UNIT 1
Basic Concepts of LAB Technology-1

Basic principles and procedure of laboratory: -

Procedure of laboratory: -

- Place the well – being and service of the sick above your own interests.
- The Laboratory should be designed in such a way so that there is enough space for free movement of the people working in it.
- A cramped laboratory is likely to lead to accidents, damaging the equipment and endangering the life of individuals working in such a laboratory.
- The room should be adequately ventilated but without strong currents of air.
- The laboratory should have ample of lighting arrangement that is necessary for accurate measurement in any form.
- Working benches that are to be used for sting should prefer ply be 12/2 feet high, and those to use while standing should be at least 3 feet high.

Basic principles: -

1) The clinical and principal workers are equally at risk of acquiring transmission diseases through the patient through the test samples.
2) Disinfect the working benches and as for as possible autoclave various glassware used in the laboratory.
3) To develop an understanding of the concept of healthy living.

   Place the well – being and service of the sick above your own interests.
4) Be loyal to your medical laboratory profession by maintaining high standards of work and strive to improve your professional knowledge.
5) Work scientifically and with complete honesty.
6) Do not measure your professional skills or knowledge for personal gain.
7) Never take anything from your place of work that does not belong to you.
8) Do not disclose to a patient or any unauthorized person that result of your investigations.
9) Treat with utmost confidentiality and personal information that you may learn about a patient.
10) Respect and work in harmony with the other members of your personal information that you may learn about a patient.
11) Be at all times courteous, patient and considerate to the sick and their relations.
12) Promote health care and the prevention and control of disease.

To develop an understanding of the concept of healthy living.

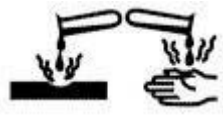
A healthy lifestyle is one which helps to keep and improve people's health and well-being. Healthy living is a lifelong effect. The ways to being healthy include healthy eating, physical activity, weight management, and stress management. Good health allows people to do many things.

-Laboratory hazard

Labeling of hazardous Regents / chemical

(1) 

Danger

(2) 

Toxic Corrosive

(3) 

Explosive

(4) 

Risk of danger

(5) 

Highly Inflammable

(6) 

Harmful/irritant

(7) 

Oxidizing

(8) 

No Smoking

(9) 

No Entry

(10) 

Not to be extinguished with water

You must remain alert and cautious while working in the laboratory. You must know that caseless handling of reagents, glassware, or specimens to be tested in the laboratory can cause serious injury and is dangerous to life.

Safety with chemical / Reagents: -

- Excepting just a couple of reagents almost all chemicals / reagents used even in the most basic laboratory are lethal poisons if consumed by anyone.
- Even if they are splashed on the skin eye, they can cause irreversible damage.
- There is an appropriate way of handling and storage of hazardous chemicals to avoid injury and damage to self and others.

Flammable chemicals

These include ether; xylene toluene, methanol ethanol, glacial acetic acid, acetone, acetic anhydride alcoholic Romanowsky stains and acid alcohol etc.

Storage

- These should be stored in a fireproof metal box at ground level, preferably in a cool store.
- A container well lined with tin foil can also be used.
- Store only small quantities of such eluents on the shelves.

Safe use

- Ensure that there is no open flame nearby while opening a bottle containing Flammable so lent.
- The nearest flame should be at least 10 feet away.

Measuring and dispensing liquids

The equipment you should choose to measure out liquids depends upon the volume to be dispensed, the accuracy required and the number of times the job must be reported. Conical flasks, beakers, measuring cylinders, and volumetric flasks, measure the volume of liquids contained in them, while burettes, pipettes, syringes and micro syringes mostly measure the volume delivered from them: think about the requirements of the experiment.

Criteria for choosing a method for measuring out of liquid

- **Pasteur pipette** Volume- 1-5ml Accuracy- low

  Usefulness for repetitive measurements – Convenient
- **Conical flask/Beaker** Volume- 25-5000 ml Accuracy- very low
- **Measuring cylinder** Volume- 5-2000 ml Accuracy – medium
- **Volumetric flask** Volume- 5-2000 ml Accuracy- High
- **Burettes**
- **Volume- 1-100 ml Accuracy- High**

- **Glass pipette** Volume- 1-100 ml Accuracy- High
- **Mechanical pipette** Volume- 5-1000 microliters Accuracy- High
- **Syringe** Volume- 0.5-20 microliter Accuracy- Medium
- **Micro syringe** Volume- 0.5-50 microliters Accuracy- High
- **Weighing** Volume- Any depends on accuracy of balance Accuracy- Very high

  Usefulness for repetitive measurements- Inconvenient
- **Safety Precaution with glass container: -**

a) When handling cool flask, grasp the neck with one hand and support the bottom with the other hand.

b) Lifts cool beakers by grasping the side just below the rim.

c) Never carry bottles by their necks.

d) Use a cart to transport large bottles of dense liquid.

Safety Precaution with plastic container: -

a) Whenever possible use plastic container safety cans, when working with an open Container

b) Use a laboratory fume hood to control the accumulation of flammable vapor. For

Example: - Open flames Electrical equipment Sources of static electricity.

- Never heat a flammable liquid over any flame.
- Use a water bath or electric not plate.

Handling acids and alkalis

I (a) diluting sulphuric acid with water.

(b) Always add sulphuric acid to the water drop by drop,

(c) Stirring the mixture after each drop.

II (a) Bottles of acids and alkalis: keep them on the lower shelves of the cupboards.

(b) When you take one out, hold it firmly upright with a dry hand.

(c) Do not keep acids and alkalis in bottles with ground glass stoppers as they may get stuck.

III (a) Pipetting: - Where possible, use small measuring cylinders for measuring acids and Alkalis

Use a pipette plugged with nonabsorbent cotton wool or with rubber tube attached.

Heating glassware and liquids.

I (a) Test tubes: - Never heat the bottom of a test tube, the liquid inside might sputter.

- Heat the middle of the tube, shaking gently
- The mouth of the tube should be facing away from the worker and any other person,

Towards an empty space or a sink

(b) Ordinary Glass and Pyrex: - Only Pyrex glassware and pro clean receptacles can be heated over a Bunsen flame. Ordinary glass will break,

(c) Inflammable liquids. Only small quantities of flammable liquids such as ether, ethanol, acetone, bemene, toluene and carbon disulphide should be kept in the laboratory.

(II) Do not use broken, cracked or chipped laboratory glassware.

(III) Put clear labels on poisons. Keep them in a locked cupboard.

(IV) Do not use nylon clothes while working as there are easily inflammable. Always use a Laboratory apron

(V) Always ensure that electrical wiring and electrical appliances are in good conditions.

Choose glass container: -

Glassware is the most common family or lab supplies.

It's provided the best sample integrity. Glass is inert and thus more chemically compatible than plastic, the only concern about the chemical resistant of lab glassware is the type of liner inside the cap.

For example: -

- Amber glass is ideal for light sensitive applications.
- Clear glass allows for maximum visibility.
- Plastic coated glass prevent against dangerous spills. If the glass breaks the plastisol coating contain the content long enough to allow proper disposal.

■ **Clean plastic container: -**

a) It is depending on the type of plastic

b) Temperature in the form of extreme heat or cold affect flexibilityand strength.

■ **Acid bath:** -

- Immerse plastic ware into a 1 M nitric acid and allow to soaked overnight for mild contamination.
- Keep in bath for about one week for heavy contamination.
- The clean plastic ware is then taken out of the bath and rinsed with distilled water and put to dry.
- rinse will acetone or placement in a glass ware dryer at low temperature can be used.

■ **Clean glass container: -**

- The glassware overnight in acid bath procedure.
- Requires scrubbing, scrub with a brush.
- Using for cleaning hot soapy water.
- Rinse thoroughly with tap water.

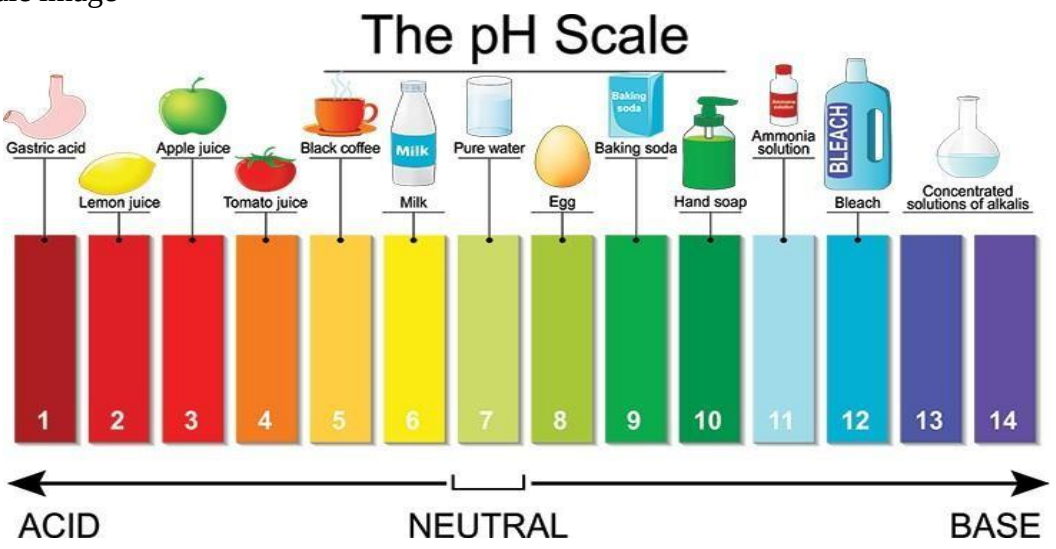
- Followed by rinse with deionized water
- Wash with hot soapy water.
- Rinse thoroughly with tap water.
- Then rinse 3-4 times with deionized water.
- Be sure the final rinses sheets of the glass.

