Tissue culture
lab # 2

Tissue Culture Equipment

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Objective

- Introduction to tissue culture
- Applications of Cell Culture
- Equipment of tissue culture
- Basic equipment
- Additional supplies

What is cell culture?

- Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment.

Applications of Cell Culture

- Cell culture is one of the major tools used in cellular and molecular biology.
- It provide an excellent model system for studying:

1. The normal physiology and biochemistry of cells (e.g., metabolic studies, aging)
2. The effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis.
3. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins).

Cell Culture Equipment

- All cell culture laboratories have the common requirement of being **free from pathogenic microorganisms** (i.e., asepsis), and share some of the same basic equipment that is essential for culturing cells.
- This section lists the equipment and supplies common to most cell culture laboratories, as well as beneficial equipment that allows the work to be performed more efficiently or accurately, or permits wider range of assays and analyses. Note that this list is not all inclusive; the requirements for any cell culture laboratory depend the type of work conducted.

Basic Equipment

- Cell culture hood
- Incubator
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter
- Inverted microscope
- Liquid nitrogen (N2) freezer or cryostorage container
- Sterilizer (autoclave)

Additional Supplies

- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi-well plates)
- Pipettes
- Waste containers
- pH meter
- Media and reagents
- Cells

Cell culture hood (biosafety cabinet)

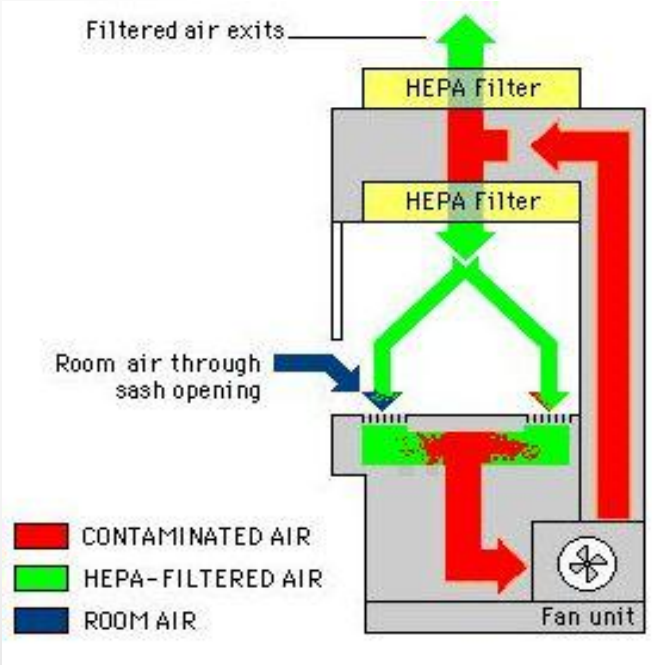
- Three kinds of cell culture hoods designated as Class I, II and III, have been classified based on effectiveness, environment, product production and airflow dynamics.

