MICROBIOLOGY LAB MANUAL

Offered to

THREE/ FOUR-YEAR MAJOR/ MINOR/MDC DEGREE COURSE OF STUDIES

UNIVERSITY OF CALCUTTA

NEP SYLLABUS

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CC101

INTRODUCTION TO MICROBIOLOGY

AND MICROBIAL

DIVERSITY(PRACTICALS)

Serial No.	Demonstration	Date	Page No.	Signature
1.	Microbiology Good Laboratory Practices and Biosafety.			
2.	To study the principle and applications of important instruments (biological safety cabinets, autoclave, incubator, BOD incubator, hot air oven, light microscope, pH meter) used in the microbiology laboratory.			
3.	Preparation and inoculation of culture media for bacterial cultivation. a. Nutrient Broth b. Nutrient Agar Slant			
4.	Study of Rhizopus, Penicillium, Aspergillus using temporary mounts			
5.	Study of Spirogyra and Chlamydomonas, Volvox using temporary Mounts			
6.	Study of the following protozoans using permanent mounts/photographs: Amoeba, Entamoeba, Paramecium and Plasmodium			
7.	Determination of size of microbial cell using Micrometry			
8.	Enumeration of microbes: Yeast by Haemocytometer			

1. Microbiology Good Laboratory Practices and Biosafety

Safety in a microbiological laboratory substantially differs from that in other (chemical or physical, etc.) laboratories because, in addition to hazardous chemicals, substances and operations that pose a laboratory work-related risk, there is a risk of infection when working with microbes. The presence of, and working with infectious agents and materials in a microbiological laboratory, i.e. the potential of acquiring laboratory-associated infection, assumes the application of hierarchical control methods. These control measures first take into account the knowingly or unknowingly (e.g. as is the case in environmental microbiology) handled infectious agents; the approval of laboratory practices and safety equipment used (good laboratory practices [GLP]; containment approach), and the level by which laboratory workers are aware of the risk of infection (behavioural factors). Therefore, safety programs and safety management are organised with these questions in mind.

Epidemiologic analysis of (laboratory acquired) infections (including not only symptomatic infections, but similarly nonsymptomatic seroconversions as well) made the constitution of risk categories among microbes possible (based on health effects, means of spreading, routes of entry, etc.); and biological safety level requirements in laboratories (containment measures combining laboratory practices, safety equipment and design) in order to prevent the exposure of the operator, their colleagues and the broader environment. The most common ways of exposure to infectious agents are percutaneous inoculation (through injuries caused by sharp contaminated objects and animal bites, scratches, etc.), aerosol inhalation (as a result of spills, or caused by sprays associated with work procedures, e.g. vortexing; the mere opening of a Petri dish culture of a sporulating fungus; work with lyophilised cultures, etc.), and ingestion (e.g. during mouth pipetting, or by eating or drinking in the laboratory). Since the infectious dose of a disease-causing agent is vital, the higher concentration of microorganisms associated with certain research procedures (e.g. cultivation) increases the risk. “Infectious dose” is the number of microbial cells that cause an acute infection in humans. E.g. certain Vibrio cholerae strains cause a disease when ingesting only 10 cells, whereas with some Escherichia coli strains, $>10^6$ cells are needed “per os” for disease induction.

In the laboratory, researchers, assistants and students are exposed to the highest risk; however, one has to take into account the exposure of the cleaning, dishwasher and maintenance staff. The aforementioned laboratory workers are usually assumed to be healthy individuals in risk assessments. However, there are health status conditions, which increase the risk of infection. Different life phases, some (even chronic) diseases and the use of certain medications influence the host's defence (e.g. pregnancy with the threat of foetal or congenital infection; allergic hypersensitivity, immunodeficiency caused by e.g. diabetes mellitus, cancer chemotherapy, etc.). Moreover, working in a laboratory can result in allergic reactions (e.g. to spore proteins of actinobacteria).

When talking about (microbiological) laboratory in its broadest context, an environmental microbiologist will also consider field trips, the collection and on-site investigation of samples in their natural environment. It is easy to imagine the risk of infection at a communal sewage treatment plant or at a waste deposition site, not to mention other obviously infectious events like the sampling of cadavers/carcasses.

Biological safety level categories and the airborne route of pathogen transmission

The grouping of microorganisms into four **biological safety level (BSL) categories** is mainly based on the severity of the disease they cause and their transmission route, since airborne transmission (i.e. transmission via aerosol) is the most difficult to control. Laboratory facilities and the required laboratory techniques and practices are similarly classified into four safety levels according to the agent. Organisms in **BSL 1 are not known to cause any disease in healthy adults.** Working with them needs practically no aerosol containment. BSL 1 facilities are adequate for teaching laboratories at post-secondary or undergraduate training level. In such laboratories, only a sink to wash hands for decontamination is required.

Microbes in BSL 2 group are transmitted with ingestion, or via contact with mucous membranes (or by accidental self-injection), however their high concentration in aerosols may result in transmission (high infectious dose at droplet infection). Thus, in the case of working with such microbes, aerosol-generating laboratory practices have to be contained with the use of an adequate biological safety cabinet (BSC). Personal protective equipment should be

used when appropriate (laboratory coats, splash-protecting glasses and goggles, gloves, etc.). Naturally, washing hands for decontamination is a requirement. Adequate waste collection and decontamination facilities must be available (biohazard waste collecting bags and boxes, containers with microbicide liquid for used pipettes and other consumables, terminal decontamination autoclave, etc.).

Microbes ranked as BSL 3 cause disease in humans and explicitly spread airborne (with low infectious dose). In this case, all of the activities with materials that are as much as suspected to be contaminated have to be performed in adequate BSC. Access to the laboratory must be controlled, and adequate ventilation systems are needed to minimise the risk of the release of infectious aerosols. Microbes or samples that are verified or only supposed to have a high risk of causing serious or even fatal disease in humans, independently of the transmission route, are categorized as BSL4.

In BSL4 facilities, the highest-level BSCs are used or/and the laboratory personnel is protected by special ventilated suites. Not only the microorganisms themselves and the infection pose biological hazard, but the metabolic products of the microorganisms are similarly of concern (e.g. toxins, biotransformation products, such as vinyl chloride). Special care has to be taken to control the (occupational) exposure to such compounds. Special safety measures regulate biotechnological applications and the use of recombinant technologies especially when large-scale (> 10 L) applications are used. When considering recombinant techniques, well-characterized non-pathogenic hosts should be used, where the presence of incidental events can be excluded. Inserts should be similarly well characterized, free of “harmful” genes. Vectors should be as small as possible in size so they are unable to transfer DNA to wild-type hosts.

Since BSL categories strictly relate to the airborne pathogens and the airborne route of pathogen transmission, it is advisable to briefly summarize the ways by which aerosol is formed in laboratories. Most bacteria and yeastgrown in the laboratory on solid media form butyrous cohesive masses, making it unlikely to form aerosol whenthe culture container is opened. On the contrary, sporulating (conidiosporeforming) bacterial and fungal colonies pose a hazard of spore aerosol formation for example with the mere opening of a Petri dish. For this reason, in the case of such cultures grown for prolonged periods, lids should be taped, not to be opened before prior

examination for sporulation (presence of aerial hyphal forms), and should only be opened in BSCs. On the other hand, the manipulation of cultures like subculturing (e.g. the ignition of an inoculating loop), preparation of suspensions (by e.g. vortexing), centrifuging suspensions/broth cultures, pipetting, using blender type homogenizers, etc. are all procedures where small liquid droplets (aerosol) containing (infectious) cells (materials) may form. The larger particles (> 150 μm) readily drop, dry and form dust and thus contaminate bench top and floor surfaces. Particles smaller than 150 μm in diameter will most possibly evaporate before reaching the ground, forming “droplet nuclei”, which may hover for long periods. Droplet nuclei may even penetrate tissue facemasks. All microbes that are desiccation resistant (e.g. Staphylococcus, Mycobacterium spp., sporulating microbes) are of stressed importance since they remain alive for longer periods. Their UV tolerance further increases the risk of infection. It is recommended to work with the risk of aerosol/droplet nuclei formation in BSCs, and used contaminated materials (e.g. pipette tips, tubes) should be carefully submerged into disinfectant. The risk of formation of “droplet nuclei” containing infectious dust, especially neccessitates the thorough, regular, disinfective cleaning of surfaces in a microbiological laboratory.

The prevention of aerosol formation is an important aspect in the development of good laboratory practice measures. Thus, when subculturing e.g. Mycobacterium tuberculosis, in spite of using ordinary loops and a gas burner, rather the use of electric incinerators or the application of disposable loops is required. Similarly, centrifugation (especially high-speed centrifugation) should be made in aerosol-proof safety tubes/containers, and even the rotors should be tightly covered.

BIOSAFETY LEVEL 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices.**Special containment equipment or facility design is neither required nor generally used.** Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in

microbiology or a related science. The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and phone number of the investigator.
10. An insect and rodent control program is in effect.

B. **Special Practices**: None

C. Safety Equipment (Primary Barriers)

1. Special containment devices or equipment such as a biological safety cabinet is generally not required for manipulations of agents assigned to Biosafety Level 1.

2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.

3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.

4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

General Laboratory Directions

1. Always read the assigned laboratory material before the start of the laboratory period.
2. Before entering the laboratory, remove coats, jackets, and other outerwear.

These should be left outside the laboratory, together with any backpacks, books, papers, or other items not needed for the work.

3. To be admitted to the laboratory, each student should wear a fresh, clean, knee-length laboratory coat.

4. At the start and end of each laboratory session, students should clean their assigned bench-top area with a disinfectant solution provided. That space should then be kept neat, clean, and uncluttered throughout each laboratory period.

5. Learn good personal habits from the beginning:

Tie back long hair neatly, away from the shoulders.

Do not wear jewelry to laboratory sessions.

