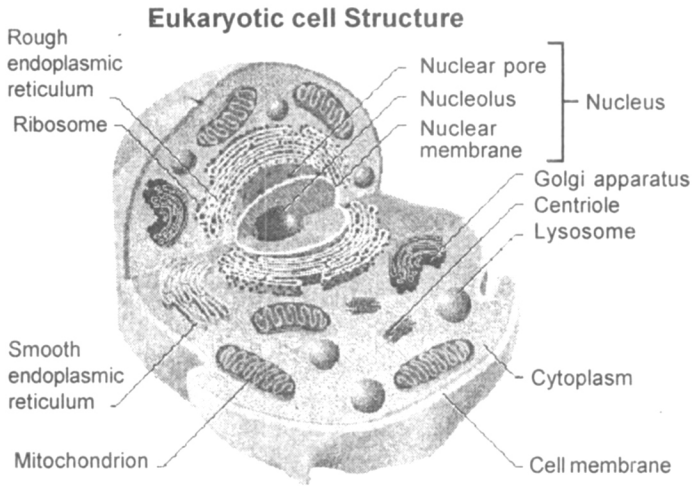

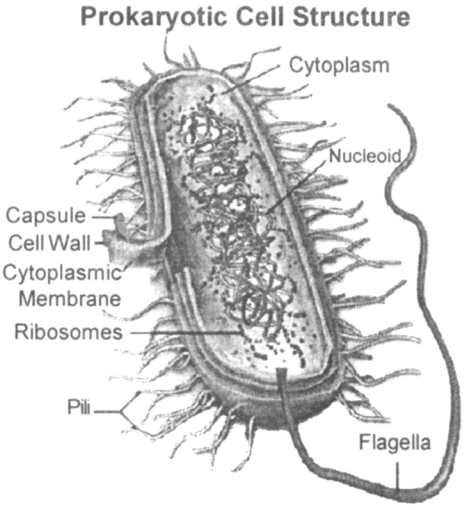

Fig 1.1: Structure of Eukaryotic Cell **Fig 1.2: Structure of Prokaryotic Cell**

2. Laboratory Safety Guidelines (Rules and Regulations)

The biology department should provide a safe environment for all. Laboratory safety is a mutual responsibility and requires full participation and cooperation of all involved persons (students and staff).

Common Instructions

- Restrict laboratory access to authorized persons only. Children are not permitted in the laboratory.
- Smoking; eating; drinking; storing food, beverages or tobacco; applying cosmetics or lip balm and handling contact lenses are not permitted in laboratories.
- Wear lab coats (knee length) and safety glasses in laboratories employing chemicals, biohazards or radioisotopes.
- Open shoes, such as cut shoes, should never be worn in the lab.
- Tie back or otherwise restrain long hair when working with chemicals, biohazards, radioisotopes.
- Keep work places clean and free of unwanted chemicals, biological specimens, radioisotopes and idle equipment.
- Avoid leaving reagent bottles, empty or full, on the floor.
- Work only with materials once you know their flammability, reactivity, toxicity, safe handling and storage and emergency procedures.
- Prepare and maintain a chemical inventory for the lab.
- Keep exits and passage ways clear at all times.
- Ensure that access to emergency equipment (eyewashes, safety showers and fire extinguishers) is not blocked.
- Working alone is an unsafe practice at any time.
- Report accidents and dangerous incidents promptly to your supervisor

Stir bar (Fig. 3.10)

A stir bar (or flea) is a magnetic bar, used to stir a liquids in a laboratory. The stir bar rotates in synch with a separate rotating magnet located beneath the vessel containing the reaction. Glass does not affect a magnetic field appreciably (it is transparent to magnetism) and most chemical reactions take place in glass vessels. This allows the magnetic stir bars to work well in glass vessels. Stir bars are typically coated with teflon, so that they are chemically inert and do not contaminate or react with the reaction mixture they are in.They are bar shaped and often octagonal in cross-section and sometimes circular. Most stir bars have a ridge around the centreon which they rotate. The smallest are only a few millimeters long and the largest a few centimeters. A stir bar retriever is a separate magnet on the end of a long stick, which can be used to get (or fish) stir bars out of the reaction vessel. Most magnetic stirrers today spin their magnets with an electric motor. Stir bars work best for relatively small reactions that are not very viscous. For larger volumes or more viscous liquids, some sort of mechanical stirring is typically needed.

