Basic Cell Culture for Parasitology research

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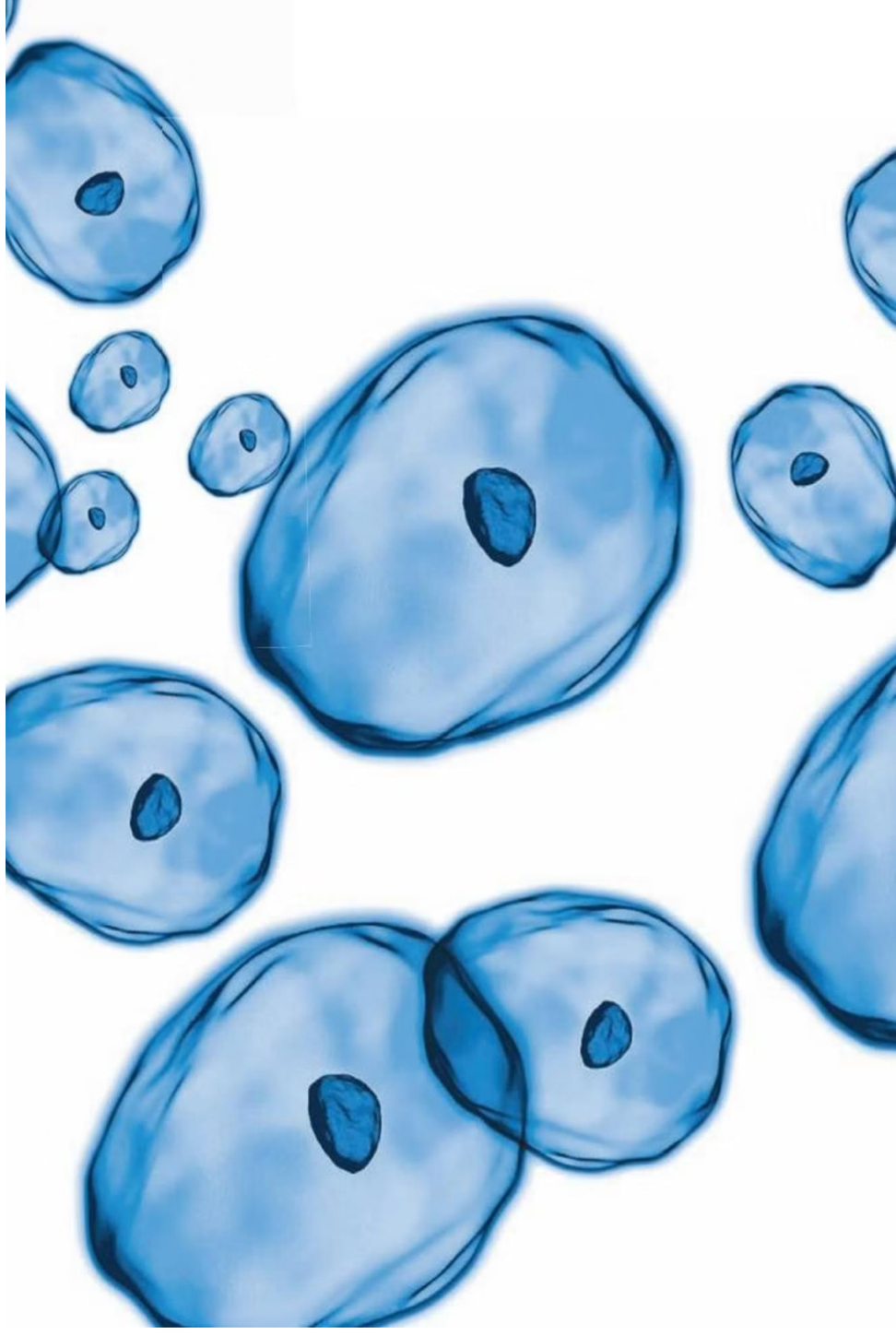


Contents

What is Cell Culture?

Cell culture involves growing cells, tissues, or organs outside their natural environment in a carefully controlled laboratory setting.

It is a fundamental part of modern biological research — allowing scientists to observe cellular behavior, analyze disease mechanisms, and test potential treatments under consistent, well-regulated conditions.

Why Cell Culture in Parasitology?

Detailed Study

Examine parasite life cycles, host-parasite interactions, and drug efficacy with precision.

Ethical Advantage

Reduces reliance on animal models, addressing ethical concerns and lowering experimental complexity and cost.

Reproducibility

Provides a controlled, standardised environment that supports rigorous, repeatable experimental outcomes.

Types of Cell Culture

Introduction to Cell Culture

Cell culture involves growing plant or animal cells in a controlled, artificial environment.

1

Primary Culture

Cells taken directly from tissue. Once they reach **confluence**, they are transferred to a new vessel with fresh medium.

2

Cell Line

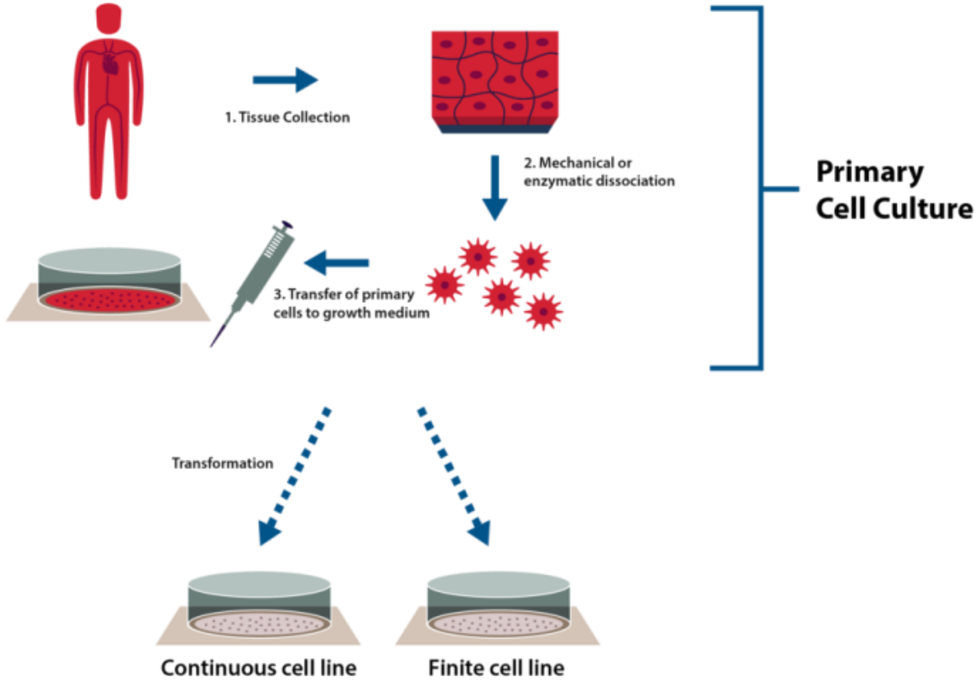
Formed after the first transfer. The fastest-growing cells dominate, creating a physically and genetically uniform population.

3

Cell Strain

A specific subset selected from a cell line, often via cloning; may develop unique genetic variations over time.

Primary Cell Cultures



Primary cultures consist of cells **directly isolated from living tissue**, offering the closest approximation to in vivo conditions.

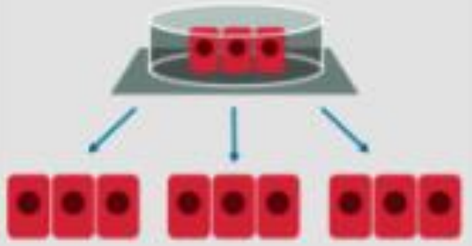
However, they have a limited lifespan and require careful handling. Their biological authenticity makes them invaluable for initial studies.

Example: Primary avian epithelial cells are used to support the intracellular development of *Eimeria tenella*, an important poultry pathogen.

https://www.eppendorf.com/ca-en/lab-academy/life-science/cell-biology/what-is-primary-cell-culture/

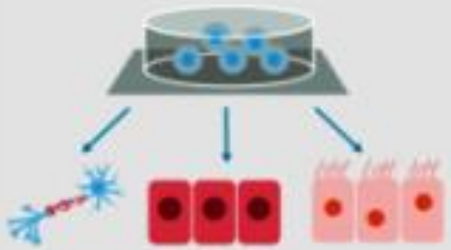
Primary cell culture: By cell differentiation

Differentiated cell culture



Differentiated cells that have lost capacity to further differentiate

Stem cell culture



Undifferentiated cells that are able to differentiate into other cell types

Primary cell culture: By adhesion

Monolayer cultures

Anchorage-dependent, usually one cell thick, with continuous cell layer on culture vessel

Suspension

Cells grow free-floating in the medium

Primary cell culture: By cell type/morphological structure

Epithelial

Polygonal-shape, appear flattened, attach to a substrate and form a continuous thin layer

Endothelial

Round outline, do not form sheets, or attach to substrates

Fibroblast-like

Angular-shape, elongated and form open network of cells that attach to substrate

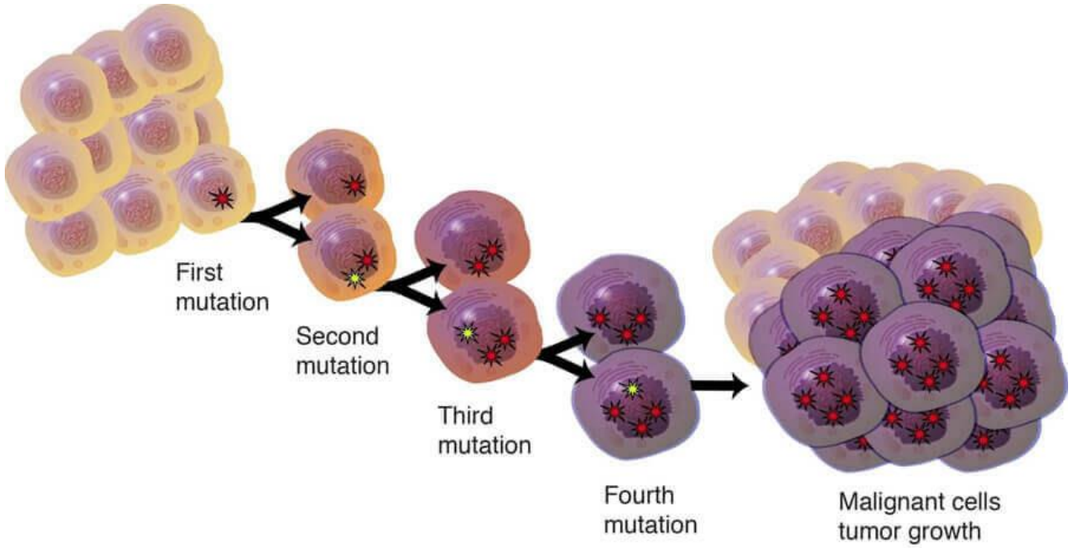
Lymphoblast-like

Spherical-shape, ranging from large cells with fine nuclear chromatin to smaller cells with condensed chromatin

Neuronal

Round, pyramidal or spindle-shaped cell body, with branching dendrites

https://www.eppendorf.com/ca-en/lab-academy/life-science/cell-biology/what-is-primary-cell-culture/



Immortalised Cell Lines

These cells can divide indefinitely, whether from tumor cells or genetically modified.

They are more consistent and easier to maintain than primary cultures, making them the mainstay of many parasitology labs.

Their consistent behaviour is ideal for large-scale drug screening and repeated experiments.

Example:

3T3 cells – a mouse fibroblast cell line derived from a spontaneous mutation in cultured mouse embryo tissue.
HeLa cells – a widely used human cell line isolated from cervical cancer patient Henrietta Lacks
HEK 293 cells – derived from human fetal cells
Jurkat cells – a human T lymphocyte cell line isolated from a case of leukemia
Vero cells – a monkey kidney cell line that arose by spontaneous immortalization.

https://www.creative-bioarray.com/support/immortalized-cell-culture-guide.htm

Finite vs. Continuous Cell Lines

Finite (Primary) Cell Lines

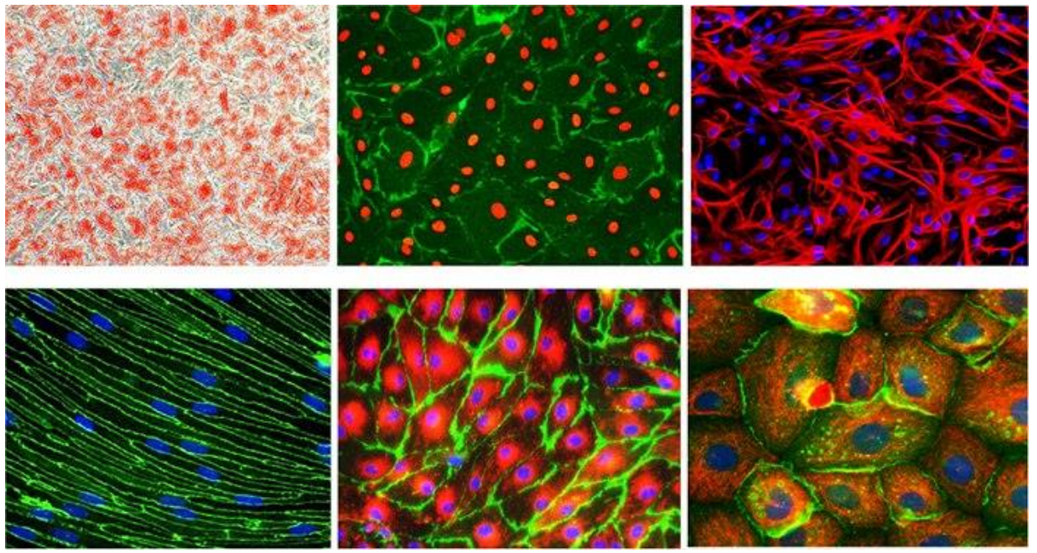
Normal cells divide a limited number of times before reaching **senescence** and stopping growth.