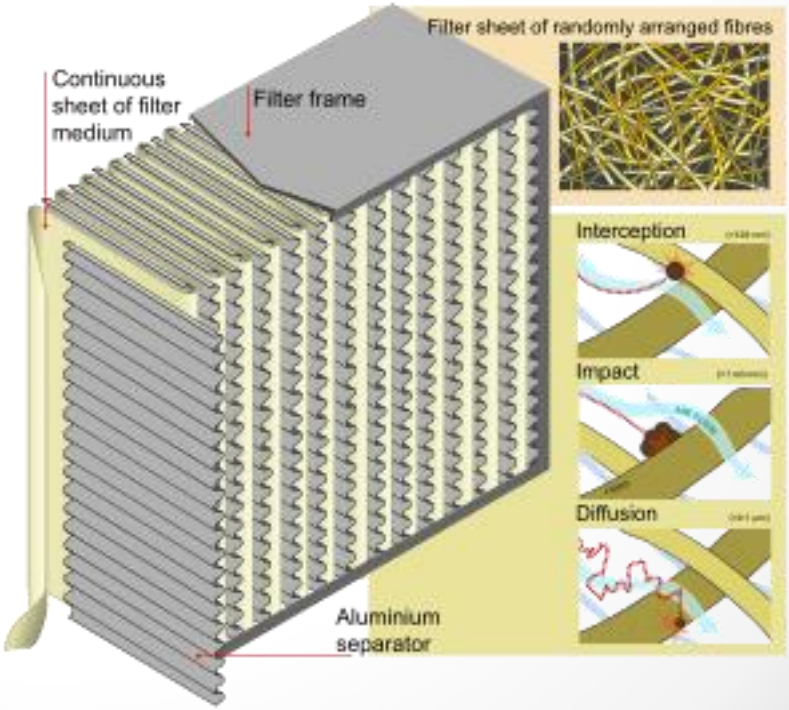
Cell culture hood

- Biological safety cabinets, provide a clean working environment to prevent contamination of cell cultures.
- The air is filtered and cleaned of particles before blown into the cabinet. Additionally, the flow of air in the hood is in smooth parallel lines which creates a "curtain" to separate inside from outside.
- Some hoods are equipped with a UV- light to sterilize the contents inside while not in use. The UV lamp must be **turned off before** working in the hood to prevent exposure to hazardous UV light.

HEPA filter

- HEPA filter stand for High-efficiency particulate air.
- It can remove 99.97% of particles (dust, mold, bacteria, and any particle with size of 0.3 µm).





Biosafety Cabinet - Class I

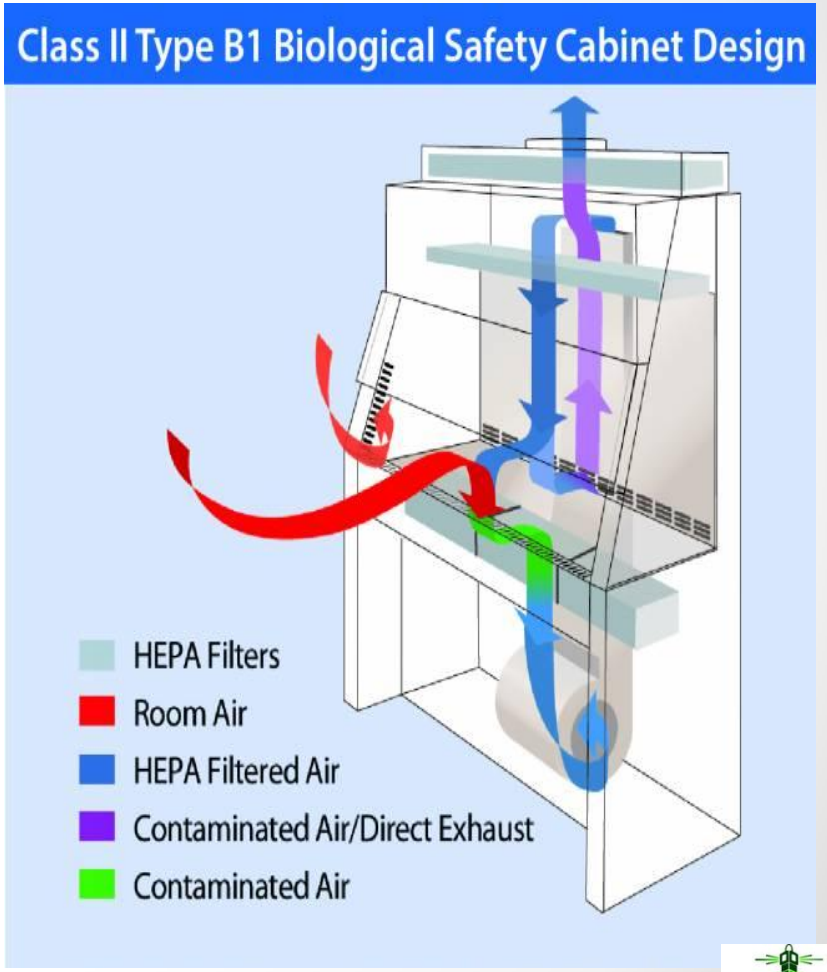
- ***Class I - Personnel and Environmental Protection Only***
- Offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques, but no product protection.
- So, they do not provide cultures protection from contamination.

