INTRODUCTION

This laboratory text is designed to guide the student through basic microbiology lab techniques, procedures and experiments. Throughout this manual, the student will learn the scientific method and its application.

Proper Safety Procedures

To insure safety of those working in the lab, as well as the integrity of each experiment, each of the following rules must be followed:

1. Clothing should be protected by a lab coat or apron. No sandals are allowed - you will be asked to return home and change if worn to lab class.
2. Hair that is long should always be tied back to avoid contamination as well as safety when working near the Bunsen burners.
3. Lab stations must be wiped down at the beginning of lab to lower contamination rates of cultures by organisms already on the stations as well as safety for the student. Stations must also be wiped down at the end of every lab session. Station cleaning is best accomplished with fresh 10% bleach. If there is visible contamination on the bench, wash with soap and water before the bleach.
4. Avoid direct contact with any microbes being tested by keeping all cultures well below mouth, nose, and eye regions. Microbial agents normally travel with gravity, so downward is the basic direction. Because of this movement, to insure integrity of cultures, avoid coughing, excessive talking, laughing, etc, while working with cultures while open. Keep cultures at a minimum of exposure to the air for best results.
5. Bunsen burners should be lit from the beginning of each session to the end as this decreases the risk of contamination of cultures and helps the safety of the lab worker.
6. Gloves must ALWAYS be worn when handling any microbial agent. Gloves are provided.
7. Lab stations should be kept clear of any extra materials (non-lab books, book bags, purses, keys, etc.) to avoid contamination as well as accidents.
8. All lab materials must be stored in the appropriate locations at the end of each session and gloves and other disposables placed in biohazard bags.
9. Tubes and racks should be placed in the appropriate location for autoclaving.
10. All spills should be reported IMMEDIATELY to the lab instructor for proper clean up. Unreported spills can result in biohazardous conditions.

Laboratory work should be fun and rewarding. To keep spills, burns, contamination and other accidents to a minimum, it is wise to stay alert and pay attention to your surroundings at all times. By following appropriate procedures, an enjoyable and safe lab can be assured.

Microbiology Laboratory Safety

A student successfully completing basic microbiology will demonstrate ability to explain and practice safe

1. **Microbiological procedures, including**
   a. reporting all spills and broken glassware to the instructor and receiving instructions for clean up
   b. methods for aseptic transfer
   c. minimizing or containing the production of aerosols and describing the hazards associated with aerosols
   d. washing hands prior to and following laboratories and at any time contamination is suspected
   e. using universal precautions with blood and other body fluids and following the requirements of the OSHA Blood-borne Pathogen Standard
   f. disinfecting lab benches and equipment prior to and at the conclusion of each lab. session, using an appropriate disinfectant and allowing a suitable contact time
   g. identification and proper disposal of different types of waste
   h. reading and signing a laboratory safety agreement indicating that the student has read and understands the safety rules of the laboratory
   I. good lab practice, including returning materials to proper locations, proper care and handling of equipment, and keeping the bench top clear of extraneous materials
2. **Protective procedures, including**
   a. wearing long pants or dresses; no sandals
   b. tying long hair back, wearing personal protective equipment (eye protection, coats, gloves; glasses may be preferred to contact lenses), and using such equipment in appropriate situations.
   c. always using appropriate pipetting devices and understanding that mouth pipetting is forbidden.
   d. never eating or drinking in the laboratory.
   e. never applying cosmetics, handling contact lenses, or placing objects (fingers, pencils, etc.) in the mouth or touching the face
3. **Emergency procedures, including**
   a. locating and properly using emergency equipment (eye wash stations, first aid kits, fire extinguishers, chemical safety showers, telephones, and emergency numbers).
   b. reporting all injuries immediately to the instructor.
   c. following proper steps in the event of an emergency

Microbiology Laboratory Skills Checklist

A student successfully completing basic microbiology will demonstrate the ability to:

