

Biological Problem

Influenza viruses are classified into types A, B, and C, with type A being the most important for humans. Influenza viruses are named based on their type, the location where they were first discovered, the year, and the subtype of two viral surface proteins (hemagglutinin and neuraminidase). For example, A/California/04/2009(H1N1) refers to a strain discovered in California in 2009 with specific H and N subtypes.

There have been at least 15 influenza pandemics in history, including one in 1918 that killed around 50 million people. Influenza viruses constantly circulate, with varying severity. Each year, global attack rates range from 5% to 10% in adults, and up to 50% in certain populations. Influenza causes an estimated 250,000 to 500,000 deaths annually, mostly in developing countries. Vaccines have been proven to reduce influenza-related illness and healthcare costs, with benefits outweighing the costs.

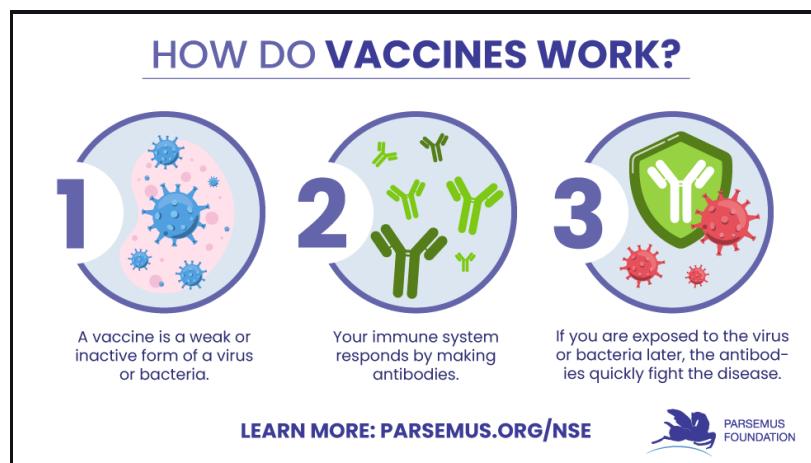
Immunity to Influenza and Vaccine Protection

Understand the approach to solve the problem:

Influenza immunity is mostly mediated by B cells and antibodies, especially immunoglobulin A (IgA) in the respiratory tract, and IgG from the blood. Antibodies targeting hemagglutinin (HA) are key for protection. Neutralizing antibodies are important for preventing infection, and levels of these antibodies are often used to assess vaccine effectiveness.

T cells, including CD4+ and CD8+ cells, also play a role in immune protection, especially in older individuals. Studies suggest that T cell responses may be better correlated with protection in these people, as their antibody responses tend to be weaker. Vaccine responses can vary depending on the vaccine type and administration method. **Inactivated vaccines, given by injection**, are good at stimulating IgG and CD4+ responses, while **live attenuated vaccines (LAV)**, given nasally, stimulate IgA and CD8+ responses more effectively.

A vaccine is a substance that stimulates your body's immune system to recognize and fight off a specific disease-causing agent (like a virus or bacteria) without causing the disease itself.

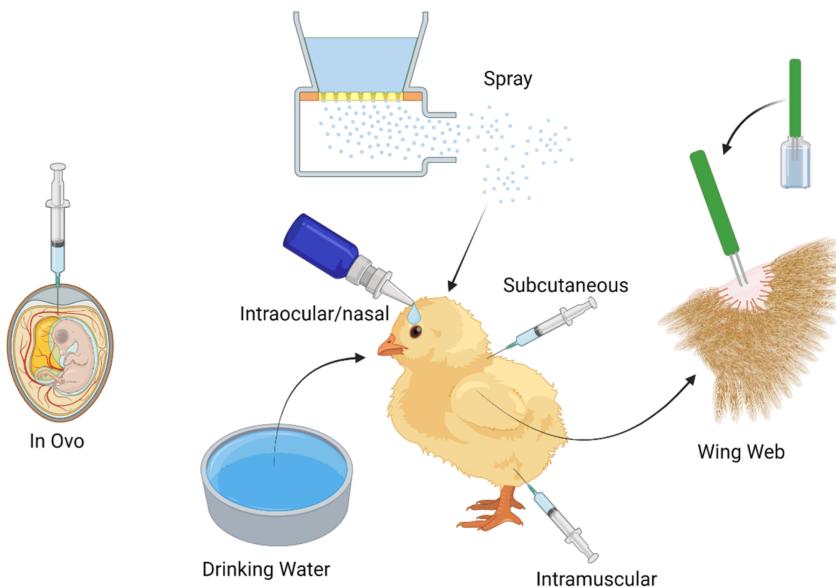


Vaccines are considered effective if they meet specific criteria for antibody levels and seroconversion (an increase in antibody levels after vaccination).

Vaccine Production Using Chicken Eggs: The Traditional Method

Since the 1940s, influenza vaccines have been produced in **embryonated chicken eggs (ECE)**, a method developed a century ago. This process allows for high yields of the virus, and the method has been highly standardized and automated. It also has a proven safety record, with billions of doses administered to people. However, there are several drawbacks:

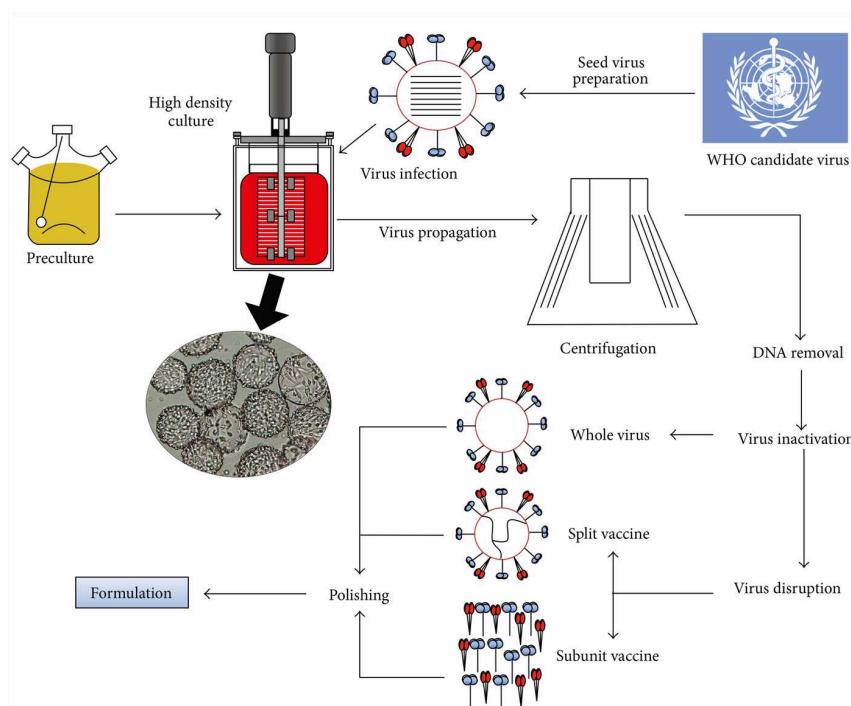
1. Egg Dependency: Producing enough vaccines **requires a large number of eggs**, which need to be available in a short time frame. The eggs also need to come from pathogen-free flocks, which can be an issue in developing countries.
2. Virus Growth Issues: Some virus strains, like recent **H3N2 strains, don't grow well in eggs, and some, like H5N1, can be deadly to the embryos**, leading to low vaccine yields.
3. Sterility Problems: Any breakdown in sterility during the vaccine production process can cause large batches to be discarded, delaying the production of vaccines.
4. Virus Mutations: Influenza viruses tend to mutate more when grown in eggs, which may affect vaccine effectiveness.
5. Allergy Risks: Despite purification, egg protein allergies are a concern in egg-based vaccines.
6. Limited Capacity: Egg-based production cannot meet global demand during epidemics or pandemics, as current capacities only produce about half a billion doses annually.



