Minimizing Contamination in Cell Culture

Overview

- Introduction
- Different types of contamination
- Sources of contamination
- Laboratory sanitation
  - Hygiene
  - Laboratory methods and skills

Contamination

- Contamination is a staple problem in cell culture
- All cell culture laboratories and workers have experienced contamination
- Affects both the use of cultures and research quality
- Most apparent consequences of contamination:
  - Time
  - Money
  - Effort
  - Erratic experimental results

Consequences of contamination

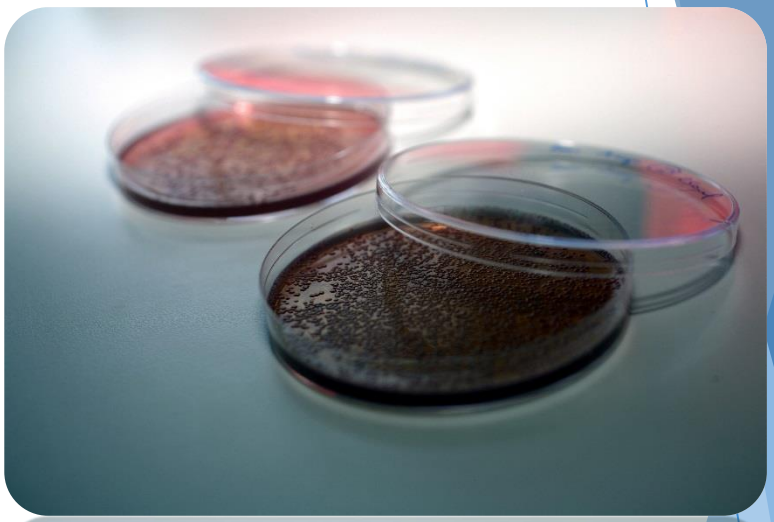
- Loss of resources
  - Time
  - Money
  - Effort

Consequences of contamination

- Reproducibility crisis
  - Mycoplasma contamination
    - very difficult to detect via ocular inspection
    - may lead to undetected contamination that may affect the current results
  - Cross-contamination
    - difficult to detect, unless PCR is done to confirm the identity of cells
    - Cross-contaminated or misidentified cells lead to erroneous and irreproducible results.

Types of Contamination

- Microbial Contamination
  - Fungi
  - Yeast
  - Bacteria
    - Mycoplasma
- Cross-contamination



Microbial Contamination

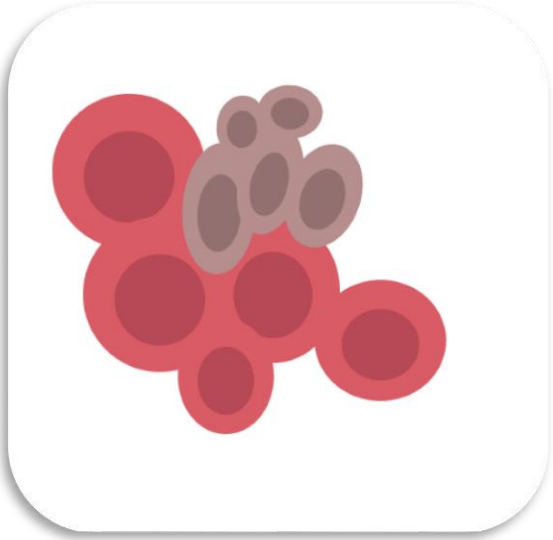
- Three most common microbial contaminants:
  - Fungi
  - Yeast
  - Bacteria
- Most common type of contamination
- Can be easily detected through microscopy by checking the medium:
  - Turbidity of the medium
  - Presence of particles in suspension
  - Rapid decline in pH (yellow color, indicates acidity)

Mycoplasma

- Smallest bacteria
- Lack cell wall
  - Resistant to common antibiotics
  - Parasitic
- Cannot be detected through microscopy
- Presence of mycoplasma must be checked through PCR
- Not benign in cell culture; can alter the host's cell function, growth, metabolism, etc.
- Can grow to high densities without any visible signs of contamination

Cross-contamination

- Cross-contamination of one cell line with another cell line
- Results from mishandling of cell lines during experiments and maintenance
- May lead to the replacement of the original cell with the contaminant, especially when the contaminant proliferates faster than the original cell line



Sources of Contamination

- Indirect sources of contamination
  - Laboratory
  - Equipment
  - Personnel
- Direct sources of contamination
  - Reagents
  - Cell lines and primary cells

Each possible source has a possible risk of transferring different types of contamination.