Keep fingers, pencils, and such objects out of your mouth.

Do not smoke, eat, or drink in the laboratory.

Do not lick labels with your tongue. Use tap water or preferably, self-sticking labels.

Do not wander about the laboratory. Unnecessary activity can cause accidents, distract others, and promote contamination.

6. Each student will need matches, bibulous paper, lens paper, a china-marking pencil, and a 100-mm ruler (purchased or provided). A black, waterproof marking pen may be used to mark petri plates and tubes.

7. Keep a complete record of all your experiments, and answer all questions at the end of each exercise. Your completed work can be removed from the manual and submitted to the instructor for evaluation.

8. Discard all cultures and used glassware into the container labeled CONTAMINATED. (This container will later be sterilized.) Plastic or other disposable items should be discarded separately from glassware in containers to be sterilized.

Never place contaminated pipettes on the bench top.

Never discard contaminated cultures, glassware, pipettes, tubes, or slides in the wastepaper basket or garbage can.

Never discard contaminated liquids or liquid cultures in the sink.

9. If you are in doubt as to the correct procedure, double-check the manual. If doubt continues, consult your instructor. Avoid asking your neighbor for procedural help.

10. If you should spill or drop a culture or if any type of accident occurs, call the instructor immediately. Place a paper towel over any spill and pour disinfectant over the towel. Let the disinfectant stand for 15 minutes, and then clean the spill with fresh paper towels. Remember to discard the paper towels in the proper receptacle and wash your hands carefully.

11. Report any injury to your hands to the instructor either before the laboratory session begins or during the session.

12. Never remove specimens, cultures, or equipment from the laboratory under any circumstances.

13. Before leaving the laboratory, carefully wash and disinfect your hands. Arrange to launder your lab coat so that it will be fresh for the next session.

2. To study the principle and applications of important instruments

A. Biological safety cabinets/ Laminar Air Flow Chamber

- Laminar air flow is an apparatus consists of an air blower in the rear side of the chamber which can produce air flow with uniform velocity along parallel flow lines. There is a special filter system of high efficiency particulate air filter (HEPA) which can remove particles as small as 0.3 mm.
- In front of the blower, there lies a mechanism through which air blown from the blower produces air velocity along parallel flow lines.
- The laminar air flow is based on flow of air current of uniform velocity along parallel flow lines which help in transferring microbial cultures in aseptic conditions. Air is passed through the filters into the enclosure and the filters do not allow any kind of microbe to enter in to the system.
- Inside the chamber one fluorescent tube and another UV tube are fitted. Two switches for these tubes and a separate switch for regulation of the air flow are fitted outside the LAF. Due to uniform velocity and parallel flow of air current, pouring of media, plating, slant preparations, streaking etc. are performed without any kind of contamination.
- Switch on the UV light for a period of 30 minutes so as to kill the germs, if any present in the area of working space.
- The front cover sheet of the apparatus is opened to keep the desired material inside. The air blower is set at the desired degree so that the air inside the chamber is expelled because the air inside the chamber may be contaminated / bring contaminants.
- Sit properly in front of the chamber and wipe the working table with alcohol to reduce the contaminants. All the work related to pouring, plating, streaking etc. are to be carried out in the flame zone of the burner or spirit lamp.
- In microbiology laboratory, horizontal type of laminar air flow is used to supply the air through filter.

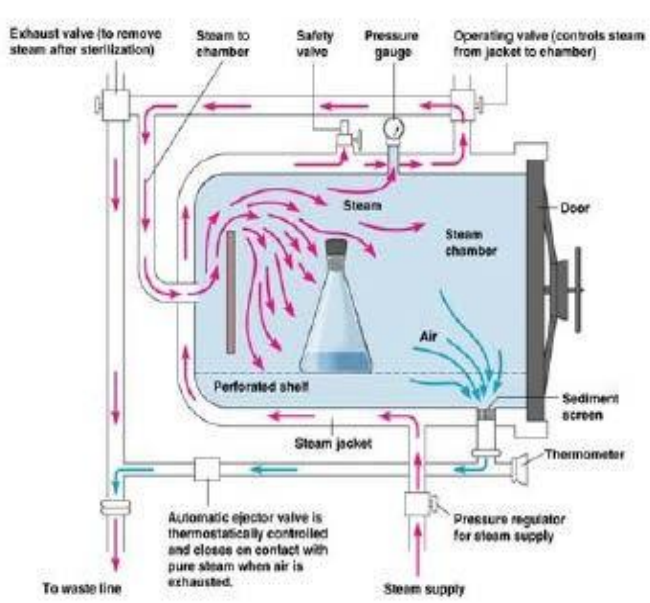
Laminar Air Flow

Precautions: Put off the shoes before entering to operate the apparatus. Wash the hands with detergents or soap. One should not talk inside the chamber while performing microbial culture transfer, failing which chances of contamination may be more which may come either through mouth, sneezing or air.

B. Autoclave

Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat because water molecules conduct heat better than air. Lower temperature and less time of exposure are therefore required than for dry heat. Moist heat readily kills viruses,

bacteria, fungi etc.



Heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure provides temperature above those obtainable by boiling. Moreover, it has advantages of rapid heating, penetration and moisture in abundance, which facilitates the coagulation of the protein of microorganisms, resulting in complete destruction of all forms of microbial life, including bacterial endospores. It is important to note that the sterilizing agent is the moist heat not the pressure. The laboratory apparatus designed to use steam under regulated pressure is called an autoclave. It is essentially a double jacketed steam chamber equipped with devices which permit the chamber to be filled with saturated steam and maintained at a designed temperature and pressure for any period of time The articles to be sterilized are placed in the sterilizing chamber and steam is maintained in the steam jacket into the sterilizing chamber, cool air is forced out and a special valve increases the pressure to 15 pounds/square inch above normal atmospheric pressure. The temperature rises to 121.7°C and the superheated water molecules rapidly conduct heat into microorganisms and will be killed. The time for destruction of the most resistant bacterial spore is reduced to 15 minutes. For denser objects, up to 30 minutes of exposure may be required.

Autoclave is an essential equipment in every microbiology laboratory. It's used to sterilize many media, solutions, discarded cultures, glass wares, metal wares etc**.**

Operation of Autoclave

1. Open the lid of the autoclave and check that there is sufficient amount of distilled or de-ionised water in it. If necessary, refill.

2. Place the correctly packaged materials (e.g. laboratory equipment, culture medium in a flask) into the chamber of the autoclave.

3. Close the lid of the autoclave.

4. Make sure that the bleeder valve is open.

5. Turn on the heating of the autoclave (the indicator lamp is lit).

6. If an intense (a thick, milky white) steam outflow can be detected through the outlet tube of the bleeder valve (100°C on the built-in thermometer), wait for 4-5 minutes and close the bleeder valve (venting).

7. With the help of a built-in manometer, check the pressure increase inside the chamber of the autoclave.

8. The sterilisation time (15 minutes or more) begins only when the pressure reaches 15 lbs above atmosphere , the temperature equalization (to 121°C) in the chamber has occurred. It is important that the operator stays with the device and controls the process of sterilization from the time it is turned on until the end of the sterilisation period.

9. Turn off the power switch of the autoclave when the sterilisation cycle/period has ended.

10. Allow the device to cool down to at least 60-70°C.

11. For decompression, slowly open the bleeder valve. Thereafter, carefully open the lid of the autoclave and remove the sterilised materials, using heat-proof gloves.

Autoclave

C. Incubator

- An incubator is an instrument that consists of copper/steel chamber around which warm water or air is circulated by electric current or by means of small gas flame.
- The temperature of the incubator is kept constant due to its control by using thermostat.
- The incubator is made up of double walled chamber adjusted to a desired temperature. It is done by using an external knob controlling the thermostat system. The gap between two walls is insulated to check heat condition. A thermometer is inserted from the top for recording the temperature.
- Temperature greatly influences the microbial growth. Therefore, instrument is generally designed that can allow the desired microorganism to grow at a particular temperature.
- It is operated to allow the microbial growth on a suitable medium under proper temperature. In an incubator, the variation in temperature should not be more than one degree.
- Small square type incubators are better than large ones. If a lower temperature than the room is required, compressor is fitted with the incubator.

Incubator

Precautions: the door of the incubator should be opened only when necessary. If the tubes are to be incubated for a long time or at higher temperature, the medium may become too dry due to excessive evaporation. In such cases cotton plug should be pushed inside the neck of the tube.

D. BOD incubator

BOD Incubator also known as Biological Oxygen Demand incubator. In microbiology laboratories, it is broadly used for cell culture and fungal growth, BOD test, fermentation, crop and physiology, and various pharmaceutical tests etc. BOD Incubator is additionally recognized as a low-temperature incubator or refrigerated incubator because it produces a temperature limit between 5°C to 60°C or including cooling and heating capacities under one unit.

Operations

1. Power is supplied by channels MCB. Temperature is fixed within a digital PID temperature controller, normally at 20°C. Before run a BOD incubator make sure the incubator is connected with the power supply.
2. After that turn on the main switch on the mainboard then turn on the switch on the cabinet.
3. After that set the desired temperature on the controller by pressing the set knob and soft key.
4. Control the temperature every day as by the following procedure.
5. Record the temperature which is displayed on the controller.
6. Monitor the temperature displayed on the digital screen. The temperature should not deviate by 2 degrees centigrade.

A BOD incubator, or biological oxygen demand incubator, is a specialized piece of laboratory equipment used to measure the amount of oxygen used by microorganisms during the process of breaking down organic matter. It is commonly used in wastewater treatment plants and environmental laboratories to assess the quality of water and the efficiency of treatment processes.

There are several advantages of using a BOD incubator:

1. It provides an accurate measure of the amount of oxygen consumed by microorganisms, which is an important indicator of the level of organic matter in water.
2. It allows for the continuous monitoring of oxygen consumption over a period of time, allowing for more accurate results.