1. Use **a compound light microscope** to view and interpret slides, including
    a. correctly setting up and focusing the microscope
    b. proper handling, cleaning, and storage of the microscope
    c. correct use of all lenses
    d. recording microscopic observations
2. Properly **prepare slides** for microbiological examination, including
    a. cleaning and disposing of slides
    b. preparing smears from solid and liquid cultures
    c. performing wet mount and/or hanging drop preparations (optional)
    d. performing Gram stains
3. Properly use **aseptic techniques** for the transfer and handling of microorganisms and instruments, including
    a. sterilizing and maintaining sterility of transfer instruments
    b. performing aseptic transfer
    c. obtaining microbial samples
4. Use **appropriate microbiological media and test systems**, including
    a. isolating colonies and/or plaques.
    b. maintaining pure cultures.
    c. using biochemical test media (optional)
    d. accurately recording macroscopic observations (Colony characters).
5. Estimate **the number of microbes in a sample using serial dilution** techniques, including
    a. correctly choosing and using pipettes and pipetting devices
    b. correctly spreading diluted samples for counting
    c. estimating appropriate dilutions
    d. extrapolating plate counts to obtain the correct CFU or PFU in the starting sample
6. Use **standard microbiology laboratory equipment** correctly, including
    a. using the standard metric system for weights, lengths, diameters, and volumes
    b. lighting and adjusting a laboratory burner
    c. using an incubator

The Scientific Method & Experimentation

Scientific experiments are designed around a question that requires an explanation. The scientific method is a guideline by which the explanation may be found and is a general procedure that is followed by all sciences. The method is modified slightly to fit each individual discipline.

General procedure for biological research:

1. Definition of the problem.
2. Review of the literature pertinent to the problem.
3. Formulation of hypothesis.
4. Statement of objectives
5. Analysis of variables
6. Design of experiment.
7. Experimental procedure.
8. Analysis of data (results).
9. Interpretation of data and conclusions from interpretations.

Some explanations and definitions of each step of the procedure are important

PROBLEM - two types:

1. Normally a fact that is assumed to be caused by some factor or factors the researcher is looking for cause and effect.
2. Involves the quantification of cause and effect. The association of the magnitude of cause related to the magnitude of effect.

HYPOTHESIS – an educated guess, a tentative theory. Anything that needs explanation is an effect. A hypothesis attempts to explain those effects by putting forth causes. A good hypothesis should:

1. Explain facts not previously explained.
2. be consistent with all known facts.
3. Account only for phenomena in question.
4. Aid in producing new facts and relations.
5. be susceptible to verification or refutation.

OBJECTIVE – This should be a carefully thought out and worded statement that Covers the cause and effect variables whose relationship is being determined. The objective should be highly specific. The attainment of the objective will support or refute the hypothesis and therefore should solve the problem under study.

VARIABLES – A thing that may vary or is able to vary. Refers to both cause and effect.

1. *Primary* – variables on which the experiment will focus. (cause and effect)

2. *Secondary* – variables which may influence or be influenced by the primary. These are not of primary interest. (side effects)
3. *Independent* – variables that are not dependent upon other variables.
4. *Dependent* – variables that depend upon other variables for magnitude.

These may be grouped together as (1) primary independent variable (cause); (2) primary dependent variable (effect); (3) secondary independent variable (extraneous variable of influence); and (4) secondary dependent variable (side effect).

The research should identify ALL variables that may concern or influence the experiment. The general procedure for handling variables is: 1. Control the secondary independent variables. 2. Manipulate and evaluate or select and evaluate the primary independent variable(s). 3. Evaluate the resulting variations in the primary dependent variable(s). 4. Ignore the secondary dependent variable(s).

EXPERIMENT – A trial or test. The process of learning through observation (Webster's, 181)

Most scientific experiments are controlled or "pure" experiments which are defined by (1) a predetermined plan; (2) objective of confirmation or refutation of a cause and effect relationship or degree of effect between two variables; (3) elimination, equalization, or evaluation of influencing, extraneous variables; and (4) assumption that the independent variable (cause) will be actively manipulated.

ANALYSIS – May or may not be statistical in nature. Often involves averages of observations.

INTERPRETATION/CONCLUSIONS – Meaning of facts, underlying causes, their affects and any other implications should be discussed. Researchers should avoid the most common errors in logic (illusions, generalizations, prejudices, biases, etc.).

Microbiology Laboratory Thinking Skills

A student successfully completing basic microbiology will demonstrate an increased skill level in

1**. Cognitive processes**, including
a. formulating a clear, answerable question
b. developing a testable hypothesis
c. predicting expected results
d. following an experimental protocol
2. Analysis skills, including
a. collecting and organizing data in a systematic fashion
b. presenting data in an appropriate form (graphs, tables, figures, or descriptive paragraphs).
c. assessing the validity of the data (including integrity and significance).
d. drawing appropriate conclusions based on the results
3. Communication skills, including
Discussing and presenting lab results or findings in the laboratory
4**. Interpersonal and citizenry skills**, including
a. working effectively in teams or groups so that the task, results, and analysis are shared.
b. effectively managing time and tasks allowing concurrent and/or overlapping tasks to be done simultaneously, by individuals and within a group
c. integrating knowledge and making informed judgments about microbiology in everyday life.

