Lab. 1: Cell culture

By

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Viral cultivation

- Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture).
- Viruses can be isolated from samples by filtration.
- Viral filtrate is a rich source of released virions.
- Bacteriophages are detected by presence of clear plaques on bacterial lawn.
- Animal and plant viruses are detected by cytopathic effects, molecular techniques (PCR, RT-PCR), enzyme immunoassays, and serological assays (hemagglutination assay, hemagglutination inhibition assay).

Cell culture

Refers to the **removal of cells from an animal or plant** and their subsequent growth in a favorable artificial environment.

The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

Primary culture

Refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence). At this stage, the cells have to be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Finite compare to continuous cell Line

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence (aging الشيخوخة); these cell lines are known as finite (محدود او ينتهي)

However, some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced.

When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.

Mammalian Cell Culture, continued

Cell Line	Cell Type	Species	Tissue	Medium*
H9	lymphoblast	human	T-cell lymphoma	RPMI-1640, 20% FBS
HaK	epithelial	hamster	kidney	BME, 10% calf serum
HCT-15	epithelial	human	colorectal adenocarcinoma	RPMI-1640, 10% FBS
HeLa	epithelial	human	cervix carcinoma	MEM, 10% FBS, and NEAA (in suspension, S-MEM)
HEp-2	epithelial	human	larynx carcinoma	MEM, 10% FBS
HL-60	lymphoblast	human	promyeolocytic leukemia	RPMI-1640, 20% FBS
HT-1080	epithelial	human	fibrosarcoma	MEM, 10% HI FBS, and NEAA
HT-29	epithelial	human	colon adenocarcinoma	McCoy's 5A, 10% FBS
HUVEC	endothelial	human	umbilical cord	F-12K, 10% FBS, and 100 µg/mL heparin
I-10	epithelial	mouse	testicular tumor	F-10, 15% horse serum, and 2.5% FBS
IM-9	lymphoblast	human	marrow from myeloma patient	RPMI-1640, 10% FBS
JEG-2	epithelial	human	choriocarcinoma	MEM, 10% FBS
Jensen	fibroblast	rat	sarcoma	McCoy's 5A, 5% FBS
Jurkat	lymphoblast	human	lymphoma	RPMI-1640, 10% FBS
K-562	lymphoblast	human	myelogenous leukemia	RPMI-1640, 10% FBS
KB	epithelial	human	oral carcinoma	MEM, 10% FBS, and NEAA
KG-1	myeloblast	human	marrow from erythroleukemia patient	IMDM, 20% FBS
L2	epithelial	rat	lung	F-12K, 10%FBS
LLC-WRC 256	epithelial	rat	carcinoma	Medium 199, 5% horse serum
McCoy	fibroblast	mouse	unknown	MEM, 10% FBS
MCF7	epithelial	human	breast adenocarcinoma	MEM, 10% FBS, NEAA, and 10 µg/mL insulin

Culture conditions

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing the following:

A substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals)

Growth factors

Hormones

Gases (O_2, CO_2)

A regulated physico-chemical environment (pH, osmotic pressure, temperature))

Culture conditions

Most cells are anchorage-dependent and must be cultured while attached to a solid or semi-solid substrate (**adherent or monolayer culture**), while others can be grown floating in the culture medium **(suspension culture**).

Cryopreservation

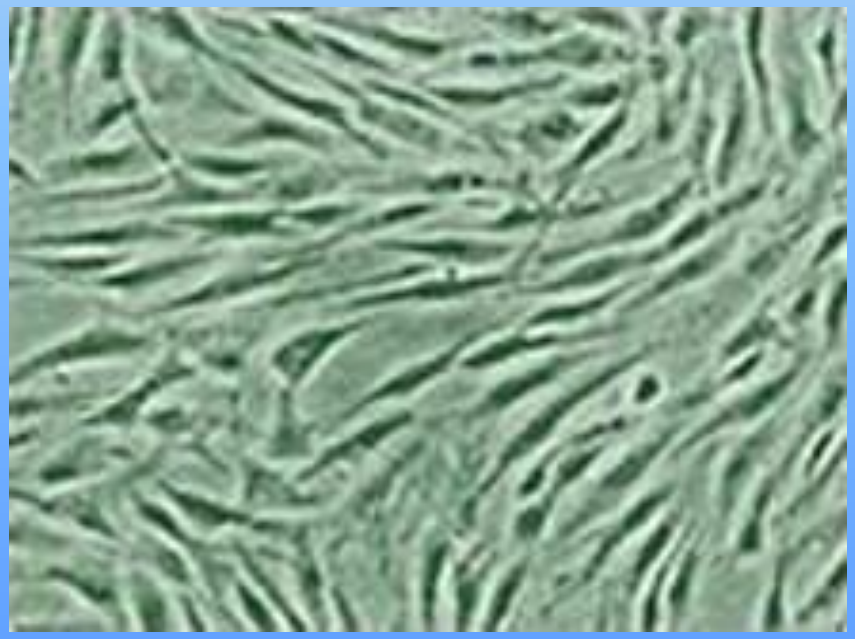
If a extra cells are available from sub-culturing, they should be treated with the appropriate protective agent (**e.g., DMSO or glycerol)** and stored at temperatures **below –130°C (cryopreservation**) until they are needed.