Continuous Cell Lines

Transformed cells gain **immortality** via natural, chemical, or viral triggers, allowing indefinite division.

Comparison between Primary cells and Continuous cell lines

Properties	Primary cells	Cell lines
Biological Relevance	High	Low
Lifespan and Proliferation	Limited/Finite	Unlimited/Infinite
Consistency	Medium	High
Genetic Integrity	Retains I*n Vivo* Tissue Genetic Makeup	Subject to Genetic Drift
Ease-of-Use	Needs Optimized Culture Conditions	Well Established Conditions and Protocols
Time & Expense	Slower Cell Growth and Higher Cost	Faster Cell Growth and Lower Cost



https://www.merckmillipore.com/VE/es/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/primary-cell-culture/primary-cell-culture

Organotypic / 3D Cultures

Structural Complexity

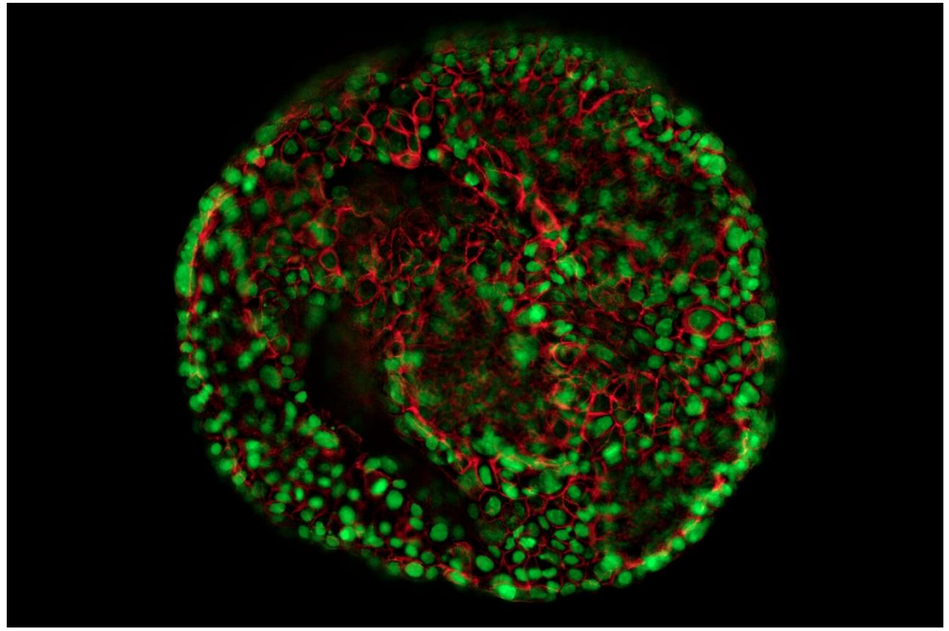
Mimic the three-dimensional architecture and physiological function of organs, providing a more realistic tissue microenvironment.

Physiological Relevance

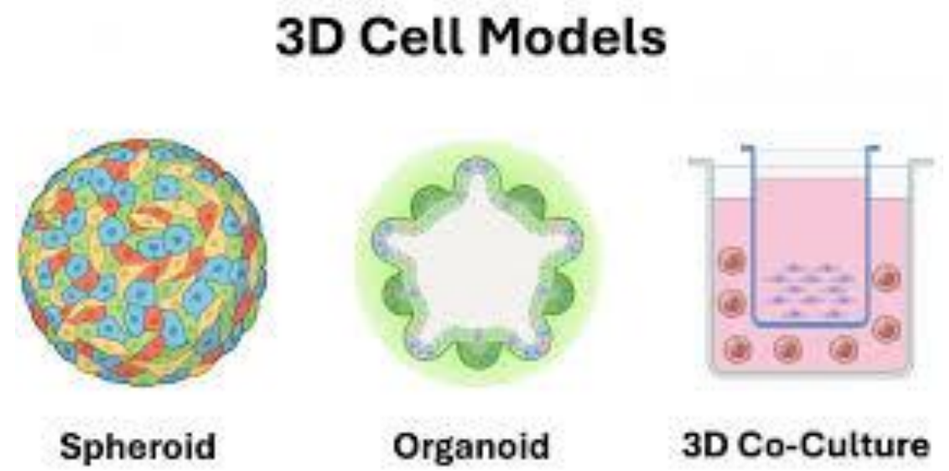
Capture cell-cell and cell-matrix interactions that are absent from conventional 2D monolayer cultures.

Emerging Applications

Increasingly applied to model complex parasite infections and study invasion dynamics in tissue-like contexts.



https://www.leica-microsystems.com/applications/life-science/organoids-and-3d-cell-culture/



https://fluorofinder.com/2d-vs-3d-cell-cultures/

Culture Conditions & Cryopreservation

Culture Environment

Cells require precise nutrients, growth factors, and controlled gas, pH, and temperature levels to thrive.

Adherent vs. Suspension

Cells grow either by attaching to a solid surface (**adherent**) or by floating in liquid medium (**suspension**).

Cryopreservation

Cells are stored long-term by adding protective agents like DMSO and freezing them below **–130°C**.

Uses for Cell Culture

Basic Research

Study cellular growth, function, aging, and disease progression.

Drug Development

Test new compounds and medicines in a controlled environment.

Biomanufacturing

Large-scale production of vaccines and therapeutic proteins.

Cell culture enables **consistent, reliable** research using identical cell populations.

https://www.snapcyte.com/everything-you-need-to-know-about-cell-culture/

Staying Safe in the Cell Culture Lab

Laboratory work involves specific biological risks, including needle pricks, skin contact, or inhalation of harmful particles. **Strict adherence to standard safety protocols is essential for a secure working environment.**

Biosafety Levels

1

BSL-1

Basic safety for agents not typically causing disease in healthy adults.

2

BSL-2

Moderate safety for agents causing human disease. Standard for most cell culture labs.

3

BSL-3

High safety for agents that spread via air and cause serious illness.

4

BSL-4

Maximum safety for rare, dangerous, incurable agents requiring specialized facilities.

Safety Equipment & PPE

Primary Barriers

Tools like biosafety cabinets and sealed containers isolate hazardous materials. The **biosafety cabinet** is essential for preventing exposure to splashes and airborne particles.

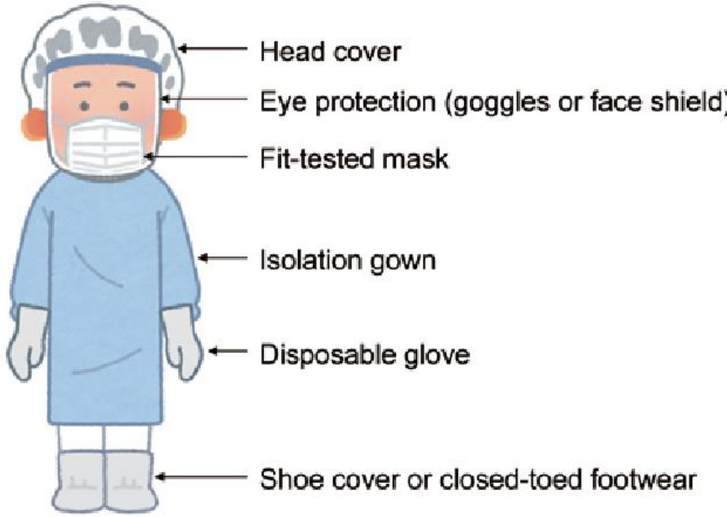
Personal Protective Equipment

- Gloves and lab coats
- Respirators and face shields
- Safety glasses or goggles

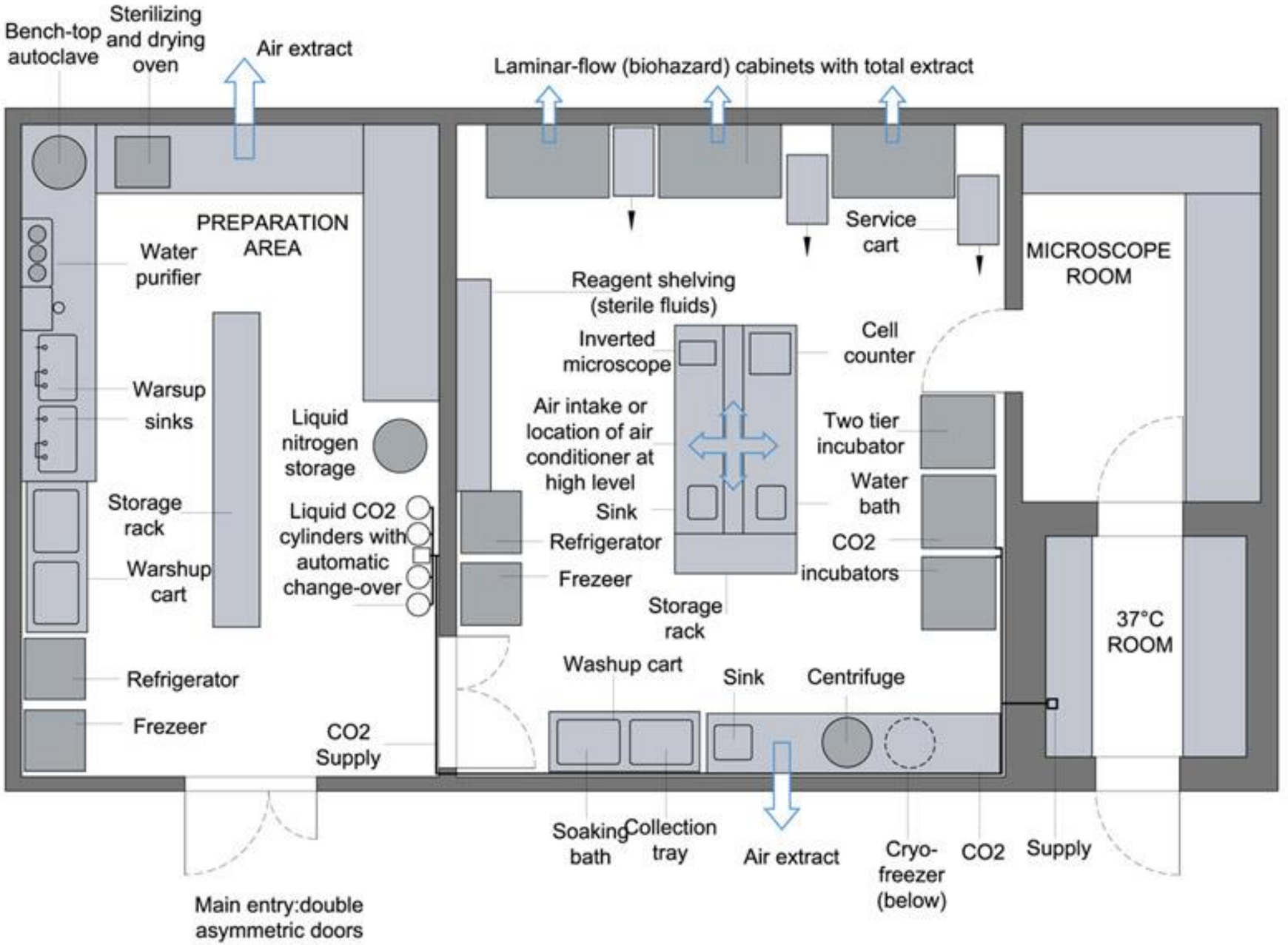
Always wear required PPE alongside biosafety cabinet use. Follow your facility's specific safety protocols.

https://www.aimplas.net/blog/new-cell-culture-laboratory-to-assess-healthcare-products/

https://www.labmanager.com/personal-protective-equipment-in-the-lab-20269



https://www.rgu.ac.uk/life-at-rgu/learning-facilities/health-science-facilities/cell-culture-lab



https://www.archweb.com/en/cad-dwg/culture-lab-02/

Safe Laboratory Practices

Personal Hygiene

Always wear required PPE. Wash hands after handling materials. No eating, drinking, smoking, or makeup in the lab.

Decontamination

Clean surfaces before and after tasks. Sanitize all materials before disposal and clean spills immediately.

Aerosol Prevention

Work carefully to minimize splashes or mists. Strictly follow protocols for handling sharps and broken glass.

Incident Reporting

Immediately report any accidents or potential exposures to your supervisor or safety officer.