- **pH**

Introduction: -

- A pH meter is a scientific instrument that measures the hydrogen-ion activity in water based solutions, indicating its acidity or alkalinity expressed as pH.
- These are the indicators and usually acids of weak strength whose molecules in solution are of a different color than their anions.

pH scale image



- pH. Neutral pH=7.0 ,less than 7.0 is acidic, more than their 7.0 is alkaline.
- The color of an indicator solution depends on the degree of dissociation of the indicators, and on the pH of the solution. Supposing the weak acid indicator is H Indic, it would dissociate as:

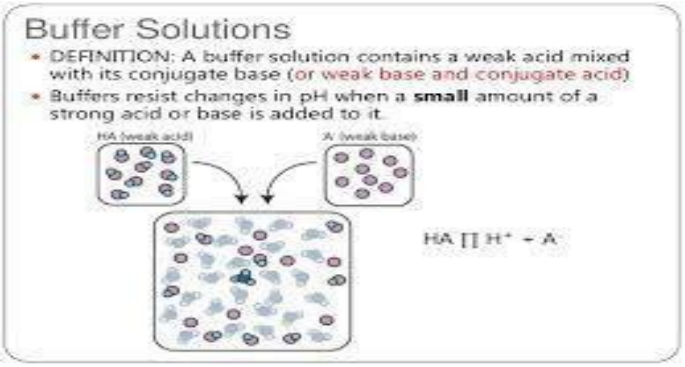
  H Indic'= H' +. + Indic-

  (Color-X)(hydrogen ion) (color Y)
- All acids contain hydrogen ions, so addition of an acid would make the reaction shift from right to left and addition of an alkaline would lead to production of water as the OH radicles will associate with and remove H+ ions causing a shift to the right of the reaction. Indicator that just show whether a solution is an acid or alkaline are called broad indicator while some indicator change color at a precise pH.

Buffer solution

-At any given temperature these solutions retain their definite pH and maintain it even after adding considerable amounts of acid or alkalis.

-These solutions generally consist of a weak acid mixed with it's sodium or potassium salt.



- **Procedure of hand hygiene**

1) Wet hand with warm running water.

2) Apply enough soap to cover all surfaces.

3) Thoroughly wash all parts of hand and fingers up to the wrists , rubbing hands together for at least 15 seconds.

4) Rinse hands under running water and dry thoroughly with paper towels.

5) Use paper towels to turn off faucet before discarding the towels in the waste receptacle.

- **To be equipped with Technique use of PPE (Personal Protective Equipment).**

1) Gloves

2) Apron/gown

3) Masks

1) **Gloves**:- Wear gloves when handling infectious materials or where there is a possibility of exposure to blood and other body fluids.

   All laboratory that work with material that is potentially infected with HIV requires a generous supply of good quality gloves.

   Discard gloves whenever they are thought to have become contaminated or performed, wash your hands and put on new gloves. Alternatively where there are economic constraints wash gloves hands whenever they get contaminated with blood/body fluids before collected further samples.

   Do not touch your eyes, nose or other exposed membranes or skin with your gloved hands. Gloves image

2) Apron or gown: -

Apron or gown should be worm to prevent soiling of clothing during procedure that may involve contact with body fluids, blood, secretions, or excretions.

Sterile apron/gown is only required for procedure that need a sterile field.

Apron/gown should be made of moisture- resistant material that provides an effective barrier to body substances.

Apron/gown should be changed after giving care to an individual person and after performing any procedure which involves contact with blood or body substances.

When removing a soiled apron/ gown, minimize contamination of your hands clothes. It should be held inside without touching the outside.

Used apron/gown must be disposed of into proper receptacle. Apron image

3) Use of Face mask: -

- A mask protects you from breathing in microorganisms from the person respiratory tract and

  Vice versa
- Disposable mask are to be won whenever there is reasonable expectation that droplets

  Transmission occurs
- A properly applied mask fit snugly over your mouth and nose, and covers the chin area, so that Infectious organism and body fluid cannot enter or escape through the sides.
- If you wear glasses, the top edges of the mask should fit below the glasses so that they will. Not cloud over as exhales.
- Taking should be kept to a minimum while wearing a mask to reduce respiratory air flow.
- Before removing a mask, remove your gloves or wash your hands if they have come in contact.

With infectious material

- A mask that has become moist is ineffective and should be discarded.
- Dispose of mask after each use. It should never be reused. Mask image

UNIT-2

Care and maintenance of glassware

1) New look for cracks: - If any soak in 2% HCL for overnight to neutralize any alkali present

   Wash in running tap water.
2) Boil in synthetic detergent for 30 minutes.
3) Rinse in tap water and finally in distilled water.

■ Used:

Glassware should be rinsed immediately after use.

Boil n detergent for 30 minutes clean thoroughly with a brush, rinse in tap water. Dry them in the oven with temperature not exceeding 80$^{\circ C.}$

Beaker: -

-It is a generally cylindrical container with a flat bottom.

-Most have a small spout to aid pouring.

-It is found in small to wide range that is one milliliter to several liters.

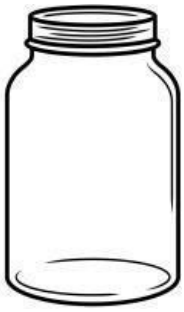
-It is made by borosilicate glass

Beaker

Jar: -

- It is a cylindrical container.
- It is a wide opening that may be sealed.
- Bell jar is used to contain a vacuum.

jar



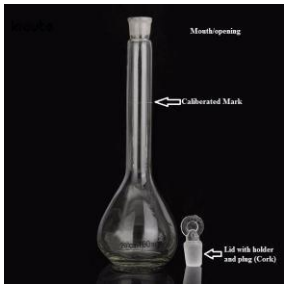
B) Flasks:-

- A flask is a type of container made of glass.
- It is volumetric measuring flasks.
- It can be used making solution or for holding, containing , or volumetrically measuring chemicals , sample solution.
- It can other process is used are mixing, heating etc. chemical.

The most common types of flask are:

- Erlenmeyer flask.
- Florence flask.
- Volumetric flask.
- Büchner flask.
- Fernbach flask.

- Erlenmeyer flask. Florence flask.



- Volumetric flask. Büchner flask.

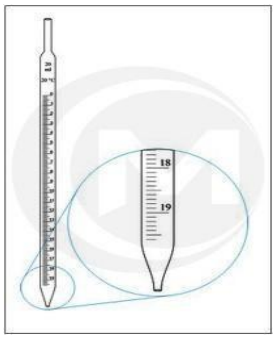
- Fernbach flask.

C) **Graduated pipette:** -

- These are of various sizes.

1) Volumetric pipette: - have a bulb shape in the stem.

- Each pipette is marked to show the given volume of fluid.



2.) **Blood pipettes**: -

These are white back and include the 0.02 ml pipette use for hemoglobin, red cell and platelet counts and also the 0.05 ml pipette for white cell count so.

3.) **Pasteur pipettes**: -

-These are multiple uses.

-they are not graduated or marked.

D) Test tubes: -

-It is clear glass or plastic container.

-It commonly U- shaped bottom and has a open top.

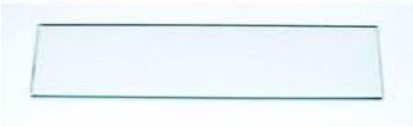
-These are used to mix, hold and heat chemical experiment. Image of test tubes.

E) Petri Dish: -

- It is shallow transparent lidded dish.
- Biologists use to cells culture such as bacteria, small microorganisms etc.
- It is most commonly culture plate.

Microscopic slide: -

- It is a thin flat piece of glass.
- It's 75 by 26 mm and about 1 mm thick.
- It is used to hold objects for examination under microscope.



Stirring rod:-

- It is made of glass.
- It's piece of glass stir rod and laboratory equipment.
- It is basically used to mix chemicals.

I) **Graduated Cylinders: -**

- It is a measuring cylinder or mixing cylinder.
- It is a common piece of laboratory equipment.
- It is used to measure the volume of a liquid.
- It has a narrow cylindrical shape.
- Each marked line on the graduated cylinder represents the amount of liquid that has been measured.