Morphology of Cells in Culture

Cells in culture can be divided into three basic categories based on their shape and appearance (i.e., morphology).

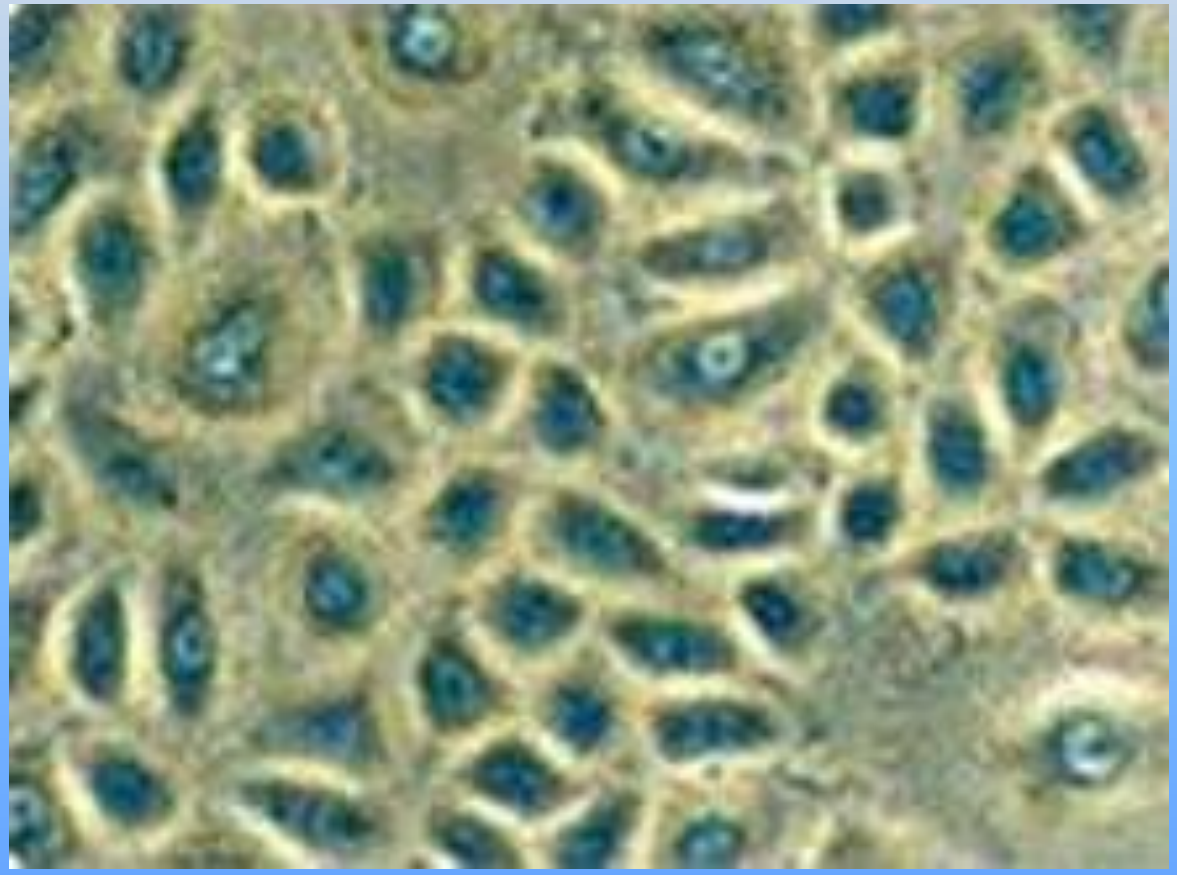
Fibroblastic (or fibroblast-like)

cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.