Basic Cell Culture Techniques

Cell Culture Equipment

Pipettes & Sterile tips

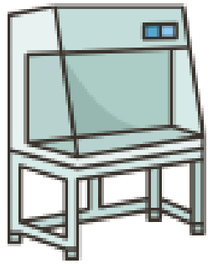
Aseptic transfer of media and cells

70% Ethanol & Gloves

Sterility and protection

Culture media (e.g., DMEM)

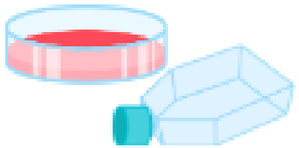
Nutrient source



Biosafety Cabinet

Sterile manipulation (~10 cm inside for airflow)

Flasks/Plates



Growth surfaces

Inverted Microscope

Observes cell morphology and confluency

FBS

Growth factors

Trypsin-EDTA

Detaching adherent cells

CO_2 incubator (37°C)

Maintains temperature and pH (5% CO_2)

What You Need

https://www.snapcyte.com/everything-you-need-to-know-about-cell-culture/

Cell Culture Hood Classes

Class I

Protects the user and environment; does not keep cultures sterile. Similar to a standard chemical fume hood.

Class II

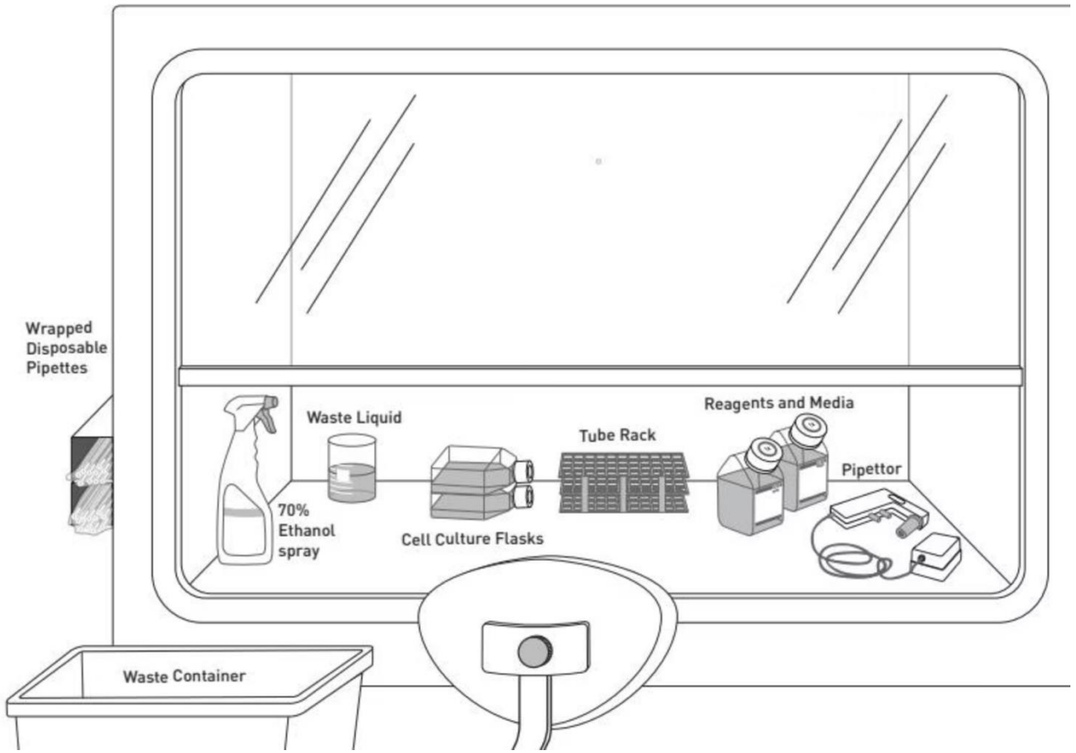
Provides sterile environment for cultures and user protection. Required for human/primate cells and biohazardous materials.

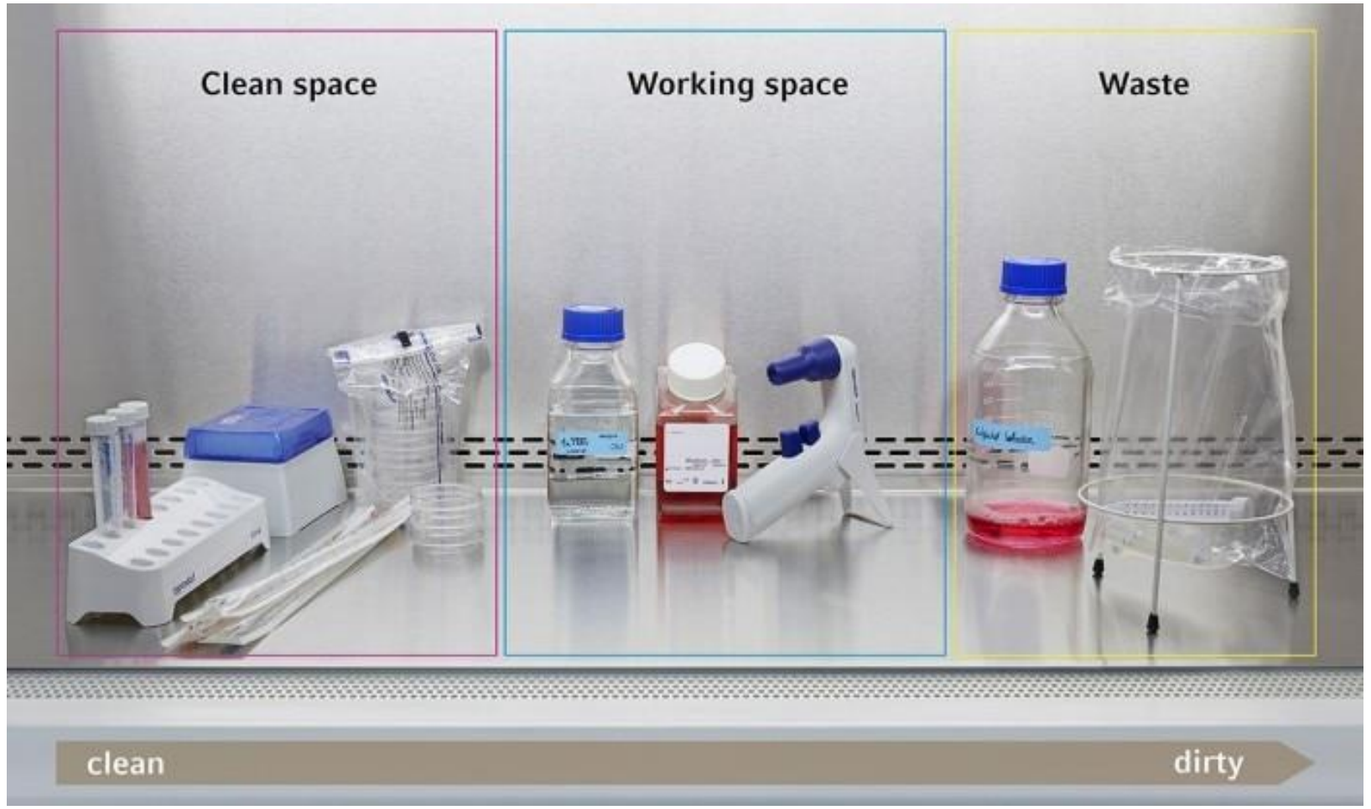
Class III

Gas-tight, sealed unit for maximum protection. Essential for handling high-risk pathogens.

All cabinets utilize constant **HEPA-filtered airflow**. Note: Standard clean benches are **not** biosafety cabinets and are unsuitable for cell culture.

Cell Culture Hood Layout





https://www.eppendorf.com/th-en/lab-academy/life-science/cell-biology/cell-culture-faq-how-to-work-at-the-bsc

Standard layout for right-handed workers. Keep workspace clear and disinfect items with 70% alcohol before entry.

01

Center: Clear space for working with cell cultures.

02

Front right: Reachable area for your pipettor.

03

Rear right: Storage for media and reagents.

04

Rear middle: Rack for extra tubes and supplies.

05

Rear left: Container for liquid waste.

Incubator & Storage

Incubator Types

Dry: Low-cost; requires sealed flasks to prevent evaporation.

Humid CO_2: High humidity/CO_2 control for dishes and plates.

Maintain stability (±0.2°C) and clean regularly to prevent contamination.

Storage Guidelines

- Follow specific label directions.
- Use dark containers/foil for light-sensitive items.
- Store media and reagents at 2–8°C (refrigerator).
- Use manual-defrost freezers (-5 to -20°C) for enzymes and antibiotics.

Cryogenic Storage & Cell Counter

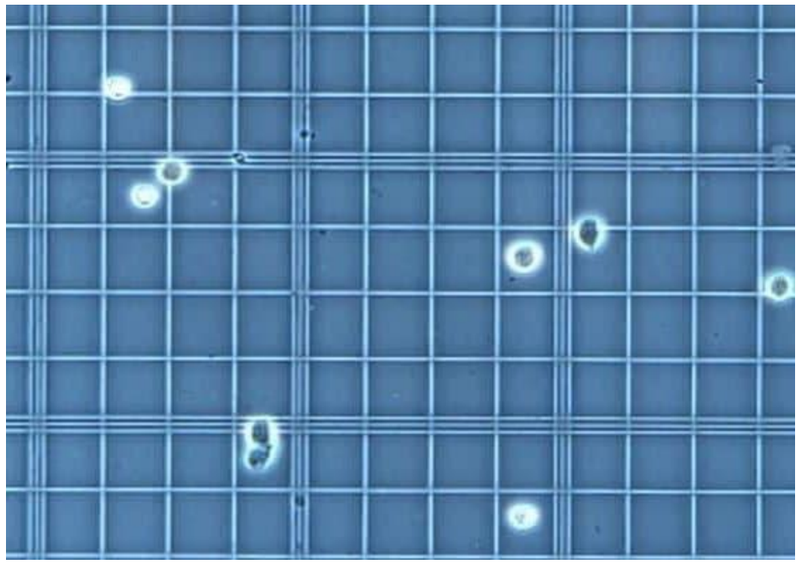
Cryogenic Storage

- Avoid standard freezers (–20°C/–80°C) to maintain cell viability.
- **Use liquid nitrogen storage**

https://www.asynt.com/news/need-liquid-nitrogen-generator/

Cell Counter

- Uses standard Trypan Blue method.
- Compatible with most common cell types.



https://www.cellseco.com/product/trypan-blue-solution/

Aseptic Technique

Maintain sterile cultures by preventing contamination from bacteria, fungi, and viruses using these four core practices:

Clean Workspace

Ensure your environment is sanitized.

Personal Hygiene

Wear proper PPE and wash hands.

Sterile Supplies

Use only pre-sterilized equipment.

Careful Handling

Execute movements slowly and precisely.

Sterile Work Area & Personal Hygiene

Cell Culture Hood Setup

- Keep your workspace clear and free of drafts.
- Wipe all surfaces with 70% ethanol before and after use, and immediately clean any spills.
- Keep the hood running during your work session.

UV Sterilization

- Use UV light to sanitize surfaces between sessions.
- Avoid using a Bunsen burner, as it is unnecessary and potentially hazardous in a cell culture hood.

Personal Hygiene

- Wash hands thoroughly before and after handling cultures.
- Wear appropriate protective gear, cover skin, and tie back long hair to prevent environmental contamination.

Sterile Handling Guidelines

Wipe everything with 70% ethanol

Clean all surfaces and items with 70% ethanol before placing them inside the hood.

Use sterile pipettes once

Use each pipette only once. Avoid pouring liquids directly from bottles.

Keep containers closed

Always cap bottles and flasks when not in use to prevent air exposure.

Work quietly and quickly

Avoid talking or unnecessary noise. Complete tasks efficiently to maintain sterility.

Aseptic Technique Checklist

Work Area

- Hood set up and draft-free?
- Surface wiped with 70% alcohol?
- Storage areas clean?

Personal Hygiene

- Hands washed/PPE worn?
- Long hair tied back?
- Pipettor used for all liquids?

Reagents & Media

- Solutions properly sterilized?
- Bottles wiped with 70% alcohol?
- Bottles capped when not in use?
- No signs of contamination?