3. It is a reliable and reproducible method for measuring BOD, with results that are consistent with standard laboratory procedures.
4. It is easy to use and requires minimal training.
5. It is suitable for use in a variety of settings, including wastewater treatment plants, environmental laboratories, and research facilities.

Overall, the BOD incubator is a useful tool for assessing the quality of water and the efficiency of treatment processes, and is widely used in a variety of settings.

E. Hot Air Oven

This is the most widely adopted method of sterilization by dry heat. The hot air oven utilizes radiating dry heat for sterilization. This type of energy does not penetrate materials easily and thus, long periods of exposure to high temperature are necessary. For example, at a temperature of 160°C, a period of two hours is required for the destruction of bacterial spores. Hot air oven is used to sterilize glassware, forceps, scissors, scalpels, glass syringes, liquid paraffin, dusting powder etc. A holding period of 160°C for an hour is used. The oven is usually heated by electricity, with heating elements in the wall of the chamber and it must be filled with a fan to ensure even distribution of hot air and elimination of air pockets. The materials should be arranged in a manner which allows free circulation of hot air in between the objects. It should not be over-loaded. Glass wares should be perfectly dry before being placed in the oven. Test tubes,

flasks etc. should be wrapped in craft paper. Oven must be allowed to cool slowly for about 2 hours before the door is opened, since the glassware may get cracked by sudden or uneven cooling.

Sterilization control: The spores of a non – toxigenic strain of *Clostridium tetani* are used as a microbiological test of dry heat efficiency. Paper stripes impregnated with 106 spores are placed in envelop and inserted into suitable packs. After sterilization is over, the strips are removed and inoculated into thioglycollate or cooked meat media and incubated for sterility test under strict anaerobic conditions for five days at 37°C.**Hot Air Oven**

F. Light Microscope

A good microscope is an essential tool for any microbiology laboratory. There are many kinds of

microscopes, but the type most useful in diagnostic work is the compound microscope. By means of a series of lenses and a source of bright light, it magnifies and illuminates minute objects such as bacteria and other microorganisms that would otherwise be invisible to the eye. This type of microscope will be used throughout your laboratory course. As you gain experience using it, you will realize how precise it is and how valuable for studying microorganisms present in clinical

specimens and in cultures. Even though you may not use a microscope in your profession, a firsthand knowledge of how to use it is important. Your laboratory experience with the microscope will give you a lasting impression of living forms that are too small to be seen unless they are highly magnified. As you learn about these “invisible” microorganisms, you should be better able to understand their role in transmission of infection.

A. Important Parts of the Compound Microscope and Their Functions

1. Look at the microscope assigned to you and compare it with the photograph provided. Notice that its working parts are set into a sturdy frame consisting of a base for support and an arm for carrying it. 2. Observe that a flat platform, or stage as it is called, extends between the upper lens system and the lower set of devices for providing light. The stage has a hole in the center that permits light from below to pass upward into the lenses above. The object to be viewed is positioned on the stage over this opening so that it is brightly illuminated from below (do not attempt to place your slide on the stage yet). Note the adjustment knobs at the side of the stage, which are used to move the slide in vertical and horizontal directions on the stage. This type of stage is referred to as a mechanical stage. 3. A built-in illuminator at the base is the source of light. Light is directed upward through the Abbe condenser. The condenser contains lenses that collect and concentrate the light, directing it upward through any object on the stage. It also has a shutter, or iris diaphragm, which can be used to adjust the amount of light admitted. A lever (sometimes a rotating knob) is provided on the condenser for operating the diaphragm.

The condenser can be lowered or raised by an adjustment knob. Lowering the condenser decreases the amount of light that reaches the object. This is usually a disadvantage in microbiological work. It is best to keep the condenser fully raised and to adjust light intensity with the iris diaphragm. 4. Above the stage, attached to the arm, a tube holds the magnifying lenses through which the object is viewed. The lower end of the tube is fitted with a rotating nosepiece holding three or four objective lenses. As the nosepiece is rotated, any one of the objectives can be brought into position above the stage opening. The upper end of the tube holds the ocular lens, or eyepiece (a monocular scope has one; a binocular scope permits viewing with both eyes through two oculars). 5. Depending on the brand of microscope used, either the rotating nosepiece or the stage can be raised or lowered by coarse and fine adjustment knobs. These are located either above or below the stage. On some microscopes they are mounted as

two separate knobs; on others they may be placed in tandem with the smaller fine adjustment extending from the larger coarse wheel. Locate the coarse adjustment on your microscope and rotate it gently, noting the upward or downward movement of the nosepiece or stage. The coarse adjustment is used to bring the objective down into position over any object on the stage, while looking at it from the side to avoid striking the object and thus damaging the expensive objective lens. The fine adjustment knob moves the tube to such a slight degree that movement cannot be observed from the side. It is used when one is viewing the object through the lenses to make the small adjustments necessary for a sharp, clear image. Turn the adjustment knobs slowly and gently, as you pay attention to the relative positions of the objective and object.

Avoid bringing the objective down with the fine adjustment while viewing, because even this slight motion may force the lens against the object. Bring the lens safely down first with the coarse knob; then, while looking through the ocular, turn the fine knob to raise the lens until you have a clear view of the subject. Rotating the fine adjustment too far in either direction may cause it to jam. If this should happen, never attempt to force it; call the instructor. To avoid jamming, gently locate the two extremes to which the fine knob can be turned, then bring it back to the middle of its span and keep it within one turn of this central position. With practice, you will learn how to use the coarse and fine adjustment knobs in tandem to avoid damaging your slide preparations.

6. The total magnification achieved with the microscope depends on the combination of the ocular and objective lens used. Look at the ocular lens on your microscope. You will see that it is marked “10” meaning that it magnifies 10 times.Now look at the three objective lenses on the nosepiece. The short one is the low-power objective. Its metal shaft bears a “10” mark, indicating that it gives tenfold magnification. When an object is viewed with the 10 objective combined with the 10 ocular, it is magnified 10 times 10, or 100. Among your three objectives, this short one has the largest lens but the least magnifying power. The other two objectives look alike in length, but one is an intermediate objective, called the high-power (or high-dry) objective.

The third objective, is called an oil-immersion objective. This objective is the most useful of the three for the microbiologist because its high magnification permits clear viewing of all but the smallest microorganisms (viruses require an electron microscope). As its name implies, this lens must be immersed in a drop of oil placed on the object to be viewed. The oil improves the

resolution of the magnified image, providing sharp detail even though it is greatly enlarged. The function of the oil is to prevent any scattering of light rays passing through the object and to direct them straight upward through the lens. Notice that the higher the magnification used, the more intense the light must be, but the amount of illumination needed is also determined by the density of the object. For example, more light is needed to view stained than unstained preparations.

7. The focal length of an objective is directly proportional to the diameter of its lens. You can see this by comparing your three objectives when positioned as close to the stage as the coarse adjustment permits. First place the low-power objective in vertical position and bring it down with the coarse knob as far as it will go (gently!). The distance between the end of the objective, with its large lens, and the top of the stage is the focal length. Without moving the coarse adjustment, swing the high-power objective carefully into the vertical position, and note the much shorter focal length. Now, with extreme caution, bring the oil-immersion objective into place, making sure your microscope will permit this. If you think the lens will strike the stage or touch the condenser lens, don't try it until you have raised the nosepiece or lowered the stage (depending on your type of microscope) with the coarse adjustment. The focal length of the oil-immersion objective is between 1 and 2 mm, depending on the diameter of the lens it possesses (some are finer than others). Never swing the oil-immersion objective into use position without checking to see that it will not make contact with the stage, the condenser, or the object being viewed. The oil lens alone is one of the most expensive and delicate parts of the microscope and must always be protected from scratching or other damage. 8. Take a piece of clean, soft lens paper and brush it lightly over the ocular and objective lenses and the top of the condenser. With subdued light coming through, look into the microscope. If you see specks of dust, rotate the ocular in its socket to see whether the dirt moves. If it does, it is on the ocular and should be wiped off more carefully. If you cannot solve the problem, call the instructor. Never wipe the lenses with anything but clean, dry lens paper. Natural oil from eyelashes, mascara, or other eye makeup can spoil the oculars badly and seriously interfere with microscopy. Eyeglasses may scratch or be scratched by the oculars. If they are available, protective eye cups placed on the oculars prevent these problems. If not, you must learn how to avoid soiling or damaging the ocular lens. 9. If oculars or objectives must be removed from the microscope for any reason, only the instructor or other delegated person should remove them. Inexperienced hands can do

irreparable damage to a precision instrument. 10. Because students in other laboratory sections may also use your assigned microscope, you should examine the microscope carefully at the beginning of each laboratory session. Report any new defects or damage to the instructor immediately. Proper handling of a microscope. Both hands are used when carrying this delicate instrument.(Note: When lifting and carrying the microscope, always use both hands; one to grasp the arm firmly, the other to support the base . Never lift it by the part that holds the lenses.