LAB: 1 THE COMPOUND MICROSCOPE

Primary Objective

Utilize all powers of magnification on the compound microscope.

Other Objectives

Be able to identify all the parts of a compound microscope. Know the rules for proper microscope care. Accurately measure appropriate specimens using Micrometer (m = 0.001 μm = 1 x 10-6 meters) scale in the scope lens.

Introduction

The most fundamental skill of microbiology is the use of the microscope. The definition of a microscope is a device for magnifying objects that are too small to be seen with the naked eye. Today's microscopes often employ more than two sets. The largest and most accurate microscope is the electron microscope that uses electrons in a focused beam to illuminate objects rather than light. The electrons pass through the specimen being viewed and are electrically focused. Normally the image is then 'broadcast' to a television monitor or photographed. A scanning electron microscope gives a three dimensional image of the specimen. An electron microscope is capable of specimen magnification millions of times the actual size. This type of microscopy has allowed the viewing of virus particles that are so small a few million could fit on the head of a pin.

A compound microscope consists of at least two magnifying lenses. One magnifying lens is in the **ocular** and one is in the **objective**. Each contributes to the magnification of the object on the **stage**. The **total magnification** of any set of lenses is determined by multiplying the magnification of the objective by the magnification of the ocular. The **turret** (**nose**) rotates allowing the objectives to change and thus change the magnification of the microscope. An **iris diaphragm** below the stage should be used to control the amount of light passing through a specimen. Less light is need at low magnification than at higher magnification. Too much light at low magnification may mask the specimen, particularly something as small as a bacterial cell. The distance between the specimen on the stage and the objective is known as the **working distance**. The coarse adjustment knob will cause the working distance to visibly change while the fine adjustment knob is for final, fine focusing. The ability to see things using a microscope is limited by the resolving power of the microscope. The resolving power of a microscope is the distance two objects must be apart and still be seen as separate and distinct. For the light microscope this is approximately 0.2 μm. Objects closer together than 0.2 μm will not be distinctly seen. Increasing the magnification will not make the objects more distinct, just bigger. **Low magnifications** are used for quickly examining the slide to find an appropriate area to examine. **Higher magnifications** allow the examination of a particular object on the slide. While looking through the microscope move the iris diaphragm lever and notice how the

brightness of the light changes. As you move the objectives to provide increased magnification you will look at a smaller section of the slide.

Calculation of Magnification
Total magnification requires multiplication of the magnification of the objective lens times that of the ocular lens (which is typically 10x).
(Objective magnification)(Ocular magnification) = Total magnification
i.e. (4x objective) (10x ocular) = 40x total magnification

The problem is, the greater the magnification of an object the more blurred it is. This is known as empty magnification. To solve these problems, the focus of light must occur at the microscopist's eye. Ernst Abbe a German mathematician and physicist determined that resolving fine detail depends upon the amount of light that is gathered by a lens. More light equals better resolution. Abbe found that immersion oil, which has the ability to bend light equivalent to that of glass, allows more light to be gathered and allows a greater amount of resolution.

If the stage is a great distance away from the objective when the higher powers are used, the microscope has been adjusted incorrectly.

Microscopic Field - this is the area one can observe while looking through the oculars. As the magnification increases this will also decrease. When you look through the ocular you will see a lighted circle. This is known as the field of view or the field.

Parfocality - this refers to the ability of a microscope to need only minor focusing adjustments after the specimen is found and focused using the lowest power**. A microscopist should use the coarse tuning knob only when originally finding and focusing the specimen. Once the original focus is made, the only adjustment should be with the fine tuning knob as the magnification is increased.** Most microscopes are considered parfocal.
Be sure you move the object you want to view into the center of the field before moving to the next objective. These microscopes are parfocal. Once you have focused on an object using one objective the object will be approximately in focus on the next objective. Use of the fine focus knob will sharpen the focus.

Parts, Care and Procedures
The parts of the microscope are labelled on the diagram on the following page. Knowing the names of the parts and their functions is an important part of working with microscopes. Many microscopes have only one ocular, these are termed **monocular**. Those with two oculars are termed **binocular**. Microscopes should always be carried with one hand under the base and the other hand holding the arm of the scope securely, keeping the cord out of the way of feet. The lenses should

always be wiped down with lens paper and the cord should be kept out of the way of the working area.

