Course Name: Laboratory skills (240 MIC)

Course Lab Session: Dr. Saleh H. Salmen

Office: 2B 20

Time: 8:00 to 12:50

Subject	Week	Hours
General precautions and safety rules.	**1**	**4**
Sterilization and Disinfection skills	**2**	**4**
Microscopy	**3**	**4**
Preparation of microbial cultivation media	**4**	**4**
Isolation and purification of microorganisms from soil	**5**	**4**
Preparation of microbial slides, Gram staining	**6**	**4**
Midterm Exam	**7**	**4**
Isolation and purification of fungi from different plant parts	**8**	**4**
Fungal staining and microscopic techniques	**9**	**4**
Physical and chemical factors affecting growth and activities of microorganisms	**10**	**4**
Isolation of microorganisms from the Human body (Throat and skin culture)	**11**	**4**
Final Exam	**12**	**4**

Lab 1

General precautions and safety rules

Objectives:

1. Learning the technical safety requirements for work in microbiological laboratories.
2. Avoiding the harmful effects of microorganisms, chemical and radiation exposure in microbiological labs.

Procedures:

1. Use your lab coat, when working in the laboratory to protect clothing from contamination or accidental discoloration by staining solutions.
2. Clean your work area (laboratory bench) with a recommended disinfectant such as 5% Lysol or 5% phenol before and after each laboratory period to avoid contamination.
3. Eating, drinking, and smoking are forbidden at all times in the laboratory.
4. Always keep the laboratory work area free from articles not actually in us and replace all reagents, cultures, and glassware in their appropriate places.
5. Keep your hands away from your mouth and eyes while in the laboratory. Do not place anything such as pencils, food, and fingers in your mouth.
6. Wash your hands thoroughly before and after each experiment, using disinfecting soap if possible.
7. Wear your musk and gloves to avoid inhalation of harmful solvents and buffer solutions as well as affecting your hands.
8. Avoid contamination of benches, floor, and wastebaskets.
9. Place all discarded cultures, infectious materials, used glass slides and contaminated glassware into the provided receptacles. Do not let unneeded materials accumulate.
10. Place all used or contaminated pipettes in appropriate glass jar filled with any disinfectant such as 5% phenol.
11. When infectious material is accidentally spilled, cover it immediately with a disinfectant such as 5% Lysol or 5% phenol and notify your instructor at once.
12. Do not move with a loop or pipette containing infectious material through the laboratory. Flame wire loops and needles before and immediately after transfer of cultures.
13. Label all experimental material properly.

Lab 2

Sterilization and Disinfection Skills

Objectives:

The purpose of this part to:-

- Define, what is term sterilization mean?
- Study the sterilization techniques and learn, how and when we use the different heat based and another sterilization tools.

Sterilization: is defined as, the complete destruction or elimination of all forms of viable microorganisms from a material by a chemical or physical methods.

Definition and explanations of Sterilization techniques:

1-Heat:

It is considering the most common and reliable method of sterilization. This method can be used for all materials that withstand heat. Sterilization by heat could be concluded as follow.

1.1. Dry Heat:

a. Direct flaming:

It is a simple and common method for effective sterilization, of materials that can be heated to redness in direct flame. Flaming could be used in microbiology labs to sterilize inoculating needles, loops, forceps tips, searing spatulas and straight-wires by exposure to Bunsen burner until become red. Loops, forceps tips and other metal object could be dipped in 70% ethanol before briefly passing over Bunsen burner flame to obtained maximum degree of sterilization. Mouth of test tubes, flasks, glass slides and cover slips are also sterilized by passing through the Bunsen burner flame for a few seconds to become free from germs.

b. Hot air ovens:

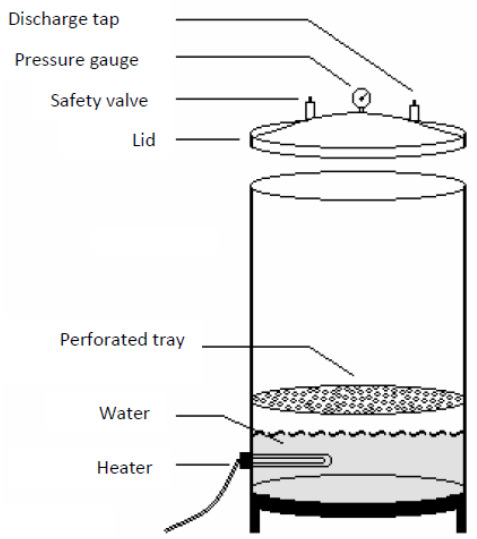
Dry heat can be used to sterilize glassware or other non-porous heat conductive materials. In dry ovens, 1 to 2 hours at 160 to 170 $^{\circ}$C is standard setting for complete sterilization. Dry heat takes much longer time than moist heat to be transferred to the organism, thus both the time and the temperature must usually be increased. Dry heat has the advantage that it can be used on powders and other heat-stable materials that are adversely affected by steam.

1.2. Moist Heat:

Steam under pressure (Autoclaving):

This technique could be used to sterilize microorganism's culture media, aqueous solutions, and contaminated items such as Petri dishes, tips, tubes, gloves and plastic vessels comprised of polypropylene.

Water boils at 100°C at atmospheric temperature, but within an autoclave, steam pressure can reach (15 psi, above atmospheric pressure) with this increased pressure (the water temperature will be increased to 121°C which will sterilize media more efficiently. Generally, we can use an autoclave or pressure cooker at 121°C; 15 psi for 15 minutes, this is the standard recommended for most microbiological media. These conditions are adequate to quickly and effectively kill all microbial forms even the hardiest spore formers.



An autoclave machine

2. Radiation:

There are two types of radiation could be employed to achieve microbe-free environment. Because radiation does not generate heat, it is termed "cold sterilization". Rays are harmful to skin and eyes. It doesn't penetrate glass, paper or plastic.

Ionizing rays but. Electromagnetic rays such as gamma rays produced by a linear accelerator from a heated cathode are high-energy with good penetrative power. It is used to sterilize disposable Petri dishes, gloves, plastic syringes, dressing packs, antibiotics, vitamins, hormones and fabrics in few seconds.

Non-ionizing rays: UV rays of wavelength longer than the visible light are low energy with poor penetrative power. Wavelengths of UV rays, ranged from 200-280 nm have a destruction effect on microbial cells, with 260 nm being most effective. UV rays use in surface disinfection to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.

3. Chemicals:

There are many chemicals that have a broad spectrum of antimicrobial activity and are fast acting. Mercuric chloride, sodium hypochlorite, formalin, phenols, ethanol and isopropanol are extensively used in microbiological labs for a variety of purposes such as swabbing a bench before and after use, for the sterilisation of surfaces, and for the disposal of used instruments such as Pasteur pipettes. Many laboratory disinfectants need to be prepared before they can be used.

When using disinfectants, it is important to ensure that they are used at the correct concentration and that they are left to work for the correct length of time.

4. Filteration:

Filtration could be done for liquids and gases that may chemically altered by heat exposure such as antibiotic and certain of culture media components. These solutions could be sterilized by passing them through filters of an appropriate pore size. While most solutions used in molecular biology will be adequately sterilized with a 0.22-μ filter, those for tissue culture should be use A 0.1-μ filter to remove mycoplasma from tissue-culture. There are much kind of filters could be used for such purpose i.e. asbestos filters, membrane filters and sintered glass.

Lab 3

Microscope

Objectives:

1. Study the different kinds of microscopes.
2. Learn how to use this device correctly.

Definitions and explanations (kinds of microscopes):

1. Optical Microscope:

The optical microscope has one or two lenses that work to enlarge and enhance images placed between the lower-most lens and the light source. It was the first device ever created.

a. Simple Optical Microscope:

It was used by Anton Van Leeuwenhoek during the late-sixteen and early-seventeenth centuries, around the time that the microscope was invented. It has one lens, the convex lens, in the magnifying process.

b. Compound Optical Microscope:

It has two lenses, work to minimize both chromatic and spherical aberrations so that the view is unobstructed and uncorrupted. The compound light microscope consists of three sets of lenses:-

- Condenser contains lenses that collect and focuses the light to upward directing through any object on the stage. The amount of light is controlled by shutter, or iris diaphragm, which adjust the amount of light admitted.
- The objectives include three or four lenses that provide a magnified and inverted image of the specimen
- The eyepiece adds further magnification

2. Stereoscopic (Dissecting) microscope:

It is two microscopes in one, and uses two separate optical shafts, which focus on the same point from different angles to produces a three-dimensional visualization of the examined sample. It provides slightly different viewing angles to the left and right eyes because using two separate optical paths with two objectives and two eyepieces.

