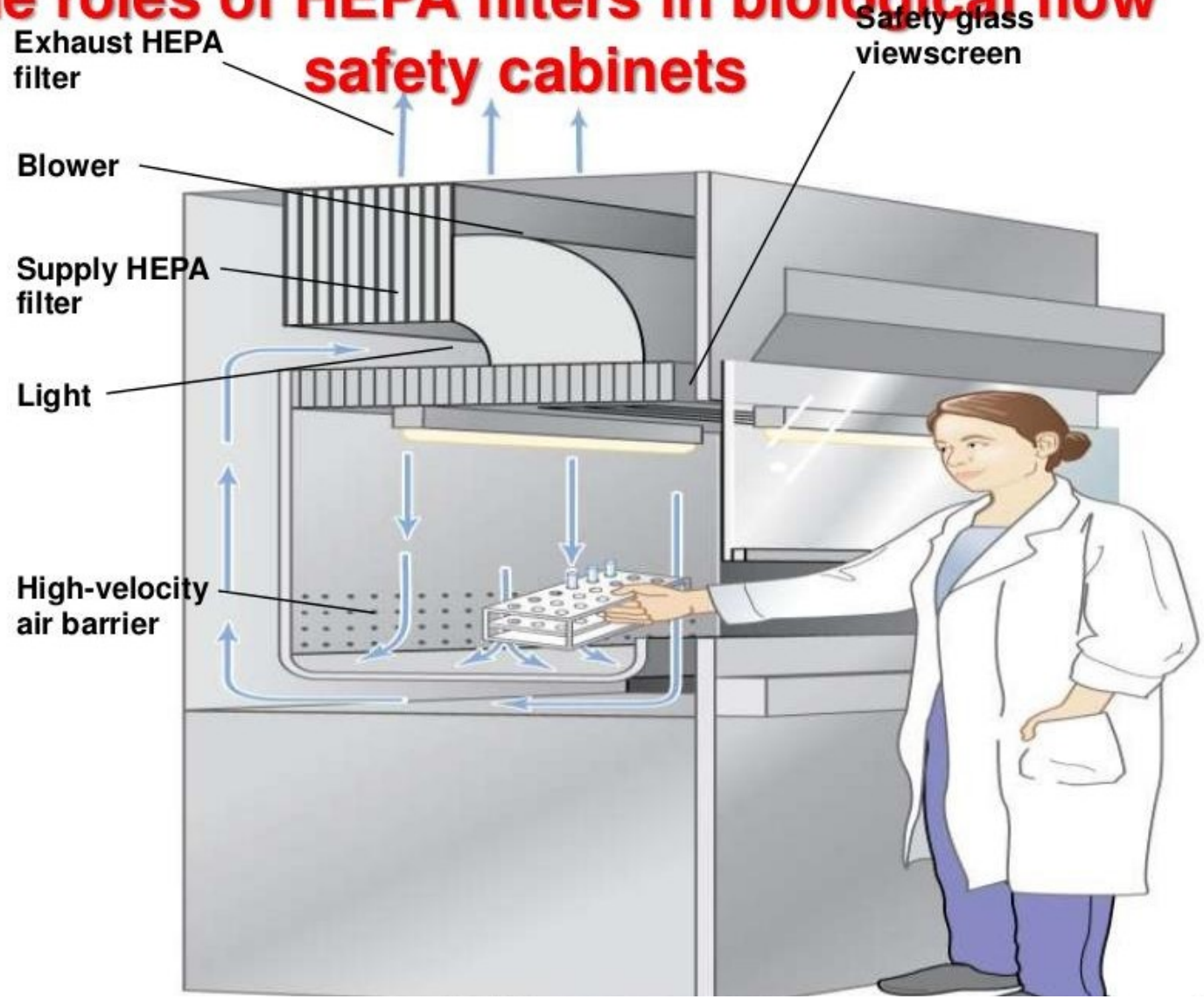
Sterilize solutions that may be damaged or denatured by high temperatures or chemical agents.



The filtering Depends on Pore Size

The pore size for filtering bacteria, yeasts, and fungi is in the range of 0.22-0.45 μm (filtration membranes are most popular for this purpose).

The roles of HEPA filters in biological flow safety cabinets



Sterilization by filters:

- The use of filters for sterilization of liquids often becomes necessary, since the constituents of these liquids may get destroyed at higher temperatures (dry heat or moist heat).
- Sterile filtration is a novel technique for heat- labile solutions. The size of micropores of the filters is 0.1-0.2 µm.
- Filters, made from several materials are in use. These materials include **nylon, cellulose acetate, cellulose nitrate, polycarbonate, polyethersulfone (PES) and ceramics.**

TABLE 33.2 Sterilization of major equipment, apparatus and liquids used in tissue culture

Sterilization device	*Items sterilized*
I *For equipment and apparatus*	
Dry heat	Glass slides
	Pipettes
	Ampoules (glass)
	Pasteur pipettes
	Instruments
	Test tubes
Autoclave	Ampoules (plastic)
	Apparatus with silicone tubing
	Filters (reusable)
	Glass bottles with screw caps
	Glass syringes
	Magnetic stirrer bases
	Screw caps
	Stoppers (rubber silicone)
II *For liquids and nutrients*	
Autoclave	Salt solutions
	Glucose–20%
	Agar
	Bacto–peptone
	Glycerol
	Lactalbumin hydrolysate
	Phenol red
	Tryptose
	HEPES
	EDTA
	Water
Filter	Serum
	Amino acids
	Vitamins
	Antibiotics
	Bovine serum albumin
	Collagenase
	Glutamine
	Drugs
	NaOH
	Trypsin
	Transferrin

Personal Hygiene

- When entering the laboratory it is important to wash hands since this will remove dry skin and loosely adherent microorganisms which could potentially contaminate cell cultures.
- **Gloves should be frequently swabbed with 70% (v/v) sterile ethanol.**
- Other personal protective equipment includes head caps and face masks, but these are not always necessary, particularly when a class 2 microbiological safety cabinet is being used.
- Long hair should be tied back to remove obstruction and reduce the risk of contamination.

- When working within the cabinet the operator should remember that the air-flow does not make the environment sterile but keeps it clean.
- Before any practical procedure is conducted the cabinet should be stocked with all the materials required for the experiment.
- **In doing so the operator restricts the number of times that their hand/arm is removed from the cabinet into a non-clean environment**

- Each item within the cabinet should be positioned to minimise movement and traffic over the area where cell culture operations are performed. Both the rear and front of the cabinet should be cleared to achieve maximum airflow.

- Flasks and dishes should be the last items to enter the cabinet.
- All items that enter the cabinet must be sprayed with 70% (v/v) sterile ethanol to prevent dust and particulates from entering the cabinet.
- Twenty minutes should elapse before any tops or containers are opened to allow the airflow to purge the work area of particulates that may have been introduced.