When beginning an examination of a specimen the scope should be on the lowest power with the stage raised as high as it will go. The slide should be placed between the stage clips and all placement of slide and stage objectives should be done BEFORE looking into the oculars. Once all placement is ready, adjustment should be done while looking through the ocular. Adjustment should begin with the coarse adjustment, once the specimen is spotted then the fine tuning adjustment can be used. Because of parfocality, once the specimen is in focus on the lowest power there should be no need to adjust the coarse adjustment even with increased magnification.

Materials

Compound light microscope
Prepared slides of 3 types of bacteria
Prepared freshly stained bacteria slides
Immersion oil
Lens paper
Hay infusion
Coverslips
Pipettes
Newsprint

Procedure 1 - Using the Microscope

1. Obtain a slide from the instructor.
2. Place the slide on the stage of the microscope.
3. Turn the revolving nosepiece so that the scope is on the 4x objective (low).
4. Roll the stage up to its highest point.
5. Move the stage until the specimen is in the middle of the stage.
6. Focus on the specimen by rolling the stage down slowly using the coarse adjustment.
7. Ask the instructor to verify that the sample is in focus and sign the report sheet.

Procedure 2 - Examining Stained Bacteria

1. Obtain one slide of stained bacteria.
2. Place the slide on the stage and follow proper procedure for focus, etc.
3. Once the high dry objective (40x) has been reached and focus is maintained, apply one drop of immersion oil to the slide and move to the oil-immersion objective (100x). Refocus if necessary and draw what is in the two fields on the report sheet.

Lab 1 Report Form

Procedure 1: Low (4x) Medium (10x) High dry (40x)
Procedure 2: (Oil immersion)

PROCEDURE 3- Examining Newsprint

1.Place a piece of newsprint on a microscope slide and cover with a coverslip. ALWAYS USE A COVERSLIP!
2.Turn the microscope on and set the light source on its highest setting.
3.Use the coarse adjustment knob to obtain maximum working distance.
4.Place the slide on the stage. The slide should fit into the slide holder but is not placed under the slide holder. Use the stage adjustment knob to move the slide the edge of the coverslip bisects the hole in the stage.
5.Rotate the scanning objective (4X) into place.
6.Use the coarse adjustment knob to obtain the minimum working distance. Develop the habit of watching this process to be sure the objective does not crash into the slide.
7.Look through the oculars. Adjust the light with the iris diaphragm lever on the condenser if necessary. Slowly turn the coarse adjustment knob until the edge of the coverslip comes into focus. Use the fine adjustment knob to sharpen the focus.
8.Use the stage adjustment knob to locate the letter “e” in the newsprint. Note the orientation of the letter “e” in the newsprint.
9.Rotate a higher power objective (10X) into place. Use the fine adjustment knob to sharpen the focus. Do not use the coarse adjustment knob. Adjust the light using the iris diaphragm lever if necessary. The image is now magnified 100X (10X ocular x 10X objective = 100X magnification). Draw the letter “e” as it appears in the microscope on the lab report sheet.
10.Place a stage micrometer on the stage and determine the diameter of the field of view for all four objectives. The micrometer is 2 mm in length. The ruler is divided into tenths. Record the distances on the lab report sheets.
11.When using the high power objective (100X) use the following procedure. Rotate the turret halfway between the 40X and 100X objective. Place a drop of immersion oil on the slide and rotate the oil immersion objective (100X) into place. The objective should be immersed in the oil on the slide. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary. Never use the coarse adjustment knob with high power.

Procedure 4: Examining the hay infusion:

1- Place a drop of water from the hay infusion on a microscope slide.
2- Cover with a coverslip and view under all four objectives.

When you are finished with the microscope clean the microscope, as described below, and return it to storage.

PROCEDURE FOR CLEANING A MICROSCOPE

1.Turn off the light and unplug the cord. Store the cord appropriately.
2.Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.
3.Using lens paper clean all the lenses starting with the cleanest first—oculars, 4X through 100X objectives.
4.Clean any oil off of the stage using Kim-wipes or paper towels.
5.Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.
6.Return the microscope to the appropriate storage area.

For the Lab. Report:

1- **Sketch the bacteria from two prepared slides under 1,000X magnification and give their approximate sizes in micrometers in the space below. Note the name and color of the bacteria.**
2- **Sketch the orientation of the letter "e" as viewed through the microscope at 100X magnification.**
3- **Sketch two (2) of the organisms of hay infusion at 400X magnification**.

LAB 2: The Common Lab. Equipment's And The Sterilization Techniques.

Sterilization:

The Process which makes things free of microbes.