Biosafety Cabinet - Class II

- ***Class II - Product, Personnel and Environmental Protection***
- They provide an aseptic environment necessary for cell culture experiments. A Class II biosafety cabinet should be used for handling potentially hazardous materials (e.g., primate-derived cultures, virally infected cultures, radioisotopes, carcinogenic or toxic reagents).

Biosafety Cabinet - Class II

- Lab air is drawn in through the front grille, then directed up the back of hood before passing through a HEPA filter, where some clean air go out to the laboratory and the rest flows back down to – and around – the work space, repeating the cycle again.



Biosafety Cabinet - Class II

- There are four types: Type A1, Type A2, Type B1, and Type B2.
- The four types are differ in the amount of air exhausted and recirculated through the HEPA filter.
- About 95% of all biosafety cabinets installed are Type A2 cabinets.
- Type A2 cabinet 70 % of the air recirculated within the hood and 30 % exhausted air back into the laboratory.
- Class II cabinets are the commonly used cabinets in clinical and research laboratories.

Biosafety Cabinet- Class III

- ***Class III –***
- Biosafety cabinets are gas-tight, and they provide the highest attainable (maximum) level of protection to personnel and the environment.
- The enclosure is gas-tight, and all materials enter and leave through a dunk tank or double-door autoclave.
- A Class III biosafety cabinet is required for work involving known human pathogens and other BSL-4 materials.

How Biological Safety Cabinets Are Classified?

Classification	Biosafety Level	Application
Class I	1,2,3	low to moderate risk biological agents
Class II	1,2,3	low to moderate risk biological agents
Class III	4	high risk biological agents

Incubator

- The purpose of the incubator is to provide the appropriate environment for cell growth.
- The incubator should be large enough for your laboratory needs, have forced- air circulation, and should have temperature control to within ±0.2°C.
- Stainless steel incubators allow easy cleaning and provide corrosion protection, especially if humid air is required for incubation.
- Frequent cleaning of the incubator is essential to avoid contamination of cell cultures.

Incubator

- **Types of Incubators**
- There are two basic types of incubators:

Dry incubators

Humid CO2 incubators

Types of Incubators

- **Dry incubators**
- more economical
- the cell cultures incubated in sealed flasks to prevent evaporation.
- Placing a water dish in a dry incubator can provide some humidity, but they do not allow precise control of atmospheric conditions in the incubator.

- **Humid CO2 incubators**
- more expensive
- allow superior control of culture conditions.
- They can be used to incubate cells cultured in Petri dishes or multi-well plates, which require a controlled atmosphere of high humidity and increased CO2 tension.

Water bath

Water bath

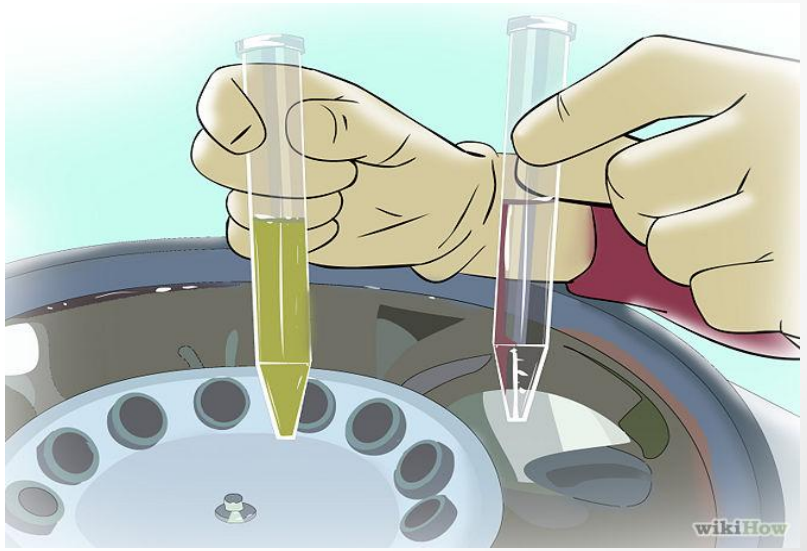
- A water bath is a laboratory equipment made from a container filled with heated water.
- Temperature may be controlled digitally.
- Utilizations include warming of reagents, melting of substrates or incubation of cell cultures.

Centrifuge

- A centrifuge is a laboratory device that is used for the separation of fluids, gas or liquid, based on density. Centrifugation is used to remove protein end products, dead cells and cell debris.