Handling

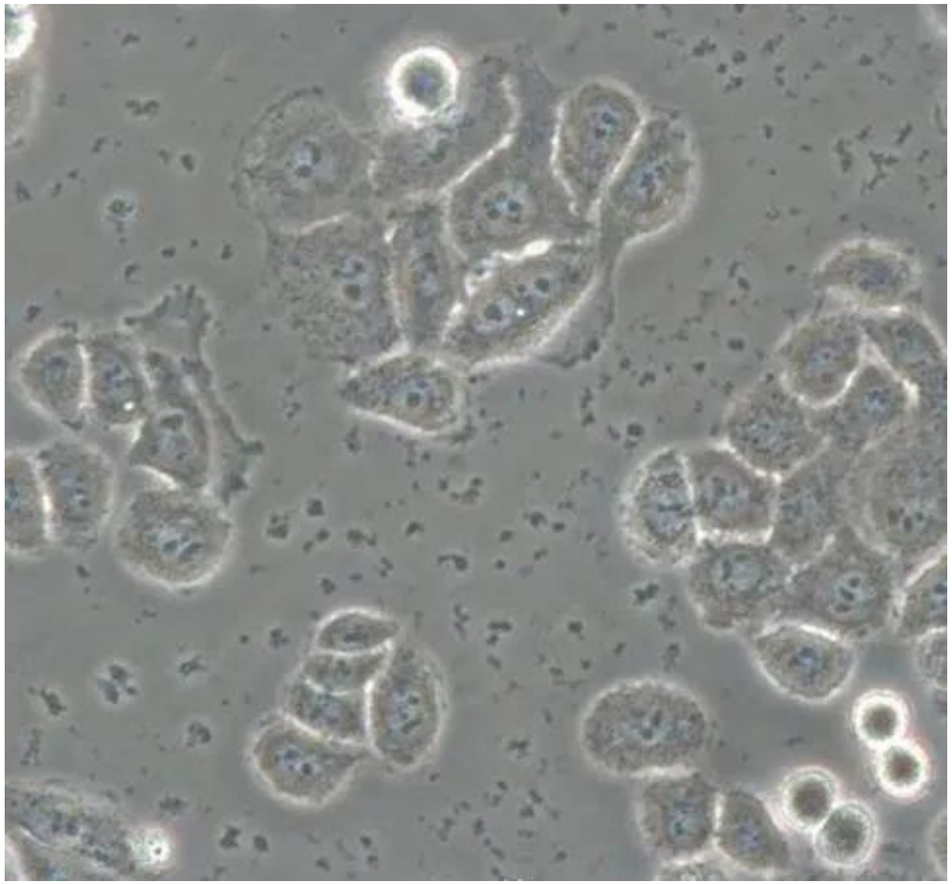
- Working slowly and carefully?
- Caps placed face-down?
- Pipettes used only once?

Biological Contamination

Contamination is a frequent challenge in cell culture labs. It generally falls into two categories:

- **Chemical:** Impurities in water, media, or equipment.
- **Biological:** Living contaminants like bacteria, mold, yeast, viruses, and mycoplasma, or cross-contamination between cell lines.

Strict cleaning habits and identifying contamination sources are essential to maintaining culture safety.



Mycoplasma Contamination

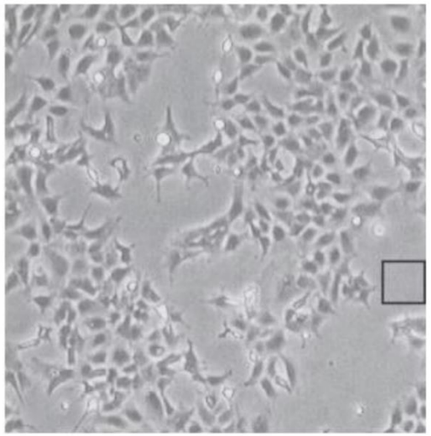
https://www.procellsystem.com/resources/cell-culture-academy/detecting-and-managing-mycoplasma-and-nanobacteria-contamination-1515

Bacteria & Yeasts

Bacteria

The most common lab contaminant due to rapid growth.

- Cloudy or murky medium; surface film.
- Sudden drop in pH levels.
- Visible tiny, moving specks under microscopy.



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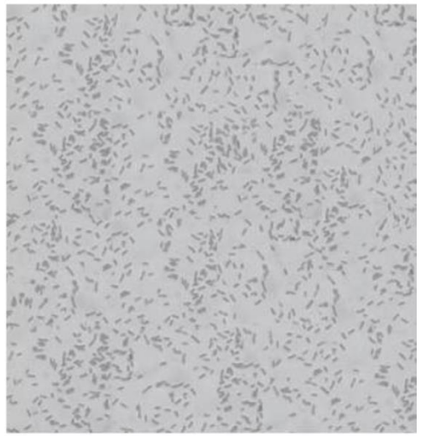
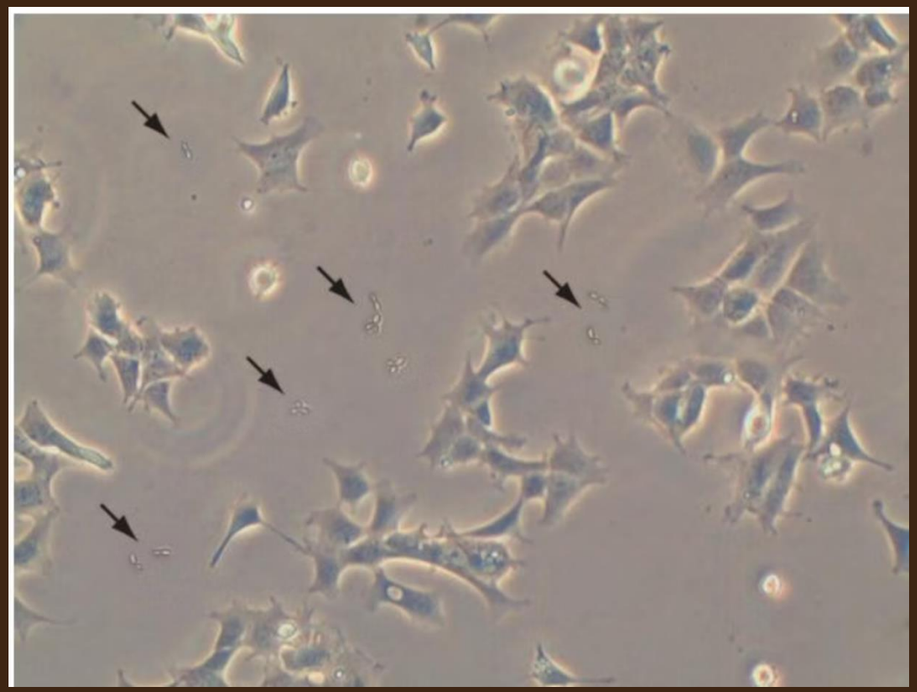


B

Yeasts

Single-celled fungi that proliferate in culture.

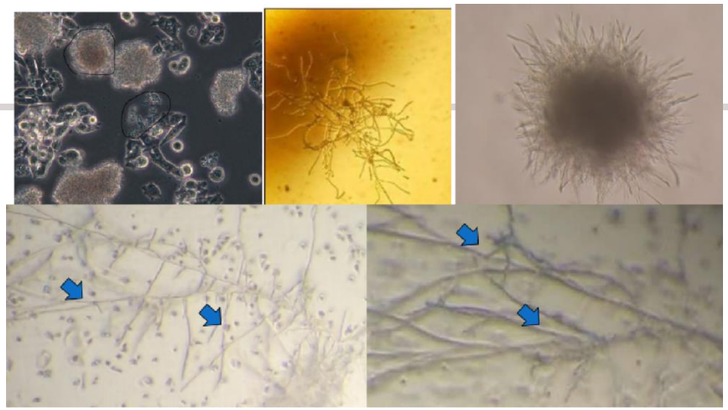
- Cloudiness appears in advanced stages.
- pH levels rise as the yeast grows.
- Round or egg-shaped particles with visible buds.



Molds & Viruses

Molds

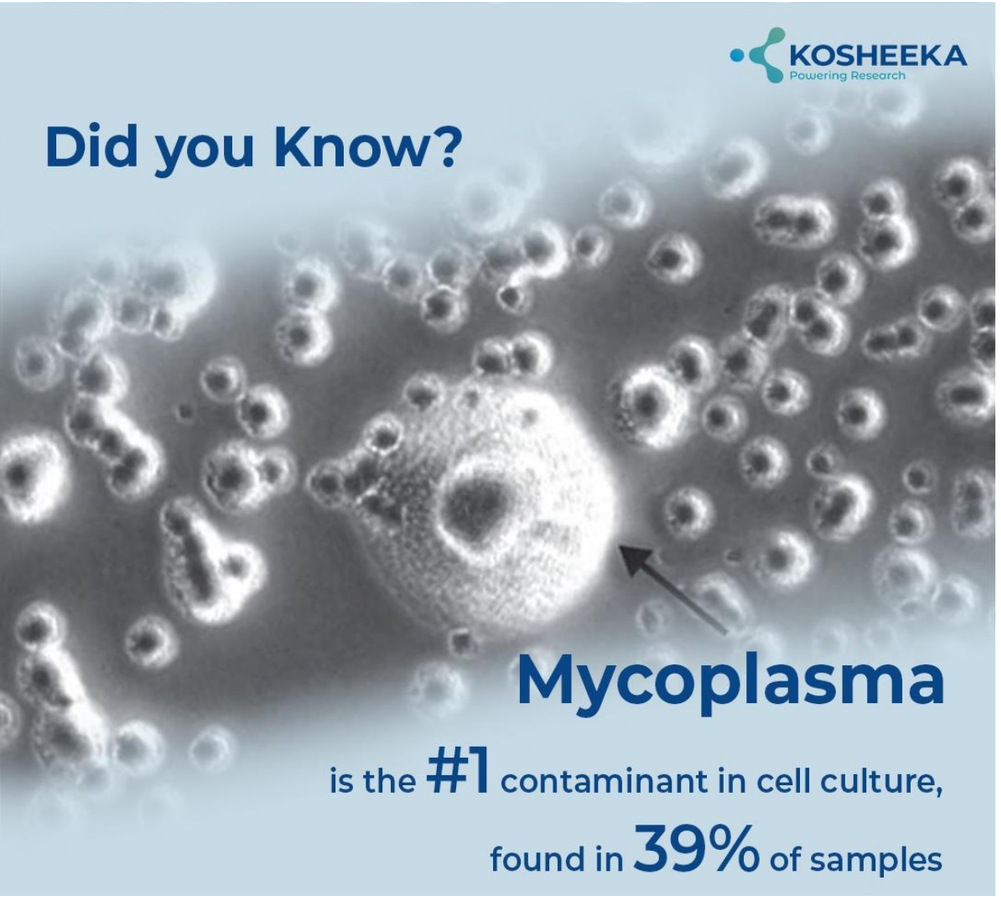
Fungi growing as thread-like strands. The pH remains stable initially but rises as contamination worsens. Microscope view shows wispy threads or clumps of hardy spores.



Viruses

Extremely small and difficult to detect. They pose a **serious health hazard** in human or primate cell cultures. Detect using electron microscopy, immunostaining, ELISA, or PCR.

https://www.yeasenbio.com/blogs/cell/troubleshooting-cell-culture-contamination-a-practical-solution-guide



https://www.kosheeka.com/

Mycoplasma Contamination

Mycoplasma are the smallest self-replicating organisms and are hard to detect before culture damage occurs.

- **Signs of infection:** Slower growth, reduced cell counts, and clumping.
- **Detection:** Regular testing is essential. Reliable methods include fluorescent staining, ELISA, PCR, and lab culture.

Choosing the Right Cell Line

Species and Biosafety

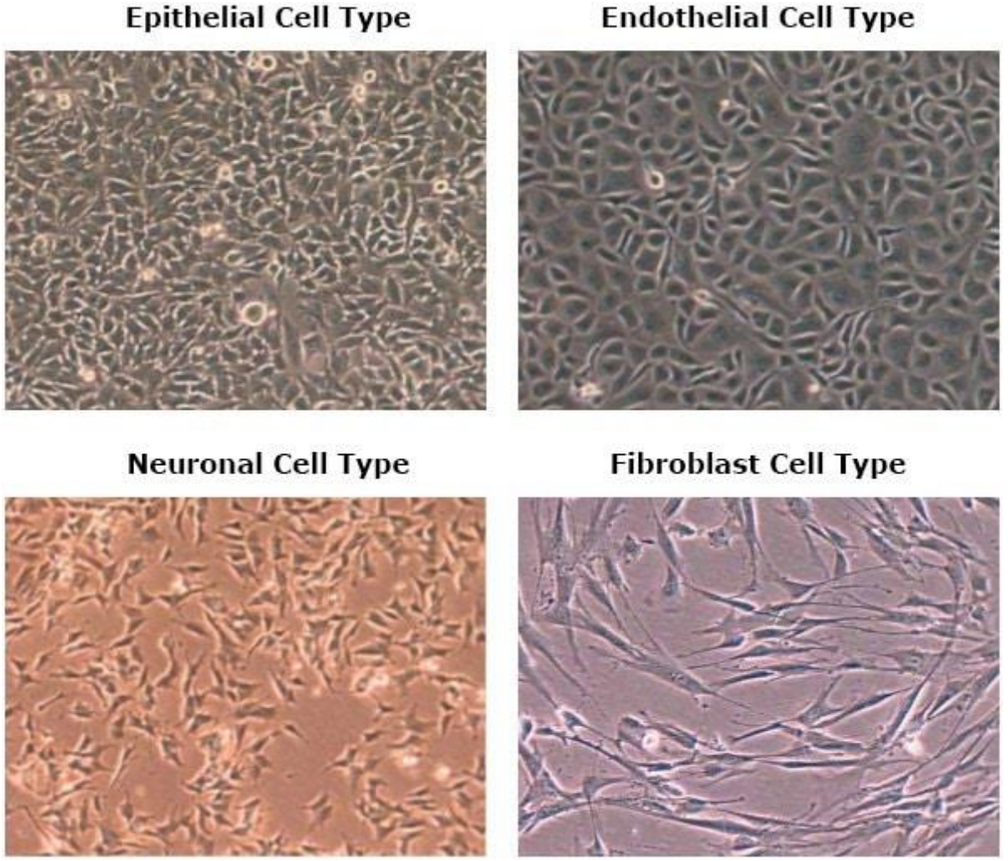
Select species based on your specific experimental requirements.