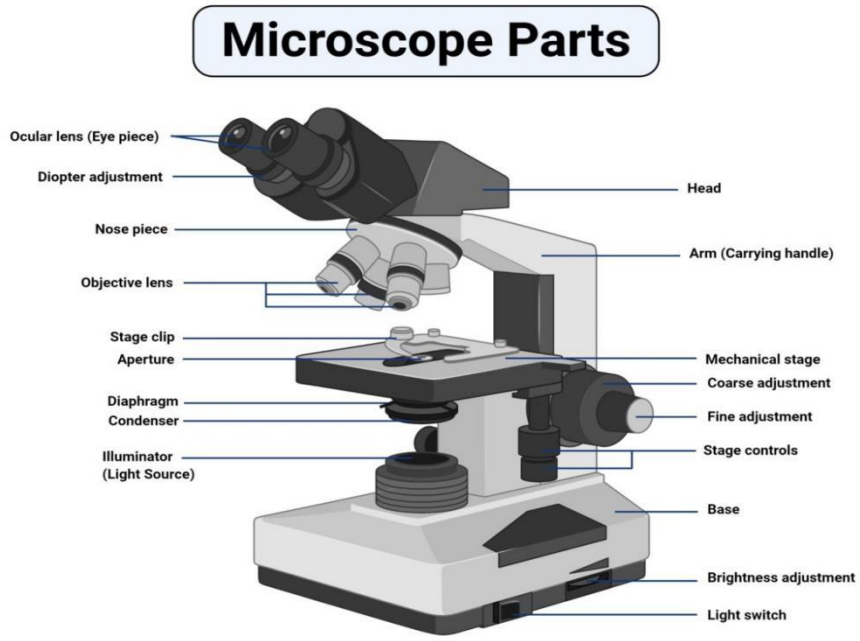


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

G. pH Meter

The pH meter is used to determine the pH (acidity and alkalinity of a solution) of solutions of unknown pH as well as for setting of pH of various media used for the cultivation and testing biochemical activities of microorganisms. pH is expressed as a number from 0 to 14. The number is an expression of the concentration of H^+ in the solution- the higher the concentration the more acidic the solution. The value is the negative exponent of the H^+ concentration. For example when $H^+ = 0.000001$ (10^{-6}) M, the pH is 6 Pure water a pH of 7 (i.e. neutral). pH values less than 7 indicate increasing acidity where as values above 7 indicate increasing alkalinity, Growth and survival of microorganisms is greatly influenced by the pH of the environment and all organisms differ as to their requirements. The specific range for bacteria is between 4 to 9 with the optimum being 6.5 to 7.5.

Fungi prefer an acidic environment with optimum being 4 to 6.

The measurement of pH with pH meter is done electrometrically and the measurement of pH depends upon the development of a membrane potential by a glass electrode. The glass electrode consists of an internal sealed tube with a metallic tip (typically of silver-silver chloride) and an external tube that contains a standard solution. A pH sensitive glass bulb forms the immersion tip of the electrode. The potential of the glass electrode is proportional to the pH of the solution in which it is immersed. In addition to the glass electrode, a pH meter consists of another electrode, the reference electrode. The only purpose of this electrode is to complete the measuring circuit with a device that is not sensitive to any of the ions in the solution.

The pH meter is equipped with a temperature-compensation circuit for introducing a known potential to balance out the potential caused by different sample temperatures. The instrument is also provided with a standardizing potential which is used to balance the circuit to indicate the correct pH of the standard used as a reference to measure the pH of a sample solution.

The procedure for the determination of pH with a simple laboratory pH meter is as follows:

(i) Set the temperature compensation dial to proper setting.

(ii) Immerse the two electrodes in a standard buffer solution of known pH (say pH 4)

(iii) Using the standardization knob, set the meter reading to the proper pH i.e. 4.

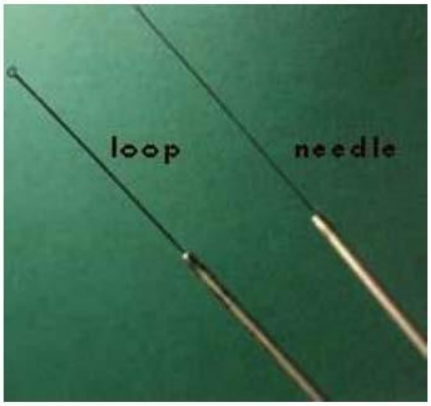
Iv) After these calibration steps. replace the buffer with the solution sample of unknown pH.

V) Read the pH directly from the scale of the pH meter in millivolts or in pH units

Other important tools in Microbiology Laboratory

1) Inoculation Needle & Inoculation Loop

- These are the most commonly used tools.
- Inoculation needle/loop is made up of a long platinum wire fixed into a metallic rod.
- A wire loop has a handle with steel screw shaft in which nichrome or platinum wire is to be fitted.
- The straight wire needle is used for transferring culture from solid medium. Even smaller amount of liquid culture can be manipulated by using straight needle.
- The loop and wire are also used for picking small quantities of solid materials from a microbial colony and can be used to inoculate either a liquid or a solid medium. Both the loop and straight wire must be flamed immediately after use to avoid contamination.



Inoculation Loop & Needle

Bunsen Burner

2) Bunsen Burner

- Sterilization of tools by using spirit lamp is called incineration.
- Gas enters the burner at the base, and its supply is regulated externally by the gas cock.
- The amount of air can be controlled by rotating a sleeve that fits over the holes in the body of the burner.
- To keep the flame from blowing out special tips are frequently used to fit over the top end of the barrel.
- The proper method of lighting the burner is to close off the air supply, turn on the gas and light. The flame will be large and yellow. Gradually open the air intake until the flame takes a blue colour.

3) Water Bath

- Water bath is an instrument that is used to provide constant temperature to a sample.
- It consists of an insulating box made up of steel fitted with electrode heating coil.
- The temperature is controlled through a thermostat.

- In some of the water baths, plate form rotates, then it is called water bath shaker. It is more useful to microbiologists because it provides a uniform heat to the sample material meant for incubation.
- The main use of water bath is the incubation of samples at a desired and constant temperature.

Water Bath

4. **Preparation and inoculation of culture media for bacterial cultivation**

a) Nutrient Broth

Aim

Preparation and inoculation of basic liquid media (Nutrient Broth) for routine culture of bacteria

Theory

Nutrient preparations in which microorganisms can be cultured in the laboratory are called 'culture medium'. Liquid culture medium is also called broth. One of the common types of broth used for routine cultivation of bacteria is 'Nutrient Broth'. It has only peptone and beef extract as its constituents. Peptone is a water-soluble mixture of polypeptides and amino acids formed by the partial hydrolysis of protein with the enzyme pepsin. Peptone can supply amino acids to microorganisms. Beef Extract Powder is a dehydrated extract of bovine tissue for use in preparing microbiological culture media in a laboratory setting. Beef extract acts as a source of organic carbon, nitrogen, vitamins and inorganic salts.

Requirements Composition of Nutrient Broth

	g/l
Peptone	5.0
Beef Extract	3.0
NaCl	3.0

Distilled water-1000 ml

pH-7.0

Procedure

1. Put the weighed amount of the constituents in 500 ml of distilled water in a conical flask.
2. dissolve the constituents.
3. Add distilled water to make the volume 1 lit.
4. Check pH (7.0) with pH paper strip. Adjust the pH if necessary by adding 1N HCl or 1 N NaOH drop by drop.

5. Dispense about 5 ml of the media in dry test tubes, plug the test tubes with nonabsorbent cotton.
6. Sterilize the tubes containing medium in an autoclave for 15 min at 15 lbs/square inch pressure.
7. After autoclave, cool it down and inoculate the broth with sterile inoculation loop in aseptic condition with known pure culture available in the laboratory. Keep one tube non-inoculated as control.
8. Incubate the tubes in incubator for 24 hrs at 37° C in shaking condition.
9. Note the observation after 24 hrs.

Observation.

b) Nutrient Agar Slant

Aim

Preparation and inoculation of basic solid media (Nutrient agar) for routine culture of bacteria and for preservation

Theory

Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms. Agar was first subjected to chemical analysis in 1859 by the French chemist Anselme Payen, who had obtained agar from the marine algae Gelidium corneum. Agar consists of a mixture of two polysaccharides: agarose and agaropectin, with agarose making up about 70% of the mixture. Agarose is a linear polymer, made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3, 6-anhydro-L-galactopyranose. Agaropectin is a heterogeneous mixture of smaller molecules that occur in lesser amounts, and is made up of alternating units of D-galactose and L-galactose heavily modified with acidic side-groups, such as sulfate and pyruvate. Agar melts at 85 °C (358 K, 185 °F) and solidifying from 32–40 °C (305–313 K, 90– 104 °F). This property lends a suitable balance between easy melting and good gel stability at relatively high temperatures. Since many scientific applications require incubation at temperatures close to human body temperature (37 °C), agar is more appropriate than other solidifying agents that melt at this temperature, such as gelatin.

Composition of Nutrient Broth 1) **ition of Nutrient Broth**

	g/l
Peptone	5.0
Beef Extract	3.0

NaCl	3.0
Agar	20

Distilled water-1000 ml

pH-7.0

Procedure:

1. Dissolve Peptone, Beef Extract, Nacl in distilled water. 2. Adjust to pH 6.8-7.0 with 1(N) NaOH or 1(N) HCl. 3. Add agar and dissolve it by heating. 4. Dispense about 5 ml of the media in dry test tubes, plug the test tubes with non-absorbent cotton. 5. Sterilize the tubes containing medium in an autoclave for 15 min at 15 lbs/square inch pressure. 6. Place the tubes in an inclined position in order to have a greater surface area of the media. 7. Allow the media to solidify by keeping undisturbed for about one hour. 8. Inoculate the surface in zigzag manner with sterile inoculation loop in sterile environment using any bacteria available in the laboratory. Keep one tube non-inoculated as control. 9. Incubate the tubes in incubator for 24 hrs at 37° C . 10. Note the observation after 24 hrs.

Observation.

5. **Study of Rhizopus, Penicillium, Aspergillus using temporary mounts**

Study of Characteristics of Some Common Fungi

1) *Aspergillus* species

Colony Morphology

Colonies are wooly at first, white to yellow, then turning dark brown to black. Reverse is white to yellow.

Microscopic Morphology

Conidiophores are smooth and colourless and turned dark toward vesicles. The vesicle was globes and bearing phialides mycelium septate.