Care and maintenance

1. All Glass wares kept after used.
2. After used wash the Glassware and sterilized.
3. Kept always same rack that i.e. Test tube in test tube rack.
4. After sterilized, kept in wrap paper,

Cleaning Methods

- Dichromate cleaning solution.
- Dissolve 25 gm potassium dichromate 25 ml of water.
- Add 50 concentrated sulphuric acid.
- Cool, store it in a stoppered bottle,

Storage of Glassware and Glass Apparatus

- Mostly glassware and Glass Apparatus are storage of according to glassware, some glassware are discarded after uses and some , cleaning and storage.

  Discard when it starts turning green.

(i) Petri Dishes

Autoclave to remove infected material

- Wash in soapy water
- Rinse in running water let dry
- Rinse in methylated spirit, let dry
- Sterilize in hot air oven.

(ii) Pipettes

- Soak in chromic acid solution overnight
- Wash in running water
- Rinse in distilled water
- Dry on suction pump using methylated spiral, or ether or methylated acetone.
- To sterilize – plug mouthpiece with non-absorbent cotton wool, wrap in kraft paper and hot air sterilize (160^0c for 1 hour)

Test Tubes

- Autoclave to remove in feted material
- Boil in detergent solution for 30 minutes.
- Clean with a brush
- Rinse in running water rinse in distilled water and place them and sterilize in the bot air over. ‘

Pasteur pipettes

- Soak in 3% Lysol for 1 hour.
- Wash as before (as test tubes)

Microscopic glass slides

- Boil in a detergent for 30 minutes.
- Place in dichromate for overnight.
- Wash in running water
- Keep in methylated spirit
- For using, take them out with a forceps and hold them only by the edges.

Types of different lab instrument and equipment:-

LAB INSTRUMENT -

- It is a general term for all kinds of instruments, vessels, and other tools needed for operations in various laboratories, synthesis, and analysis
- Therefore, laboratory instruments have to have a high quality and be durable in order to meet the high standards in laboratory technology

LAB EQUIPMENT

- It refers to the various tools and equipment used by scientists working in a laboratory:
- The classical equipment includes tools such as Bunsen burners and microscopes as well as

  Specialty equipment such as operant conditioning chambers, spectrophotometers, andcolorimeters.

1) Centrifuge Machine.
2) Lab Incubator.
3) Laboratory Stirrer.
4) Laboratory Shakers.
5) Centrifuge Tube.
6) Needle Destroyers.
7) Electronic Balance.
8) Bunsen Burner
9) Funnel
10) Pipette bulb
11) Autoclave
12) Laminar Air Flow
13) Hot air oven 14) Water Bath
15) Cell Counter 16)Microscope

1) **Centrifuge Machine**: -

Introduction

- It is a laboratory device that is used for the separation of fluids, gas or liquid, based on density.
- Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel. Mar 17, 2017

Principle of Centrifuge

Centrifuge is used to sediment or deposit rapidly particles such as cells which may be suspended in a fluid. The speed is expressed as RPM i.e., revolutions per minute.

Care and Maintenance

- Use tubes made of strong glasses and they should not be too long.
- The opposite tubes should be balanced properly.
- The centrifuge should be increased gradually.
-. The instrument should be kept clean. It something spills over inside it should be cleaned and the instrument disinfected if necessary.

2) Lab Incubator.

Introduction: -

- It's providing a controlled, contaminant-free environment for safety.
- It's give a reliable work with cell and tissue cultures by regulating conditions such as temperature, humidity, and CO2.
- Microbiological incubators are used for the growth and storage of bacterial cultures.

Principle of Incubator: -

Work on electricity and regulates temperature thermostatically.

- Necessary for various investigations where body temperature 37^0C incubation are required.

Care and Maintenance

1) Always kept on flat base.
2) Before and after use clean the incubator.
3) After use switch off incubator.
4) After on the incubator, to set the temperature according to requirement systematically.
5) Prevent from water,
6) After uses, covered the incubator.

3) Laboratory Stirrer.

- A magnetic stirrer or magnetic stir plate/
- It is commonly used in laboratories to ensure liquid samples are homogeneous in consistency and temperature.
- A magnetic stirrer can use magnetic stirrer bars or inductive agitators to complete the mixing process.

4) Laboratory Shakers

- A shaker is a piece of laboratory equipment used to mix, blend, or agitate substances in a tube or flask by shaking them.
- It is mainly used in the fields of chemistry and biology.
- A shaker contains an oscillating board that is used to place the flasks, beakers, or test tubes.

5) Centrifuge Tube.

- Centrifuges tubes are precision-made, high-strength tubes of glass or plastic made to fit exactly in the rotor cavities.
- Laboratory centrifuges are used for the separation of fluids, gas or liquid, based on density.
- Centrifugation separates solid particles dispersed in liquid medium, such as blood cells and plasma.

6) Needle Destroyers.

- Needle Destroyer is electrically operated compact equipment designed to destroy.
- The used disposable needles and cut the disposable syringe nozzle easily.
- It is an accepted practice worldwide to destroy the needles and disposable syringes immediately after use on the spot.

7) Electronic Balance

- An electronic balance is a device used to find accurate measurements of weight.
- It is used very commonly in laborites for weighing chemicals to ensure a precise measurement of those chemicals for use in various experiments.

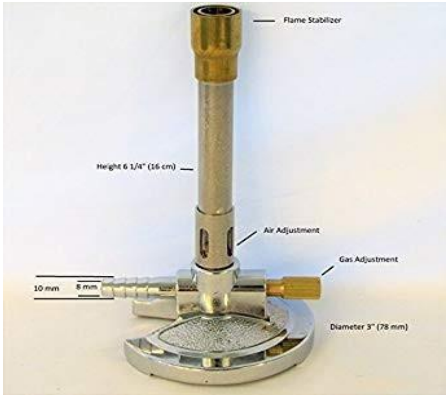
- Electronic balances may also be used to weigh food and other grocery items from home.

Care and Maintenance

a) The weighing equipment must be placed on a film bench, away from vibration, draughts, direct sunlight, and dust.
b) It should be kept perfectly horizontal but altering the screws on which the equipment stand.

8) Bunsen Burner

- A Bunsen burner, named after Robert Bunsen.
- It is a common piece of laboratory equipment that produces a single open gas flame.
- Which is used for heating, sterilization, and combustion.



- The gas can be natural gas (which is mainly methane) or liquefied petroleum gas, such as propane, butane, or a mixture of both.

Care and maintenance

- Make sure you are working on a fireproof bench.
- Connect the gas tube to the gas outlet.
- Make sure there are no cracks in the gas tube.
- Make sure that the collar at the base of the chimney is turned so that it is closed.
- Before turning on the gas, light your match/splint and hold it a bit above the top of

9) Funnel

- A funnel is a laboratory instrument used to pour liquids into another container without the risk of spilling the liquid.
- This is made possible by the funnel's shape, which includes a wide mouth and a narrow tube. -
- The tube can be inserted into the container where the liquid will be poured into.

Care and maintenance

- When treated with proper care laboratory apparatus will give long and satisfactory.
- Glass stopcocks on Burettes.
- Separating Funnels should be lubricated ...

10) Pipette bulb

- Rubber bulbs are used in chemistry laboratories.
- By placing them on top of a glass or plastic tube.
- It serves as a vacuum source for filling reagents through a pipette or Pasteur pipette.

- These also help control the flow of liquid from the dropping bottle.

11) Autoclave Introduction: -

- An autoclave is used to sterilize surgical equipment.
- Laboratory instruments, pharmaceutical items, and other materials.
- It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes.
- Autoclaves vary in size, shape and functionality.
- They are made of strong metal jackets; strong enough to withstand high pressure required.

- The autoclave door is hermetically sealed.
- It has a safety valve set to blow off at a predetermined pressure.
- Principle: - Water boils when its vapour pressure is equal to the pressure of the surrounding atmosphere.

Working Principle:

-Autoclaves use pressurized steam as their sterilization agent. The basic concept of an autoclave is to have each item sterilized -whether it is a liquid, plastic ware, or glassware- come in direct contact with steam at a specific temperature and pressure for a specific amount of time.

12) Laminar Air Flow

- Laminar flow cabinet.
- Laminar flow cabinet or tissue culture hood is a carefully enclosed bench designed to prevent

  Contamination of semiconductor wafers, biological samples, or any particle sensitive materials.
- Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user.

An air flow unit is a clean room equipment meant for protecting the work zone or equipment from noxious particulate matters. It is very important to maintain a clean work zone in order to churn out maximum efficiency from the available machine. Additionally, the contaminated have a tendency to ruin the components of machine thus affecting their workability and efficiency.

The horizontal laminar Air flow hood maintains a clean environment by making it free from bacteria, and then air flow is in horizontal direction. It requires a single-phase power supply of220V, 50Hz.