Vaccine Production Using Cultured Cells: The New Alternative : Cell based Vaccine

Cell-culture-based vaccine production has gained attention over the last 20 years due to the issues with egg-based production. It has several advantages:

1. No Egg Dependency: Cells can be characterized and stored for future use, avoiding reliance on eggs for raw material.
2. Better Virus Growth: Some viruses grow better in cells, and reassortants can be generated directly in the cells.
3. More Control: The cell culture process is more standardized and controlled, which improves tracking and quality.
4. Better Scalability: Cell culture allows for better scalability than egg-based methods.
5. No Egg Allergies: Cell culture avoids the risk of egg protein allergies.
6. More Accurate Virus Strains: Viruses grown in mammalian cells are more similar to the field virus, leading to better matching in vaccines.
7. Cross-Reactivity: Vaccines from mammalian cells often generate broader immune responses, though efficacy may not differ.
8. Flexible Facilities: The same facilities can be used for different vaccines when not producing influenza vaccines, offering greater flexibility.



What is Cell Culture?

Cell culture is the process of growing cells **outside of their natural environment**, typically from animals or plants, in a controlled artificial setting. Cells can be taken directly from tissues or derived from already established cell lines. This technique has been important in many fields, like disease modeling, stem cell research, and making medicines.

- 
- A vertical timeline chart showing the evolution of cell culture from 1665 to 2010+. The timeline is represented by a large blue arrow pointing downwards, with each milestone marked by a blue circle on the left side of the arrow. The text for each milestone is aligned to the right of its corresponding circle.
- 1665 – Hooke's *Micrographia* is published and the term „cell“ is used for the first time
 - 1676 – A. van Leeuwenhoek presents results of his microscopic observations in a letter to the Royal Society
 - 1838-1839 – Schleiden and Schwann formulated the „cell theory“
 - 1855 - Virchow's theory of tissue formation – '*omnis cellula e cellula*'
 - 1885 – Roux's first method of cell culture
 - 1907 – Harrison establishes method of cell culture in hanging drop and maintains frog embryo nerve fibers *in vitro*
 - 1910 – Carrel, Burrows and Montrose explants of tissue fragments for 2-3 months
 - 1912 – Carrel establishes aseptic techniques for cell cultures
 - 1916 – Rous and Jones work out trypsinization and subculture methods
 - 1920 – The ECACC is established for cell culture preservation
 - 1925 – The ATCC is established for cell culture technique evaluation
 - 1925 – 1926 – Strangeways & Fell describe differentiation *in vitro* in organ culture
 - 1930's – Carrel & Lindbergh's new cell cultures devices
 - 1940s – Keilova, Cruikshank& Lowbury introduce antibiotics in tissue culture
 - 1943 – Establishment of the first continuous mouse fibroblast cell line (L-cells)
 - 1948 – Sanford derives clone 929 from the L cell line
 - 1949 – Enders uses cell cultures for growth of virus
 - 1952 – 1955 – Gey establishes the first human cell line
 - 1955 – Eagle develops defined cell culture media
 - 1970s – Kruse develops of laminar-flow cabinets
 - 1965 – Hayflick defined finite life span of human cells
 - 1975 – Kohler & Milstein develop first hybridoma cell lines
 - 1983 – Genentech produces the first therapeutic protein in cell culture and conducts the human clinical trial
 - 1992 – SkinEthic produces human tissue and neural stem cells cultured *in vitro*
 - 1998 – Thomson & Gearhart isolate and culture human embryonic stem cells
 - 2002 – Atala & Lanza exploit tissue engineering
 - 2006 – Yamanaka obtains induced pluripotent stem cells (iPS)
 - 2010+ – Atala demonstrates 3D tissues and organs bioprinting techniques

In 1676, the Royal Society (RS) received a letter from Antonie van Leeuwenhoek, in which the microscopist had described his exciting discoveries—observations and records of small living particles.

It began after the idea of "spontaneous generation" was proven wrong by Louis Pasteur in the 1800s. **By the early 1900s, scientists learned that cells come from other cells and developed ways to grow cells in the lab.**

One of the key moments in the development of cell culture happened in the early 1900s when **Ross Harrison created a method for growing cells in the lab, and other scientists like Burrows and Carrel improved it.** These breakthroughs allowed scientists to grow animal and human cells outside of the body.

This led to advances in research, including studying diseases, making medicines, and producing proteins for treatments. Today, cell culture is used in areas like stem cell research, cancer studies, and tissue engineering, offering new possibilities for medical treatments and healing injuries.

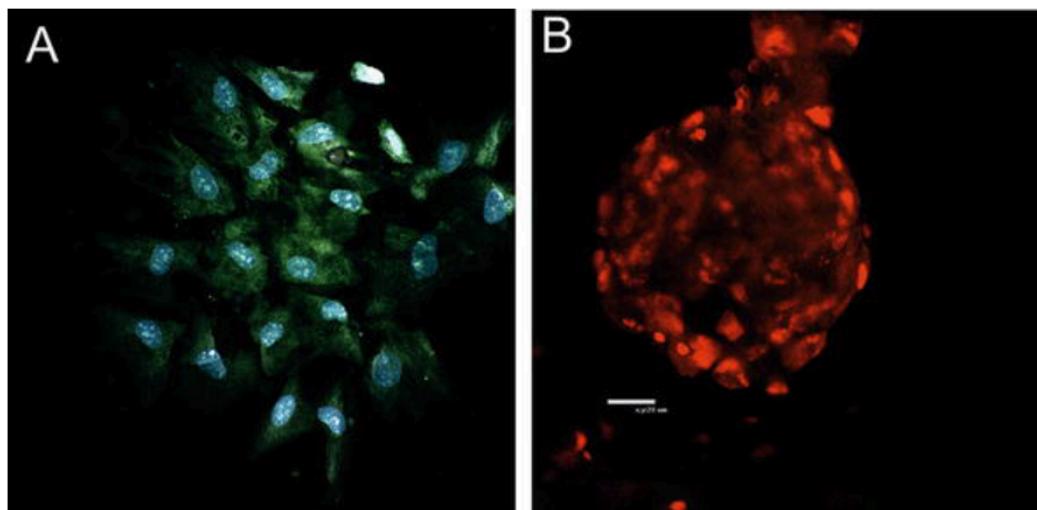


Figure 2.

Bovine mammary epithelial cells immunostained against cytokeratins and DAPI-stained nuclei (confocal laser scanning microscopy, 400 \times) (A). Dome structures stained with propidium iodide (confocal laser scanning microscopy; magnification, 600 \times) (B) [14].

Development of Cell Line Cultures

The first cell line, known as the "L" cell line, was created by Earle in 1948 from **mouse tissue. During the 1950s and 1960s, additional diploid cell lines were developed, such as HeLa (from human cervix), MRC-5 (human lung), WI-38 (human lung), and Vero (simian kidney).** These early cell lines are summarized in Table below.

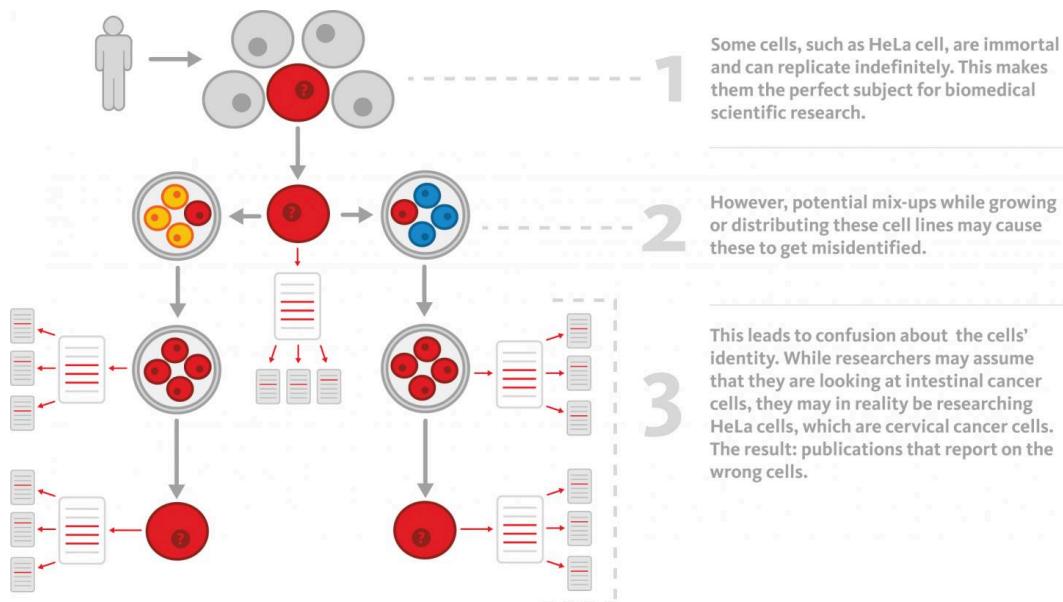
Cell lines differ from primary cell cultures, which are directly obtained from tissues and only considered primary until the first subculture. While primary cultures contain mixed cell types,

cell lines are derived from serial subcultures. Cell lines from normal tissues have finite growth, whereas those from cancerous tissues can proliferate indefinitely.