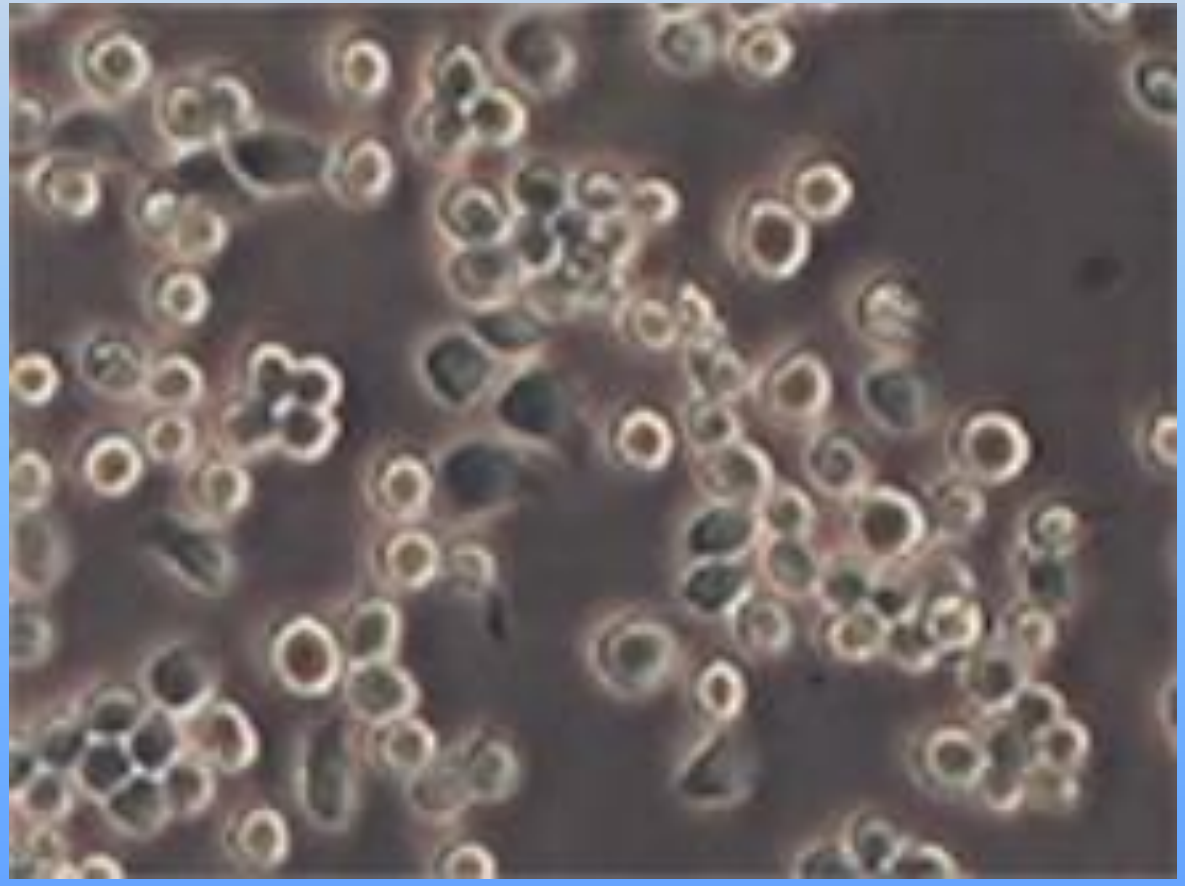
Epithelial-like

Cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



Lymphoblast-like

cells are spherical in shape and usually grown in suspension without attaching to a surface.



Applications of Cell Culture

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

Video 1: Introduction to Cell Culture

This video provides an overview of the basic equipment used in cell culture and proper laboratory set-up. Guidance on how to work safely and aseptically in a cell culture hood is introduced and demonstrated

https://www.thermofisher.com/iq/en/home/references/gibco-cell-culture-basics/introduction-to-cell-culture.html