There are four main types of an air flow workstation, horizontal laminar airflow, vertical laminar Air flow, a mobile air flow, It is accompanied with a LED/UV light. This machine can be accommodated with polycarbonate or glass side panel as per the requirement.

Working Principles:-

Laminar Air Flow provides a work area with aseptic/sterile conditions for the tissue culture. Laminar Air Flow has continuous displacement of air (it provides streamline flow of air) that passes through HEPA (High Efficiency Particulate Air) filter that removes particulates from the air.

Care and maintenance

- The laminar airflow hood shall have routine contract maintenance performed every six (6) months.
- or when the LAFW is relocated, to assure proper function to HEPA filters.
- The laminar airflow hood shall be cleaned every shift and PRN with disinfectant solution.
- All surfaces should be cleaned adequately.

13) Hot air oven Introduction

- A hot air oven is used to sterilize equipment and materials used in the medical field.
- A hot air oven is a type of dry heat sterilization.
- Dry heat sterilization is used on equipment that cannot be wet, and on material that will not melt, catch fire, or change form when exposed to high temperatures.

Working principle

Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, then passes towards the centre of the item, layer by layer. The forced air hot air oven works by heating the oven and using a fan to move the hot air around.

Care and Maintenance

7) Always kept on flat base.
8) Before and after use clean the oven.
9) After use switch off oven.
10) After on the oven, to set the temperature according to requirement systematically.
11) Prevent from water,
12) After uses, covered the oven.
14) Water Bath

- A water bath is a device used in the laboratories to incubate samples in water maintained at a constant temperature.
- Temperature may be controlled digitally or by a dial and once set, the water bath cycles on and off to ensure constancy of the temperature.

Working principle

The Cu50 sensor transfer water temperature to resistance value, amplified and compared by integrated amplifier, then output the control signal, efficiently control the average heating power of electric heating tube and maintain water in constant temperature.

It is electrically heated and has a thermos tic temperature regulator. It can provide temperature ranging from room temperature to 100^0c.

- Various size to suit various workloads are available.

Care and Maintenance

- Water bath should be drained.
- It is cleaned and refilled weekly to avoid contamination and buildup of salt.
- Use of oxygenated water to avoid rust.
- Regular heating at a temperature of >60 C for 30 minutes for biological application
- Avoid running the water bath to dry.

15) Cell Counter: -

Introduction

It is any of various methods for the counting or similar quantification of cells in the life science.

It is an important subset of cytometry, with application in research and clinical practice. For example: - complete blood cell can help a physician to determine why a patient feels unwell and what to do to help. Cell counts within liquids media are usually expressed as a number of cells per unit of volume, thus expressing a concentration.

Image

- Automated cell counters are machines that automatically count cells.
- Used in medical and research labs, automated cell counters.
- It can be used on blood or urine samples to determine the number.
- Its types of cells present or to check the viability of a cultured cell line for research purposes.

Working principle

Automated cell counters are machines that automatically count cells. The sample is loaded into an automated cell counter and it is forced through a small tube while the automated cell counter uses optical or electrical impedance sensors to count how many cells go through the tube.

16) Microscope

Introduction:

- Micro – small, scope – to view
- It is an instrument used to see objects that are too small to be seen by the naked eye.
- Microscopy is the science of investigating small objects and structures using such an instrument.

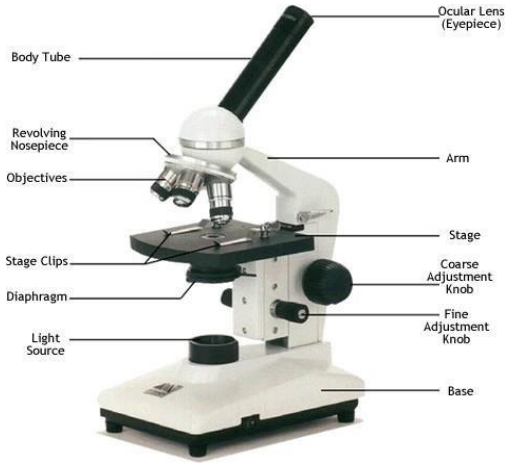
Principle of Optical Microscope (Compound Microscope)

An optical microscope creates a magnified image of an object specimen with an objective lens and magnifies the image furthermore with an eyepiece to allow the user to observe it by the naked eye. In short, the last image to be observed is an inverted virtual image.

It magnifies the image of the object to be visualized through it.

Normally the laboratory microscope provide a magnification of X 40 (scanner) x100 (low power), X400 (high power) and 1000 (oil immersion).

The total magnification is obtained by multiplying the magnification of the objectives with that of the eyepiece.



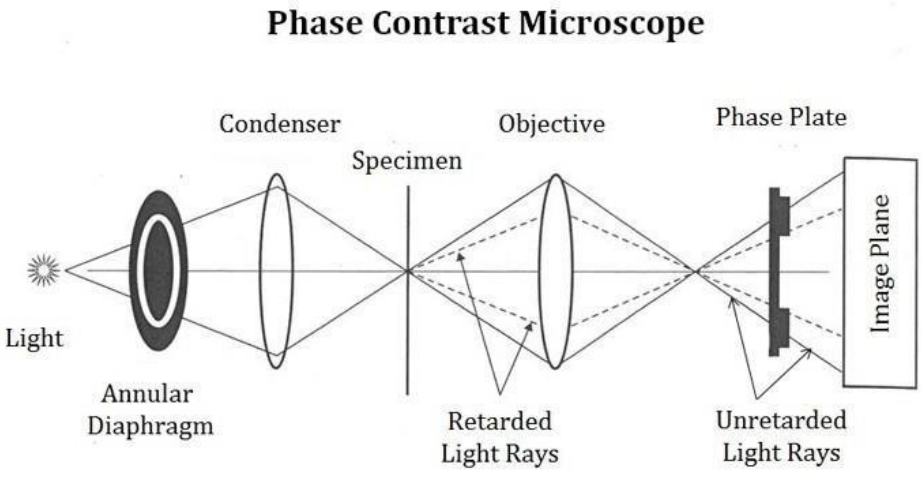
Types of Microscope

1. Phase contrast microscope.

Phase-contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations.

Working **Principle** of **Phase Contrast Microscopy**

The **phase contrast microscopy** is based on the **principle** that small **phase** changes in the light rays, induced by differences in the thickness and refractive index of the different parts of an object, can be transformed into differences in brightness or light intensity.

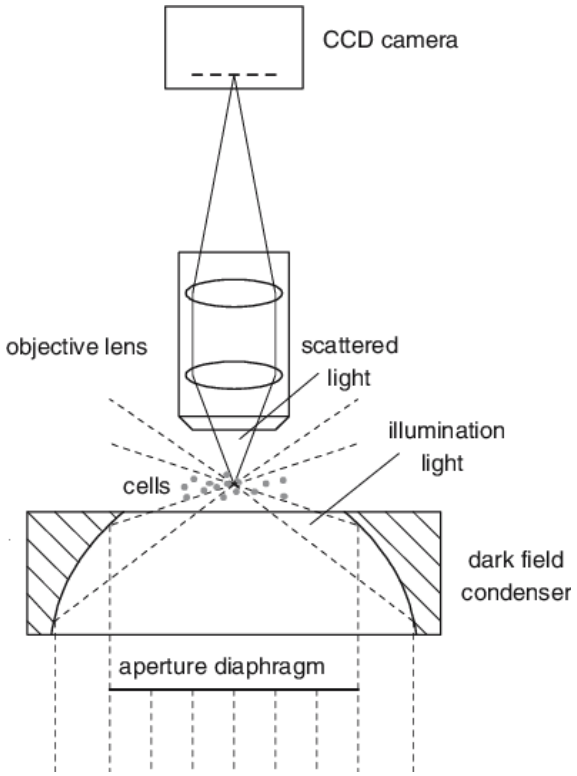


2. Dark Ground illumination

Dark-field microscopy describes microscopy methods, in both light and electron microscopy, In optical microscopy, **dark-field** describes an **illumination** technique used to enhance the contrast in unstained samples. It works by illuminating the sample with light that will not be collected by the objective lens and thus will not form part of the image. This produces the classic appearance of a dark, almost black, background with bright objects on it.

Principle of **dark ground** microscopy,

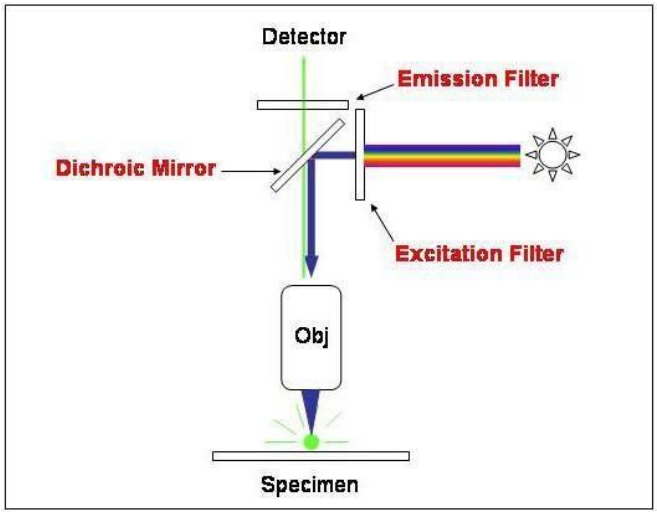
Only oblique scattered light reaches the specimen and passes onto the lens system causing the object to appear bright against a **dark background**.