Name	Species and tissue	Morphology	Author and year of origin
L929	Mouse connective tissue	Fibroblast	Earle, 1948
HeLa	Human cervix	Epithelial	Gay, 1951
CHO	Chinese Hamster ovary	Epithelial-like	Puck, 1957
MDCK	Canine kidney	Epithelial	Madin and Darby, 1958
WI-38	Human lung	Fibroblast	Hayflick, 1961
BHK-21	Syrian Hamster kidney	Fibroblast	Macpherson and Stoker, 1961
Vero	African Green Monkey kidney	Epithelial	Yasumura and Kawakita, 1962
NIH 3T3	Mouse embryo	Fibroblast	Todaro and Green, 1962
MCR-5	Human lung	Fibroblast	Jacobs, 1966
SH-SY5Y	Human neuroblastoma	Neuroblast	Biedler, 1970

However, using cell lines has limitations, including:

- Genetic changes with increasing passage numbers
- Genotypic and phenotypic drift in long-term cultures
- Different drug responses compared to patient reactions
- Differences in the microenvironment of original tumors vs. cell cultures (2D vs. 3D)
- **Risk of cross-contamination** (e.g., with HeLa cells)
- Morphological, gene expression, and pathway changes due to culture conditions
- Infections (e.g., mycoplasma) that alter cell behavior
- Difficulty in creating long-term cancer cell lines for certain tumor types
- Loss of tumor heterogeneity in cultures.



Contaminated cell lines have been extensively used in research without knowledge of their true character. For example, most if not all research on the endothelium ECV-304 or the megakaryocyte DAMI cell lines has in reality been conducted on bladder carcinoma and erythroleukemia cells, respectively. Thus, all research on endothelium- or megakaryocyte-specific functions utilizing these cell lines has been misguided.

In the 1920s, **salt solutions for cell cultures were formulated** by researchers like Pannett and Compton (1924), Gay (1936), Earle (1943), and Hanks (1948), which helped **define the basic needs for cell culture media, including amino acids, salts, vitamins, hormones, and glucose**. By 1962, about 60 chemically defined media were developed, including essential and non-essential amino acids, with notable examples like Earle's protein-free media for L cells and the development of Essential Medium (EM) and Dulbecco Modified Eagle's Medium (DMEM). Media were also categorized into long-term and short-term cultivation media, such as Trowell's medium for organ cultures.

By the 1940s, antibiotics were added to cell cultures to prevent contamination. Penicillin and streptomycin were studied for their effects on cell growth, though some antibiotics showed toxic effects at higher concentrations. Antibiotics like aminoglycosides and tetracyclines were found effective for mycoplasma elimination, though their use can lead to antibiotic resistance.

Cell culture environments also require proper conditions. Incubators, first used in the late 1800s, became essential for maintaining the temperature, humidity, and CO₂ levels needed for cell growth. Automated incubators are now standard, typically maintaining 37°C, 5-10% CO₂, and 95% humidity.

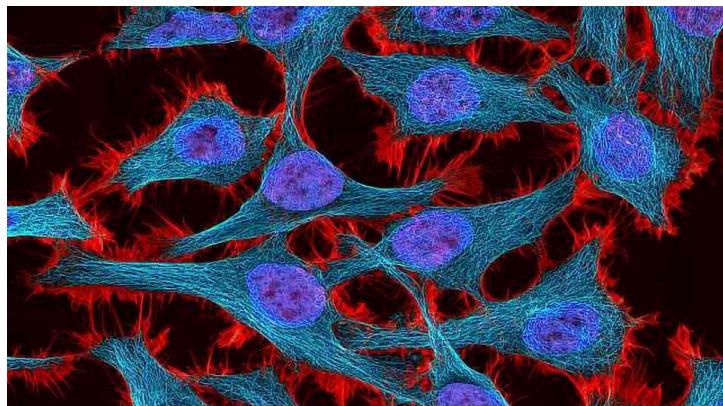
To protect cultures from contamination, **safety techniques** have evolved. In the early 1900s, safety cabinets were developed to prevent exposure to harmful bacteria. In the 1960s, laminar flow clean rooms with HEPA filters became common, and later, Class II biological safety cabinets (BSCs) were introduced. These cabinets protect both the culture and personnel by ensuring clean air flow and sterilizing the work surface with UV light.

To avoid contamination, good aseptic techniques, copper CO₂ incubators, regular mycoplasma testing, and routine media filtration are essential. Using antibiotics should be minimized to prevent resistant microbial strains, and cross-contamination of cell lines should be monitored through methods like karyotyping, electrophoresis, and DNA fingerprinting.

1950s:

- **1951:** Henrietta Lacks, a 31-year-old African American woman, seeks treatment for cervical cancer at Johns Hopkins. Cells from her tumor are taken and become known as HeLa cells.
- **1952:** HeLa cells grow continuously in the lab for the first time, contributing to over 110,000 research studies over the years.
- **1953:** HeLa cells are used to grow large amounts of the poliovirus, laying the groundwork for the polio vaccine.
- **1956:** HeLa cells are used to study the effects of X-rays on cells, helping scientists understand radiation's impact on human health.

In 1951, **Henrietta Lacks' cervical cancer cells were cultured by Dr. George Gay at Johns Hopkins, becoming the HeLa cell line.** These cells grew rapidly and were used in research, including the development of the polio vaccine. HeLa cells became a key resource for cancer studies.



HeLa cells are considered "immortal" because they can divide indefinitely in a laboratory setting, unlike normal human cells which have a limited lifespan, due to the overactive telomerase enzyme preventing telomere shortening and cell aging. **These cells could grow and multiply endlessly in labs, unlike most cells that die quickly after being removed.** Johns Hopkins did not profit from HeLa cells and freely shared them for research.

1960s:

- **1964:** HeLa cells are sent to space to study how radiation affects human cells in space.
- **1964:** HeLa cells help scientists understand the benefits of Hydroxyurea for treating blood cancers and sickle cell anemia.

1970s:

- **1973:** HeLa cells are used to study how *Salmonella* infects the body, leading to better treatments.

1980s:

- **1985:** HeLa cells help discover that HPV causes cervical cancer, leading to the development of an HPV vaccine.
- **1985:** HeLa cells show that the drug Camptothecin slows cancer growth, eventually leading to FDA approval for cancer treatment.
- **1988:** HeLa cells contribute to understanding how HIV infects cells.
- **1989:** HeLa cells help discover how telomerase prevents aging by protecting chromosomes.

1990s:

- **1993:** HeLa cells help scientists understand how tuberculosis infects humans, aiding in treatment development.

2000s:

- **2001:** HeLa cells help develop single-cell imaging techniques, winning a Nobel Prize.
- **2001:** Research with HeLa cells reveals how Ebola and HIV enter cells, aiding vaccine development.
- **2008:** Dr. Harald zur Hausen wins the Nobel Prize for proving that viruses like HPV can cause cancer, leading to the HPV vaccine.
- **2009:** Dr. Elizabeth Blackburn, Dr. Carol Greider, and Dr. Jack Szostak win the Nobel Prize for their work with HeLa cells on telomeres, explaining cellular aging.