Successful cell culture
depends upon many factors,
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including the
quality of the cells
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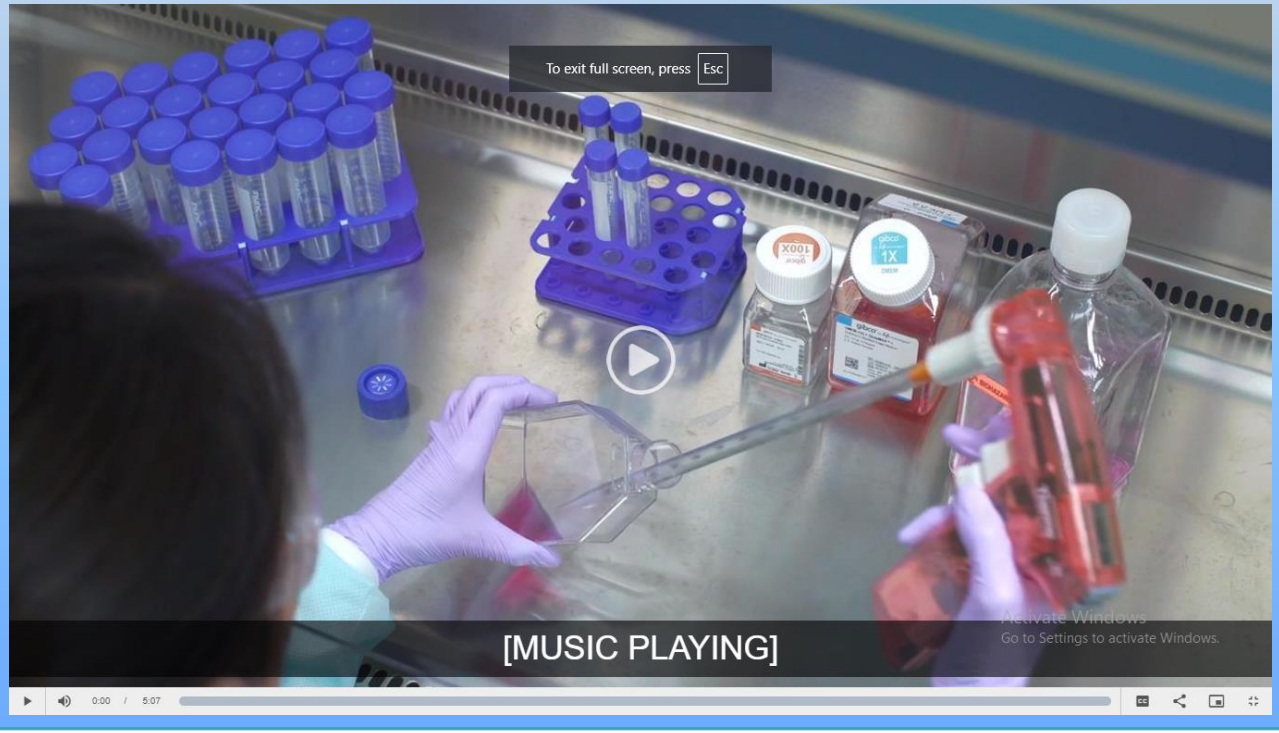
Video 2: aseptic techniques

This video is focused on the steps you should take to prevent contamination of your cell culture. All of the basic actions required to perform cell culture using best practice sterile techniques are demonstrated.



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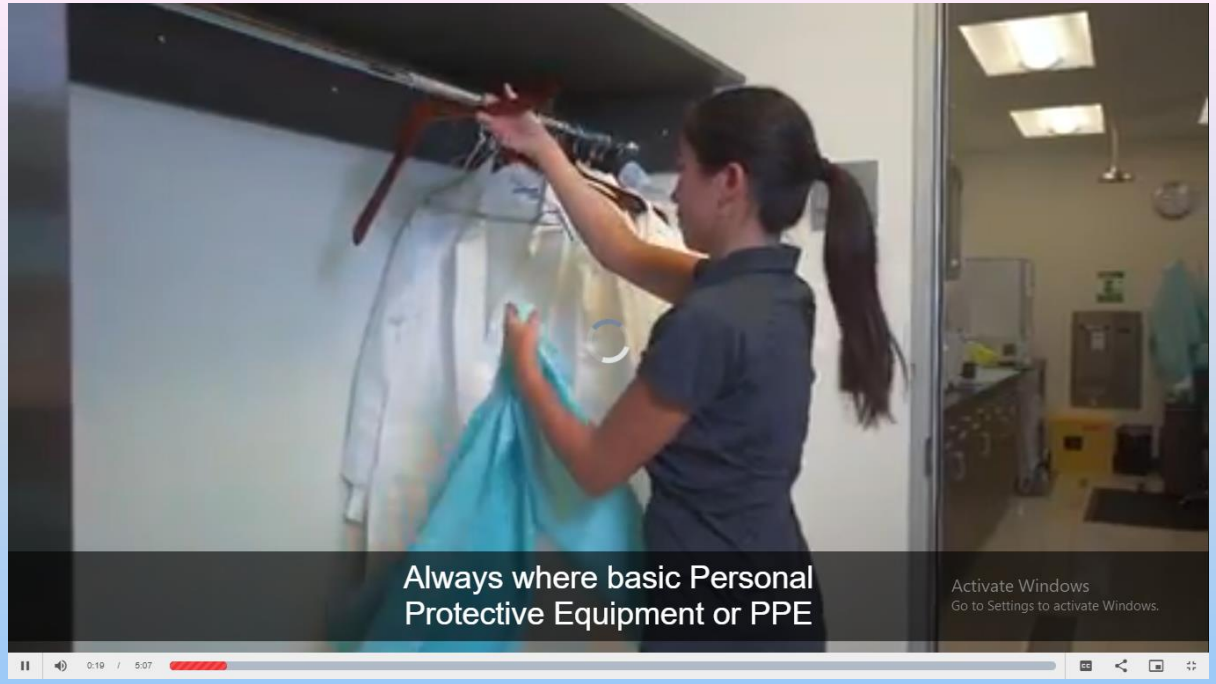
Always where basic Personal
Protective Equipment or PPE
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when working in the laboratory.
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Talk to the safety
team at your institute
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Rosalind
Franklin
Laboratory
1818
for your required PPE.
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And cover your legs then wear protective eye glasses