Growth Traits

- Assess growth rates and media requirements.
- Transformed" lines typically grow rapidly with lower serum needs.

Functional Needs

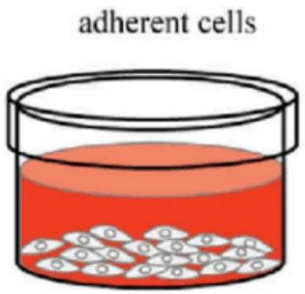
- Choose cells (e.g., liver or kidney) that align with your study goals.
- Continuous lines offer simpler maintenance and propagation.

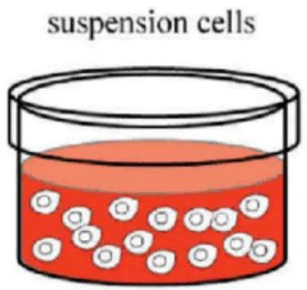


https://www.sigmaaldrich.com/

Adherent vs. Suspension Culture

Adherent Culture	Suspension Culture
Best for primary cultures and standard research.	Ideal for blood-derived or floating cell types.
Requires regular subculturing and surface-treated dishes.	Easier subculturing; requires daily viability checks.
Cells detached via enzymatic or mechanical methods.	Cells grow freely; no detachment required.
Limited by surface area availability.	Limited by cell density; needs mixing for aeration.
Suited for structural studies and imaging.	Optimal for large-scale protein production.





https://www.accegen.com/recent-posts/a-brief-overview-of-human-primary-cells/

Culture Media

Basal Media

Provides essential nutrients (amino acids, vitamins, salts, glucose). Typically requires serum supplementation for optimal cell growth.

Reduced-Serum Media

Basal media enriched with additional nutrients.
Allows for reduced serum usage to minimize unwanted serum-related effects.

Serum-Free Media (SFM)

Uses specialized nutrients and hormones.
Provides consistency and better purification, though often cell-specific with slower growth rates.

pH, CO_2 & Temperature

pH & CO_2

- Mammalian cells require **pH 7.4**
- Insect cells prefer **pH 6.2**.
- A **5–7% CO_2** environment stabilizes pH; fluctuations in CO_2 levels directly impact media pH.

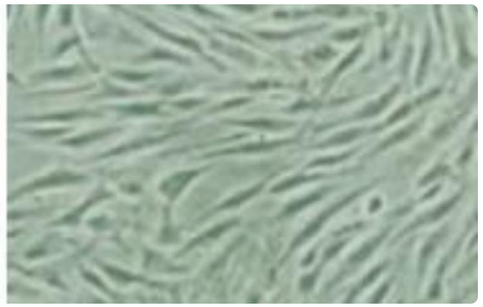
Temperature

- **Mammalian:** 36–37°C
- **Insect:** 27°C (avoid >30°C)
- **Avian:** 38.5°C
- **Cold-blooded:** 15–26°C

Overheating is more damaging than cooling. Incubate slightly below the optimal setpoint.

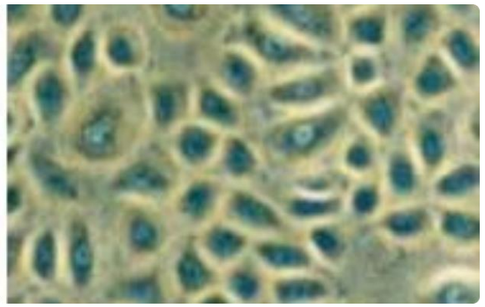
Morphology of Cells in Culture

Cells are grouped into three primary types based on their appearance.



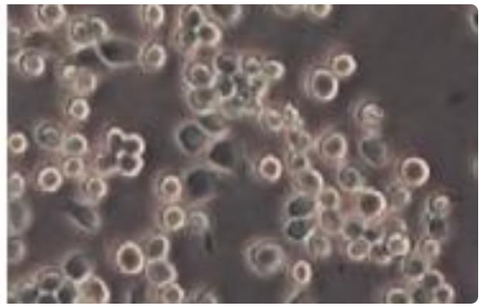
Fibroblastic

Thin, spindle-shaped cells that grow by adhering to surfaces.



Epithelial-like

Polygonal cells that grow in clusters attached to surfaces.

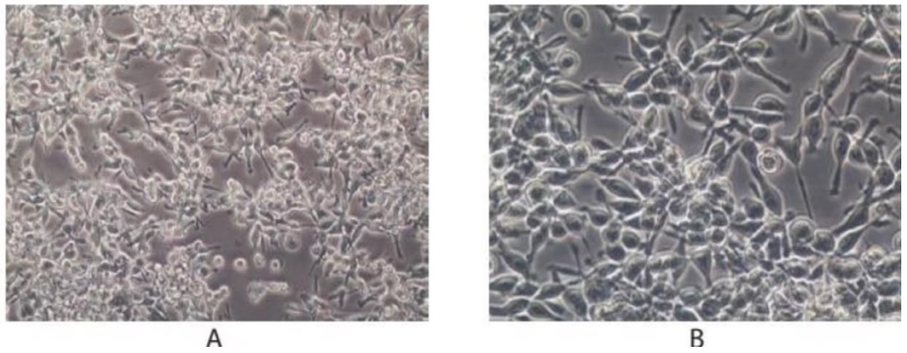


Lymphoblast-like

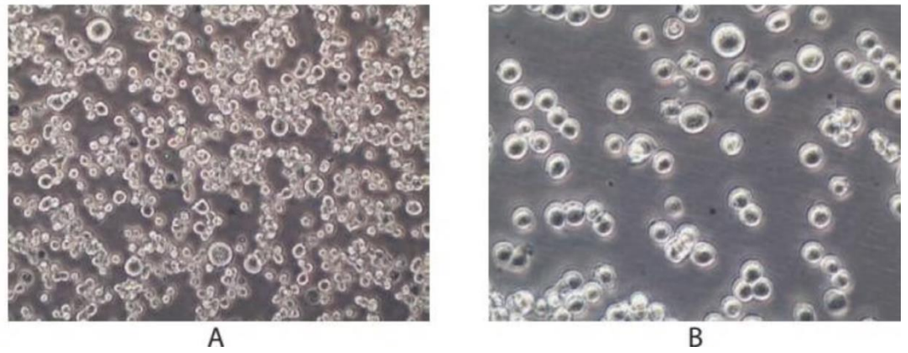
Round cells that grow in suspension, floating in the medium.

Morphology of 293 Cells

293 cells are human embryonic kidney cells modified with adenovirus type 5 DNA for high protein expression. Available variants include serum-free suspension strains for large-scale production.



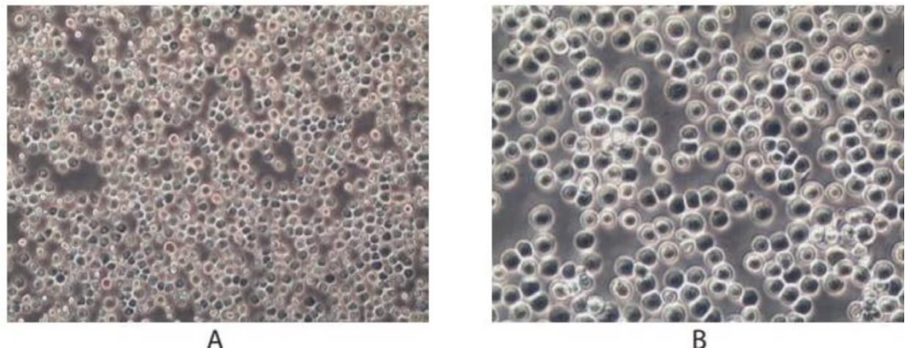
293 cells attached at 80% confluence (5×10^4 cells/cm^2).



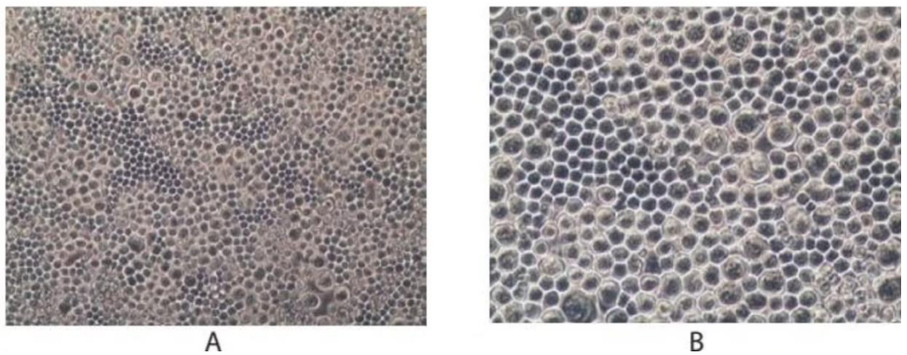
293F cells in suspension (2×10^5 cells/mL).

Morphology of Sf21 Insect Cells

Derived from Fall Armyworm ovaries, Sf21 cells are round, granular, and vary in size. They thrive in both suspension and monolayer cultures, making them highly versatile for laboratory research.



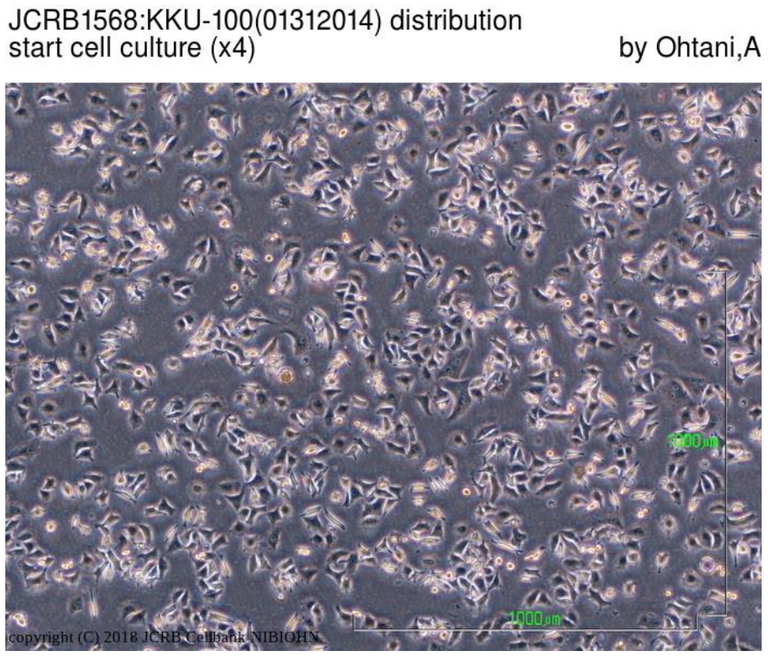
Sf21 cells in suspension, grown at 3×10^5 cells/mL in Sf-900 II SFM at 28°C (Day 3).



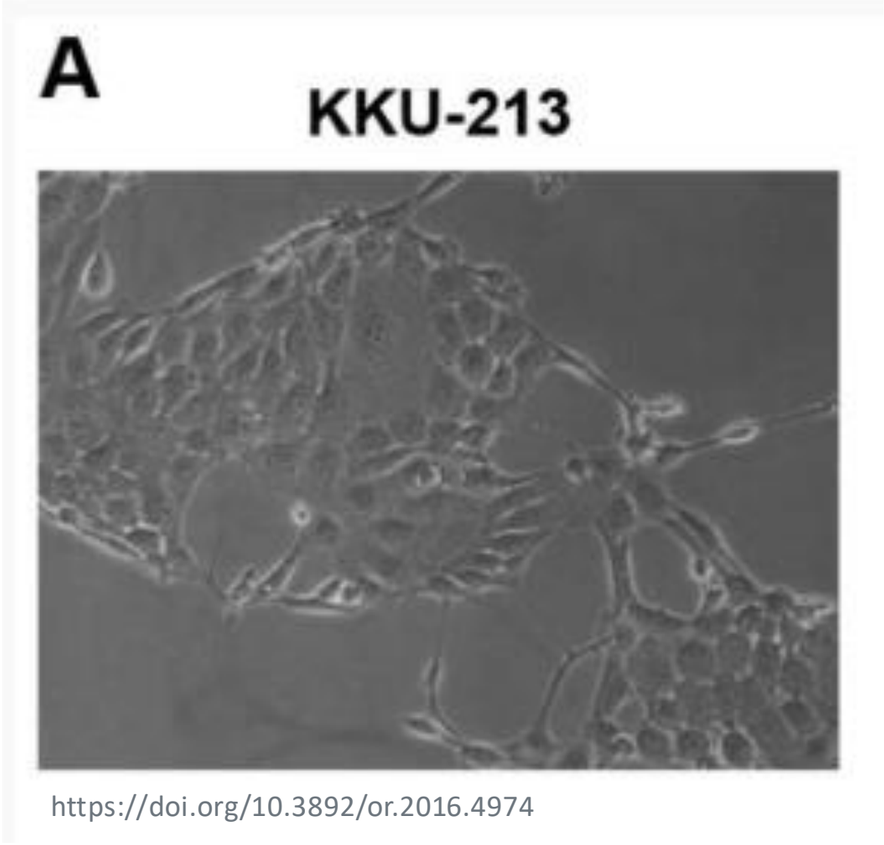
Sf21 cells in monolayer, seeded at 5×10^4 cells/cm^2 at 28°C (Day 7, confluent).