2010s:

- **2010:** HeLa cells help repurpose thalidomide to treat cancer.
- **2013:** NIH reaches an agreement with Henrietta Lacks' descendants to allow controlled access to the HeLa cell genome for research.
- **2014:** HeLa cells are used to develop a new microscope technique for live viewing of cellular processes, winning the Nobel Prize.

In the 1950s, Initially, rhesus monkey cells were used for vaccine testing, but since large quantities were hard to obtain, HeLa cells were chosen as an alternative. In October 1952, Dr. H.M. Weaver of the NFIP met with Dr. Brown to discuss creating a HeLa production lab at Tuskegee, which would be supported by an NFIP grant. Drs. Brown and Henderson received training in cell culture techniques and set up the necessary equipment and infrastructure. By April 1953, they received the original HeLa cell strain from Dr. George Gey at Johns Hopkins. They aimed to ship at least 10,000 cultures per week to various labs.

By 1955, they had shipped 600,000 cultures. Tuskegee's work led to the creation of a similar facility in Maryland, starting a multibillion-dollar industry in biomedical specimen production. The Tuskegee HeLa factory was eventually closed due to competition, but the university's contributions to biomedical research, especially in the fight against polio, remain significant.

HeLa cells were infected with HPV18, which degrades the p53 tumor suppressor gene, and have multiple genetic mutations. In 2013, their genome was sequenced without the family's consent, sparking controversy. Studies revealed chromosomal rearrangements and gene expression changes, leading some to suggest HeLa cells may have evolved into a new species.

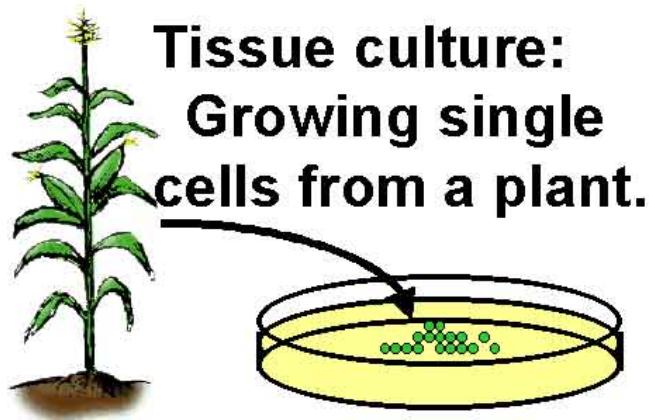
Why study cell culture today?

Cell culture is used as a model system in biology and medicine to study cells in a controlled environment, enabling researchers to investigate cell behavior, drug effects, and disease mechanisms, while also being crucial for manufacturing biopharmaceuticals and developing vaccines.

Cell culture is widely used for studying cell functions, drug effects, genetic changes, and cancer. It's also key in drug testing, vaccine production, and manufacturing biological products like therapeutic proteins, offering consistent and reproducible results.

Primary Culture

Primary culture refers to **cells that have just been isolated from tissue** and are growing in culture. When the cells grow to cover the surface of the vessel (confluence), they must be subcultured, meaning they are transferred to a new vessel with fresh growth medium to continue growing.



Primary Culture: This is the first successful growth of cells after isolation from tissue. To maintain optimal growth, cells are subcultured through several passages.

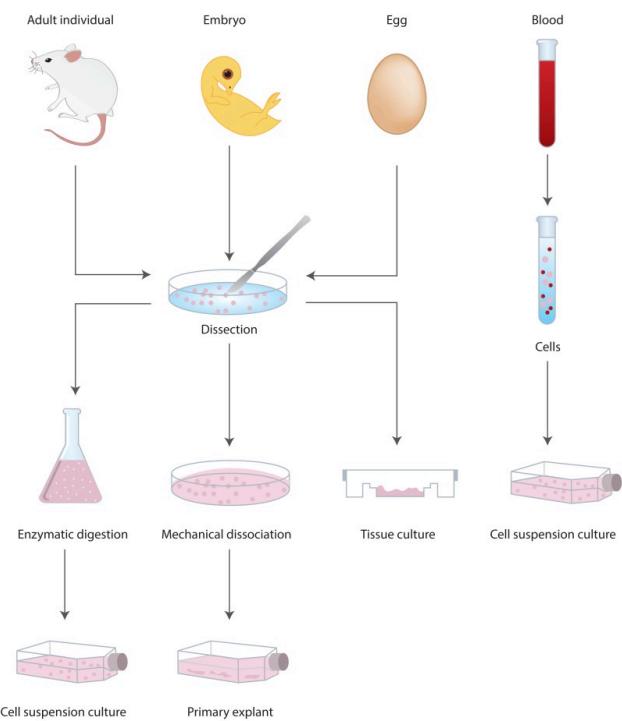
However, cells eventually go into senescence after about 30 divisions. To preserve cells long-term, they are stored in a cell bank. In some cases, cells can be immortalized (e.g., B cell lymphocytes can be immortalized with the Epstein-Barr virus), allowing them to grow indefinitely. These immortalized cells have unlimited availability but may lose their original characteristics.

Cell sampling and preparation involve obtaining cells from tissues, organs, or fluids for analysis. It's crucial to ensure sample quality, preventing degradation. Blood samples require careful collection, considering factors like nutritional state, hormone levels, and circadian rhythms, which affect cell behavior. The choice of anticoagulant also matters, as some can impact cell function. Various cell separation methods, such as differential and

density gradient centrifugation, are used to isolate specific cell types. These techniques separate cells based on size, density, or sedimentation rate, ensuring efficient recovery and preserving cell viability for further analysis.

Cell Lines and Strains

- **Cell Lines:** Cells that are cultured repeatedly over time.
- **Cell Strain:** A specific genetic variation within a cell line.



<https://m.youtube.com/watch?v=8vnpmRANztU>

Finite vs Continuous Cell Line

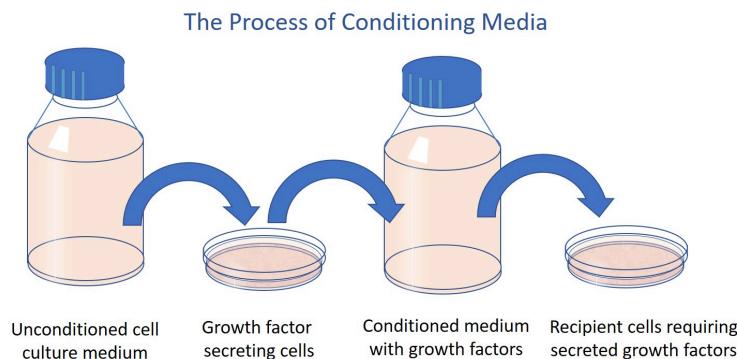
- **Finite Cell Line:** Normal cells that divide a limited number of times before they stop, a process called senescence.
- **Continuous Cell Line:** Cells that have become "immortal" and can divide indefinitely, often due to a transformation caused by chemicals or viruses.

Culture Conditions

Cells need specific conditions to grow, which include:

- **A substrate or medium** providing nutrients like amino acids, vitamins, and minerals.
- **Growth factors and hormones.**
- **Gases** like oxygen and carbon dioxide.
- **A controlled environment** (temperature, pH, osmotic pressure).

Some cells need to attach to a surface to grow (adherent culture), while others can grow in suspension (suspension culture).



Cryopreservation

Excess cells from subculturing can be frozen with protective agents (like DMSO or glycerol) at temperatures below -130°C for long-term storage, called cryopreservation, until needed.

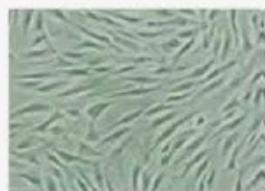
Morphology of Cells in Culture

Cells can be classified by their shape:

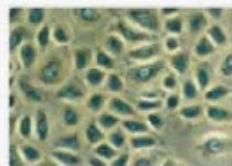
- **Fibroblastic cells:** Elongated, grow attached to a surface.
- **Epithelial-like cells:** Polygonal, grow in patches on a surface.
- **Lymphoblast-like cells:** Spherical, grow in suspension without attachment.