Things that can be sterilized:

- Glass wares: (Petri dishes – pipettes – Beakers – Flasks).
- Media
- Inoculating Needles (or loops), etc...
- Apparatus
- Clothes
- Benches
- Skin

There are 3 different sterilization principles:

1. Heat sterilization (dry & wet)
2. Chemical sterilization
3. Radiation sterilization

1. Heat sterilization

There are 2 procedures depending on the tolerance of the material used:

- Steam sterilization (Autoclaving) or wet.
- Dry heat sterilization (hot air oven).

Advantages: It is the simplest, most effective and inexpensive method.

1.A- Incineration:

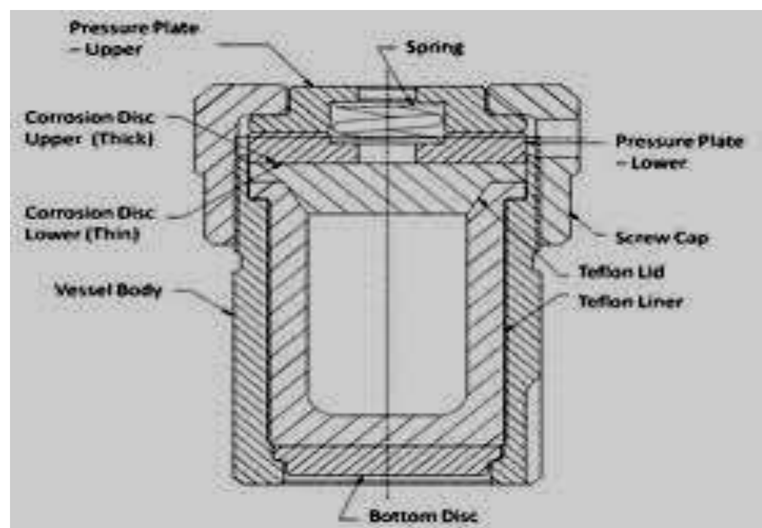
The most common method of treating infectious waste. Hazardous material is literally burned to ashes at temperatures of 870ºC to 980ºC. It is the safest method to ensure that infective materials remain in samples or containers.

Prions, infective proteins, are not eliminated using this method/

1.B- Wet sterilization (Moist heat):

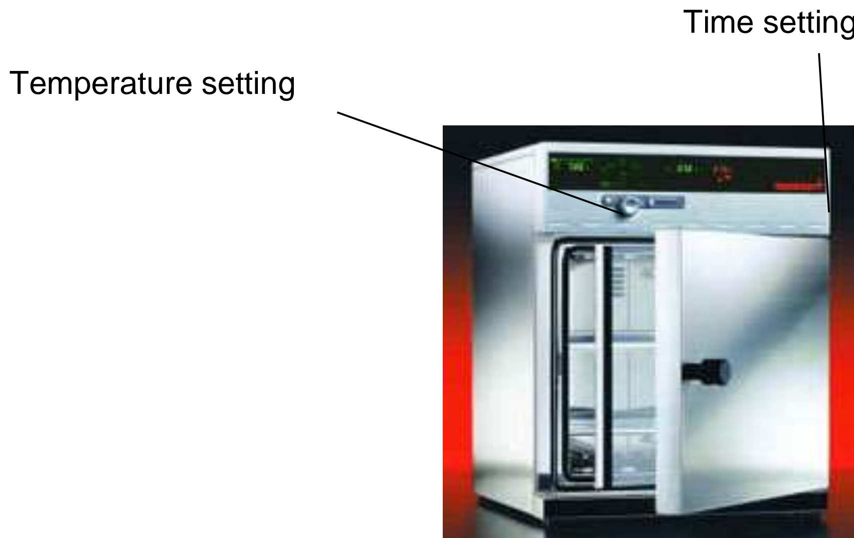
- It includes autoclaves.
- Used to sterilize culture media, glassware etc.
- Autoclave sterilizes items by heating them with steam to a very high temperature at certain atmospheric pressure. It sterilize the tools at 121 ° C/15 p.si (at 15 lb/sq.inch) for 15 min.

Advantage of using an autoclave: it can reach temperatures higher than boiling water alone, so it can kill not only bacteria but also bacterial spores, which tend to be resistant.



1.C- Dry heat sterilization

- Used to free the glassware from microbes.
- It uses dry air by heating Such as hot oven.
- It is recommended when it is undesirable that steam make contact with the material to be sterilized. This is true for glassware's – glass petri plates, Pipettes as well as for substances like oil, powder, etc.
- After/during sterilization wrap them in aluminium foil to avoid recontamination.