Morphology of KKU-100, KKU-213L Human cholangiocarcinoma cell line

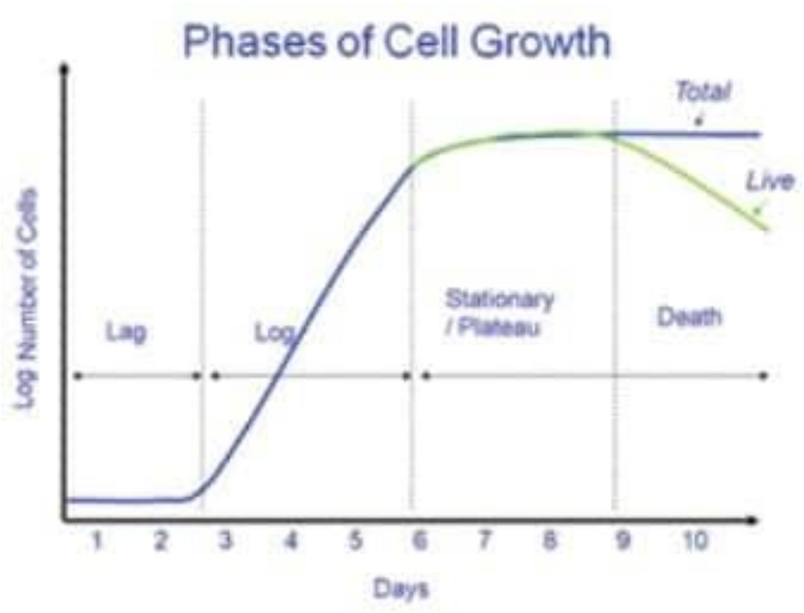
Derived from opisthorchiasis-associated cholangiocarcinoma cell line (KKU-100), established from biliary tract.

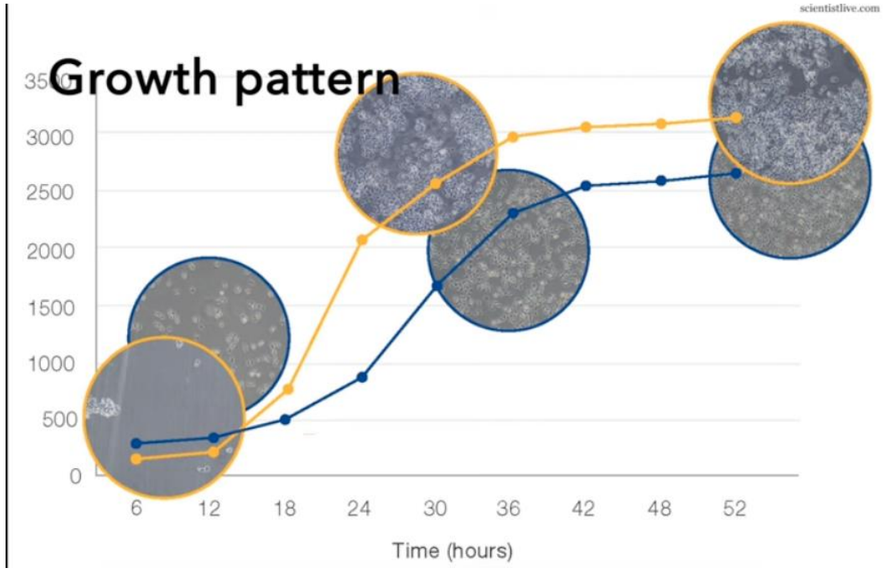


https://cellbank.nibn.go.jp/~cellbank/en/search_res_det.cgi?ID=7096



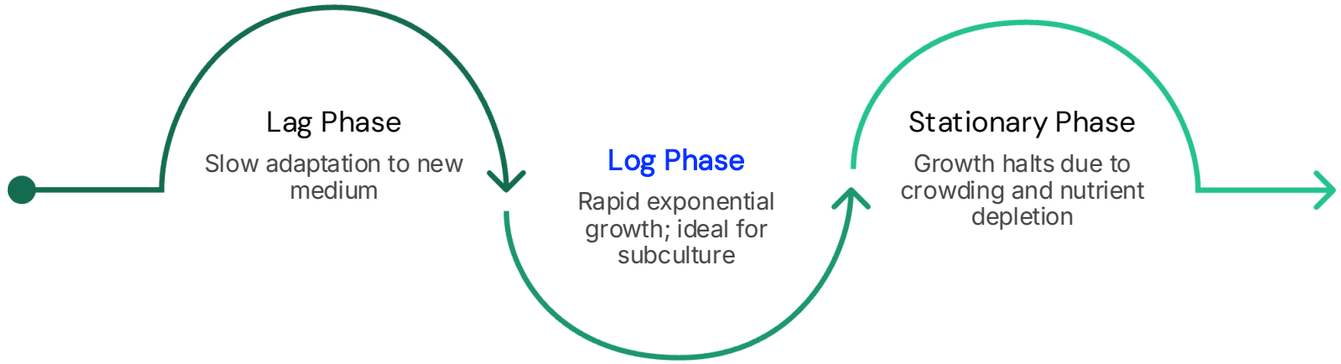
https://doi.org/10.3892/or.2016.4974





What is Subculture?

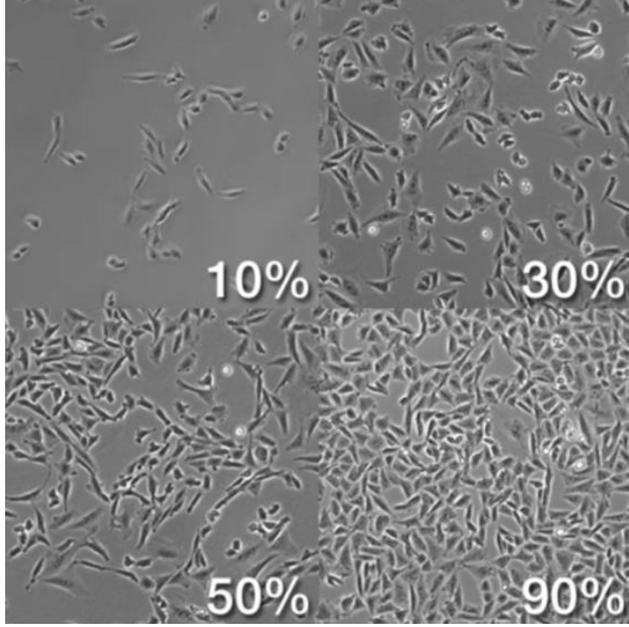
Subculturing (or passaging) involves moving cells to a new container with fresh medium to sustain growth. When cells fill their container, they deplete nutrients and stop dividing, necessitating a transfer.



Subculture during the **Log Phase** while cells are actively dividing.

https://www.sigmaaldrich.com/

When to Subculture?



Mammalian Cells

- Passage adherent cells while actively growing and before over-confluence.
- For suspension cells, passage before clumping occurs to maintain culture health.
- Subculture if media pH drops rapidly due to high cell density.

Insect Cells

- Passage during active growth to prevent slowed division and poor attachment.
- Monitor density; avoid overcrowding to ensure high survival rates.
- Media pH typically shifts as density peaks, signaling the need for subculture.

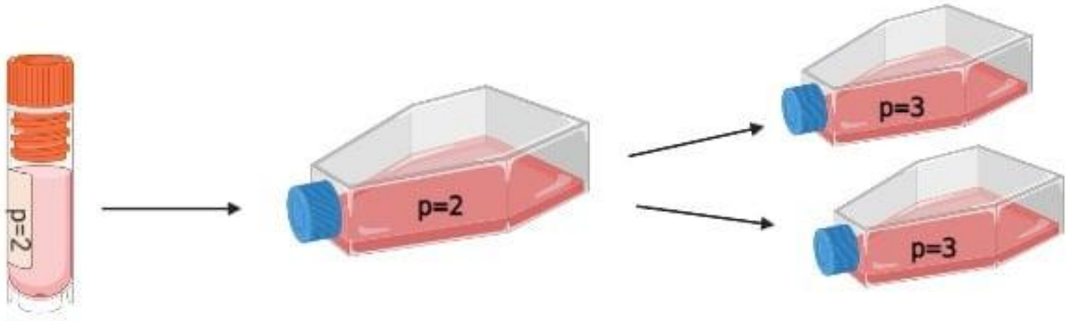
Video Mammalian Cell Culture Technique

Recommended Media for Common Cell Lines

Cell Line	Type/Species	Tissue	Medium
293	Fibroblast (Human)	Embryonic kidney	MEM + 10% FBS
A549	Epithelial (Human)	Lung cancer	F-12K + 10% FBS
CHO-K1	Epithelial (Hamster)	Ovary	F-12 + 10% FBS
HeLa	Epithelial (Human)	Cervix cancer	MEM + 10% FBS + NEAA
Jurkat	Lymphoblast (Human)	Lymphoma	RPMI-1640 + 10% FBS
MCF7	Epithelial (Human)	Breast cancer	MEM + 10% FBS + Insulin
Sf9/Sf21	Insect	Pupal ovary	TNM-FH (+ FBS or serum-free)

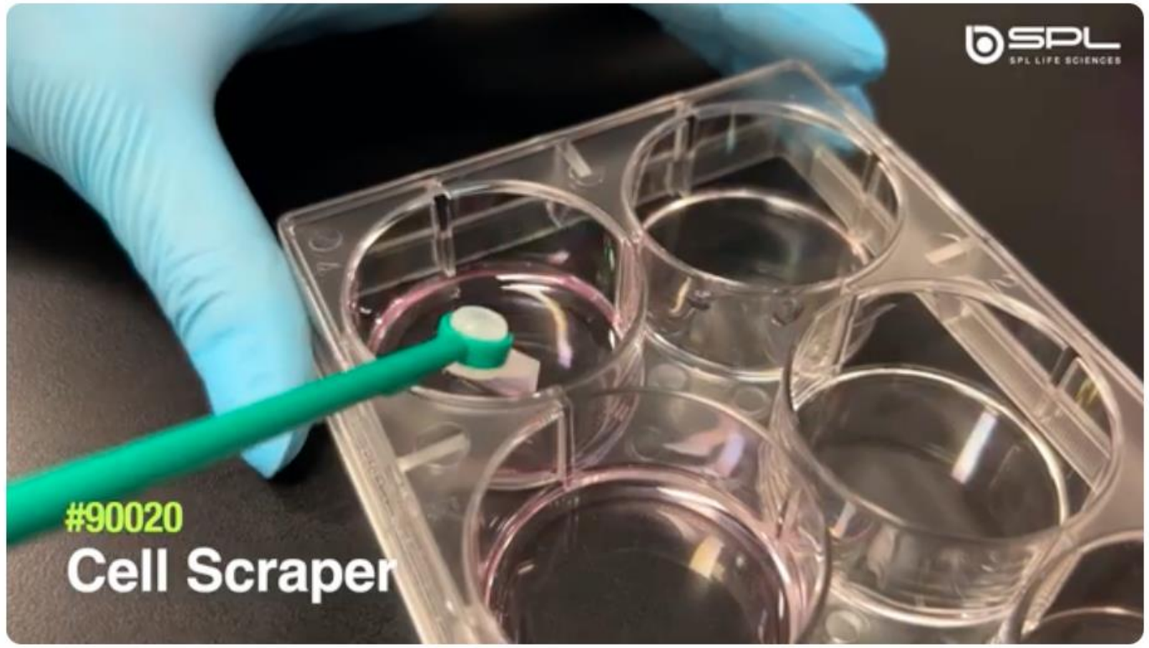
Abbreviations: MEM (Minimum Essential Medium), FBS (Fetal Bovine Serum), NEAA (Non-Essential Amino Acids).