4) Fluorescence microscopy

A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, scattering, reflection, and attenuation or absorption, to study the properties of organic or inorganic substances.

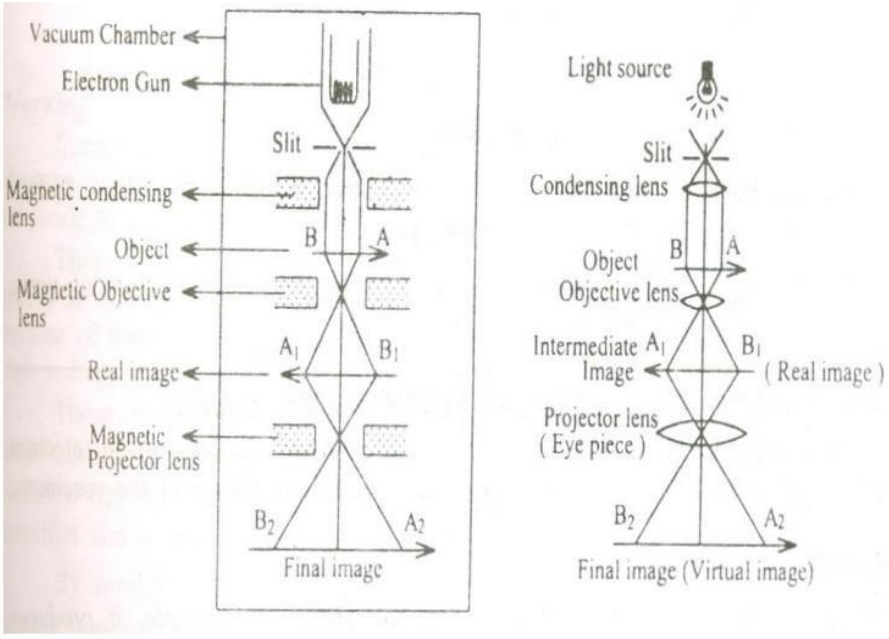
Principle. The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light).



4. Electron microscope

An **electron microscope** is a **microscope** that uses a beam of accelerated **electrons** as a source of illumination.

Working **Principle**: An **electron microscope** uses an '**electron** beam' to produce the image of the object and magnification is obtained by 'electromagnetic fields'; unlike light or optical **microscopes**, in which 'light waves' are used to produce the image and magnification is obtained by a system of 'optical lenses'.



Care and Maintenance

- Clean the objective lens with lens cleaning tissue only.
- Never clean lenses with alcohol, ordinary tissues, cleaning paper, toilet paper, cotton wool or hand towels, which will scratch the lens surface.
- Cover the microscope with a dust cover when not in use.

UNIT-3

Introduction to different reagents, solutions, stains:-

- Many of the reagents used in science are in the form of solutions possible.
- The Flynn Laboratory Solution Preparation reference section.
- The section is divided into several parts for your convenience.
- Basic (stain and fixative), good for.

1. **Carbol fuchsine**: -

Introduction:-

- It is a mixture of phenol and basic fuchsin,
- It is used in bacterial staining procedures.
- It is commonly used in the staining of mycobacteria as it has an affinity for the mycolic acids found in their cell membranes.
- It is a component of Ziehl–Neelsen stain, a differential stain.
- Carbol fuchsin is used as the primary stain dye to detect acid-fast bacteria because it is more soluble in the cells wall lipids than in the acid alcohol. If the bacteria is acid-fast the bacteria will retain the initial red color of the dye because they are able to resist the destaining by acid alcohol (0.4–1% HCl in 70% EtOH).
- Carbol-fuchsin is also used as a topical antiseptic.

Composition:-

Basic fuchsin. – 10 gms Alcohol. – 100 ml

5% aqueous phenol – 1000ml

Uses:- used for leprosy where the bacteria are less acid fast. Store at room temperature filter before use

2. **Gram iodine**:-

Introduction:-

- It is used in Gram staining , When iodine is applied for staining with Crystal violet or another stain of that group a compound is formed which is insoluble in water, but soluble in alcohol or acetone.
- Gram's iodine is used in Gram staining procedure to differentiate gram positive and gram negative organisms. Gram's iodine acts as a mordant that causes the crystal violet to penetrate and adhere to the gram –positive organisms.

Composition:-

Iodine. – 1.0 grams Potassium iodide 2.0gms Distilled Water- 50 ml

Dissolve the potassium iodide in 250 water and then add 10 gm of iodine. When dissolved make up to 1000 ml with distilled water.

3) Geimsa stain:- Introduction:-

- Giemsa stain is a type of Romanowsky stain, named after Gustav Giemsa, a German chemist who created a dye solution.
- It was primarily designed for the demonstration of malarial parasites in blood smears, but it is also employed in histology for routine examination of blood smear.

Principle of Giemsa Stain

Giemsa stain is a differential stain and contains a mixture of Azure, Methylene blue, and Eosin dye. It is specific for the phosphate groups of DNA and attaches itself to where there are high amounts of adenine-thymine bonding. Methanol act as a fixative as well as the cellular stain.

Composition:-

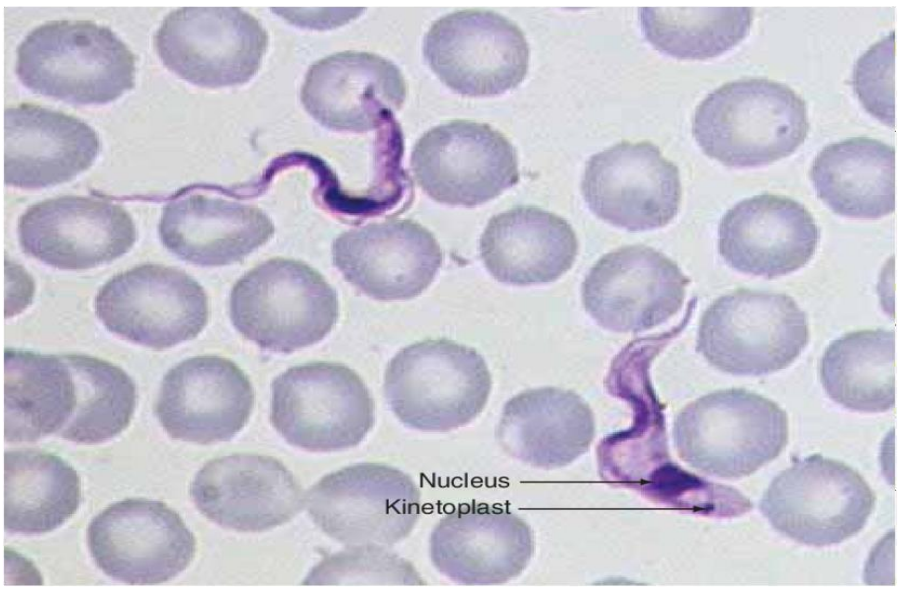
Giemsa powder -. 0.3 gms

Glycerin -. 25.0 ml

Acetone free methyl alcohol 25.0 ml.

This make stock solution and before use it has to be diluted by adding 1 ml to 9 ml of buffered distilled water.

Uses: - This stain is used to peripheral blood smear thick and thin films.



The kinetoplast contains the mitochondrial DNA, and the nucleus contains genomic DNA.

4) Crystal violet:-

Introduction:-

- It is a triarylmethane dye used as a histological stain.
- It in Gram's Method of classifying bacteria.
- Crystal violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic.
- The medical use of the dye has been largely superseded by more modern drugs, although it is still listed by the World Health Organization.
- The name *gentian violet* was originally used for a mixture of methyl pararosaniline dyes (methyl violet), but is now often considered a synonym for *crystal violet*.
- The name refers to its colour, being like that of the petals of certain gentian flowers; it is not made from gentians or violets.

Composition:-

Crystal violet – 0.5 % Solution in Distilled water In histopathology:-

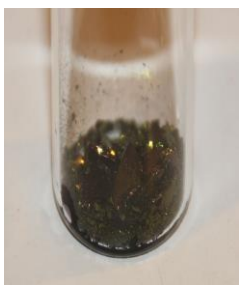
Crystal violet:- it is used in Gram stain. Solutions:-

Stock crystal violet solution crystal violet to saturate approximately – 14.0 gm.

Alcohol, 95% - 100.0 CC

Crystal violet; stock solution – 10.0CC

Distill water - 300. cUses :- To give a pink color of a specimen in slide. This stain used in gram stain procedure in this first stain used of Crystal violet.