Aspergillus is a common laboratory contaminant. Its conidia are usually present in air. *Aspergillus* species are used industrially for manufacturing citric, gluconic and gallic acid and for fermenting soy sauce. Wide range of enzymes is produced by *A. niger* and *A*. *oryzae*. *Aspergillus* sp. also causes various diseases of plants. Different species of *Aspergillus* like, *Aspergillus fumigatus* is responsible for aspergillosis in human. Certain *Aspergillus* species e.g. *A. flavus* produces aflatoxins, which are highly toxic, and carcinogens in human, causing liver cancer. We have already studied about this in Unit 5 in the Food Microbiology and Safety theory Course. *Aspergillus* species are also responsible for spoilage of food, leather, cotton fabric etc., thus

reducing their commercial value. It can grow on decaying vegetables, butter, ghee, bread, rice, jam, jellies etc. easily by producing large number of enzymes.

The identifying features include:

1. Macroscopically *Aspergillus* colonies are powdery and are of different colours like green, blue, black, yellow, brown etc.
2. Microscopically mycelium consists of branched, bright or pale coloured hyphae some of which grow within the substrate while others grow on the substrate.
3. From these vegetative hyphae, long, unbranched, nonseptate erect hyphae arise called *conidiophores*. The cell from which conidiophore arise is called *foot cell*. It is thick walled and Tshaped and one conidiophore arises from each foot cell.
4. Conidiophores terminate into a globular structure called *vesicle*, as you can see in the Figure 8.7.
5. Around the vesicle, there are 1-2 layers of flask shaped structures called *phialides* or *sterigmata*.
6. At the tip of the sterigmata, a chain of small unicellular spores called *conidia* arises. These conidia are formed in basipetal manner (oldest is at the top). These are arranged compactly side by side. The whole structure consisting of the foot cell, the upright hypha, the vesicle, the metullae and the phialides constitutes the conidiophore.
7. Sexual Reproduction occurs by formation of ascus and ascospores.

Note your observation.

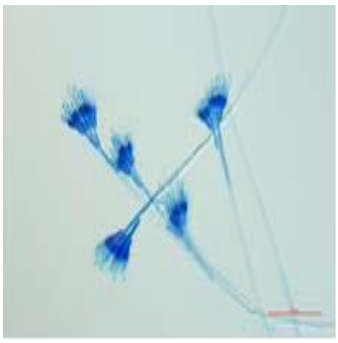
2) *Penicillium* species

Colony Morphology

Colony appeared as bluish green mycelium was septate and ridged

Microscopic Morphology

Penicillium has brush like appearance formed of chains of spores extending from the ends of phialides borne on short branches of conidiophores on the hyphae.



Penicillim is cosmopolitan in distribution. It is called green or blue mould (look of growth or media above) though exists in different colours. *Penicillium* is used in industries for production of organic acids like oxalic, fumaric and citric acid. It is also a source of antibiotics like *Penicillin* and *Griseofulvin* which are produced by *P. chrysogenum* and *P.griseofulvin*. In cheese industries *Penicillium* (example: *P. camemberti* and *P.roqueforti*) is employed to impart distinctive flavour and odour to the product. On the other hand, *Penicillium* damage leather goods, fabrics and wood furniture. It also spoils bread, cheese, butter, jam, jelly and other food stuffs. Some are plant pathogens, e.g., soft rot disease of citrus fruit is caused by *P.italicum* and *P.digitatum*. Mycotoxins and ochratoxins are produced on cereal grains by *P. viridicatum*. Let us next study the identifying features of *Penicillium*.

The identifying features of *Penicillium* are:

1. Mycelium consists of colourless, septate and branched hyphae, some of which grow inside the substratum to get nutrients and the rest spread on the surface. Former are called *haustaria hyphae*.

2. Erect, tubular septate hyphae called conidiophores grow outward in the air from any cell of the mycelium. No foot cells are present in *Penicillium*. Only one conidiophores arises from one cell.
3. Unlike *Aspergillus*, conidiophores branches once, twice or even more times to produce primary, secondary or tertiary branches. The ultimate branches bear's tufts of flask shaped structures called *sterigmata* (phialides). These branches are called the *metulae* while lower branches which support the metulae are called *rami.* At the tip of the sterigmata, a long chain of conidia arise in a basigenous arrangement. The conidia are shed continuously. The conidiophore along with remi, metulae, sterigmata and conidia gives artist's brush or broom like appearance and the structure is called penicillus, as illustrated in the Figure 8.8.
4. The conidia are tiny, uninucleate and unicellular, globose, solid, elliptical or pyciform structures. These may be smooth or rough.
5. Sexual reproduction is observed in a few species by formation of asci containing ascospores.

Ascospores are uninucleate, lens shaped structures.

From our discussion above you may have noticed that *Penicillium* like *Aspergillus* also grow conidiophore structure. But *Aspergillus* and *Penicillium* differ in their conidiophores structure. Former has a conidiophore which is nonseptate, unbranched and arising from a foot cell. It ends in a vesicle at its tip bearing sterigmata and conidia. Instead in *Penicillium*, a conidiophore is septate, branched and forms a broom-like structure – *Senicillus*. There is no foot cell and vesicles.

Note your observation:

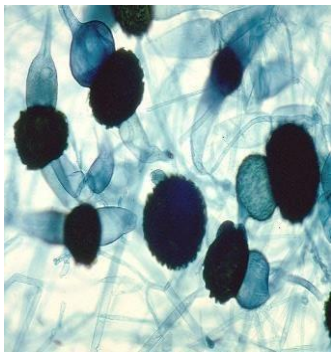
3) *Rhizopus* species

Colony Morphology

Colonies are columnar, fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.

Microscopic Morphology

Sporangiophores up to 1500 µm in length and 18 µm in width, smooth walled, non - septate, simple or branched, arising from stolons opposite rhizoids usually in groups of 3 or more. Sporangia are globose, often with a flattened base, grayish black, powdery in appearance, up to 175 µm in diameter and many spored.



Classification
Kingdom Mycetae
Division Amastigomycota
Class Zygomycetes
Order Mucorales
Family Mucoraceae
Genus *Rhizopus*

Rhizopus is a common laboratory contaminant. It is a spoiling mould and found frequently on the surface of bread, fruits and vegetables. It can grow as weak facultative parasite under certain

conditions, causing soft rot of sweet potato and leak of peach, raspberry, strawberry and some other fungal diseases in animals and human. Various species of *Rhizopus* are exploited industrially for production of cortisone (*R. stolonifer)*, alcohol (*R. oryzae*), lactic acid and fumaric acid (*R. oryzae, R.*
stolonifer).

Next let us look at the identifying features.

Identifying features of Rhizopus:

1. Macroscopically *Rhizopus* appears as a white cottony mass (look at rhizopus growth on media above) growing rapidly and spread over entire plate during vegetative phase. Later black pinhead like structures formed in reproductive phase giving blackish appearance as shown in Figure 8.5. Therefore, *Rhizopus* is also called *black mould.*

2. Microscopically, hyphae are aseptate and coenocytic. There are 3 kinds of hyphae:

(a) *Stolon* – These grow horizontally on substratum surface. Look at the Figure and identify the stolon. Stolon runs horizontally for some distance and then arches over so that its tip touches the substratum and forms node-like structure from which fresh stolon arise. These hyphae help in the spread of the mycelium.

(b) *Rhizoids* – These are brown slender root like structures, as you may have seen in Figure which arise in cluster from each node of the stolon. These penetrate the substratum and helps in anchorage and the absorption of nutrients from the substratum. Rhizoids and stolons are present during vegetative phase.

(c) *Sporangiophore* – Tufts of special, erect unbranched, hyphae growing in air arise from stolon just opposite to rhizoids, as illustrated in the Figure. These are sporangiophores which appear during reproductive phase

3. Sporangiophores swell at the tip into a spherical knob like structure called *sporangium*. It has two zones.

- Central dome shaped zone called *columella*, and

- Peripheral sporiferous zone in which black spores called *sporangiospores* are formed. Because of sporangiospores, sporangium appears black at maturity.

4. Sporangiospores are non-motile, colourless or brown, multi-nucleated, globose to oval structure.
5. *Rhizopus* usually reproduce asexually, but under unfavourable conditions, sexual reproduction (isogamous) occur resulting in formation of zygospores – thick, black, rough structures as you have already seen in Figure. Zygospore on germination produces a hyphae that bears an asexual sporangium and the cycle begins anew.

Note your observation:

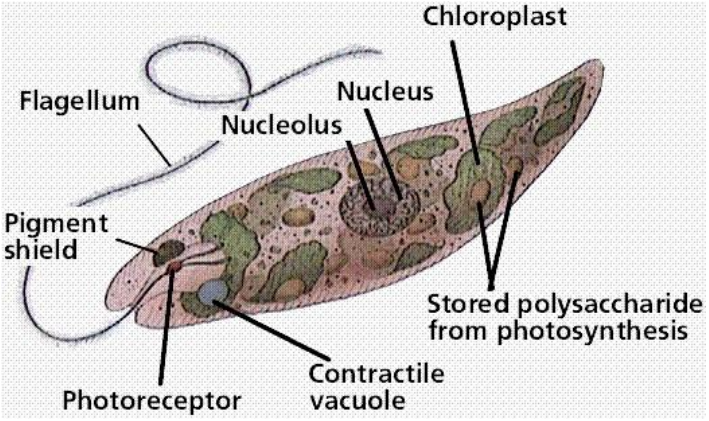
6. **Study of Spirogyra and Chlamydomonas, Volvox using temporary Mounts**

The Protists –

Subkingdom Algae

Subkingdom Algae includes all the photosynthetic, eukaryotic protists. Again, they lack the tissue differentiation found in plants. Algae may be unicellular, colonial, or filamentous. Algae have distinct, visible nuclei and chloroplasts. **Chloroplasts** are organelles that contain photosynthetic pigments which harvest the energy of sunlight to form carbohydrates through a process known as **photosynthesis**. There are 7 different divisions of algae, but we will examine only a selection of them:

- Euglenophyta (the euglenoids) – ex. *Euglena*
- Chlorophyta (the green algae) – ex. *Volvox, Ulothrix,* and *Spirogyra*
- Chrysophyta (the golden brown algae) – ex. Diatoms



Euglena are unicellular protozoans that almost always have **chloroplasts**. Although they photosynthesize (autotrophy), *Euglena* can also eat food by heterotrophy (like animals). They use a **flagellum** for locomotion.