1.D- Direct heat (Flame sterilize):

Bunsen burner and flames produce gas flame witch used to sterilize needles and loops

1.E- Alcohol inflammation:
To sterile metals like scissors, cork borer, glass rods, or forceps. It can be done using alcohol 50% with the flame to ensure the total incineration of the tool.

2- Chemical sterilization

- Ethylene oxide and formalin
- For sterilizing disposable materials (that cannot tolerate high temperatures).

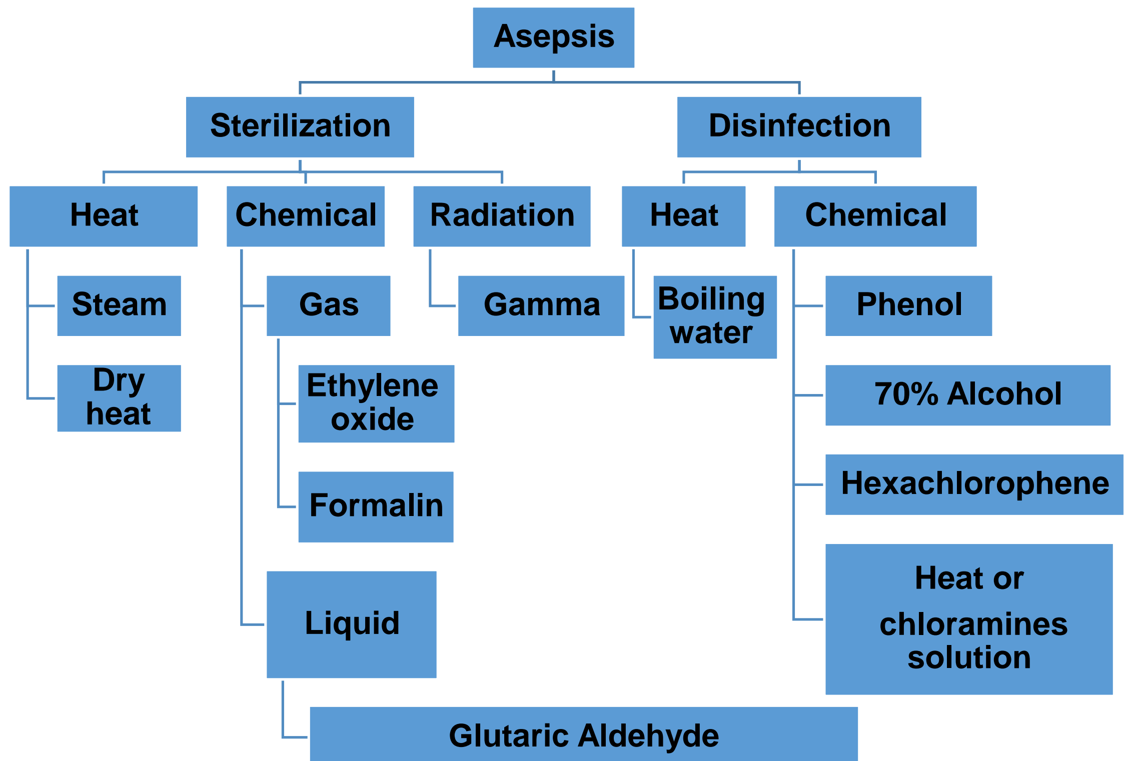
i.e. Benches /tables can be sterilized with 50% Dettol, or fresh 10% bleach.

Radiation

- Gamma rays,U.V Rays

Other sterilization techniques

- Filtration
- Boiling
- Cold temperature



LAB 3: Culture Media Preparation

Objective:

To prepare solid and Liquid media

Understanding concept

-Microorganisms need food to grow

-Primary ingredients required by all living organisms include:

a carbon source, water, minerals, and a nitrogen source.

These nutrients together make a media

-Different microbes need different amounts of these nutrients.

Culture media may be found in one of three states:

- liquid (called broth)
- semi-solid
- solid.

Agar--Media is made solid by adding **Agar**

- ***Solid Media (Nutrient agar)*** consist of Nutrient agar: 23g and Distilled water: 1000ml
- ***Liquid Media (Nutrient Broth)*** consist of Nutrient broth: 23g and Distilled water: 1000ml

Examples of general media for Fungal growth and cultivation:

1- Solid Media

Malt extract agar: 20g

Water: 1000ml

2- Liquid Media/ Broth

Malt extract : 20g

Water: 1000ml

Materials:

- Nutrient broth
- Nutrient agar
- PDA/malt extract
- Distilled water
- Agar
- Test tubes
- Petri plates
- Burners
- Autoclave

Procedure:

1.Weigh the proper amount of selected media by balance.
2.dissolve the powder in the selected amount of D.W.
3. Heat the mixture until homogenized (become transperant) by boiling water or 2 minutes in the microwave.
3.sterilize by autoclave for 30 min at 121C/15
4.cool until you can pour it at 45 C
5. Under aseptic conditions, pour the cooled media in either **labelled*** plates or tubes (or make stabs / slants).
6.refrigerate till use.