Stereo microscope is relatively low power compared with compound microscopes (below 100 X). It is often used to study the surfaces of solid specimens or to carry out close work such as sorting, dissection, microsurgery, small circuit board manufacture or inspection, and the like.

3. Inverted Microscope:

This kind of microscope views objects from an inverted position than that of regular microscopes. It used to the study cell cultures in liquid media.

4. Fluorescence microscopy:

Fluorescence microscope is widely used device in the life sciences and biology. It is a light microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence. Both of an excitation and emission filters used in the fluorescent microscope.

5. Digital microscope:

Traditional optical, stereoscopic and inverted microscopes have been recently modified into digital microscopes. These modified microscopes constructed from computer units attached to camera devices. Computer software converts the images to be displayed on a high resolution LCD monitor instead of direct viewing.

6. Electron Microscopes:

Electron microscopy employs electron waves running parallel to a magnetic field providing higher resolution. Electron microscopy allows one to visualize objects that are as small as 1 nm. Electron microscopy is a high-cost technology use very expensive materials such as osomium gold-palladium or carbon or platinum.

a. Scanning electron microscope (SEM):

Scanning electron microscope used to visualize the surface of tissues, macromolecular aggregates.

b. Transmission electron microscope (TEM):

Transmission electron microscope used to study the inner structure of objects (tissues, cells, viruses).

Lab 4

Preparation of microbial cultivation of media

Objectives:

1. Study the different kinds of media.
2. Learn how to use them during isolation and purification of microorganisms.

Medium*:* a nutrient blend used to support microbial growth.

A medium is sterilized (living organisms removed) before usage in the lab.

Important points:

Sterilization methods include; autoclaving, dry-heat, filtration, UV exposure and ethylene oxide.

Culture: Is part of specimen grown in culture media.

Culture Media: is a medium (liquid or solid) that contains nutrients to grow bacteria *in vitro*. Because sometimes we cannot identify with microscopical examination directly, and sometimes we do culture for antibiotic sensitivity testing.

History

- The original media used by Louis Pasteur – meat broth
- Liquid medium – diffuse (spread out over a large area) growth
- Solid medium – discrete (individually separate and distinct) colonies.

- Cooked cut potato by Robert Koch – earliest solid medium
- Gelatin – not satisfactory
- -liquefy at 24^{o}C

Properties of agar:

- Angelina Fanny Hesse
- Used for preparing solid medium
- Obtained from seaweeds.
- Comes as sold powder and then you add water to it.
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98^{o}C & sets at 42^{o}C
- 2% agar is employed in solid medium

Properties of Media

- Support the growth of the bacteria.
- Should be nutritive (contains the required amount of nutrients).
- Suitable pH (neutral to slightly alkaline 7.3-7.4).
- Suitable temperature, and suitable atmosphere. (Bacteria grow at 37^0C)
- ***Note:*** media are sterilized by autoclaving at 121^0C and 2 atmosphere for 15-20 minutes. With the autoclave, all bacteria, fungi, viruses, and spores are destroyed. Some media can't be sterilized by autoclaving because they contain eggs or carbohydrates.

Types of culture media

I. Based on their consistency

a) solid medium

b) liquid medium

c) semi solid medium

II. Based on the constituents/ ingredients

a) simple medium

b) complex medium

c) synthetic or defined medium

d) Special media

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

III. Based on Oxygen requirement

- Aerobic media

- Anaerobic media

Lab 5

Isolation and purification of microorganisms from soil

Objective:-

Learning how to isolate soil fungi.

Materials:

Soil sample.
Acidified Czapek–Dox + 0.5 % yeast extract agar medium.
Sterilized Petri plates.
Sterilized distilled water in test tubes (9cm water/each).
Golf shaped like glass road.

Procedures:-

Simple plating technique (Direct isolation):

The direct inoculation method may be best for isolating various and general soil fungi simply, readily, and economically.