Morphology of Cells in Culture

Fibroblastic (or fibroblast-like)



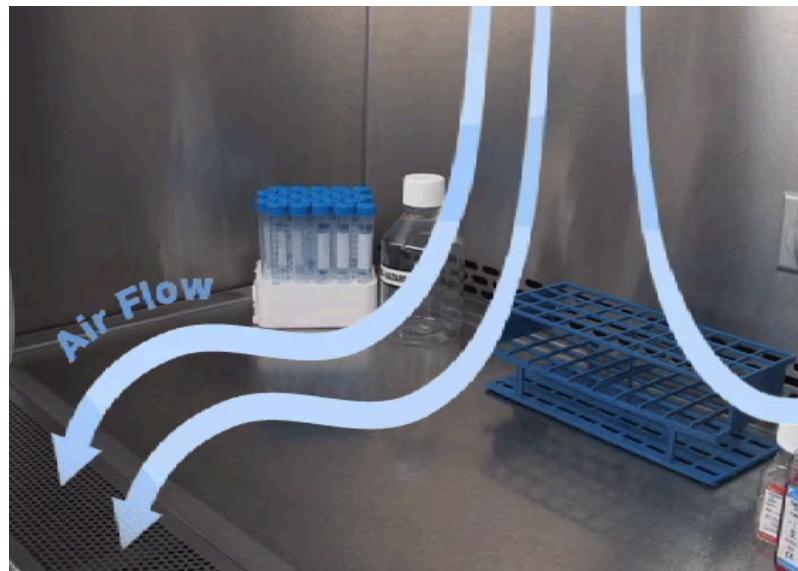
Epithelial-like cells

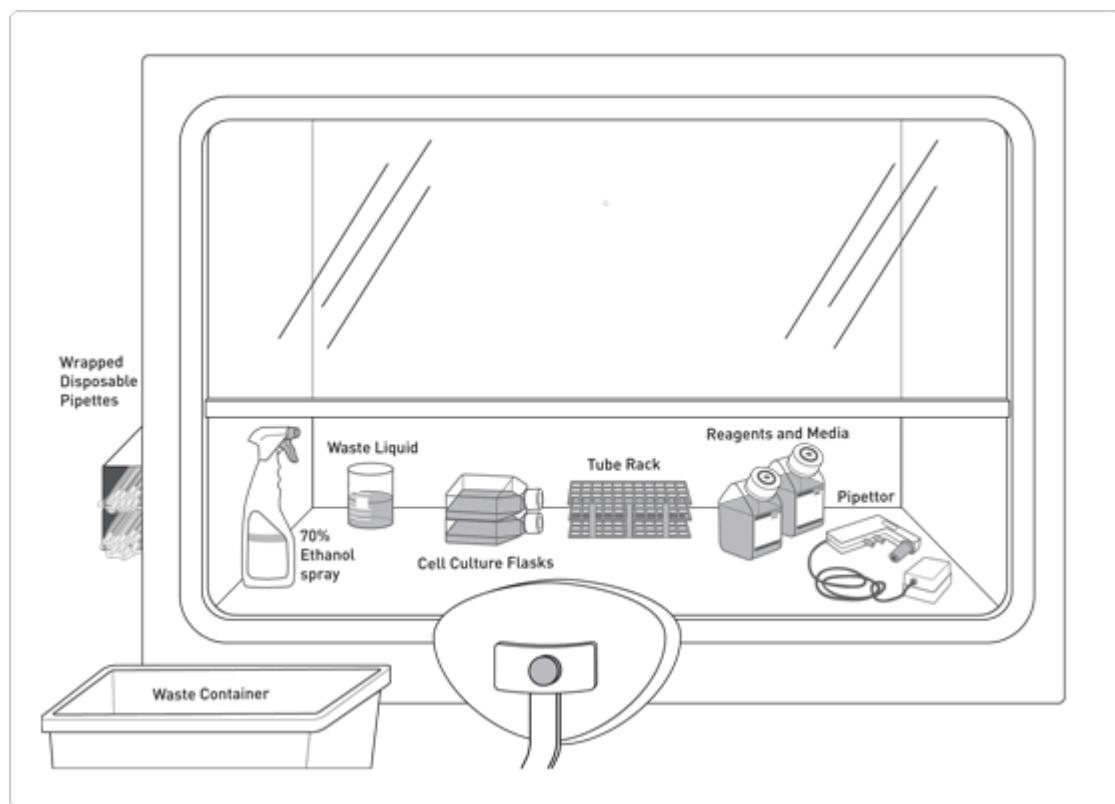


Lymphoblast-like cells



Webinaria





The basic layout of a cell culture hood for right-handed workers. Left-handed workers may switch the positions of the items laid out on the work surface.

Cell Culture Hood vs. Biosafety Cabinet

Both cell culture hoods and biosafety cabinets are types of laminar flow hoods, but not all biosafety cabinets are suitable for cell culture. Class I biosafety cabinets, for example, don't protect cultures from contamination and should not be used for cell culture work.

For cell culture labs, a **Class II** or higher biosafety cabinet (often called a cell culture hood) provides proper protection for the culture, personnel, and environment. This protection is crucial for preventing contamination in experiments and ensuring valuable time and resources aren't wasted.

Clean Bench

A clean bench, whether horizontal or vertical, is not a biosafety cabinet. It only protects the product and can expose the user to hazardous materials, making it unsuitable for mammalian cell culture. Clean benches can be used for certain tasks like sample protection in hospitals and cleanrooms but should never be used for handling cell cultures.

Biological Safety Levels and Classes of Laminar Flow Hoods

It's important to match the appropriate biosafety level (BSL) with the class of laminar flow hood for safe handling of materials:

- **BSL-1:** Basic protection for agents that don't cause disease in healthy humans.
- **BSL-2:** For moderate-risk agents that can cause human disease through ingestion or exposure.

- **BSL-3:** For agents that may cause serious infections, especially through aerosol transmission.
- **BSL-4:** For highly dangerous agents with no known treatments, requiring high containment.

Classes of Laminar Flow Cabinets

- **Class I:** Protects the environment, not the culture. Suitable for low-risk cultures and aeration tasks.
- **Class II:** Provides protection for personnel, cultures, and the environment. Ideal for handling potentially hazardous materials.
- **Class III:** Highest level of protection, gas-tight, for handling dangerous pathogens in BSL-4 labs.

Cell Culture Hood Layout

A cell culture hood should be spacious, easy to clean, and have good lighting. Items should be arranged in a way that's easy to reach and clean. For a right-handed worker, the layout often follows this structure:

- Center: Cell culture vessels
- Right front: Pipettor
- Right back: Reagents and media
- Rear middle: Tube rack with extra reagents
- Left rear: Liquid waste container

Disinfect all items with 70% ethanol before placing them in the hood.

The equipment needed for a cell culture laboratory depends on the type of research being conducted. However, all labs require the same basic equipment to maintain aseptic conditions and support the cell culture process. Here is a simplified list of essential equipment used in a mammalian cell culture lab:

1. **Cell Culture Hood:** A sterile area, usually a laminar flow hood or biosafety cabinet, is essential to prevent contamination and protect both researchers and cells. This hood filters air and contains splashes to maintain aseptic conditions.
2. **Incubators:** These provide the right environment for cell growth. Incubators should have precise temperature control ($\pm 0.2^\circ\text{C}$). There are two types: dry incubators, which prevent evaporation by sealing culture flasks, and humid CO₂ incubators, which provide better control of humidity and CO₂ levels.
3. **Storage:** Labs need spaces to store media, reagents, chemicals, cultureware, and PPE. It's important to store sensitive items like media in dark conditions to avoid degradation from light.
4. **Refrigerators and Freezers:** Proper cold storage is critical. Lab refrigerators maintain temperatures between 2–8°C, while freezers can go as low as –20°C for storing supplements. Ultra-low freezers (–40 or –80°C) are used for long-term

storage and cryopreservation.