A 70% ethanol wash kills
microorganisms that
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Always clean the hood
before and after use.
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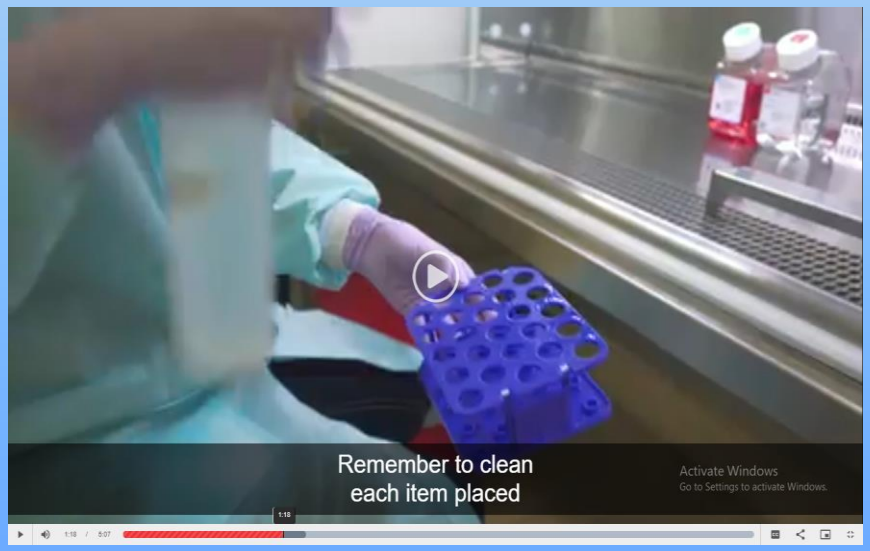
Remember to clean
each item placed
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As you clean the item and place
it into the cell culture hood,
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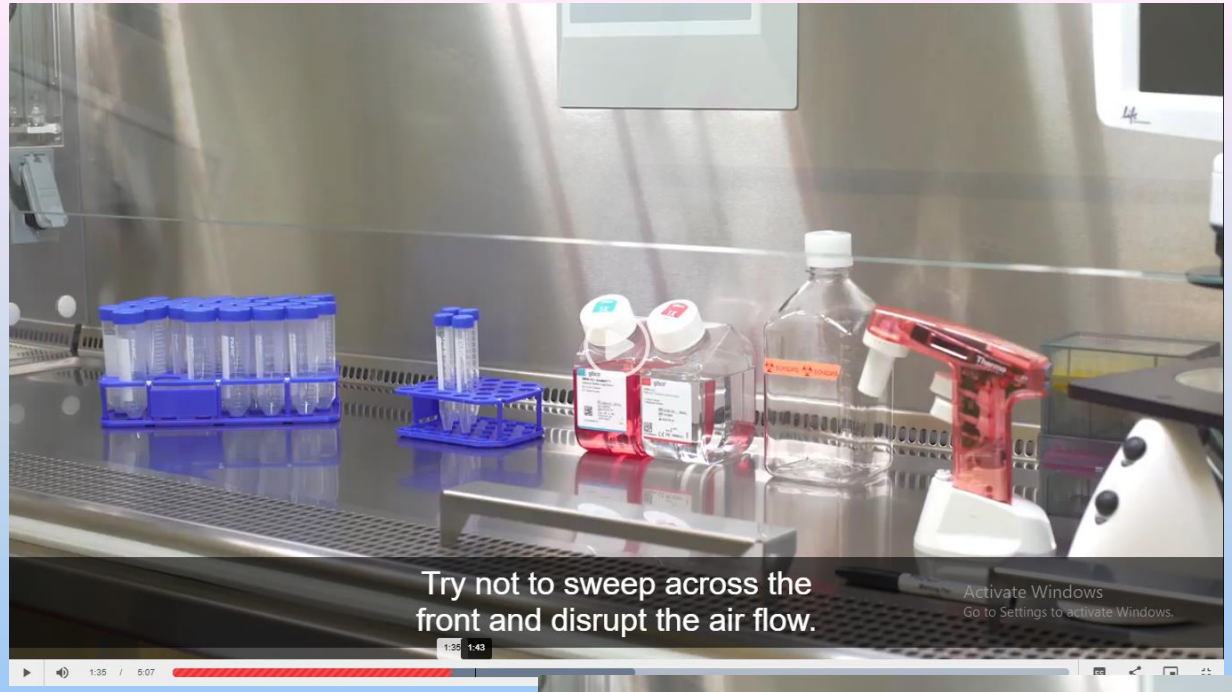
Try not to sweep across the
front and disrupt the air flow.
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includes not overcrowding
and the work surface.
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Not only does this increase
the risk of contamination
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through accidental touching, but
it also interrupts the air flow
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The basic techniques for cell culture are:

An air-conditioned room of cleanroom class 10,000 (FS209E) equipped with lights and ventilation.

A laminar flow hood, a CO_2 incubator, sterilizers with both saturated steam and dry heat, a low speed centrifuge with refrigerator.

Upright and inverted phase contrast microscopes.

A freezer at −20, a deep freezer at − 80℃, a refrigerator at 4℃.

A sink, disposable sterile plasticware (flasks, dishes, tubes, and pipettes), balance, ultrapure water, and a supply of media, and other reagents needed for the cell environment.

Further helpful apparatuses are a pH meter, a cell counter (hemocytometer), a vacuum pump, a pipette-aid, micropipettes, a liquid nitrogen tank, a fluorescent microscope, and so on.

Attention to safety and the maintenance of equipments is essential to understand the significance, the reasons, and the mechanisms.

Contamination of microbials such as bacteria (e.g. *Staphylococcus)*, yeast, fungus, and *Mycoplasma* should be strictly avoided in cell culture.

Simultaneously careful attention not to overgrow but grow with sufficient cell density and to avoid passages for a long time because the phenotype of the cell may subject to change.

This knowledge will help researchers with even a little prior experience to set up a suitable laboratory for basic cell culture

https://www.youtube.com/watch?v=tCNtKrxlZPs

Freezing cell culture to go back for early passage if it needed

- A typical workflow for sub-culturing an adherent cell line with a step-by-step description will be discussed here. Biomedical research areas, such as cancer, drug development, tissue engineering, stem cells, or cellular and molecular biology of disease, rely on *in-vitro* mammalian cell culture assays, essential tools for clinical and pharmaceutical applications.

- For successful cell-line maintenance, controlled growth conditions are needed to keep physiological and phenotypical stability.

- Cell growth is monitored at regular intervals and cells are subcultured to ensure continuity.

Thank you for listening

Lecture. 1: West Nile Fever

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West Nile Fever

West Nile is a virus that spreads through mosquito bites. Most people infected with West Nile virus don't have symptoms. About 1 in 5 people have symptoms like fever, rash and muscle aches. Rarely, West Nile can cause serious brain and spinal cord inflammation (encephalitis and meningitis).

These are the most common symptoms of West Nile fever:

- **Fever.**
- **Headache.**
- **Body aches.**
- **Skin rash on trunk of body.**
- **Swollen lymph glands.**

Your body has to fight the infection on its own. In mild cases of West Nile, symptoms usually last for 3 to 6 days, and you can recover at home (weeks to months).

West Nile Fever

West Nile is named after the West Nile district of Uganda, where it was first identified

How common is West Nile virus?

- **West Nile virus is found in many parts of the world, including North America, Europe, Africa, the Middle East, Australia and Asia. It's the most common mosquito-transmitted virus in the U.S., with cases reported in 49 states.**
- **There have been over 51,000 symptomatic cases in the U.S. since the first cases in the country in 1999.**

Common symptoms of West Nile fever include:

Fever, headache, muscles aches, Nausea and vomiting, Diarrhea.
Rash (usually concentrated around your chest and back).
Swollen lymph nodes, Sore throat. Pain behind your eyes.