Phylum Chlorophyta: green algae

• Ancestors of Green Plants ***Have cellulose cell walls***

Chloroplasts with Chlorophyll

Store food as starch

• Types of Algae

Chlamydomonas, single cells

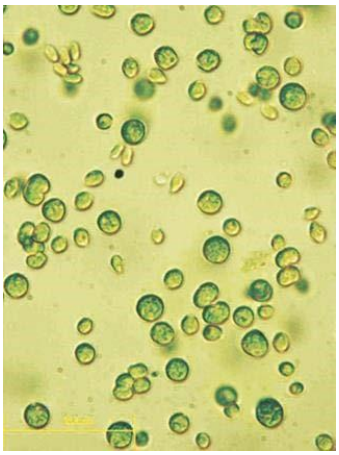
Spirogyra, filamentous

Volvox, colonial

Like vascular plants, green algae have chlorophylls a and b in addition to a variety of carotenes and xanthophylls that act as accessory pigments. Nutrition is autotrophic, with the reserve carbohydrates stored in plastids in the form of starch. Green algae exhibit a wide variety of thallus forms, ranging from single cells to filaments to parenchymatous thalli. In tropical and subtropical waters, many forms may be calcified. Morphology The green algae are well represented in the marine plankton and damp terrestrial environments, with many species occurring as unicellular organisms. These include motile vegetative cells that produce vegetative spores and/or gametes (e.g. Chlamydomonas), non-motile vegetative cells that produce flagellated reproductive structures (spores/gametes) (e.g. Chlorococcum), and lastly nonmotile vegetative cells without motile reproductive structures (e.g. Chlorella). Colonial green algae (e.g. Volvox) are not commonly encountered in the marine environment, though they are common residents of soil and standing freshwater. Colonies (synobia) are made up of distinct single cells held together by mucilage. A giant evolutionary step was made in the green algae when the cells that resulted from a mitotic event remained contiguous. Connected cells enabled both multicellularity and the complex functions this required. Filamentous green algae are well represented in the sea either as unbranched, simple uniseriate (single thread) filaments of cells that show little or no thallus differentiation (e.g. Chaetomorpha, Ulothrix), or as branched filaments (e.g. Cladophora). Unicellular Forms Key Organism—Chlamydomonas, Chlorella Unicellular forms can be motile or non-motile.

Chlamydomonas is a single-celled motile alga commonly found in damp soil, ditches, and tide pools and occasionally in salt marshes. The organism is typically 9 egg-shaped and has a large cup-shaped chloroplast containing a proteinacious body—the pyrenoid, which functions in starch

polymerization. The nucleus is often difficult to see because of the prominent chloroplast. Chlorella is a single-celled nonmotile alga that often forms a symbiosis with aquatic invertebrates and protozoa. The cells contain a stigma (eyespot) that functions in the absorption of light. Reproduction in **Chlamydomonas** is usually asexual except during times of environmental stress, when the organism produces identically sized and shaped gametes (isogamy) for sexual reproduction. egg-shaped and has a large cup-shaped chloroplast containing a proteinacious body—the pyrenoid, which functions in starch polymerization. The nucleus is often difficult to see because of the prominent chloroplast.

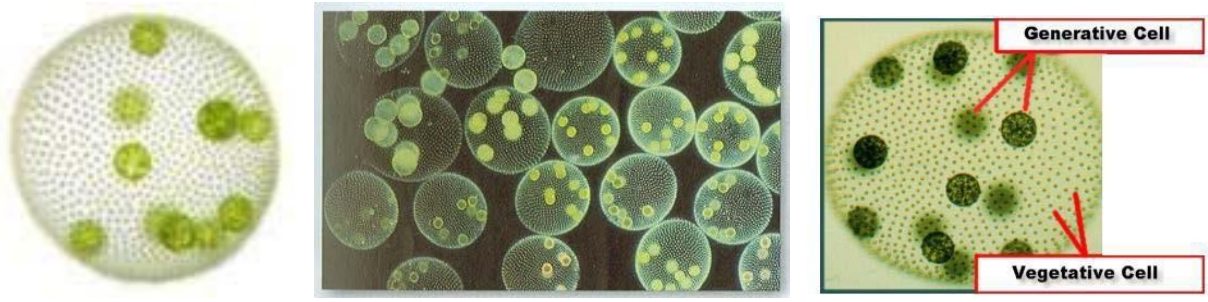


Chlamydomonas

Chlorella is a single-celled non-motile alga that often forms a symbiosis with aquatic invertebrates and protozoa. The cells contain a stigma (eyespot) that functions in the absorption of light. Reproduction in Chlamydomonas is usually asexual except during times of environmental stress, when the organism produces identically sized and shaped gametes (isogamy) for sexual reproduction.

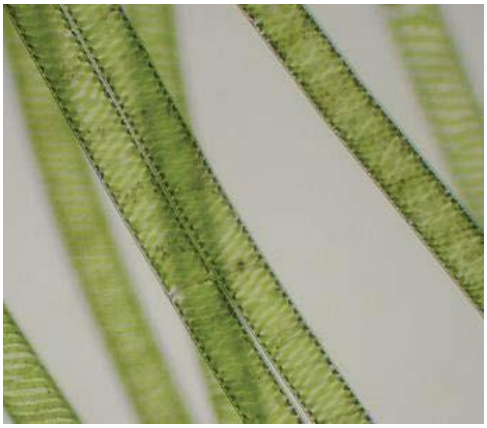
Colonial Forms **Volvox** is a type of green algae. It forms spherical colonies of up to 50,000 cells. They live in a variety of freshwater habitats. Each mature Volvox colony is composed of numerous flagellate cells embedded in the surface of a hollow sphere. The cells swim in a coordinated fashion, with distinct anterior and posterior poles. The cells have eyespots which enable the colony to swim towards light. The spheres will break up with advanced age. The generative cells then grow into new colonies. A Volvox colony can have up to 50,000 individual cells. The cells are arranged in a layer around the periphery of the colony. The two flagella of

each cell are oriented out into the surrounding water. The inside of the colony is a mixture of water and mucilage. New daughter colonies form within the colony. Volvox can reproduce asexually and sexually.



Volvox

Spirogyra is a filamentous green algae of the, named for the helical or spiral arrangement ofthe chloroplasts. It is commonly found in freshwater areas. Spirogyra measures approximately 10 to 100μm in width and may stretch centimeters long. This particular algal species, commonly found in polluted water, is often referred to as "pond scum". The cell wall has two layers: the outer wall is composed of pectin that dissolves in water to make the filament slimy to touch while the inner wall is of cellulose. The cytoplasm forms a thin lining between the cell wall and the large vacuole it surrounds. The chloroplasts are ribbon shaped, serrated or scalloped, and spirally arranged. In spring Spirogyra grows under water, but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubbles between the tangled filaments. The filamentous masses come to the surface and become visible as slimy green mats. This picture shows part of a gigantic single cell of Spirogyra. This organism is unusual because the chloroplasts form a long spiral inside the cylindrical cells. Spirogyra is called a filamentous alga, because it grows as long, filament-like chains of cells. This body plan provides a ratio of surface area to volume, which is important because each cell must independently absorb nutrients and sunlight directly from the environment. Although the cells are linked together, they live more or less independently. Examine the live and prepared slides of Spirogyra provided. Draw what you see.



Key points about green algae:

- Photosynthetic eukaryotes.
- Closely related to green plants.
- Many are unicellular, but some have simple multicellular body plans. Do not have differentiated tissues other than reproductive cells.

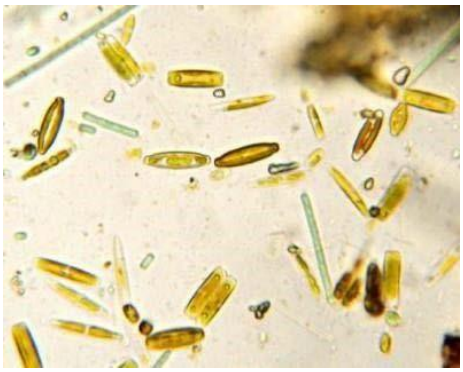
Because they lack differentiated tissues and other adaptations to life on land, they must live and reproduce in the water.

Chrysophyta: Diatoms

- Most numerous Algae in Oceans
- Cell wall contains silica dioxide, "silica shell"
- Similar Biochemically to Brown Algae above
- Economic Importance Abrasive surfaces, used in toothpaste, scouring powder etc.

Diatoms are a major group of **golden brown algae**, and are one of the most common types of **phytoplankton** (microscopic plants). Diatoms are **primary producers** within the food chain. Most diatoms are unicellular, although they can exist as colonies in the shape of filaments or ribbons, fans, zigzags, or stellate colonies. Diatom cells are characteristically encased within a

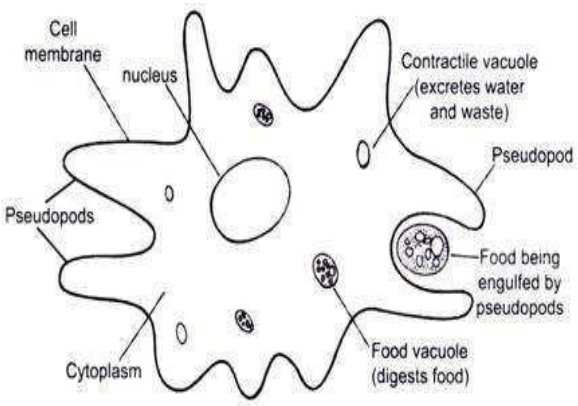
unique cell wall made of **silica** (hydrated silicon dioxide) called a **frustule**. These frustules show a wide diversity in form, but **usually consist of two symmetrical sides with a split between them**, hence the group.