5. **Cryogenic Storage:** For long-term cell preservation, cryogenic storage is essential. Cells should be frozen with a cryoprotectant (like DMSO or glycerol) and slowly cooled to avoid damage. Cryopreservation can be done in either vapor-phase or liquid-phase liquid nitrogen systems.
6. **Cell Counter:** This tool counts live and dead cells, checks cell viability, and measures average cell size. It's essential for accurate growth tracking, especially when working with multiple cell lines. While manual counting with a hemocytometer is possible, automated cell counters like the Countess 3 are faster and more precise.
7. **Water Bath:** Used to heat media and reagents to the right temperature, especially for thawing frozen samples. However, it can become a contamination risk if not properly maintained. An alternative is using Lab Armor Beads, which replace water to reduce contamination risk.
8. **Centrifuge:** Important for processes like cell subculturing, freezing, and thawing. Cells are separated from liquids by spinning them at high speeds, forming a pellet for the next steps. Benchtop centrifuges are commonly used in cell culture labs, but care is needed with delicate cell lines.
9. **Imaging Microscopes:** Used to observe cell behavior, morphology, and growth. They are essential for checking cell health, detecting contamination, and monitoring cell detachment during passage. Advanced systems like the EVOS provide high-resolution images.
10. **Consumables:** These are disposable items like culture plates, pipettes, culture tubes, gloves, and protective clothing. They ensure sterile conditions and reduce contamination risks in the lab.

Aseptic vs. Sterile Technique

- **Sterile technique** ensures an environment is completely free of microorganisms to prevent contamination.
- **Aseptic technique** focuses on avoiding contamination in a previously sterile environment. For example, a cell culture hood is sterilized before use (sterile technique), and during experiments, aseptic techniques are used to maintain its sterility.

What are Aseptic Techniques?

Aseptic techniques are procedures designed to **protect cell cultures from contamination by microorganisms**. Key elements include a sterile work area, personal hygiene, and sterile reagents and handling.

Why is Aseptic Technique Important?

Aseptic techniques minimize contamination risks from bacteria, fungi, viruses, and other sources like nonsterile supplies, unclean surfaces, and airborne particles. Poor aseptic practices can lead to altered growth, compromised cell viability, and wasted resources.

Aseptic Techniques Checklist

- Maintain a **sterile work area**, ideally in a designated tissue culture room or lab section.
- Use a **laminar flow hood** (biosafety cabinet) for sterile handling.
- Follow PPE guidelines (gloves, coats, face shields) to prevent contamination from personnel.
- Sterilize reagents, media, and solutions properly before use.

Sterile Handling

Sterile handling includes using PPE, following sterilization protocols for reagents, and taking care not to introduce contaminants during experiments.

Safe Lab Practices

Adhere to aseptic techniques to protect cultures and minimize safety hazards in the lab. Risks include accidental punctures, spills, and exposure to harmful agents. Always follow institutional safety guidelines and standard microbiological practices.

How to Identify Contamination in Cell Culture

Contamination in cell culture can be identified by knowing the normal cell morphology and potential contaminants. Regular testing and monitoring help detect contamination early. Some contaminants, like mycoplasma, are harder to detect and need special tests.

Tests to Identify Contamination:

- **Microscopy:** Visual inspection under a microscope can reveal bacteria, fungi, or viral infections.
- **Microbial Testing:** Techniques like PCR, ELISA, and immunostaining can help identify specific contaminants.
- **Cell Line Authentication:** Verifying the identity of cell lines ensures they are not contaminated or misidentified.

Types of Contamination:

- **Bacterial Contamination:**

- Bacteria are common and grow quickly. They can make cultures cloudy and cause a drop in pH.
- Bacteria appear as small, moving granules under a microscope, and higher magnification can show their shape.

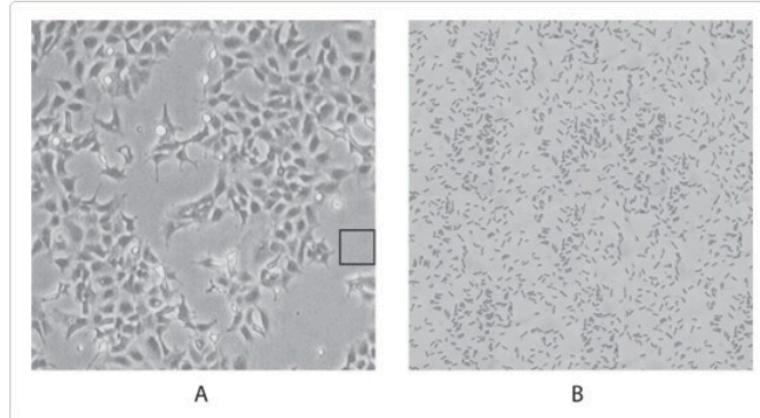


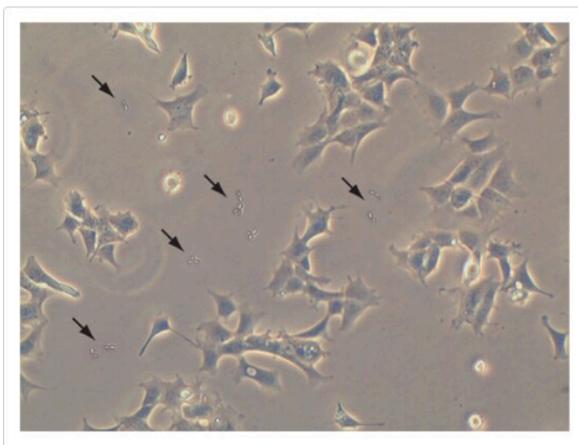
Figure 1. Simulated phase contrast images of adherent 293 cells contaminated with *E. coli*. The spaces between the adherent cells show tiny, shimmering granules under low power microscopy, but the individual bacteria are not easily distinguishable (panel A). Further magnification of the area enclosed by the black square resolves the individual *E. coli* cells (panel B), which are typically rod-shaped and are about 2 μm long and 0.5 μm in diameter. Each side of the black square in panel A is 100 μm .

- **Viral Contamination:**

- Viruses are difficult to detect due to their small size. They may not affect cell cultures from different species but can be dangerous to lab personnel.
- Detection requires electron microscopy or PCR with viral primers.

- **Fungal Contamination:**

- Fungi (like molds and yeasts) can grow in culture media.
- Yeast contamination turns the medium turbid, with pH changes occurring later. Yeast appears as oval or spherical cells under the microscope.
- Mold contamination causes a rapid increase in turbidity and shows as thin, wisp-like filaments under a microscope.



Using Antibiotics and Antimycotics:

- Antibiotics and antimycotics should be used cautiously, as they can hide contamination and create resistant strains.

- Use them only as a last resort and for short periods, ensuring they don't interfere with your experiments.

Decontaminating Cell Cultures:

- **Identify the Contamination:** Determine if the contamination is bacterial, fungal, or mycoplasma.
- **Isolate the Contaminated Culture:** Separate it from healthy cultures.
- **Clean the Lab Equipment:** Disinfect incubators and laminar flow hoods.
- **Use Antibiotics Carefully:** Test the right antibiotic concentration to avoid toxicity to cells, then culture cells with the antibiotic at safe levels.
- **Monitor the Cultures:** Observe for signs of toxicity and repeat the process until contamination is eliminated.

Cross-Contamination:

- Cross-contamination occurs when one culture contaminates another.
- Prevent this by obtaining cell lines from reliable sources and regularly checking their characteristics. Methods like DNA fingerprinting can help identify cross-contamination.

Types of Culture Media

The culture medium is crucial for cell growth as it provides essential nutrients, growth factors, hormones, and regulates pH and osmotic pressure. Over time, the development of defined media has helped standardize experiments and meet growing demands. There are three main types of culture media: basal media, reduced-serum media, and serum-free media, which vary in their use of serum supplementation.

1. Basal Media:

- Basal media contains amino acids, vitamins, salts, and glucose, but needs additional serum for cell growth.

2. Reduced-Serum Media:

- This media contains additional nutrients and animal-derived factors to reduce the amount of serum needed.