What causes West Nile virus?

West Nile is an arbovirus, or a virus you get from an arthropod (arthropods are a large group that includes insects). It's an RNA virus in the genus Flavivirus. Similar viruses cause dengue fever, yellow fever and Zika.

How do you get West Nile virus?

Infected mosquitos transmit West Nile virus.

They usually get the virus by biting an infected bird (there's no evidence humans get it directly from birds).

The virus multiplies inside the mosquito, and it's transmitted to you (or another animal) when it bites you.

The incubation period, how long before you have symptoms:
is usually two to six days after getting bitten (but can be up to 14 days).

Symptoms of West Nile virus

Fever.

Headache.

Body aches.

Swollen lymph nodes.

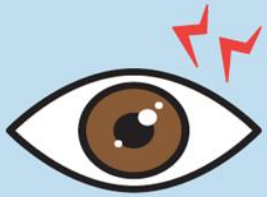
Nausea and vomiting.

Diarrhea.

Rash.

Joint pain.

Sore throat.



Pain behind your eyes.

Symptoms of severe West Nile virus

Symptoms of severe illness include neck stiffness, disorientation, muscle weakness and paralysis.

Cleveland Clinic

Serious symptoms in a few people. About 1 in 150 people who are infected develop a severe illness affecting the central nervous system such as encephalitis (inflammation of the brain) or meningitis (inflammation of the membranes that surround the brain and spinal cord).

Symptoms of severe illness include high fever, headache, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness and paralysis

Severe illness can occur in people of any age. However, people over 60 years of age are at greater risk for severe illness if they are infected (1 in 50 people). People with certain medical conditions, such as cancer, diabetes, hypertension, kidney disease, and people who have received organ transplants, are also at greater risk.

Recovery from severe illness might take several weeks or months. Some effects might be permanent.

About 1 out of 10 people who develop severe illness affecting the central nervous system die

Diagnosis

If you think you or a family member might have West Nile, talk with your health care provider.

Healthcare providers diagnose West Nile virus infection based on:

Signs and symptoms.

History of possible exposure to mosquitoes that can carry West Nile virus.

Laboratory testing of blood or spinal fluid.

Your healthcare provider can order tests to look for West Nile virus infection or other infections that can cause similar symptoms.

Treatment of West Nile Fever

No specific medicines available to treat West Nile. Antibiotics do not treat viruses

Rest, fluids, and over-the-counter pain medications may relieve some symptoms.

In severe cases, patients often need to be hospitalized to receive supportive treatment, such as intravenous fluids, pain medication, and nursing care.

Immunity agaist West Nile Fever

Most people infected with West Nile virus are believed to have lifelong immunity or protection from getting the disease again.

Some people who have weakened immune systems from certain conditions or medications might not have a strong immune response to the initial infection or their immunity may wane over time.

However, most people are protected from getting West Nile again once they have had it.

Summary of West Nile Fever

West Nile virus can result in febrile illness or neurologic disease, including meningitis or encephalitis

If you think you or a family member might have West Nile virus disease (West Nile), talk with your health care provider.

There is no specific treatment for West Nile.

Rest, fluids, and pain medications may relieve symptoms.

No symptoms in most people. Most people (8 out of 10) infected with West Nile virus do not develop any symptoms.

West Nile isn't contagious. You can't get it from another person who has it.

Mosquitos can bite anyone. But certain people are at a higher risk of getting severely ill if they get West Nile. You might be at a higher risk if you:.

Are over the age of 60.

Are an organ transplant recipient

Have cancer.

Have diabetes

Have high blood pressure (hypertension).

Have kidney disease.