Laboratory

- Air-borne contaminants
  - Ventilation, doors, windows
- Water
  - Sink, water bath, incubator pan
    - Water microbes
    - Air-borne water microbes
- Dirt and dust
  - House germs

Personnel

- Potential transfer of contaminants
  - Mouth and nose
    - Mycoplasmas, bacteria, viruses
  - Clothes
    - Bacteria, fungi, yeast
  - Hands
    - Bacteria, fungi, yeast
    - Mycoplasma
  - Shoes
    - Bacteria, fungi, yeast

Reagents

- Culture medium is sterile when delivered
- FBS was one of the main sources of mycoplasma contamination
- Growth factors, peptides, antibodies could also be contam sources
- Secondary contamination may be present in aliquots prepared in the laboratory.

Cell lines

- Newly bought cell lines might be delivered contaminated
  - Mycoplasma and other microbes
  - Cross contamination
- Untested old cell lines might have contamination
  - Mycoplasma and other microbes
  - Cross contamination
- Primary cells may be contaminated from “semi-sterile” tissue preparation
  - Mycoplasma and other microbes

Laboratory sanitation

- Indirect sanitation
  - Laboratory and equipment
  - Personnel
- Direct sanitation
  - Reagents
  - Cell lines and primary cells

Cell culture facilities and labs must be sterile.

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Cell culture laboratory

- Dedicated air filtration system
  - Positive pressure to keep microbes out of the lab
- PPE
  - Lab gown, gloves, shoes
  - Gowning rooms
- High cleaning frequency
- Sufficient sanitizers and disinfectants

CO_2 Incubators

- Fast recovery time after door openings
- Sterile surface
- Minimize door openings
- Inner doors
  - Reduce entry of microbes
  - Keep pH, temperature, humidity constant

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Cleaning and Maintenance of CO_2 Incubators

- Decontaminate incubators at least once a month
- Clean the incubator once a month
  - Disassemble and clean the inner parts
  - Clean the inner walls
  - Rinse parts with distilled water
- Consider the use of copper chamber
  - Inhibits bacterial growth better than stainless steel

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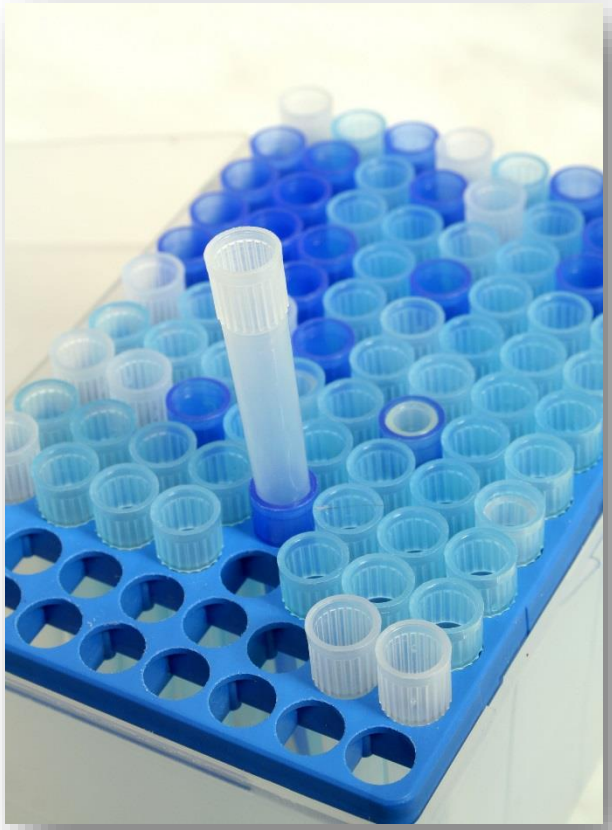
Humidity pan – CO2 Incubators