3. Serum-Free Media:

- Serum-free media replaces serum with specific nutrients and hormones. It is used for specific cell lines, such as CHO cells or hybridomas, and offers better control over cell type-specific growth factors.

- **Advantages of Serum-Free Media:**

- More consistent performance
- Easier purification
- Precise evaluation of cell functions
- Increased productivity
- Better control over cellular responses

- **Disadvantages:**

- Requires cell-type-specific formulations
- Higher reagent purity needed
- Slower cell growth

Best Practices for Cell Culture Media:

- Warm refrigerated media before use.
- Protect media from light to prevent degradation of essential vitamins.
- Store supplemented media at 2–8°C and use within 2–4 weeks.

Serum in Culture Media:

- Serum provides growth factors, hormones, and lipids. Fetal Bovine Serum (FBS) is the most commonly used serum due to its high growth factor content and low risk of cell binding or lysis.
- **Drawbacks of Serum:**
 - Expensive
 - Variability and lack of standardization
 - Can affect cell growth and function
 - Risk of contamination if not sourced properly

FBS is ideal for cell culture but should be obtained from reputable sources to ensure quality, safety, and consistency.

Types of Cell Culture

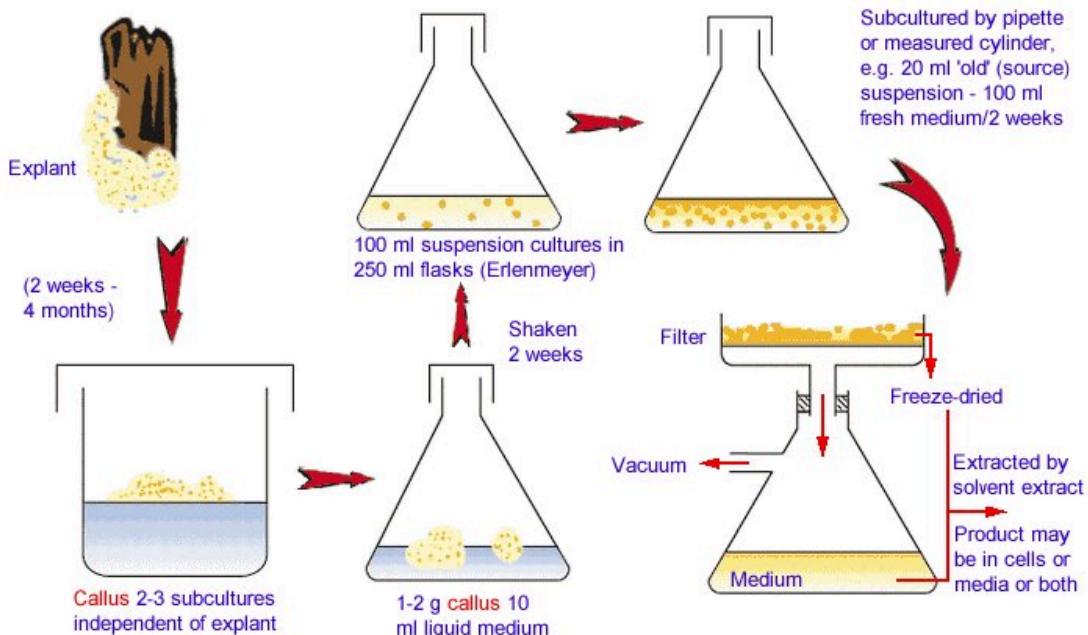
1. **Adherent Cultures:** Cells must attach to a surface (e.g., plastic dishes or flasks). These cultures are typically used for most cell types and require periodic passaging.
2. **Suspension Cultures:** Cells float in the medium and do not require attachment to a surface. These cultures are useful for cells that grow in suspension and for large-scale production.



Plant Cell Culture

Plant cell culture involves **growing single cells or small clusters in suspension** by stirring. By adjusting plant growth factors, the cells can be made to form embryos, which can then develop into whole plants. However, this process can cause genetic instability in the new plants. More commonly, cell cultures are used to produce enzymes, drugs, pigments, and flavors.

Plant Cell Culture



Procedure for obtaining **digoxin** from cell suspension of *Digitalis lanata*

Adherent vs. Suspension Cultures

Adherent Culture

Best for most cell types, including primary cultures

Requires periodic passaging and easy visual inspection

Growth limited by surface area

Requires tissue culture-treated vessels

Suspension Culture

For cells adapted to suspension or non-adhesive cells (e.g., hematopoietic)

Easier to passage, but requires daily monitoring

Growth limited by cell concentration in medium

Can be maintained in non-treated vessels with agitation for gas exchange

Cell Morphology Cell morphology (shape, size, appearance) helps determine cell health. Regular inspection ensures early detection of contamination and can indicate the need for medium changes.

Types of Cell Morphologies

- **Fibroblastic Cells:** Elongated shapes, often bipolar, and adhere to a substrate.
- **Epithelial Cells:** Polygonal shapes that grow in patches.
- **Lymphoblast-like Cells:** Round shapes, typically grow in suspension.

Choosing the Right Cell Line for Your Experiment

Selecting the right cell line for your experiment depends on factors like your research goal, the experiment's duration, and the need for consistency. Assess your objectives to pick the cell line that aligns best with your needs.

Types of Cell Lines

1. **Species-Specific:** Non-human and non-primate cell lines have fewer biosafety restrictions but may not always fit your research needs.
2. **Functional Characteristics:** Choose cell lines based on the function needed for your experiment (e.g., liver or kidney cells for toxicity testing).
3. **Normal vs. Transformed:** Transformed cells grow faster, require less serum, and are continuous, but have undergone genetic changes.
4. **Growth Conditions:** Consider growth rate, cloning efficiency, and whether the cells need to grow in suspension (for high protein yields, for example).

Considerations Before Selecting a Cell Line

- Is the cell line well-characterized, or will you need to validate it yourself?
- Do you have a normal cell line as a control for comparison?
- Is the cell line stable, and can you create sufficient frozen stocks?

You can either establish your own cell culture from primary cells or buy established lines from reputable suppliers, ensuring the cells are tested for contamination.

Mammalian Cell Lines These are commonly used for human disease research and therapeutic development, requiring specific conditions for optimal growth in culture.

Examples of Cell Lines and Morphologies

There are many types of immortalized cell lines, each with different properties. They are usually named based on the cell type they come from or resemble:

- 3T3 cells – mouse fibroblasts from a spontaneous mutation in mouse embryo tissue.
- A549 cells – lung cancer cells from a cancer patient.
- HeLa cells – widely used human cells from a cervical cancer patient.
- HEK 293 cells – human fetal cells.
- Huh7 cells – liver cancer cells.
- Jurkat cells – human T lymphocyte cells from leukemia.
- OK cells – opossum kidney cells.
- Ptk2 cells – long-nosed potoroo kidney cells.
- Vero cells – monkey kidney cells from spontaneous immortalization.
- Human Epidermal Keratinocytes (HEKn) – used in 3D cell cultures and toxicology studies, with a rounded, cobblestone appearance.
- Human Umbilical Vein Endothelial Cells (HUVEC) – used in cardiovascular research, with a cobblestone appearance.
- Human Dermal Fibroblasts (HDFa) – used in 3D cultures and toxicology studies, with a spindle shape.

Understanding these characteristics will help you choose the right cell line and culture method for your research.