Further information via following videos

West Nile Fever

https://www.youtube.com/watch?v=rfxGO6wxe1o

West Nile Fever

https://www.youtube.com/watch?v=zAQt9wk45bE

West Nile virus: What you need to know

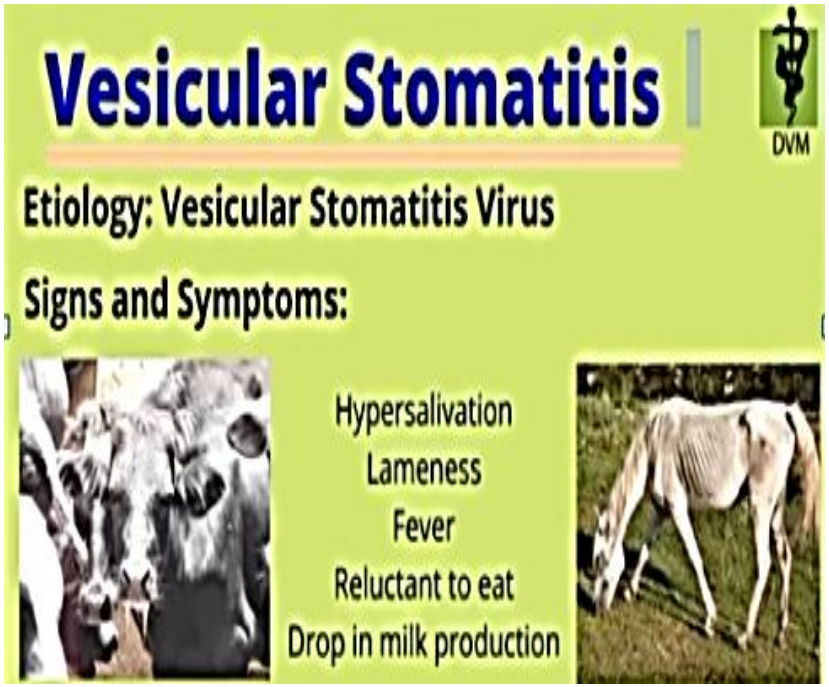
https://www.youtube.com/watch?v=TQTfuAvJwaQ

Mosquito season: How to identify symptoms of West Nile virus | Just The FAQs

https://www.youtube.com/watch?v=qmmObpvzqe8

Thank you for listening

Lecture 2: Vesicular stomatitis



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Vesicular stomatitis

Vesicular stomatitis is a contagious disease of livestock, mainly affecting horses and cattle. Occasionally, this disease can infect swine, sheep, goats, llamas, alpacas, and even people. It is primarily transmitted by biting flies and midges. The disease causes blister-like sores, among other side effects

In the past decade, the southwestern and western United States experienced a number of vesicular stomatitis outbreaks. Outbreaks usually occur during the warmer months, often along waterways. In some years, only a few premises in a single state were affected. However, in other years, multiple states and many premises were involved.

It is essential that veterinarians and livestock owners in these regions are aware that vesicular stomatitis occurs in their area – and be on the alert for animals displaying clinical signs of the disease.

Vesicular stomatitis

Doctor of veterinary medicine

Vesicular Stomatitis

DVM

Etiology: Vesicular Stomatitis Virus

Signs and Symptoms:

Hypersalivation
Lameness
Fever
Reluctant to eat
Drop in milk production

https://www.youtube.com/watch?v=sUlw5_pw0ZA

Vesicular stomatitis

https://www.youtube.com/watch?v=1OQgm5k9qyM

Vesicular stomatitis

https://www.youtube.com/watch?v=CMRKKl9XSDU

Vesicular stomatitis

Clinical signs may appear 2 to 8 days after exposure. Here's what to look for:

Drooling or frothing at the mouth

- **The first sign of illness is often excessive salivation. If you look inside the mouth, you'll see blanched and raised blisters on the inner surfaces of the lips, gums, tongue, or dental pad**

Lesions

- **Blister-like lesions can form around the mouth, nose, sheath, udders, ears, and coronary band (where an animal's hairline meets their hooves). If lesions develop around the coronary band, lameness may occur.**

Fever

- You may notice a rise in body temperature before or at the same time lesions first appear..

Reluctance to eat

The blisters swell and break open, which causes mouth pain, discomfort, and reluctance to eat or drink. This can cause weight loss.

How to prevent this disease

Although experimental vaccines have been developed, none have been approved for use in horses. The best way to prevent the disease is by:

- Isolating new horses before introducing them to your herd.
- Controlling insects on your property with fly traps, sprays, and clean horse pens.

- Separating sick horses from healthy horses on your property

- Handling healthy animals before sick animals..

- Washing and disinfecting your hands and boots after working with sick animals. If possible, change and wash your clothes as well.

How to treat Vesicular stomatitis

- Vesicular stomatitis is treated with supportive care. Since the lesions may be quite painful, your veterinarian may prescribe anti-inflammatory drugs. If your animal is having trouble eating, softening their grain in warm water could encourage them to eat more. Softening hay cubes can also help if they're having trouble eating grass and hay. If secondary infections develop around lesions, your veterinarian may prescribe antibiotics..

Thank you for listening