Labelling should include,
Your initials, type of media, and the date.

Select the chemicals

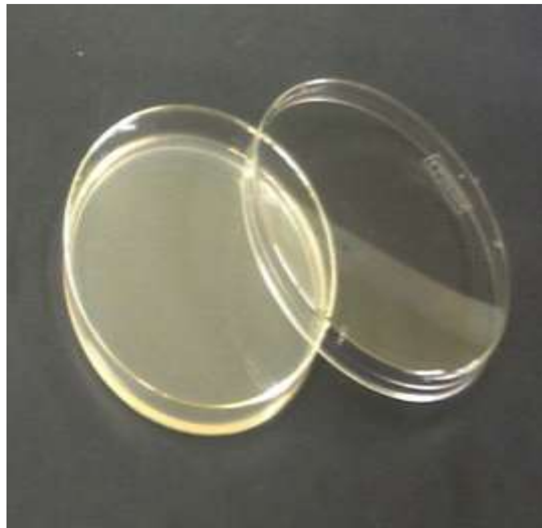
Weigh them

Add each dry culture medium ingredient to the culture flask.

Allow it boil

Sterilize the media using Autoclave.

- Petri plates



Pouring of Solid media:

1- **Making petri plates**

- Under aseptic conditions, remove the lid slightly.
- Pour the media near bunsen burner.
- Wait until the media solidify then Invert the plate.
- Write date and time on the sides of plates.

2- Making of Stabs / Slants:

- Place the test tube near a burner and remove the cap
- Pour the media in the tube: fill until ($^{2}/_{3}$) of the tube to make stab and fill ($^{1}/_{3}$) of the tube to make a slant.
- Flame the tube opening and close the cap at once
- Place the tubes in slanting manner with the help of a book (slant) or left in upright position on the rack or test tube stand (stab).
  Or (pour the media before sterilizing (after boiling) in test tubes in Autoclave).
- Place in slant position till the media solidify.

An Agar Slant

3- Making of test tubes with broth media

- Pour the media near bunsen burner. **Don't forget flaming the opening of both flask and tube!**
- Place the test tube near a burner and remove the cap
  Pour the media in the tube and close the cap at once (a $(^1/_3)$ will be enough).
- Place the tube in upright position in the test tube stand.

LAB4: Isolation of microbes from different environments

Objective: To Isolation of different micro-organisms from various environments

Materials:

- Petri plates with solid media including: (Nutrient Agar (NA) – Potato Dextrose Agar (PDA))
- Samples: (Soil, Rotten fruit, Yoghurt, water, Juice, milk, Skin …… etc)
- Isolating tools: (inoculating loops, Swabs, forceps, scalpels, … etc)
- Incubators for fungi at 25-28ºC and for bacteria at 37ºC.
- Sterilizing tools: (Bunsen burner, lighter, disinfectant (such as, alcohol 70% or Dettol 50%), cotton)

Procedure

Expose plates to air
or Place diseased part /healthy of fruit on media aseptically
or Rub the swab on solid media

Isolate Microorganisms:

To provide aseptic conditions. First, disinfect the working area using Bunsen burner and alcohol 70% or Dettol 50%. Then, write the labels on the intended (Test short name, initials and date). In this case the source of isolation. The procedure should be done as the following:

- **To isolate Microorganisms from Air:**
  - Expose the prepared plate of solid media (NA / PDA) in air for 5 – 10 min.
  - Close the lid and incubate at 25-28ºC and 37ºC, respectively.
  - After 2-5 days, observe bacteria, and /or fungus for grow.