1- Transfer a small amount (0.005–0.015 gm) of soil to a sterilized Petri dish.
2- Added 8–10 ml. of semi-cooled (45°C) nutrient medium and shake the plate to let the soil particles dispersed throughout the thin layer of agar medium before it solidify.
3- If the soil is very dry, or contains a high proportion of clay, it is preferable to mix the particles with a drop of sterile water in the plate, before adding the medium.
4- Incubate treated plates at 20-30°C, investigate the colonies appearance after 48 and record the results.

-Dilution (Plate) Method:-

- Take a proper amount of airy dried soil sample after saving it to remove any undesirable materials (plant duperies and beg granuls).
- Prepare cereal dilution (i.e. 1:10, 1:100, 1:1000…….etc) from the soil sample.
-Transfer one drop from each of the last two dilution samples to plated isolation media using a sterile pipette.
- Use golf shaped like glass road to spread the droplets onto the agar surface.

- Incubate treated plates at 20-30°C, investigate the colonies appearance after 48 and record the results.

There are many selective media that could be used such as:-

- Pepton-pentachloronitrobenzene (PCNB) agar and V-8 juice-dextrose-yeast extract agar (VDYA)-PCNB, for the selective isolation of *Fusarium* spp.
- Pimaricin-vancomycin-PCNB (P 10 VP) and hymexazol (3-hydroxy-5- methylisoxazole, HMI) containing P 10 VP or PDA at concentrations of 25 to 50 µ m/ml, together with various antibiotics for isolation of *Phytophthora*

Isolation of soil bacteria

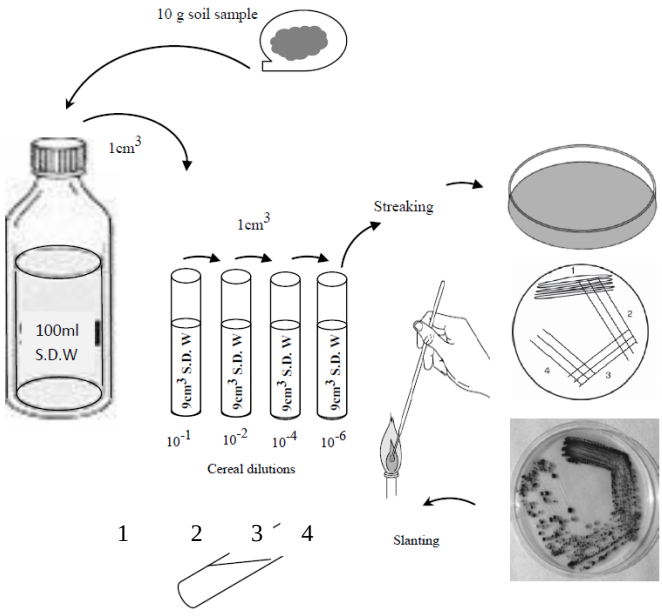
Objective:-

Learning how to isolate soil bacteria.

Materials:

Soil samples.
Water agar medium.
Sterilized Petri plates.
Bacterial loop.
Sterilized distilled water in test tubes (9cm water/each).

Procedure:



Isolation of soil bacteria using dilution technique.

1- Suspend 10 grams of air-dried powdered soil in 100 ml sterilized distilled water and shaken for 15 minutes.
2- Make cereal dilutions of previous stock in glass test tubes.
3- Take one loop of the soil dilution to inoculate agar plates.
4- Use the latest two dilutions.
5- Incubate the inoculated plates at 30°C for 24 hours.
6- Investigate your plates and write a detailed report.

Pure culture technique

A pure culture is the basis for diagnosis, identification and all further work of microbiological studies. For pure culture, all works should be performed under aseptic conditions (in a flow cabinet, if possible) to prevent any contaminating microorganism to reach the medium.

Fungal isolates:

Objective:-

Learning the purification techniques of fungal isolates.

Materials:

Fresh fungal culture.
Water agar medium.
Sterilized Petri plates.
Bacterial loop.
Sterilized distilled water in test tubes (9cm water/each).

Procedures:

There are two different methods to obtain fungal pure culture from the wild isolates.