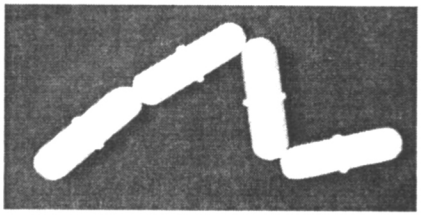

Fig. 3.10 : Stir Bar

Vortex mixer (Fig. 3.11)

A vortex mixer is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an electric motor with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-centre. As the motor runs the rubber piece oscillates rapidly in a circular motion. When a test tube or other appropriate container is pressed into the rubber cup (or touched to its edge) the motion is transmitted to the liquid inside and a vortex is created. Most vortex mixers have variable speed settings and can be set to run continuously, or to run only when downward pressure is applied to the rubber piece. Vortex mixers are common in bioscience laboratories. In cell culture and microbiology laboratories they may be used to suspend cells. In a biochemical or analytical laboratory they may be used to mix the reagents of an assay or to mix an experimental sample and a dilutant. An alternative to the electric vortex mixer is the "finger vortex" technique in which a vortex is created manually by striking a test tube in a forward and downward motion with one's finger or thumb. This generally takes longer and often results in an inadequate suspension, although it may be suitable in some cases when a vortex mixer is unavailable or the forces involved in vortexing would damage the sample.

Fig. 3.11 : Vortex Mixer

Petri dish (Fig. 3.12)

A Petri dish is a shallow glass or plastic cylindrical dish used for the cultivation of microorganisms. It was named after the German bacteriologist Julius Richard Petri (1852–1921) who invented it in 1877 when working as an assistant to Robert Koch. It consists of two shallow glass dishes, the upper half or lid and the lower half or bottom half. Glass petri dishes can be re-cycled after use by dry heating in a hot air oven at 160°C for an hour. Plastic petri-dishes are not recycled. The dish is partially filled with warm liquid agar along with a particular mix of nutrients, salts and amino acids and optionally, antibiotics. After the agar solidifies, the dish is used for microbial inoculation.

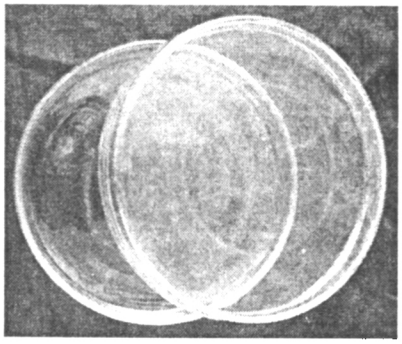

Fig. 3.12 : Petriplate

Haemometer (Fig. 3.35)

It is a compact kit used for detecting the haemoglobin content of the blood by diluting an acidified sample and comparing it with a coloured standard. Its reliability, simplicity and rugged construction makes this kit suitable for simplified services are unavailable. The kit consists of a Sahli haemometer, graduated dilution tube, dropping pipette, brush, glass rod, amber coloured acid vial, a blood pipette and a suction tube.

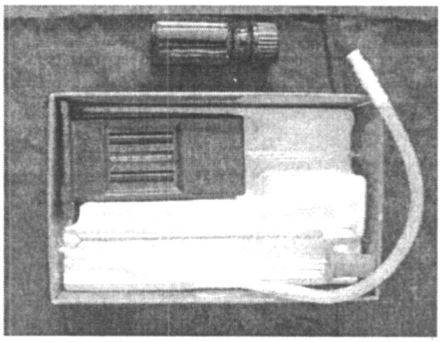

Fig. 3.35: Haemometer

Chemicals

Good laboratory grade chemicals also required as per experimental requirement. For exact chemical need, refer materials required section of individual practicals and also appendix.

Laboratory instruments, glasswares, plasticwares, chemicals, stationery items only do not satisfy the good performance of the laboratory. Laboratory performances rely only on laboratory chief, workers / students. Laboratory personnels should have the following, Analytical bent of mind, Aptitude for research, Keen power of observations, Mathematical and computational skills, Good at technical and report writing, Mind of Upgradation of knowledge, hard working nature, maintanence of good laboratory practices, following good disposal procedures for disposing chemicals, biological etc.,

When performing experiments students should have the following accessories, clean laboratory coat, record note book, glass marking pencil, glass slide & cover slip, labeling slips, match box, cloth, slide box, scissors, forceps, needle, mask, gloves, scale, eraser etc.,

4. Microscopy

Aim

To understand the nature and types of microscopes.
To know the basic principles and theory of microscopes.
To understand the working procedures of microscope.
To visualize the microorganisms by microscope.