SOLID FORM OF CRYSTAL VIOLET AQUEOUS LIQUID FORM OF CRYSTAL VIOLET

5) Fleishman stain:-

INTRODUCTION

- It is a neutral stain for blood smears which was devised by the British surgeon W. B. Leishman (1865–1926).
- It consists of a mixture of eosin (an acidic stain), and Methylene blue (a basic stain) in Methyl alcohol and is usually diluted and buffered during the staining procedure.

Principle of Leishman staining

It consists of a mixture of eosin (an acidic stain), and Methylene blue (a basic stain) in Methyl alcohol and is usually diluted and buffered during the staining procedure. It stains the different components of blood in a range of shades between red and blue.

Composition

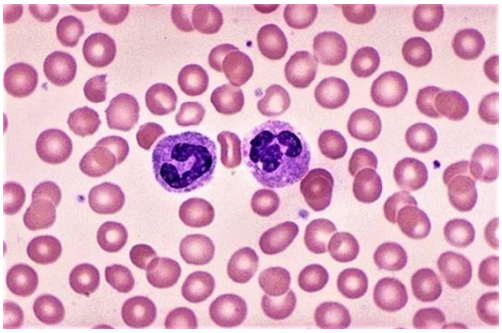
Powdered Leishman's stain- 0.15gm Acetone-free methyl alcohol 133ml

All the stain should be dissolved, keep the in a glass stoppered bottle. Do not filter. Like that for Wright' stain but with double dilution.

Fleishman stain: - It is used for the peripheral smear (blood). It is used mostly readymade and found in market easily.

Procedure:-

1. Take a peripheral blood smear.
2. Add 2-4 drop of leishman stain.
3. Wait for 15 minute. After 5 minute
4. Add 4 to 6 drop distill water.
5. Wait for 10 minute.
6. After 10 minute wash the slide.
7. Dry it. 8.Ready for microscopic



6) Safranin Introduction

- Safranin (also Safranin O or basic red 2) is a biological stain used in histology and cytology.
- Safranin is used as a counterstain in some staining protocols, coloring cell nuclei red.
- This is the classic counterstain in both Gram stains and endospore staining.

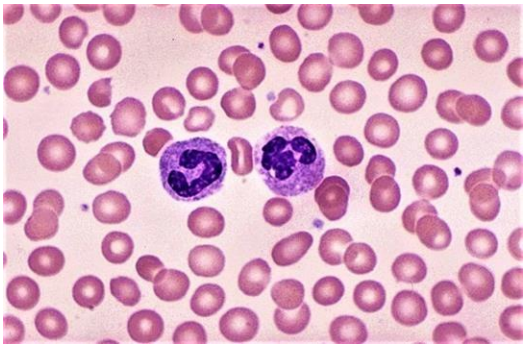
Principle

Safranin O staining highlights cartilage, mucins and mastocyte granules on histological slides. Safranin-O is a basic stain which binds with proteoglycans (acids) in cartilage with a strong affinity forming a red/orange complex. The intensity of the staining depends on proteoglycan's quantity contained in cartilage.

Composition:-

1.7 gm Safranin 50 ml alcohol
Distilled water to make 500 ml
-It is a biological stain used in histology and cytology.

Safranin is used as a. Counterstain in some staining protocols coloring cell nuclei red; this is the classic counter stain in both gram stains and endospore staining.
It can also be used for the detection of cartilage, mucin and mast cell granules.



7) Hypochlorite solution:-

Introduction:-

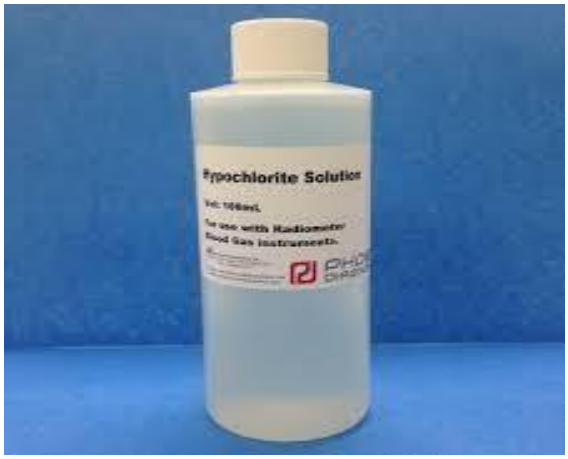
- Sodium hypochlorite is a clear, slightly yellowish solution with a characteristic odor.
- Sodium hypochlorite has a relative density of is 1,1 (5,5% watery solution).
- As a bleaching agent for domestic use it usually contains 5% sodium hypochlorite (with a pH of around 11, it is irritating).

The **principle** of on-site **hypochlorite** production by electrolysis is theoretically simple. A **solution** of sodium chloride (brine **solution**) has a direct current passed through it generating chlorine at the anode and both hydrogen and hydroxide ions at the cathode (Eqs 31.14– 31.15).

Composition:-

Hypochlorite solution with a concentration of at least 0.1% available chlorine (1g /liter, 1000 ppm)

- It is a chemical compound with the formula NaOCl or NaClO, comprising a sodium cation and a hypochlorite anion. It may also be viewed as the sodium salt of hypochlorous acid. The any clouds as a pentahyarate. A pale greenish YELLOW SOLID It can be eddectively used for water purification, bleaching odor removal and water do injection.



9) **ETHANOL**:-

Introduction:-

Ethanol (also called ethyl alcohol, grain alcohol, drinking alcohol, or simply alcohol) is a chemical compound, a simple alcohol with the chemical formula C. $_2$H. $_6$O. Its formula can be also written as CH. $_3$−CH.

Pharmacological class: Solvent

It's also called ethy (alcohol grain alcohol, drinking alcohol. It is a chemical compound. A simple alcohol with chemical formula C_2H_6O . It is a volatile, hummable colorless liquid with a slight characteristic odor. It is a psychoactive substance and is the principal active ingredient and is the principal active ingredient and is the principal active ingredient found in alcoholic drinks.

It is used in medical wipes and most commonly in antibacterial hand sanitizer gels as antiseptics.

It as an antiseptic for its for its bacterial and antifungal effects. Ethanol kills organisms y lipid and is effective against most bacteria and fungi and money viruses. However ethanol is in

effective against bacterial spores 70% ethanol is the most effective concentration, particularly because of osmotic pressure.

9) **Formaldehyde: - Introduction**

- Formaldehyde is a simple chemical compound made of hydrogen, oxygen and carbon.
- All life forms – bacteria, plants, fish, animals and humans – naturally produce formaldehyde as part of cell metabolism.

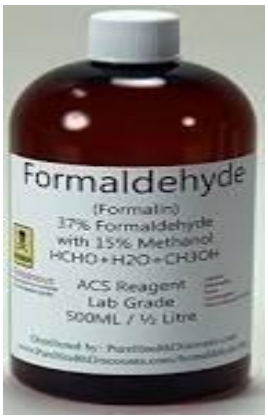
In **principle**, **formaldehyde** could be generated by oxidation of methane, but this route is not industrially viable because the methanol is more easily oxidized than methane.

Composition:-

10% Formalin solution

37-40 % formaldehyde. - 100cc Tap water – 900 cc

It is fixative used to the save organ, tissue cell to prevent the death of cell 10% formalin is most widely used fixative because it is compatible with most stains , length of fixation depends on the size of blocks.



Preparation of different types of media agar:-

Anything used for preparing culture media should be free from living organisms.

All media prepared should be sterilized according to prepared should be sterilized according to instructions for each type of media. pH adjustment should be correct for all media.

Since most organisms grow at a slightly alkaline pH -7.2-7.6.

Time can be saved by using dehydrated culture media: as per the manufacturer's instructions, weigh the dehydrated medium, add the requisite amount of boiled distilled water, mix the two and sterilize the solution.

The method for the preparation of basic microbiology media is given below. In situations where preparation is uneconomic in time, prepared, sterilized media (liquid and solid) are available from the major school science equipment suppliers. Sterilization is at 121

°C (15 lb in $^{-2}$) for 15 minutes. pH values are 7.0 unless stated otherwise.

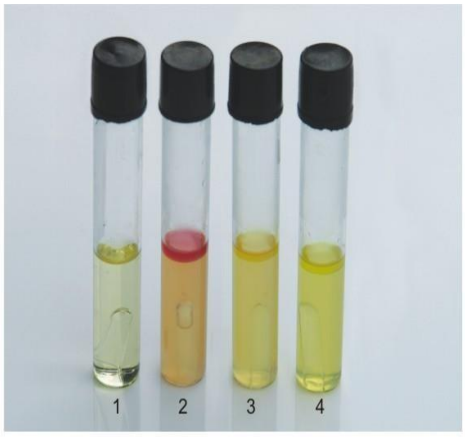
Given below is method for preparation of culture media:-

1) Peptone water:- Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the medium is 7.2±0.2 at 25 °C and is rich in tryptophan. **Peptone water** is also a non-selective broth medium which can be used as a primary enrichment medium for the growth of bacteria.