Note your observation

7. **Study of the following protozoans using permanent mounts/photographs: Amoeba, Entamoeba, Paramecium and Plasmodium**

Amoeba:-



Systematic position

Phylum: Protozoa
Subphylum: Sarcomastigophora
Class: Rhizopoda
Order: Amoebida
Genus: Amoeba
Species: proteus

Habit and Habitat:

Amoeba proteus is widely distributed. It is commonly found on the bottom mud or on underside of aquatic vegetation in fresh water ponds, lakes, springs, pools and slow running streams. It is rarely found in, free water as it requires a substratum to glide on from place to place.

Distribution: - It is found all over the world.

Identifying Characters:-

1 Animal is irregular shape, with simple or branched pseudopodia measuring 250 to 600 microns in diameter.

2 Cytoplasm is differentiated into ectoplasm and endoplasm. Ectoplasm contains ectoplasmic ridges.

3 Body of the animal is covered by a thin, delicate and permeable plasma membrane, called as plasma lemma.

4 Endoplasm contains nucleus, food vacuole, contractile vacuoles, water globules and crystals.

5 Permanent posterior end is called as uroid.

6 Withdrawal of pseudopodium and new pseudopodium containing endoplasm is present 7 Feeding may be studied by giving carmine. Nutrition is holozoic.

8 Reproduction by binary fission & Multiple fission

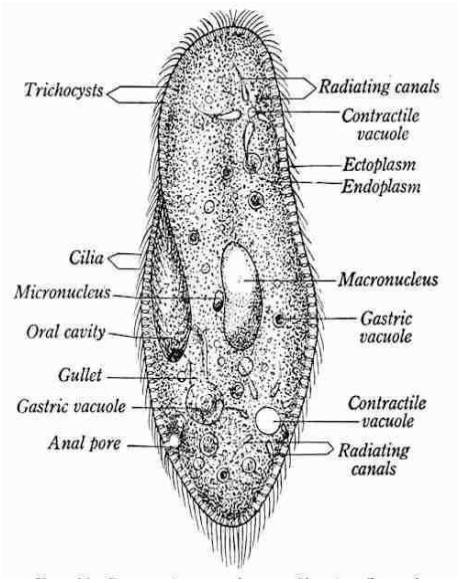
9 Amoeba proteus move by the formation of pseudopodia.Pseudopodia are blunt, finger like extensions of the ectoplasm containing endoplasm (lobo podium).

Special significance: Amoeba has unique phylogenetic significance and it is referred as immortal. Recently certain free living Amoeba has been found to be pathogenic causing meningeo encephalic.

Identification: Since the animal has pseudopodia and above feature, hence it is Amoeba proteus.

Note your observation:

Paramecium:



Classification

Phylum …	Protozoa
Subphylum….	Ciliophora
Class …	Ciliata
Subclass…	Euciliata
Order……	Holotricha
Suborder……	Trichostomata
Family…..	Paramecidae
Genus……	Paramecium

Habit and habitat: - Paramecium is best known ciliate, found in fresh water ponds, rivers, lakes, streams and pools, etc.

Distribution: - It has cosmopolitan distribution.

Identifying Characters:-

1 Commonly called as slipper animalcule, being microscopic, elongated, slipper- shaped cigar-shaped or spindle shaped.

2 Most familiar and extensively studied protozoans.

3 Pellicle covers the body. It is clear firm and elastic cuticular membrane. Pellicle has series of polygonal or hexagonal depressions for trichocysts.

4 Paramecia propel themselves by whiplash movements of their cilia, which are arranged in tightly spaced rows around the outside of their body.

5 The beat of each cilium has two phases: a fast "effective stroke," during which the cilium is relatively stiff, followed by a slow "recovery stroke," during which the cilium curls loosely

to one side and sweeps forward in a counter-clockwise fashion.

6 The densely arrayed cilia move in a coordinated fashion, with waves of activity moving across the "ciliary carpet," creating an effect sometimes likened to that of the wind blowing across a field of grain.

7 Paramecia live mainly by heterotrophy, feeding on bacteria and other small organisms.

A few species are mixotrophs, deriving some nutrients from endosymbiontic algae (chlorella) carried in the cytoplasm of the cell.

8 Osmoregulation is carried out by contractile vacuoles, which actively expel water from the cell to compensate for fluid absorbed by osmosis from its surroundings

9 The number of contractile vacuoles varies from one, to many, depending on species 10 Paramecia reproduce asexually, by binary fission. During reproduction, the macronucleus splits by a type of amitosis, and the micronuclei undergo mitosis. The cell then divides transversally, and each new cell obtains a copy of the micronucleus and the macronucleus.

11 Fission may occur spontaneously, in the course of the vegetative cell cycle. Under certain conditions, it may be preceded by self-fertilization (auto gamy), or it may follow

conjugation, a sexual phenomenon in which Paramecia of compatible mating types fuse temporarily and exchange genetic material.

12 During conjugation, the micronuclei of each conjugant divide by meiosis and the haploid gametes pass from one cell to the other. The gametes of each organism then fuse to form diploid micronuclei. The old macronuclei are destroyed, and new ones are developed from the new micronuclei.

13 Auto-gamy or conjugation can be induced by shortage of food at certain points in the Paramecium.

Identification: - Since the animal contains slipper- shaped body and two contractile vacuoles while are star –shaped and has all above features hence it is Paramecium.

Note your observation:

Plasmodium:-

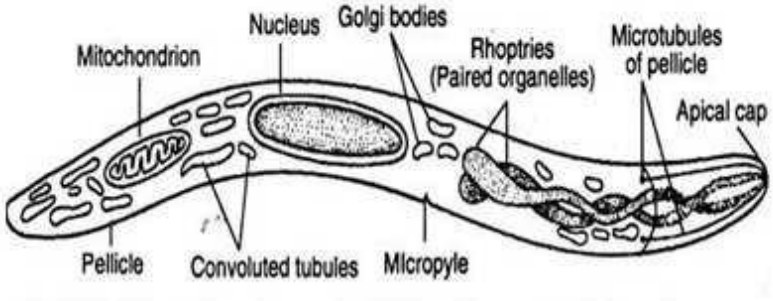


Fig. 8 Ultra structure of sporozoit of Plasmodium

Classification:

Phylum: Protozoa

Subphylum: Plasmodroma

Class: sporozoa

Subclass: Telosporidia

Order: Haemosporidia

Genus: Plasmodium

Comments:

- Plasmodium is an intracellular blood parasite of man and other vertebrates and causes malaria.
- The life history of plasmodium (Fig.8.40) is completed in two hosts, viz., partl in definitive, host the man and partly in intermediate host, the female anopheles mosquito.
- When an infected female anopheles mosquito bites a man, sporozoites are introduced in the blood from where they reach in live cells through blood streams and multiply to form merozoites.
- After a few schizogenous cycles in the liver, the merozoites enter the red blood corpuscles (R.B.C) and feed on the contents of R.B.C.

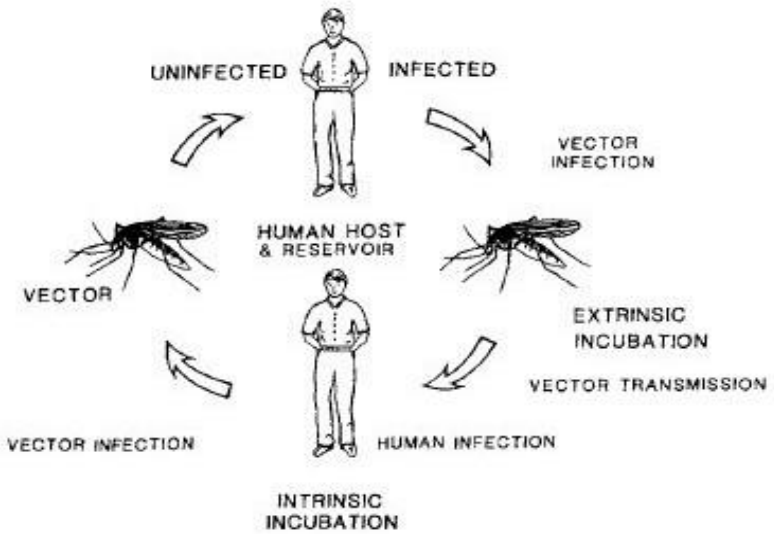
- After 2-8 schizogenous changes in the main blood stream, the merozites assume different shapes and known as gametocytes.
- Gametocytes cannot develop further in the blood of man; therefore they wait for female anopheles mosquito to suck them with the blood.
- When gametocytes are sucked in by the female anopheles with the blood of man, they undergo sporogony for further development.

There are four species of Plasmodium causing different types of fever:

1. Plasmodium vivax causes benign tertian fever.
2. P. Falciparum causes malignant tertian, fever;
3. P. Malariae causes quratan fever.
4. P. Ovale causes ovale or mild tertian fever.

Habit and Habitat:

Plasmodium is found in as an intracellular parasite in the blood of vertebrates. It is widely distributed in tropical & temperature countries of world but they are no longer a problem in colder countries of the world. Countries like India, Sri-Lanka, Bangladesh Nepal & Pakistan etc are worst affected. In India state like Bihar & Uttar Pradesh suffer a great setback by the infection of this parasite. In fact the infection of plasmodium is a global problem.