Introduction

The study of Micro organisms / cells requires appropriate methods for observation. Microscopy is the use of a microscope to view objects too small to be visible with the naked eye. Microorganisms are the tiny particles seen through microscope.

In 1676, Antony Von Leuvenhoek observed minute objects and named as animalcules through his ground pieces of glasses. His microscope magnification is around 50 - 300 times. The common initial microscope may be invented by Zacharias Janseen from Netherlands or Galielo Galielei of Italy. Advanced compound microscope was invented by Robert Hook in 18th century.

Two key characteristics of a reliable microscope are *magnification,* or the ability to enlarge objects, and *resolving power* or the ability to show detail. Lenses act like a collection of prisms operating as a unit. When a light source is distant so that the parallel rays of light strikes the lens, a convex lens will focus these rays at a specific point, the focal point. The distance between the centre of the lens and the focal point is called focal length. Many different types of microscopes have been developed over the past two centuries. Each has its own characteristics features that provide it with a specific value in microscopy. Basically there are two kinds of microscopes light and electron.

Introduction

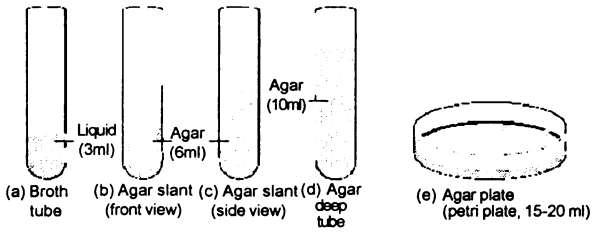

Fig 6.1: Agar deep, Agar Slant and Agar Plate

Any material that support the growth of organisms are called culture media. It must contain many nutrients. Micronutrients and macronutrients are required for the growth of microorganisms. Microbial culture medium is basically classified into two types. They are Defined medium and Complex medium. Medium, which contains known chemical constituents are called *defined medium*. Eg., Minimal Medium. Those medium which contains unknown chemical constituents are called *complex medium* Eg., Nutrient Agar. Most essential culture media are available commercially in readymade dehydrated form. Simple medium, Enriched and enrichment medium, Selective medium, Differential medium and Transport medium are the types of culture medium.

Simple medium

These are simple nutrient medium that will support the growth of microorganisms that do not require special nutrition. They are often used to prepare enriched media, storing of stock cultures and sub culturing. Eg., Nutrient Agar.

Enriched and enrichment medium

These are the medium that enriched with whole blood, lysed blood, serum, extra peptones, special extracts or vitamins to support the growth of fastidious organisms. Eg., *Haemophillus influenzae* require X and V factor for growth that is given in the form of chocolate agar. The term enrichment is used to describe a fluid medium that increases the number of pathogens by enhancing the growth and discouraging the multiplication of unwanted pathogens or bacteria. Eg., GN broth discourage the growth of Enterobacteriaceae members other than *Salmonella, Shigella* and *Escherichia.*

Selective medium

These are the media, which contains substances that prevent or slowdown the growth of microbes other than pathogens for which the media are intended. Eg. XLD medium selects Salmonella and Shigella. Now a day antimicrobial agents have became increasingly used as selective agents. Eg. New York City Agar medium used to select *Neisseria gonorrhoea* which contains Colistin, Nalidixic acid, Nystatin and Trimethoprim Sulphate. These antibiotics inhibit the growth of all gram positive, gram negative except Neisseria.

Differential medium

This type of the medium is used to differentiate various pathogens. Main differential part of the medium is indicators and dyes. Eg., TCBS medium contain bromo thymol blue which differentiate sucrose fermenting Vibrio from others.

Transport medium

These are mostly semisolid media that contain ingredients to prevent the over growth of commensals and ensure the growth of aerobic and anaerobic pathogens. Eg. Cary Blair medium used for preserving enteric pathogens.

Classification of media based on solidification

Culture media used in three forms they are Solid, Semisolid and Fluid medium.