This medium is used for the testing of indole production, for the preparation of sugar media, and when made highly alkaline (pH 8.0-8.4) is used for the cultivation of vibrio cholerae.

Composition

Peptone -10
Sodium chloride- 5 gms Distilled water -1000ml
Dissolve by steaming. Adjust the pH to 7.5. Filter through paper. Distributed in test tubes or bottles. Sterilize at 15 lb. Pressure for 20 minutes. The commercially available Peptone water consist of water soluble product obtained from lean meat or other protein material by digestion mainly with a proteolytic enzymes like pepsin , trypsin or papain .The important, proteases, amino acids and inorganic salts.



Peptone Water (M028)
(with added Kovac's Reagent - R008)
1. Control
2. Escherichia coli ATCC 25922
3. Salmonella Typhimurium ATCC 14028
4. Staphylococcus aureus ATCC 25923

2) Nutrients Broth Introduction

Nutrient Broth is a basic media composed of a simple peptone and a beef extract. Peptone contributes organic nitrogen in the form of amino acids and long-chained fatty acids. Beef Extract provides additional vitamins, carbohydrates, salts and other organic nitrogen compounds.

Peptone

- 10gms Sodium chloride – 5gms Meat extract -. 10gms Distilled water – 1000ml
- Mix the ingredients and allow dissolving. Adjust pH to 7.6. Phosphate may precipitate out and should be extricated by filtration.
- Distribute the medium in large bottles and then sterilized at 15 lb. for 20 minutes.
- Clear with white of egg . Autoclave and filter.
- Distribute into flasks and sterilized at 15 lb for 20 minutes.

Different type of media and agar:

Types of agar plates

- Blood agar - contains blood cells from an animal (e.g. a sheep). ...
- Chocolate agar - this contains lysed blood cells, and is used for growing fastidious (fussy) respiratory bacteria.
- Neomycin agar - contains the antibiotic neomycin.
- Sabouraud agar - used for fungi.

A) Nutrient Agar

Nutrient agar is a general-purpose medium supporting growth of a wide range of non-fastidious organisms. It typically contains (mass/volume): 0.5% Peptone - this provides organic nitrogen. 0.3%

beef extract/yeast extract - the water-soluble content of these contribute vitamins, carbohydrates, nitrogen, and salts.

- Agar-Agar, is a long chain polysaccharide substance from certain seaweeds. It forms a firm gel in watery solution at concentration of about 2%. Agar alone has no nutritive properties. It melts at about 95°C and solidifies only when cooled.
- To the nutrient broth add 2% of agar – it then becomes nutrient agar. After addition of 2% agar, autoclave at 15 lb for 20 minutes.
- Clear with white of egg. Autoclave and filter.
- Distribute into flasks and sterilize at 15 lb for 20 minutes.

B) Blood Agar

Blood agar is an enriched, bacterial growth medium. Fastidious organisms, such as streptococci, do not grow well on ordinary growth media.

Blood agar is a type of growth medium (trypticase soya **agar** enriched with 5% sheep **blood**) that encourages the growth of bacteria, such as streptococci, that otherwise wouldn't grow.

- Melt the nutrient agar and cool to 50°C. Aseptically add 5-10% sterile defibrinated blood.
- Mix and pour into Petridis or tubes which are sloped. Bank blood or rabbit blood may be used.

C) Chocolate Agar

It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80°C. Chocolate agar is used for growing fastidious respiratory bacteria, such as Haemophilus influenzae and Neisseria meningitidis.

- Add blood to nutrient agar as for blood agar.
- Mix well and raise the temperature to 80°C keeping well mixed.
- Leave at 80°C for 10 minutes.
- Pour into petri dishes or tubes as needed.
- Sugar Media.
- These are used to study the biochemical reactions of bacteria.
- To sterilized Peptone water add1%of the required sugar and 1% Anrades indicator.

- Distribute into sterile tubes containing inverted Durham's fermentation tubes.
- Indicator is used to study the acid formation by bacteria.
- If acid is produced media becomes reddish pink.
- Instead of Anrades indicators can also be used
- Neutral red 0.25% to 1% solution --- if acid is produced --- pink color .
- Phenol red 0.01%---if acid is produced --- yellow color.
- The sugar media are sterilized by fractional sterilization or tyndallisation.
- The sugar may be caramelized or charred at a temperature higher than 100°C, so
- it is steamed on three consecutive days in Arnold'ssteam sterilizer.

Agar: - It is used to grow of bacterial cell.

c) Blood Agar plates: - There are made by adding 5 to 10 % sheep or horse blood to the nutrients medium.

d) Nutrient Agar:- Nutrient Agar Grams the largest variety of microbes, typically fungi and bacteria.

- Mac conkey Agar :-
- Chocolate Agar.

Media: - It gives artificial environment simulating natural conditions necessary for growth of bacteria.

Fluid media: - Bacteria gown very well in feud media in 3 to 4 hours hence they are used as enriched media before plating on solid media.

Type of liquid media

1. Broth: - It is a clear transparent straw cultured fluid prepared from meat extract or peptone.

2. Peptone: - It is a protein partially digested with hydrolytic enzymes. Like peprin, trypsin etc.

3. Yeast extract: - It is prepared by extracting autolysis yeast with water It has high contents of vitamin B

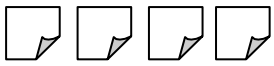
- Solid media: - They are used to study colonies of individual bacteria. They are esentialfor isolation of organism is pure form.

(a) Agar: - It is important constituent of solid media. It is complex pohysarch aride obtained from seaweeds (Agar geledium species).

It melts at 80 to 100^0C and solidifies at 35 to 42^0 C

(b) Gelatin: - It is protein protein prepared by hydrolysis of collagen with boiling water.

It is in liquid form at 37^0 C. It forms transparent get below 25^0C.



UNIT-4

Infection control and prevention: -

Infection prevention and control Infection prevention and control (IPC) is a scientific approach and practical solution designed to prevent harm caused by infection to patients and health workers. It is grounded in infectious diseases, epidemiology, social science and health system strengthening.

a. Infection control is the discipline concerned with preventing nosocomial or healthcare associate infection.

b. A practical sub discipline or epidemiology.

c. It is an essential, though often under recognized and under supported, part of the infrastructure of health care

d. Infection control and hospital epidemiology are skin to public health practice.

■ Understand practices to curb infection:-

a. Aseptic Technique used.

b. Hand hygiene: - The spread of pathogens is effective hand washing. The most important way to reduce

The spread of infection always regularly washes with soap with fresh water.

c. Sterilization:- It is the process of intended to kill all microorganisms and is the highest level of microbial

Kill that is possible sterilizers may be heat, steam or liquid chemical etc.

d. Cleaning: - It can be prevented from occurring in homes as well as. In order to reduce their chances to contract an infection. Individuals are recommended to maintain a good hygiene by washing their hands after every contact with questionable areas or bodily fluids and by disposing of garbage at regular intervals to prevent germs from growing

e. Disinfectant: - Uses as liquid chemical on surfaces and at room temperature to kill diseases causing

Microorganism's ultraviolet light has also been used to disinfect the rooms of patients infected with clostridium difficult after discharge

f. Personal protective equipment are used like as gloves, mask, apron, speckles etc.

Understand practices to curb infection

The most important way to reduce the spread of infections is hand washing - always wash regularly with soap and water. Also important is to get a vaccine for those infections and viruses that have one, when available. See the OSH Answers Hand Washing - Reducing the Risk of Common Infections for more details. **Hospital borne infection:-**

A hospital-acquired infection (HAI), also known as a nosocomial infection, is an infection that is

acquired in a hospital or other health care facility .....Health care staff also spread infection, in addition to contaminated equipment, bed linens, or air droplets

- Nosocomial Infection:- Definitions:- Infections which are acquired from hospitals are called nosocomial infections.
- It the organism come from another patient it is called cross infection and if the patient himself carries the infection to some other site then it is autoinfection.

  Infection may become apparent during the stay of the patient in the hospital or after become disc hang from the hospital.

Hospital infection and prevention:-

- We should be aware of some important hospital infections and about their prevention.

1. Wounds and bores: - It is important to remove all tissue debris accidental wounds and burns as bacteria can establish more easily in damaged
2. Urinary tract infection: - Catheter or other instruments into the bladder may cause urinary tract injection. Used catheters are difficult to sterilize and may be the cause of cross infections also hence disposable sterilized catheter should be used aseptically.
3. Alimentary tract infection: - Outbreak of E. coli gastroenteritis in children and of shield sonnet, dysentery does occur quite often in hospital. Isolation, general hygiene and exclusion of carriers are important preventive measures.
4. Bath as means of cross infection: - It is commonly seen that series of babies are made to have bath in a same sink thus resulting in dispersal of pathogenic organisms especially staphylococcus aureus through murrery. Hence it is emphasized that if new born babies need to be bathed this should be done in stainless steel bowls which can early be autoclaved after the bather of each baby.