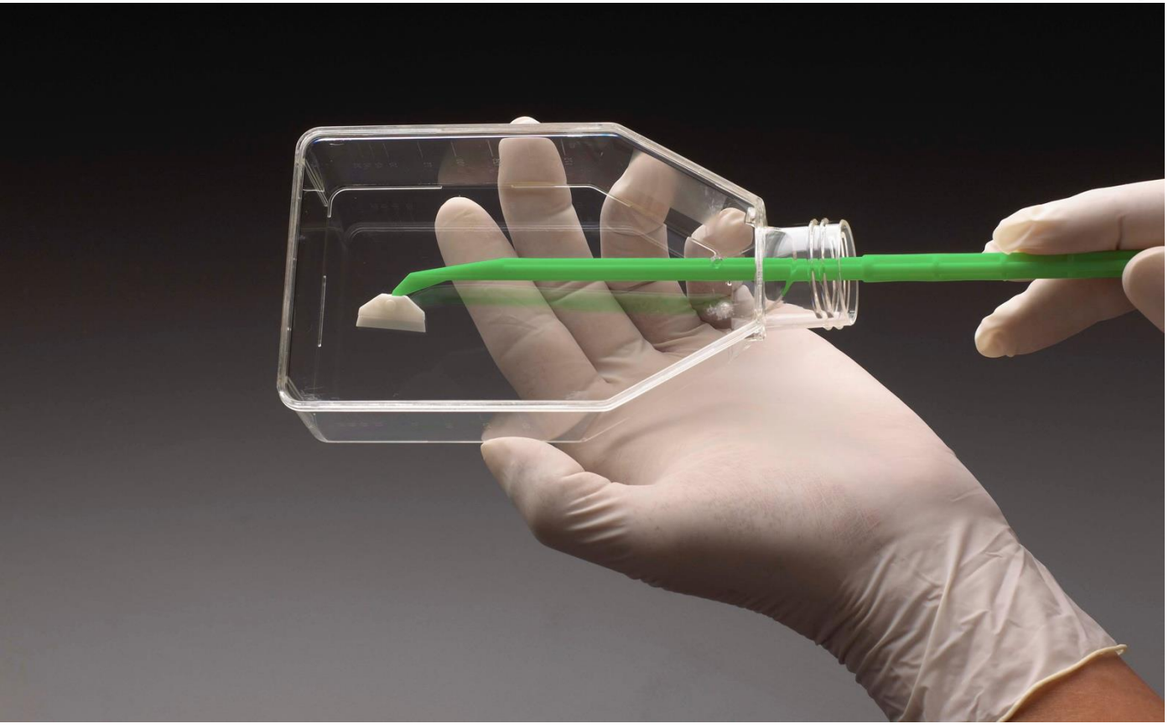
Removing Adherent Cells

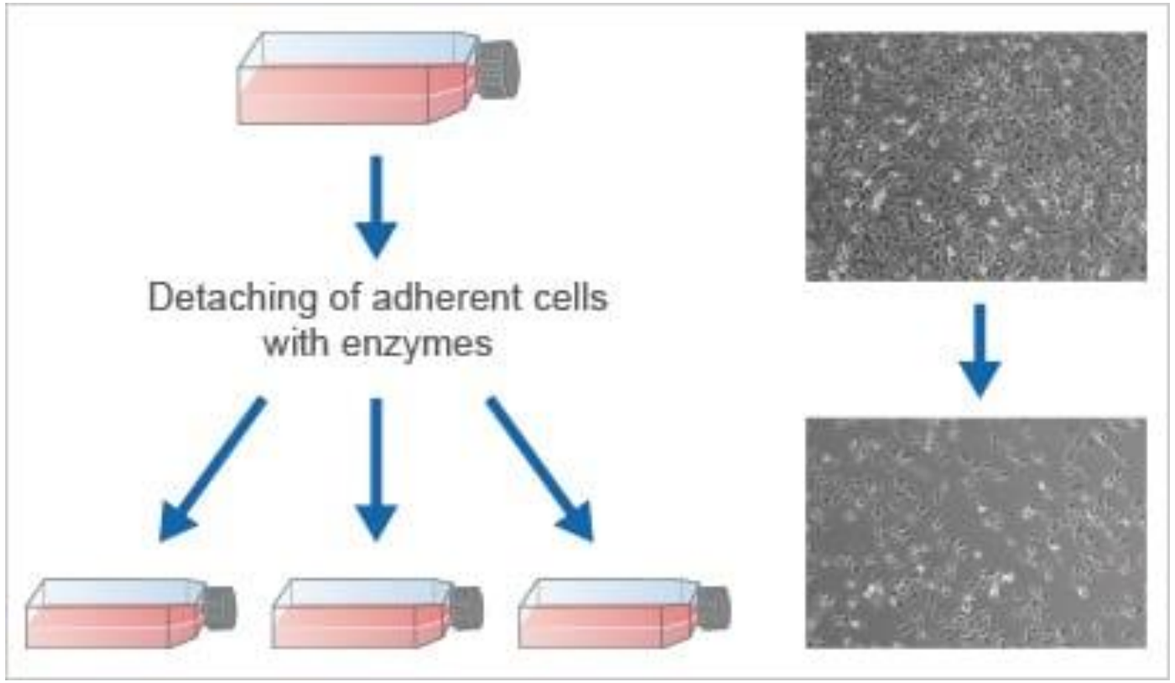


Method	What to Use	Best For
Shake-off	Gentle shaking/pipetting	Loosely attached/dividing cells
Scraping	Cell scraper	Enzyme-sensitive cells (may harm)
Enzymatic	Trypsin/Collagenase	Strongly adherent/thick layers
Enzymatic	Dispase	Removing sheets of skin cells
TrypLE™	TrypLE™ Express/Select	Strong attachment; animal-free

TrypLE™ enzymes are stable at room temperature, do not require quenching, and are free of animal/human ingredients.



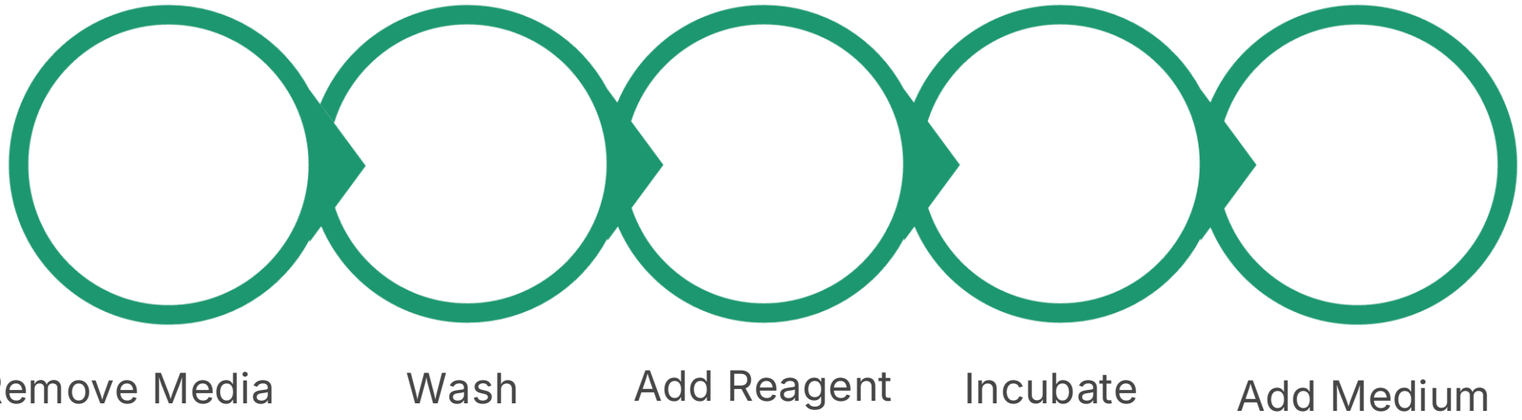




Passaging / subcultivation = transferring cells into fresh cultureware and growth medium

https://www.youtube.com/watch?v=9D1u7sknz9c

Protocol: Passaging Adherent Cells



Key Notes

Wash to remove inhibitors; incubation and centrifuge times vary by cell type. Use vented caps or loosen lids for gas exchange.

https://youtu.be/mjCZjw4KTYc?si=A_9uUczN4D9E_3vu

Subculturing Suspension Cells

Suspension cells are simpler to passage than adherent cells as **they require no enzymatic detachment**. Feed cultures every 2–3 days and dilute to desired density; cultures typically recover quickly.

Shaker Flasks

- Use non-baffled flasks.
- Loosen caps one turn for aeration.
- Centrifuge at 100×g every 3 weeks to remove debris.

Spinner Flasks

- Fill to maximum 50% capacity.
- Built-in mixers enhance oxygenation.
- Ideal for larger culture volumes.

Cryopreservation

Freezing cells is the essential method to preserve valuable stocks against drift, aging, or contamination.

Seed Stock

Create a master backup immediately. Store safely and avoid using for routine experiments.

Working Stocks

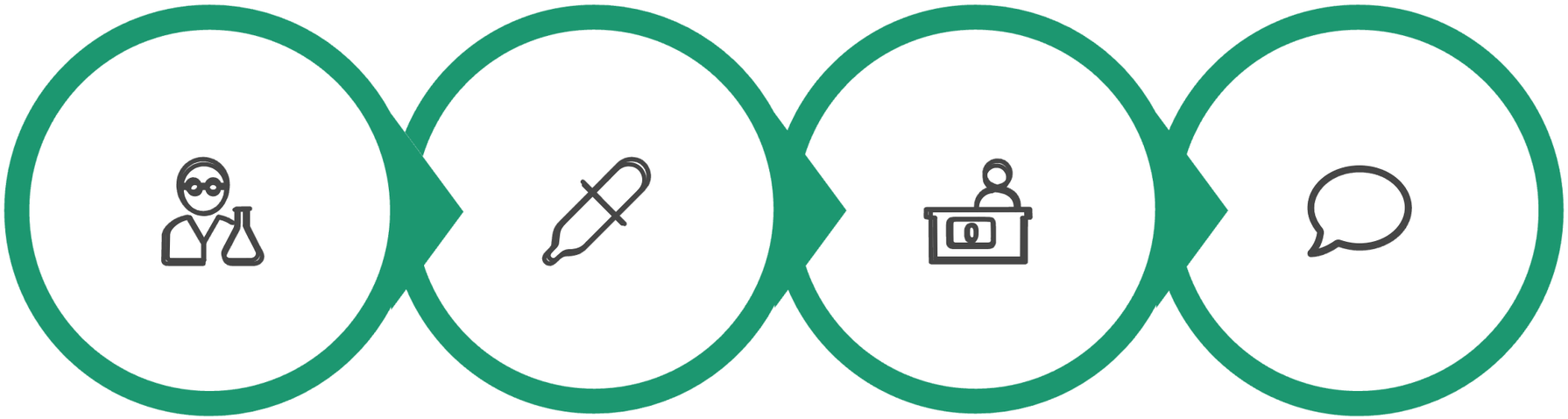
Used for regular experiments, generated from seed stocks to preserve the master reserve.

Cryoprotective Agents

Compounds like DMSO lower the freezing point to prevent lethal ice crystal formation.

https://www.thermofisher.com/th/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/freezing-cells.html

Protocol: Cryopreserving Cultured Cells



Prepare Medium

Detach Cells

Count Cells

Centrifuge

Guidelines for Cryopreservation

1

Freeze healthy cells

Use low passage, high density, and >90% viability.

2

Cool gradually

Cool at ~1°C/min using a controlled-rate freezer.

3

Use correct media

Use recommended medium with DMSO or glycerol.

4

Store properly

Keep below -70°C; avoid temperatures above -50°C.

Thawing Frozen Cells

1

Remove from Storage

Transfer vial directly from liquid nitrogen to a 37°C water bath.

2

Thaw Quickly

Swirl gently until only a small ice crystal remains (under 1 minute).

3

Add to Medium

Sterilize vial with 70% alcohol and slowly add cells to warm growth medium.

4

Spin and Plate

Centrifuge (200×g) for 5–10 minutes; remove supernatant and re-plate.

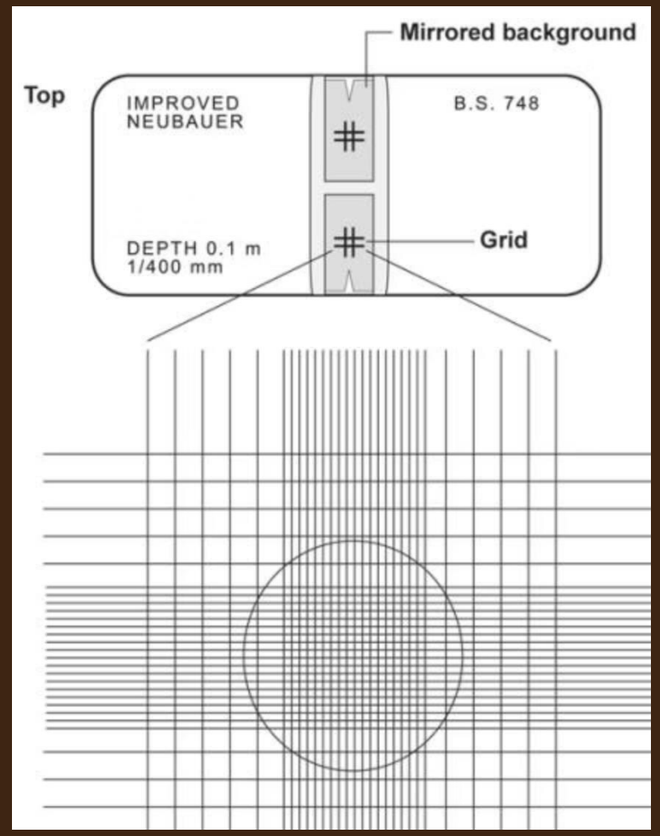
Safety: Always wear face protection as vials may burst. **Process:** Thaw rapidly, transfer slowly, and ensure high seeding density.