Life cycle of Plasmodium

Note your observation

8. Determination of size of microbial cell using micrometry

Micrometry is the measurement of microorganisms. Since microorganisms are small, the dimensions are usually expressed in units of micrometrer (μm) i.e. 10^{-3} of milimeter or 10^{-6} of a meter, nanometer (nm, 10^{-9} of meter) or angstrom (A, 10^{-10} of meter). Determination of the size of a microorganism is one of the properties useful for identification in the laboratory. As they can be only seen under microscope their size can be measured with an ocular micrometer which is calibrated against stage micrometer. Ocular micrometer is simply a glass disc with etched lines on its surface. It has 100 equally spaced divisions marked 0-10. The distance between the gradations of an ocular micrometer does not have any standard value and varies depending on the objective used. This distance is found out by calibrating it with a known scale, the stage micrometer. Stage micrometer is a special glass slide having in its centre a known distance one millimeter, which is encircled and mounted by a cover glass. This one mm distance is etched into 100 equally spaced divisions. Since there are 1000 micrometers in one millimeter. Thus one division of stage micrometer equals to **10 μm** or **0.01 mm**. The distance of each stage micrometer's division becomes correspondingly enlarged under high-power and oil-immersion objectives of the microscope.

Ocular micrometer after putting inside the eye piece is calibrated by superimposing the gradations of ocular micrometer over graduations of the stage micrometer, which is accomplished by rotating the ocular lens. By determining the number of ocular micrometer divisions coinciding with the number of divisions on stage micrometer, the calibration factor for one ocular division is calculated for the particular combination of objective and ocular lens used by applying the formula:

One ocular division (in μm) = Number of divisions on stage micrometer/No. Of divisions on ocular micrometer×10

After calibrating an ocular micrometer can be used to determine the size if an organism.

Size(in μm) = No. Of ocular divisions×calibration factor of the objective used

Procedure

1. Remove the eye piece of the microscope, unscrew it and place micrometer disc with engraved side down into it. Screw the eye piece back and insert in the microscope.
2. Place the stage micrometer on the microscopre stage and bring its scale in the microscope's field centre and focus.

3. Turn the ocular lens until the parallel lines of the ocular be parallel with those of stage.

4. Make lines coincide at the left end and find another line set which coincides.

5. Now count the number of divisions in both ocular and stage micrometers between two coinciding lines.

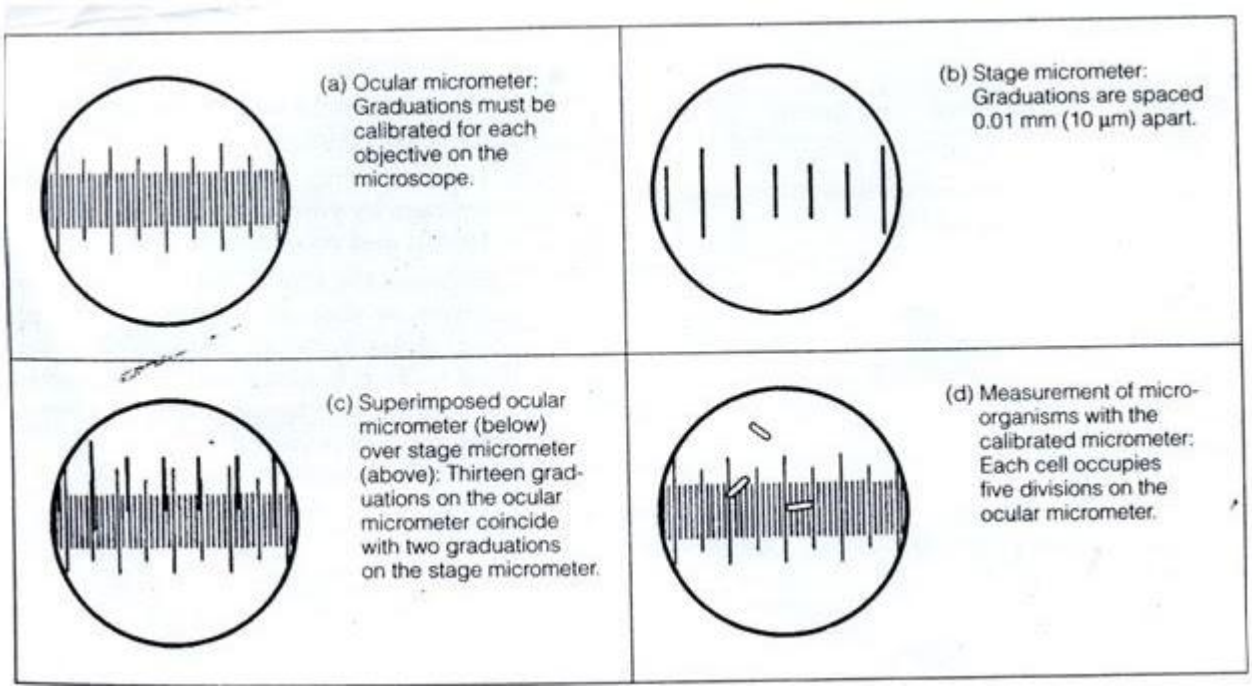
6. Take at least 3 readings in **low power** and **high power** objective lens seperately.

For example

In low power objective

20 Ocular Division =30 Stage Division

One division of ocular micrometer = 30/20×10 µm (1SD= 10µm) = 15 µm



Result (Table)

Measurement of microorganisms

A slide is prepapred with yeast cells. A drop of suspension of yeast culture can be taken, covered with cover slip and can be observed under microscope (prepared slide available in the laboratory can be used as substitute). Measure the diameter of the yeast cell by the ocular micrometer already placed in the eyepiece in low power or high power objective. Multiply the size in Ocular Division (OD) with calibration factor. Take atleast 3 readings in low power and high power each.

For example

If the diameter is 1 OD then the size will be (1×15) µm = 15µm

Result

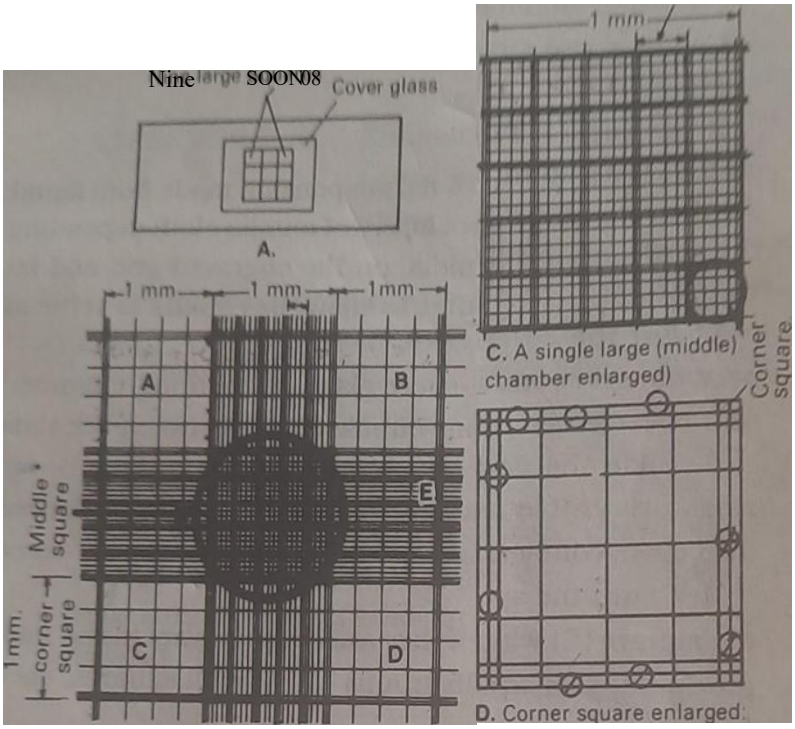
7. Enumeration of microbes: Yeast by Haemocytometer

Microbiological analysis of food, water, milk and air requires quantitative determination i.e. total population of microorganisms in these substrates. The density of cells, spores/ conidia of microorganisms can be measured in the laboratory by several methods either by direct or indirect counts. In the direct microscopic count, a known volume of liquid is added to the slide and the number of microorganisms are counted by examining the slide with the brightfield microscope. For direct microscopic counts Neu bauer or Petroff-Hausser counting chamber, breed smears or electronic cell counter (as coulter counter) are used. The hemocytometer (because it was originally devised for counting blood cells) is used for counting of microbial cells in liquid suspension. It is a special microscope slide with a counting chamber 0.1 mm deep so that volume of liquid over a one sq. mm is 0.1 cubic mm. The counting chamber has a total of 9 squares, each of 1 mm x 1 mm engraved over it. but only one square per field is visible under 100X (10x ocular and 10x objective). A one mm square is divided into 25 medium sized squares (0.2mm x 0.2 mm each). Each of which is further subdivided into 16 small squares (0.05x 0.05 mm each), thus a total of 400 squares in 1 mm. Each medium sized square is seperated by triple lines, the middle one act as the boundary. Each square has a volume of 1mm x 1mm x 0.1 mm (1/10 cm x 1/10 cm x 1/100 cm) = 1/10000 cm^3 or 10^{-4} cm^{3}. One ml of cell suspension is put into the counting chamber, number of cell is counted and the total number is determined mathematically.

Procedure

1. Place a drop of suspension of yeast cells on the engraved grid.
2. Put the cover glass (special cover glass of counting chamber) over the grid carefully so that no air bubble enters between the slide and cover glass.
3. Count the Yeast cells in the medium sized square.

4. Count cells in 4 corner medium squares (A, B, C, D) and in the middle one (E) or any five squares.



Hemocytometer used to determine (counts) spores of microorganisms and total count of cells. (A) Hemocytometer (B) Standard hemocytometer chamber. The circle indicates the approximate area covered at 100X microscope magnification (10X ocular and 10X objective). (C) A single large (middle) square Corner square enlarged. Count cells on top and left touching middle line (O) and do not count cells touching middle line at bottom and right

Results:

Cells / ml =

Precautions:

1. Always count cells on top and left touching middle line of the perimeter of each square and do not count cells touching the middle line at the bottom right sides.
2. Do not overfill or underfill the chambers of hemocytometer.
3. A minimum of 5 counting should be done to avoid error.