Centrifuge

- Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel.
- There are many types of centrifuges.



Refrigerators

- **Refrigerators**
- For small cell culture laboratories, a domestic refrigerator is an adequate and inexpensive piece of equipment for storing reagents and media at 2–8°C.
- For larger laboratories, a cold room restricted to cell culture is more appropriate. Make sure that the refrigerator or the cold room is cleaned regularly to avoid contamination.

Freezers

- **Freezers**
- Most cell culture reagents can be stored at –5°C to –20°C; therefore an ultradeep freezer (a –80°C freezer) is optional for storing most reagents.
- A domestic freezer is a cheaper alternative to a laboratory freezer. While most reagents can withstand temperature oscillations in an autodefrost (self-thawing) freezer, some reagents such as antibiotics and enzymes should be stored in a freezer that does not autodefrost.

Cryogenic Storage

- Cell lines in continuous culture are likely to suffer from genetic instability as their passage number increases; therefore, it is essential to prepare working stocks of the cells and preserve them in cryogenic storage.
- Do **not** store cells in –20°C or –80°C freezers, because their viability quickly decreases when they are stored at these temperatures.
- For that reason liquid nitrogen method is used.
- Liquid nitrogen is a cryogenic method that can cause rapid freezing on contact with living tissue.

Cryogenic Storage

- Liquid nitrogen is a cryogenic method that can cause rapid freezing on contact with living tissue.
- Cryopreservation helps survival of pure cultures for long storage times.
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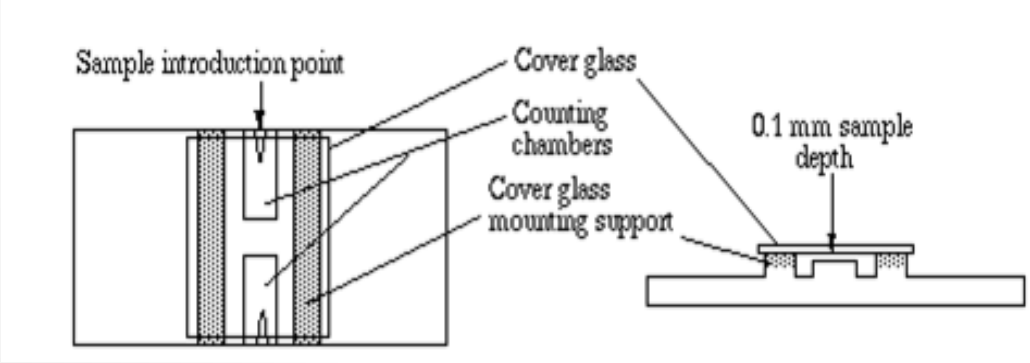
Cryogenic Storage

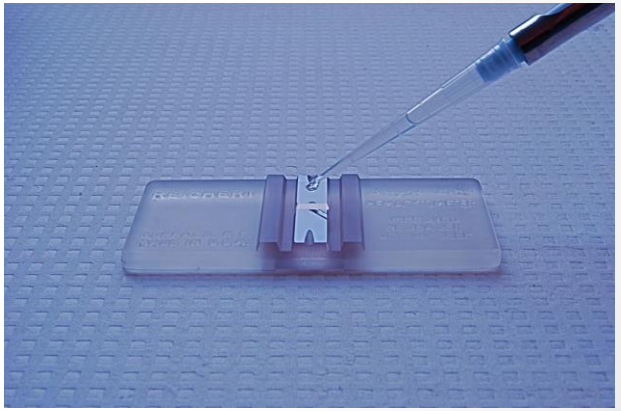
- There are two main types of liquid-nitrogen storage systems (cryopreservation):
- **Liquid phase at -196°C**
- **Vapor phase at -150°C**

- They come as wide-necked or narrow-necked storage containers.

Cell counter (Hemocytometer)

- A cell counter is a device used to count cells. It was originally designed for the counting of blood cells.