Treatment:-

1. Antibiogram and resist gram.
2. Bio typing.
3. Phage typing
4. Bacteriocin typing
5. Serotyping
6. Serum opacity factor (analysis of marker proteins, analysis of enzyme production e.g. staphylococcus aureus).
7. RNA electrophoresis as in done in Rota virus.
8. Cytoxcity assay e.g. protease mirabills.
9. Reverse phage typing e.g. staphylococcus. Aureus.
10. Plasmid profit.

Prevention:-

1. Proper washing of hands.
2. Isolation of patients e.g. plague, influenza, measles etc.
3. Careful and appropriate use of instruments.
4. Use of antibiotics only if required. It may be given to carrier staff or patient.
5. Use of blood transfusion only if must disinfectant of excreta and infected material.
6. Surveillance of infection properly and regularly.
7. Use of vaccine e.g. tetany gas gangrene, hepatitis B, etc.

Treatment of needle sticks injury

1. Investigation of patient and whose needle sticks injury for cure.
2. If patient suffering from HIV than needle stick injury person immediately goes to Anti-retroviral clinic.
3. Immediately washed out needle stick injury. Understand the management of blood and body substance **spillage in the health care setting :-**

It include number of patients involved with their distribution in wards, times of onset, their symptoms., whether all or majority of cares followed operation of so whether they were operated in same operation theatre and other clues as to the way in which they became infected. It their infections is by identical bacteria effect should be made to trace human carrier or other sources of infection. Outbreak of infection in the hospital is generally become of detective ventilation in the word of theatre, in aseptic technique or in sterilization of dressings or instruments and importer cleanliness of hospital kitchen plus its workers.

- Understand the management of blood and body substance spillage in the health care setting:-

a) Standard precautions apply, including use of personal protective equipment.

b) Spills should be cleared up before the area is cleaned.

c) Generation of aerosols from spilled material should be avoided.

- Different type of spills:-

a. The nature of the spill for example Sputum, commit, faces, urine, blood or laboratory culture.

b. The pathogens most likely to be involved in different type of spills for example stool sample may be contain viruses, bacteria or protozoa whereas Sputum may contain mycobacterium tuberculosis.

c. The size of spills for example spot (few drop), small.

d. Type of surface – for example carpet or impervious flooring.

e. The location involved that is whether the spill occurs in a contained area such as microbiology laboratory or in public area or clinical area of a health services.

f. Whether there is any like hood of bare skin contact with the soiled ((contaminated) surface.

Understand the management of blood

Hemorrhage remains the primary cause of preventable death on the battlefield and in civilian trauma. Hemorrhage control is multifactorial and starts with point-of-injury care. Surgical hemorrhage control and time from injury to surgery is paramount; however, interventions in the prehospital environment and perioperative period affect outcomes. The purpose of this review is to understand concepts and strategies for successful management of the bleeding military patient. Understanding the life-threatening nature of coagulopathy of trauma and implementing strategies aimed at full spectrum hemorrhage management from point of injury to postoperative care will result in improved outcomes in patients with life-threatening bleeding.

RECENT FINDINGS:

Timely and appropriate therapies impact survival. Blood product resuscitation for

Life-threatening hemorrhage should either be with whole blood or a component therapy strategy that recapitulates the functionality of whole blood. The US military has transfused over 10000 units of whole blood since the beginning of the wars in Iraq and Afghanistan. The well recognized therapeutic benefits of whole blood have pushed this therapy far forward into prehospital care in both US and international military forces.

Multiple hemostatic adjuncts are available that are likely beneficial to the bleeding military patient; and other products and techniques are under active investigation.

Body substance spillage in the health care settings

Health services should have management systems in place for dealing with blood and body substance spills. Protocols should be included in procedural manuals, and emphasized in ongoing education or training programs.

The basic principles of blood and body fluid/substance spills management are:

- standard precautions apply, including use of personal protective equipment (PPE), as applicable
- spills should be cleared up before the area is cleaned (adding cleaning liquids to spills increases the size of the spill and should be avoided)
- Generation of aerosols from spilled material should be avoided.

Using these basic principles, the management of spills should be flexible enough to cope with different types of spills, taking into account the following factors:

- the nature (type) of the spill (for example, sputum, vomit, faces, urine, blood or laboratory culture)
- the pathogens most likely to be involved in these different types of spills – for example, stool samples may contain viruses, bacteria or protozoan pathogens, whereas sputum may contain *Mycobacterium tuberculosis*
- the size of the spill – for example, spot (few drops), small (<10 cm)="" or="" large="">10cm)
- the type of surface – for example, carpet or impervious flooring

- the location involved – that is, whether the spill occurs in a contained area (such as a microbiology laboratory), or in a public or clinical area of a health service, in a public location or within a community premises
- Whether there is any likelihood of bare skin contact with the soiled (contaminated) surface.

Cleaning spills – equipment

Standard cleaning equipment, including a mop, cleaning bucket and cleaning agents, should be readily available for spills management. It should also be stored in an area known to all. This is particularly important in clinical areas.

To help manage spills in areas where cleaning materials may not be readily available, a disposable 'spills kit' could be used, containing a large (10 L) reusable plastic container or bucket with fitted lid, containing the following items:

- appropriate leak-proof bags and containers for disposal of waste material
- a designated, sturdy scraper and pan for spills (similar to a 'pooper scooper')
- about five sachets of a granular formulation containing 10,000 ppm available chlorine or equivalent (each sachet should contain sufficient granules to cover a 10-cm diameter spill)
- disposable rubber gloves suitable for cleaning (vinyl gloves are not recommended for handling blood)
- eye protection (disposable or reusable)
- a plastic apron
- a respiratory protection device, for protection against inhalation of powder from the disinfectant granules or aerosols (which may be generated from high-risk spills during the cleaning process).
- destroyed by incineration
- Immersed in sodium hydroxide or sodium hypochlorite for 1 hour, rinsed and placed in a pan of clean water, and sterilized on an 18-minute cycle.

Single-use items in the spills kit should be replaced after each use of the spills kit.

With all spills management protocols, it is essential that the affected area is left clean and dry.

Sodium hydroxide (caustic soda) spills kits should be available for areas at risk for higher-risk Creutzfeldt–Jakob disease (CJD) spills, such as in neurosurgery units, mortuaries and laboratories.

Cleaning spills – procedures

In clinical areas, blood and body fluid/substance spills should be dealt with as soon as possible. In operating rooms, or in circumstances where medical procedures are under way, spills should be attended to as soon as it is safe to do so.

Care should be taken to thoroughly clean and dry areas where there is any possibility of bare skin contact with the surface (for example, on an examination couch).

PPE should be used for all cleaning procedures, and disposed of or sent for cleaning after use. Hands should be washed and dried after cleaning.

Where a spill occurs on a carpet, shampoo as soon as possible does not use disinfectant. Steam cleaning may be used instead.

Wash hands thoroughly after cleaning is completed.

Cleaning spots or small spills

Spots or drops of blood or other small spills (up to 10 cm) can easily be managed by wiping the area immediately with paper towels, and then cleaning with warm water and detergent, followed by rinsing and drying the area. Dry the area, as wet areas attract contaminants.

A hospital-grade disinfectant can be used on the spill area after cleaning.

Cleaning large spills

Where large spills (more than 10 cm) have occurred in a ‘wet’ area, such as a bathroom or toilet area, the spill should be carefully washed off into the sewerage system using copious amounts of water and the area flushed with warm water and detergent.

Large blood spills that have occurred in ‘dry’ areas (such as clinical areas) should be contained and generation of aerosols should be avoided.

Granular formulations that produce high available chlorine concentrations can contain the spilled material and are useful for preventing aerosols. A scraper and pan should be used to remove the absorbed material. The area of the spill should then be cleaned with a mop, and bucket of warm water and detergent. The bucket and mop should be thoroughly cleaned after use and stored dry.

Sodium hypochlorite (bleach)

It is generally unnecessary to use sodium hypochlorite for managing spills, but it may be used in specific circumstances. It is recognized, however, that some healthcare workers and members of the public may feel more reassured that the risk of infection is reduced if sodium hypochlorite is used. Healthcare workers and members of the public should be aware that there is no evidence of benefit from an infection control perspective.

Hypochlorite’s are corrosive to metals and must be rinsed off after 10 minutes and the area dried.

Cleaning spills that contain Creutzfeldt–Jakob disease prions

If a spill of tissue that is definitely or potentially infected with CJD prions occurs (for example, brain tissue), the contaminated item should either be:

The items should then be cleaned following routine cleaning and sterilization procedures.

Surface spills should be cleaned up using paper towels before the surface is wiped with either sodium hydroxide or sodium hypochlorite, left for 1 hour (if possible, or as long as possible, with the area cordoned off), the solution wiped off and the surface cleaned by following routine cleaning procedures.