Methods & Cell Culture Protocols

1. **Concentrating Cells:** Procedure to concentrate cells from suspension culture or resuspend them from a monolayer.
2. **Counting Cells in a Hemocytometer:** Method for counting and calculating cell numbers manually using a hemocytometer.
3. **Counting Cells in a Countess II:** Automated method for counting and calculating cell numbers using the Countess II cell counter.
4. **Counting Cells in a Countess II after TrypLE Dissociation:** How to count cells after using TrypLE reagent and an automated cell counter.
5. **Cryopreservation of Mammalian Cells:** Protocol for freezing and storing cells at low temperatures.
6. **Cell Dissociation from Culture Vessels - Buffers:** Using cell dissociation buffers to detach cells from culture surfaces.
7. **Cell Dissociation from Culture Vessels - Other Reagents:** Alternative reagents for cell dissociation.
8. **Growth Factor Supplementation for Specific Cells:** Reference chart for adding growth factors to specific cells.
9. **Sera Supplementation for Advanced Media:** Recommendations for adding serum to advanced media (e.g., Advanced DMEM, RPMI 1640).
10. **3D Cell Culture Protocols:** Guidelines for generating spheroids from common cancer cell lines using Nunclon Sphera plastics and Gibco Media.
11. **Guidelines for Maintaining Cultured Cells:** Instructions on subculturing, media recommendations, dissociation techniques, and using Gibco TrypLE as an alternative to trypsin.
12. **Media Preparation from Powder and Concentrates:** Instructions for preparing media from powders or concentrates.
13. **Preparing Salt Solutions from Powder Concentrates:** How to prepare salt solutions from powdered concentrates.
14. **Subculturing Adherent Cells:** Procedure for subculturing adherent mammalian cells.
15. **Subculturing Suspension Cells:** Procedure for subculturing mammalian and insect cells in suspension culture.
16. **Freezing Cells:** Proper procedure for cryopreserving cell lines.
17. **Thawing Frozen Cells:** Correct method for thawing frozen cell lines.
18. **Trypan Blue Exclusion:** Quick test to determine cell viability using trypan blue.
19. **Use of Antibiotics and Antimycotics:** Guidelines for using antibiotics and antimycotics in cell culture.
20. **Useful Numbers for Cell Culture:** Key numbers and measurements for effective cell culture management.
21. **Red Blood Cell Lysis Using ACK Lysing Buffer:** Procedure for lysing red blood cells using ACK buffer.

Cell Selection (Plant Genetic modification technique other than Genetic Engg)

Cell selection is a method used to **develop new crop varieties**, such as soybeans, canola, and flax. It involves isolating cells **from a plant with desirable traits**, growing them in a lab, and then selecting cells that show the desired characteristics, such as resistance to herbicides. For example, **if herbicide is added to the culture, only the herbicide-resistant cells will survive, and they can be regenerated into full plants.**

This method has the advantage of quickly screening large numbers of cells in a lab, rather than conducting expensive field trials. However, it cannot be used to select for traits like increased yield, as the mechanism for yield is not understood at the cellular level.

Principle

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (**totipotency**).



Fig. a) callus b) Micropropagated apple roots after 4 weeks
c) Apples' root in media

Explant

- Preparation of plant tissue for tissue culture is performed under aseptic conditions under **HEPA** filtered air provided by a laminar flow cabinet.
- The part which is cultured is called **explant**, i.e., any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media.

HEPA stands for "High-Efficiency Particulate Air" and refers to a type of air filter designed to capture at least 99.97% of airborne particles 0.3 microns or larger.

Choice of Explant

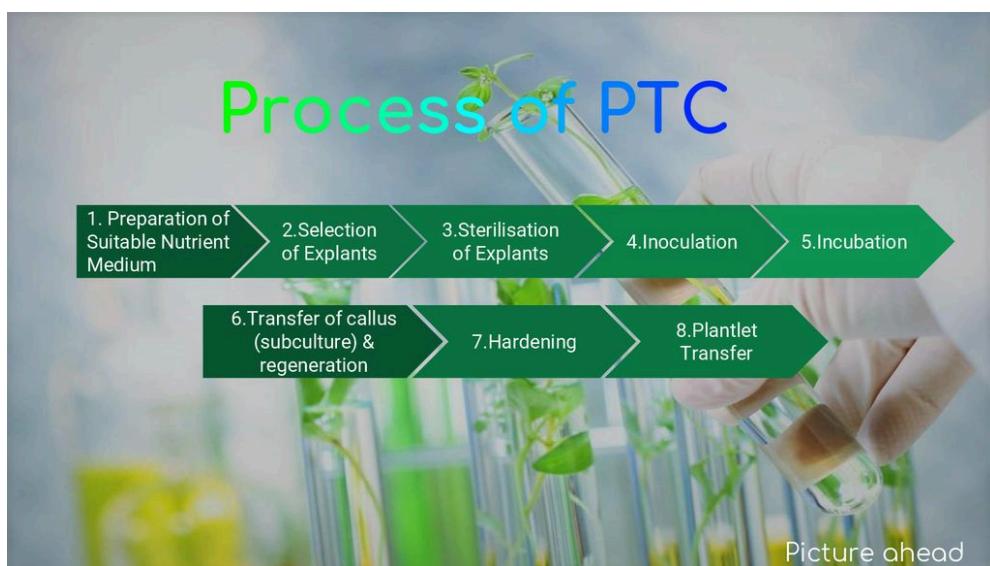
- Explants can be taken from many different parts of a plant, including portions of shoots, leaves, stems, flowers, roots, single undifferentiated cells.
- It also determines if the plantlets developed via tissue culture are **haploid** or **diploid**.

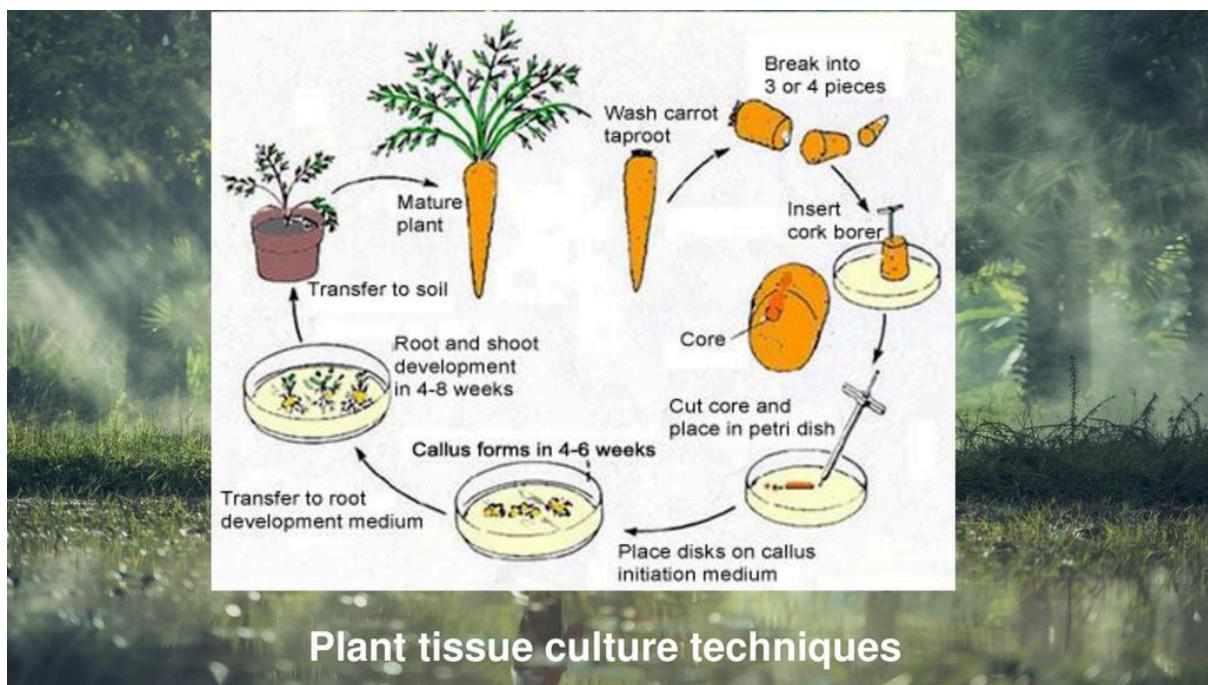
Types of Plant Tissue Culture

- 1. Seed Culture
- 2. Embryo Culture
- 3. Meristem Culture
- 4. Bud Culture
- 5. Callus Culture
- 6. Cell Suspension Culture
- 7. Anther Culture
- 8. Protoplast Culture.



Fig. Protoplast Solution Cells of Petunia leaves.





Today, cell selection is less commonly used because **newer genetic engineering methods** are more precise, successful, and have fewer unknown mutations.