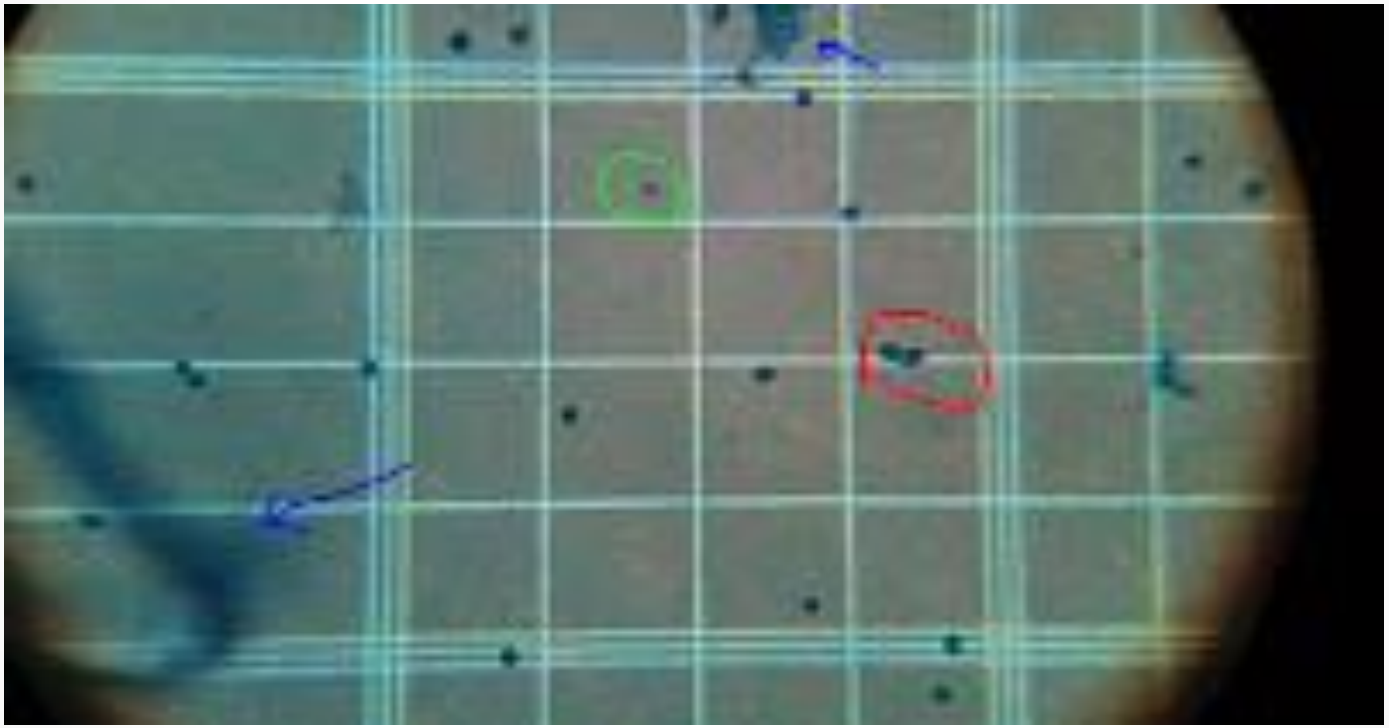




Cell counter (Hemocytometer)

- It is a modified microscope slide, containing two identical wells or chambers, into which small volume of cell suspension is pipetted.
- 100 µl of cell suspension is diluted with 100 µl of Trypan blue, which is a dye helps to distinguish between living and dead cells.
- The dye passes the membrane of the dead cell so, they appear blue under the microscope.
- The living cells appear clear.

Cell counter (Hemocytometer)