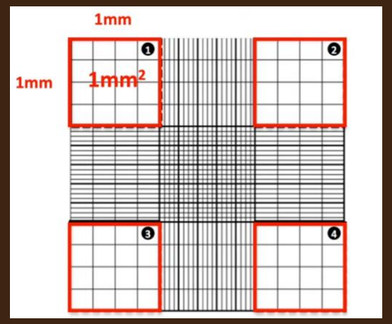
Thawing Frozen Cells

https://youtu.be/4HxqQOHifkU?si=7dKUR7ZcPzda94Cx

Counting Cells with a Hemacytometer

Clean chamber and cover with alcohol. Load 10 µL of sample. Count cells in the 1 mm² square using a 10X lens. Multiply count by 10,000 for cells/mL. Average two samples.





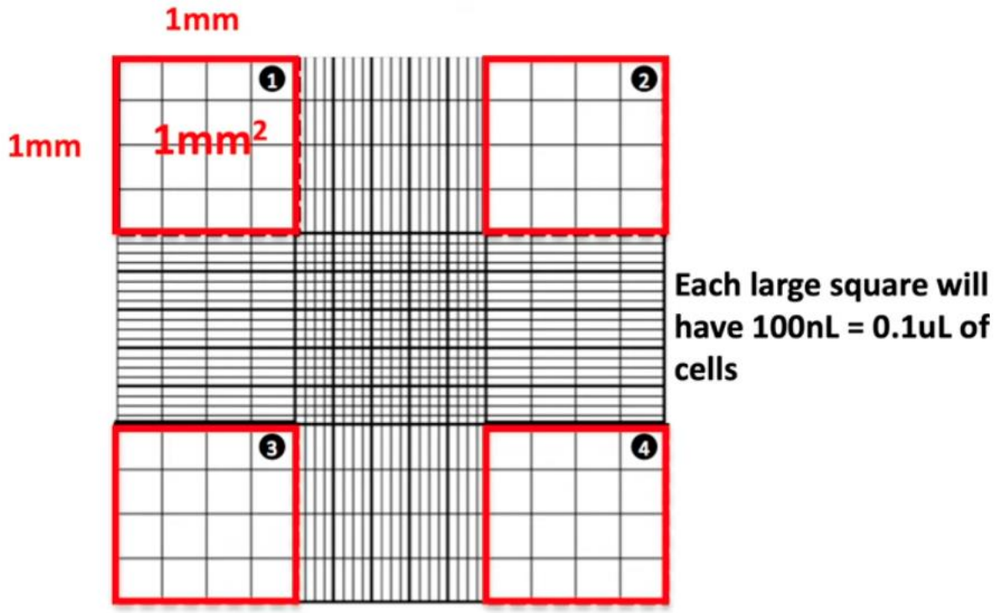
Checking Cell Health

Mix 0.1 mL cell sample with 0.4% trypan blue. Blue cells indicate death; target >95% viability.

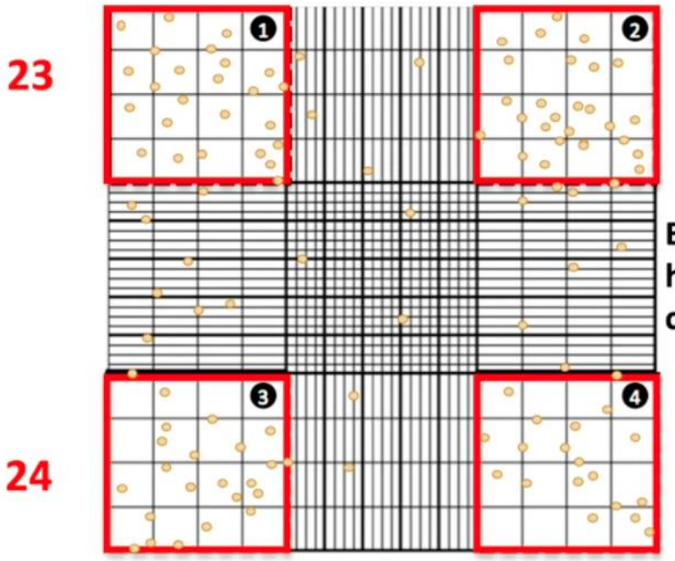
$$\% \text{ viable} = [1 - (\text{blue} \div \text{total})] \times 100$$

Concentrating Cells

Centrifuge at 800×g for 10 minutes. Remove supernatant and resuspend cells in fresh growth medium.



Each large square will have 100nL = 0.1uL of cells



Each large square will have 100nL = 0.1uL of cells

Avg.#cells x dilution factor x 10^4 = #cells / 1mL

*Multiply by volume to get total # cells

*0.1uL x 10^4 = 1,000uL or 1mL

Obtain average

23 + 27 + 24 + 18= 93/4 = 23

Obtain average

23 + 27 + 24 + 18= 93/4 = 23

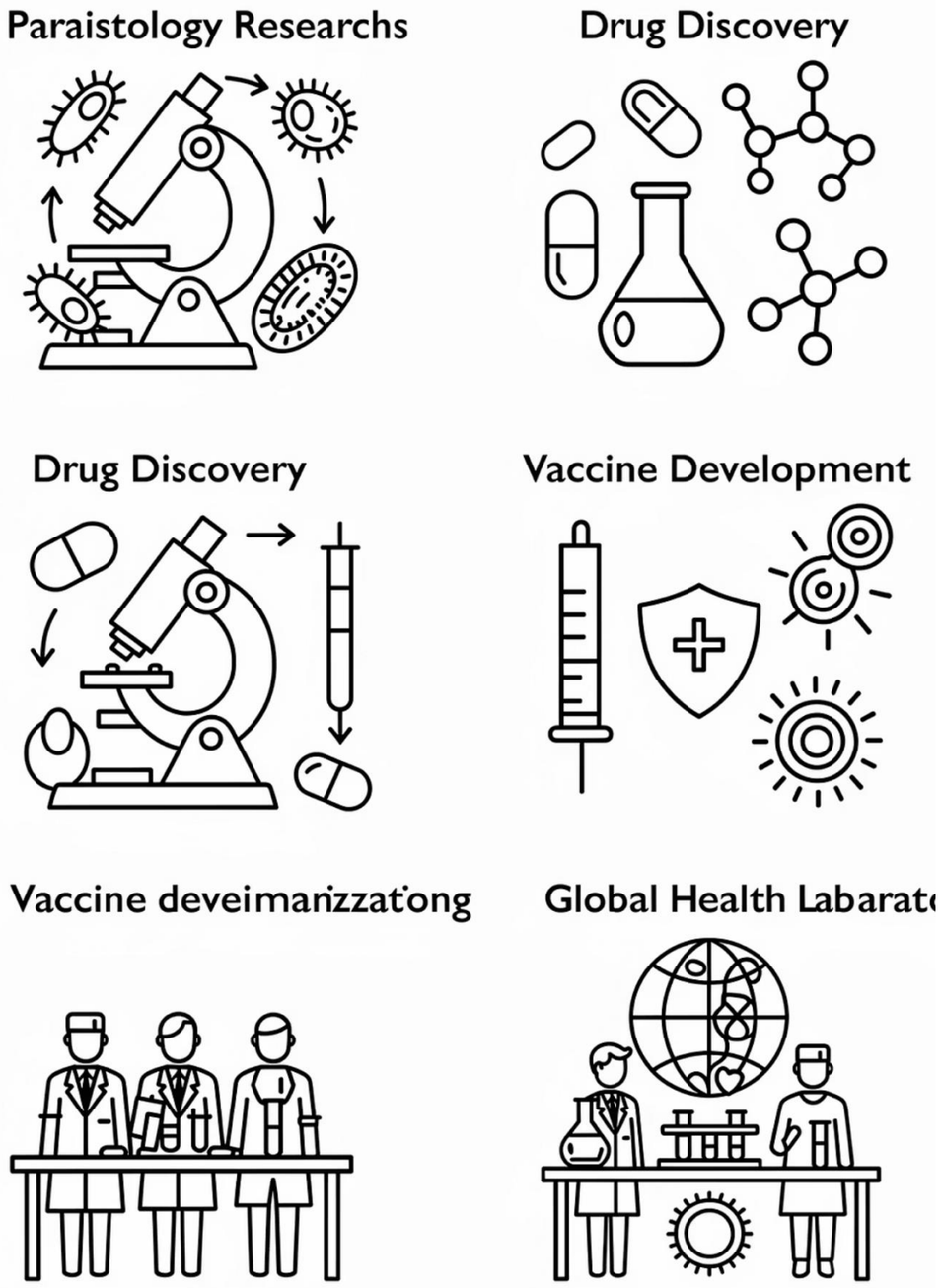
Multiply by dilution factor 10

23 x 10 = 230

Multiply by 10^4

230 x 10,000 = 2,300,000/mL

https://www.youtube.com/watch?v=-A5gLHSj2UA



Application in Parasitology Research

Key Takeaways

Keep things clean

Aseptic technique is essential. Maintain a sterile work environment and handle all materials with care.

Know your cells

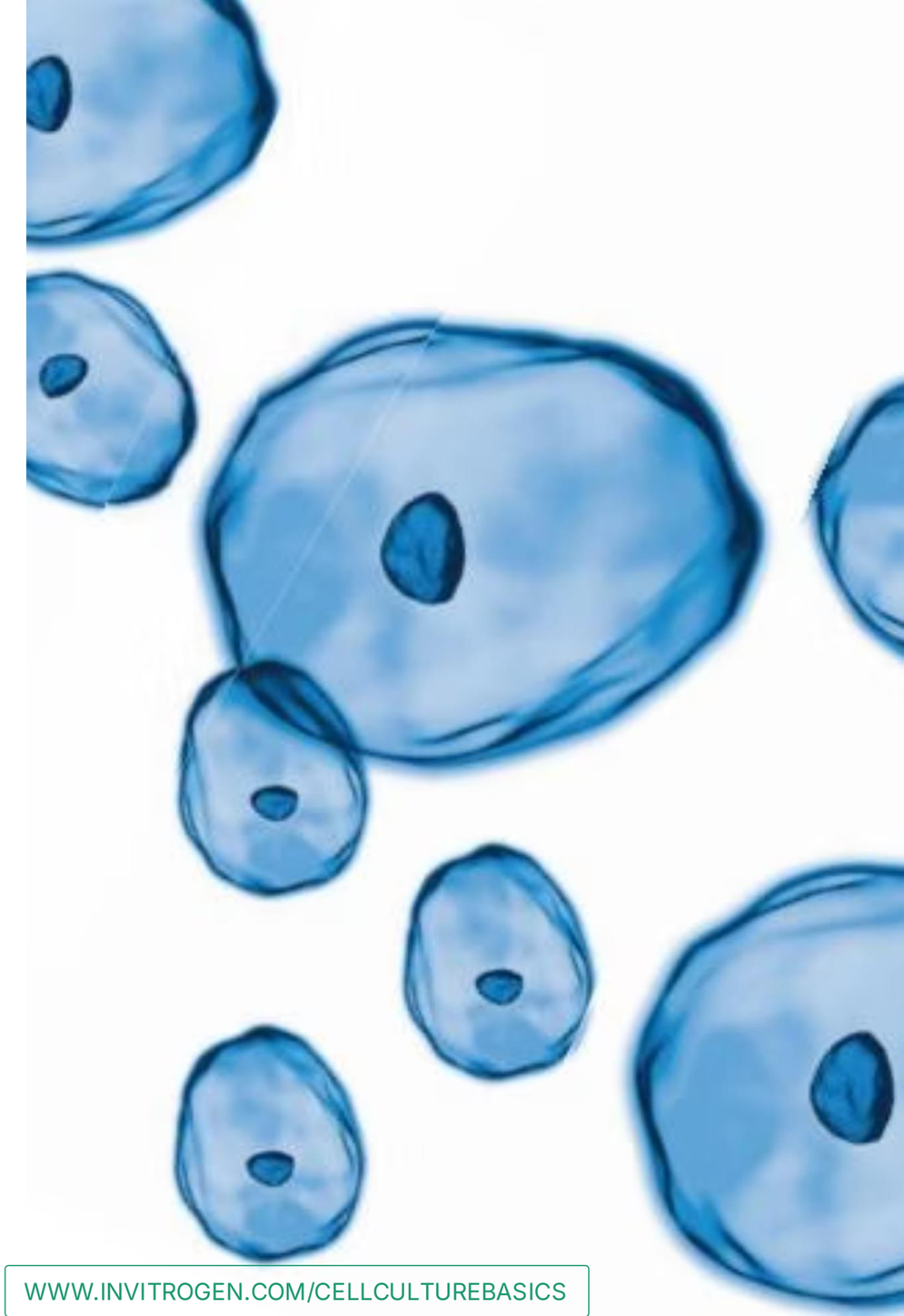
Every cell line is unique. Always follow the specific protocol designed for your cell type.

Freeze samples early

Secure your resources by freezing healthy cells early for long-term storage in liquid nitrogen.

Check cells often

Monitor your cultures daily for early signs of contamination or changes in cell health.



Assignment

Students are required to select **one parasite of interest.** Then, conduct a literature search to identify: **one original research articles**

Students should critically analyze the following aspects:

1 **Research question**

Example: Does parasite X induce M2 macrophage polarization?”

2 **Diagram of experimental design**

3 **Cell culture model/system use?**

e.g. cell line, primary cell line, Monoculture or co-culture....

4 **Readout**

e.g. ELISA, qPCR, flow cytometer etc.

Presentation 4-5 slides