- Air-borne microbes might proliferate in the humidity pan
- Fan-less design can minimize the distribution of microbes in the chamber
- Autoclaved water must be used
- Antibiotics may be added to the water to inhibit germ growth

Water bath

- Used for thawing cells, warming of media and other reagents
- A significant hub of microbes
  - Ensure that the water bath has lid
  - Clean regularly – once a week
  - Use supplements such as antibiotics to prevent growth of germs
  - Disinfect flasks, tubes, bottles well after removal from water bath and before using for aseptic techniques

Pipettes and pipette tips



- Designate pipettes to be used in the cell culture lab only
- Wipe pipettes before using inside the biological safety cabinet
- Disassemble and clean the pipettes regularly to prevent germ growth
- Check for the accuracy of the pipettes through calibration at least once every year
- Dispose pipette tips properly
  - Do not leave used tips inside the biological safety cabinet

Other lab equipment

- Wipe off surfaces daily if possible
- Check if the lids of equipment not in used are closed
- Immediately clean all spills
  - Autoclave rotors of centrifuges

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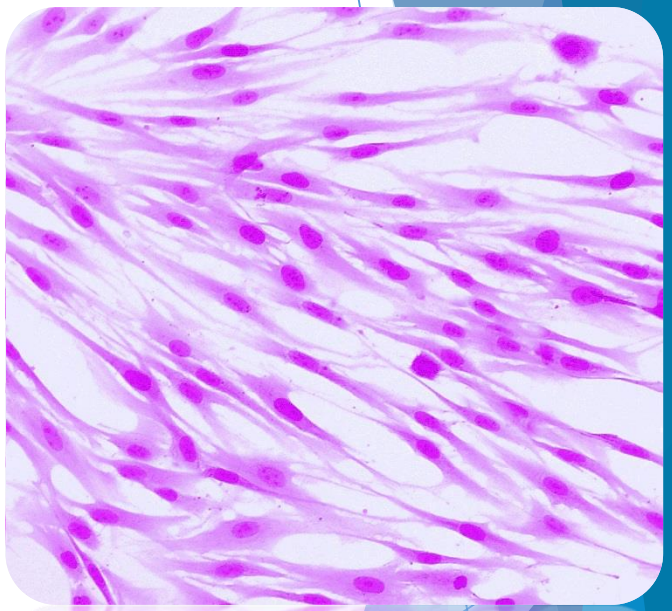
Personnel

- Wash hands thoroughly before entry into the cell culture lab
- Ensure PPE (Personal Protection Equipment) immediately
  - Lab gown
  - Gloves
  - Shoes
  - Mask (if needed, depends on BSC design)
- Wash hands before leaving the cell culture lab

Cell lines and primary cells

- Acquire cell lines only from authenticated and reliable sources
  - European Collection of Authenticated Cell Cultures (ECACC)
  - American Type Culture Collection (ATCC)
- Quarantine cells when testing
  - Bacteria, fungi, yeast (in culture)
  - Mycoplasma (PCR)
  - Cross-contamination (STR service for human cell lines)
- Primary cells must be maintained in a separate incubator



Biological safety cabinets

- Regular cleaning and maintenance
- Disinfect the surface before use
- Disinfect materials before transfer
- Arrange materials inside the BSC strategically
  - Only needed materials should be placed inside the BSC
  - Enough working space must be maintained
- Spray disinfectant after use

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Proper pipetting

Aspirate liquid

- Vertical hold
- Aspirate slowly for precision and to avoid pipette contamination

Release liquid

- Maintain a 45°C for sterility
- Release at medium speed to avoid droplets

Filtered micropipette tips

- Filter tips for increased protection
  - Protects both pipette and culture from contamination
  - Use particularly for important cell culture steps, such as cell banking

Vessels

- Lids of flasks can be an easy contamination route
  - Place lids safely when working outside the BSC
  - Upside up or upside down both allowed inside the BSC when done properly

Labeling

- Label flasks, dishes, plates, and tubes clearly
  - Prevents cross-contamination
- Ethanol-resistant pen must be used
  - Prevents loss of label when disinfecting

Safe transport

- Slightly tilt the culture flasks upwards to avoid medium contact
- Avoid spillage when transporting plates and dishes

Prevent contamination before it's too late!

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