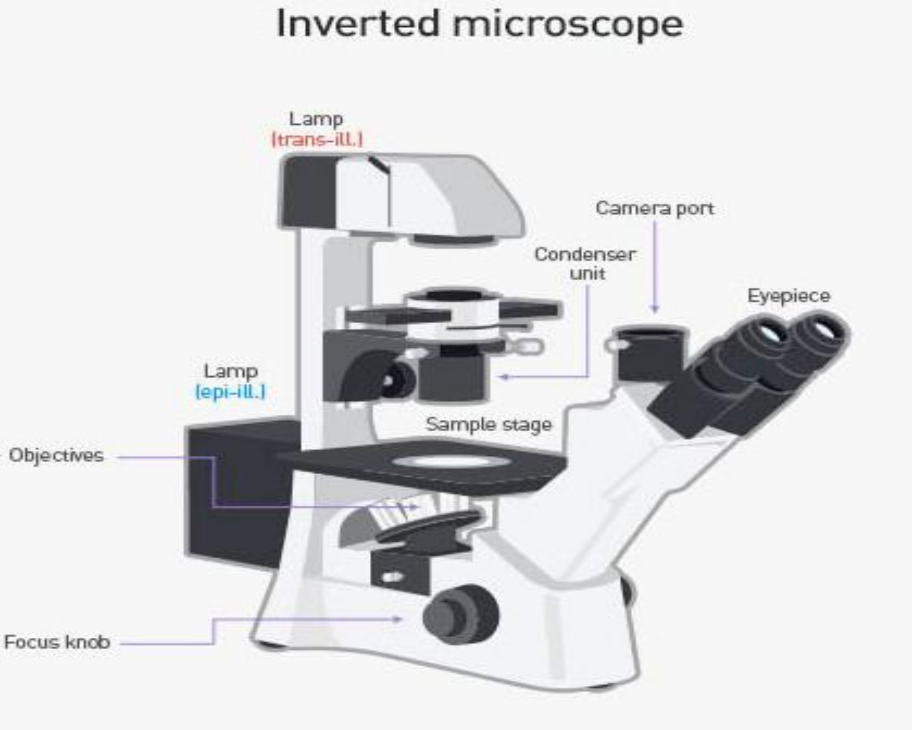
Loading the Hemocytometer Cell counting
Live cell circled in green.
Dead cells circled in red.

Inverted microscope

- This type of microscope is used for visualizing cell cultures. The cells in culture vessel remain at the bottom of the vessel and the medium floats above the growing cells.
- It is impossible to observe these cells under the ordinary microscope, therefore, the inverted microscope is used for such purposes.

Inverted microscope

- The inverted microscope has the optical system at the bottom and the light source at the top, this arrangement helps to observe the cultured cells in the plates.



WET HEAT (Autoclaving)

- The method of choice for sterilization in most labs is autoclaving; using pressurized steam to heat the material to be sterilized.
- This is a very effective method that kills all microbes, spores and viruses.

WET HEAT (Autoclaving)

- Autoclaving kills microbes by hydrolysis and coagulation of cellular proteins, which is efficiently achieved by intense heat in the presence of water.

- All of the glassware, glass pipettes, pipette tips and tubes used for cell culture must be sterilized.

Biohazard waste containers

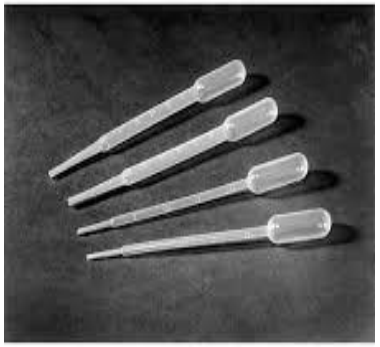
- Liquid waste from the cell-culture media can be aspirated directly into the disinfectant inside the vacuum trap container. The liquid must be in the disinfectant for at least 20 minutes before being disposed.

Biohazard waste containers

- Pipette tips, disposable glass pipettes, and any other sharps must be in sharp containers, not in trash cans.

Pipettes

- A **pipette** is a laboratory tool commonly used to transport a measured volume of liquid.



Cell culture vessels

- The vessels are flat at the bottom to provide a surface for cell growth. The bottom surface of the culture vessels are coated by molecules, such as polylysine, laminin, gelatin etc. to mimic the natural extracellular matrix and allow cell attachment.
- Three types of culture vessels are used:
- flasks
- dishes
- multi- well plates

pH Meter

- A **pH Meter** is an electronic device used for measuring the pH which is either the concentration of Hydrogen ions in a solution.
- If the H+ concentration is higher than hydroxyl ion OH- so the solution is acidic.
- If the OH- concentration is higher than H+ so the solution is basic .


THANK
YOU !

Reference

- https://www.bakerco.com/introduction-biological-safety-cabinets
- http://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf
- http://www.hettweb.com/cell-culture-description.html
- http://www.bio-link.org/home/sites/files/instructor_manualv8.pdf