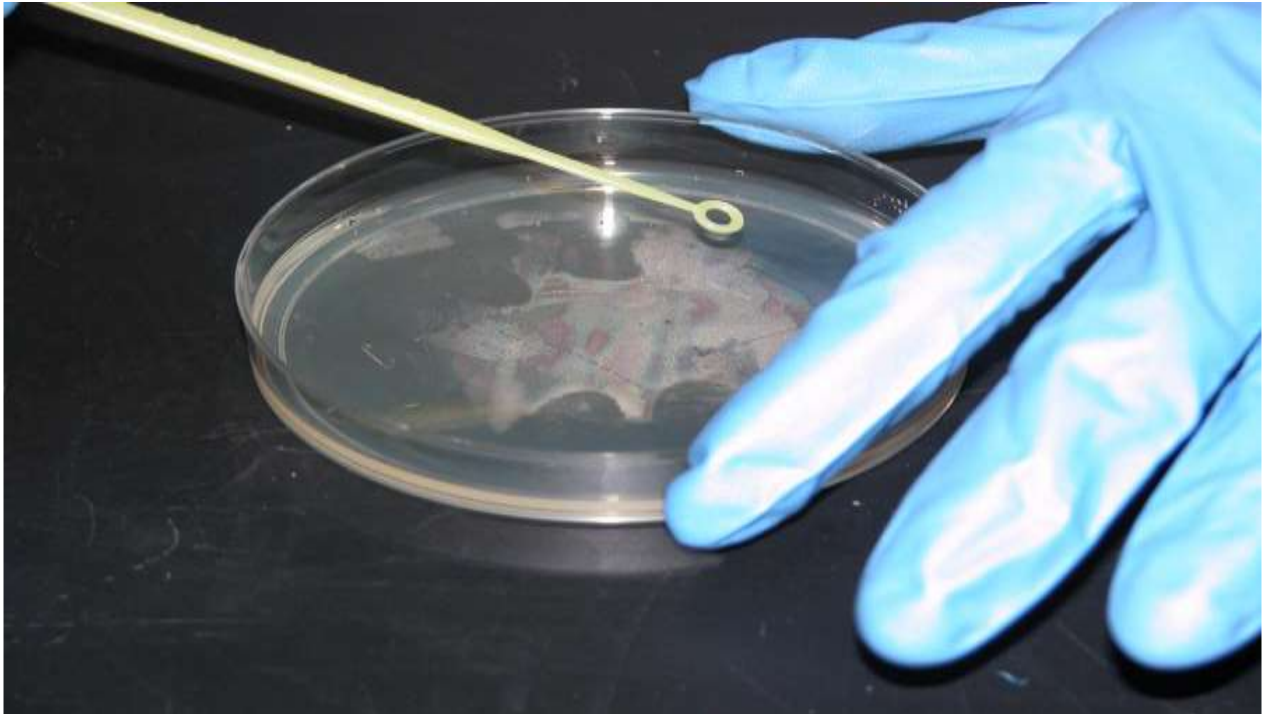
- **To isolate Microorganisms from Soil :**
  - Sprinkle a pinch of soil on solid media plate (NA /PDA).
  - Close the lid and incubate at 25-28ºC and 37ºC, respectively.
  - After 2-3 days, observe bacteria and /or fungus for grow.

- **To isolate Microorganisms from Water:**
  - Pour one or two drops of water on solid media plate (NA/ PDA).
  - Incubate at 25-28°C and 37°C, respectively.
  - Observe after 3-5 days, observe bacteria and /or fungus for grow.

- **To isolate Microorganisms from Rotten fruit:**
  - Clean the rotten part of fruit with alcohol swab.
  - Cut a piece and inoculate it on the media plate (NA/ PDA).
  - Then incubate at 25-28°C and 37°C, respectively.
  - Observe after 3-5 days, observe bacteria and /or fungus for grow.

To isolate Microorganisms from Yoghurt / Juice/ Milk:

- Put a drop of diluted solution on the solid media plate (NA/ PDA).
- Spread the drop on the media and incubate at 25-28°C and 37°C
- After 24-48 hours and 5 days, bacteria and /or fungus will grow.



- **Mouth**
- Take some saliva with the help of a wooden spatula or cotton swab
- Inoculate it on the media and incubate

Inoculation (Inoculate v.)

To introduce (microorganisms) into surroundings suited to their growth, as a culture medium. This can be done by putting the spores/cells on the media under aseptic conditions.

Incubation

The controlled laboratory conditions used to multiplying microbial organisms by letting them reproduce in predetermined culture media. That mainly include temperature, oxygen requirement, and duration of growth.

- Fungal Incubation (at 28^0C for 2-5 days).
- Bacterial Incubation (at 37^0C for 24 hours).

The essential five I'S in microbiology lab:

1. **Inoculation – introduction of a sample into a container of media to produce a culture of observable growth**
1. **Isolation –separating one species from another**
2. **Incubation – conditions that allow growth e.g. temperature ,humidity,,, etc.**
3. **Inspection**
4. **Identification**