Single spore culture:

This method used in sporulating fungi such as *Fusarium*, *Cladosporium* and *Cercospora* as a following:-

1. Cereal dilution of spore suspension is prepared from previously plated fungal isolate using sterile distilled water.

2. Solidified water agar plates are inoculated by droplets from the last two dilutions.
3. Golf stick like glass road is used to spread the spore suspension droplet onto the agar surface. Bacterial loop could be also used to streak spore suspension onto the agar surface.
4. Inoculated plates are investigated under light microscope to locate single spore.
5. Marked single spore is transferred to the surface of new agar plate and incubated at 25-30 °C for 12-24h to germinate and start to grown into new single colony.
6. Obtained single spore isolates are immediately slanted and preserved.

Hypal tip culture:

This method used in unsporulating fungi or that producing very few spores.

- By the aide of dissecting microscope, most freely grown hyphal tip are cut from the margin of previously plated fungal isolate and transfer to new water agar plates.
- Inoculated plates are incubated at 25-30 °C for 24-72h until reaching a new single colony.
- Obtained colonies are immediately slanted and preserved as pure cultures.

Bacterial isolates:

Objective:-

Learning the purification techniques of bacterial isolates.

Materials:

Fresh bacterial culture.
Water agar medium.
Sterilized Petri plates.
Bacterial loop.
Selective media.
Sterilized distilled water in test tubes (9cm water/each).

Procedure:

Bacterial pure culture could be obtained from mixed culture by using:-

- Single colony selection.
- A selective medium such as mannitol salt agar is used to select *Staphylococcus* as a selective medium, MacConkey agar is a selective medium for *E. coli*
- A differential medium is designed to show visible differences between micro-organisms such as different bacteria producing different colours of colonies.

Single colony selection:

1. To obtained pure culture isolate, one separate colony must choose from the latest streaked plate of such bacterial isolate.
2. Separate chosen colony is transferred to a new nutrient agar plate and streaked by sterilized loop.
3. One loopful is used for each plate and streaking must be in one way direction. Streaked plates are incubated at 37 °C for 12-24h or until colony appear. If this colony was developed from one cell, only one type of colony will develop few days after streaking onto the nutrient agar surface.
4. If only one type of colony was developed onto the nutrient agar plate, pure culture was obtained and must transfer to a fresh nutrient slant.

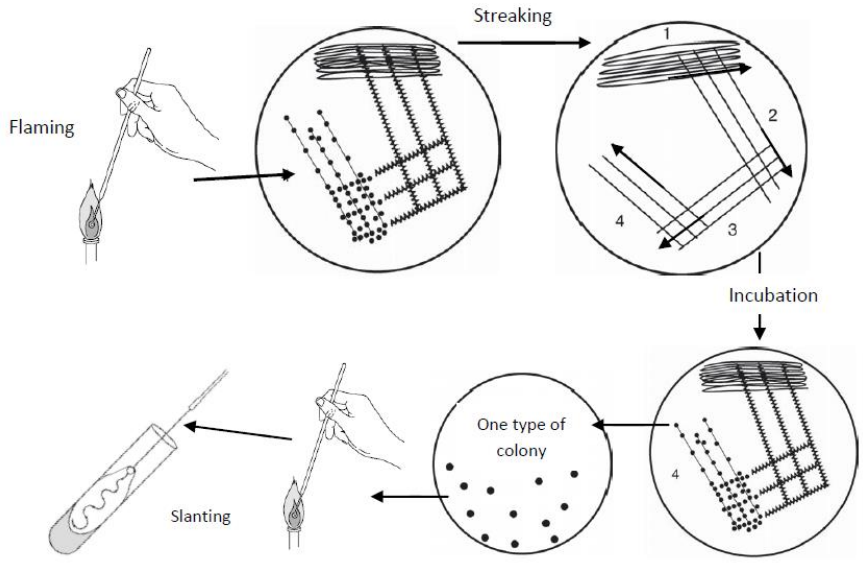


Diagram explain how can pure bacterial culture obtain

Lab 6

Preparing of microbial slides, Gram Staining

Objective: Microscope examinations of microorganisms require some preparation to get a clear and informative vision. Prepare of stained slide is one of the most common tool in this particular.

Bacterial staining:

Simple Stains

Bacterial shapes and activities could be observed under a cover glass without staining, but forming a complete idea about their morphology is so difficult. Bacterial cells are minute and tend to be transparent, even when magnified. So the simplest method for examining them is to make stain preparations for microscopic study.

Objective:

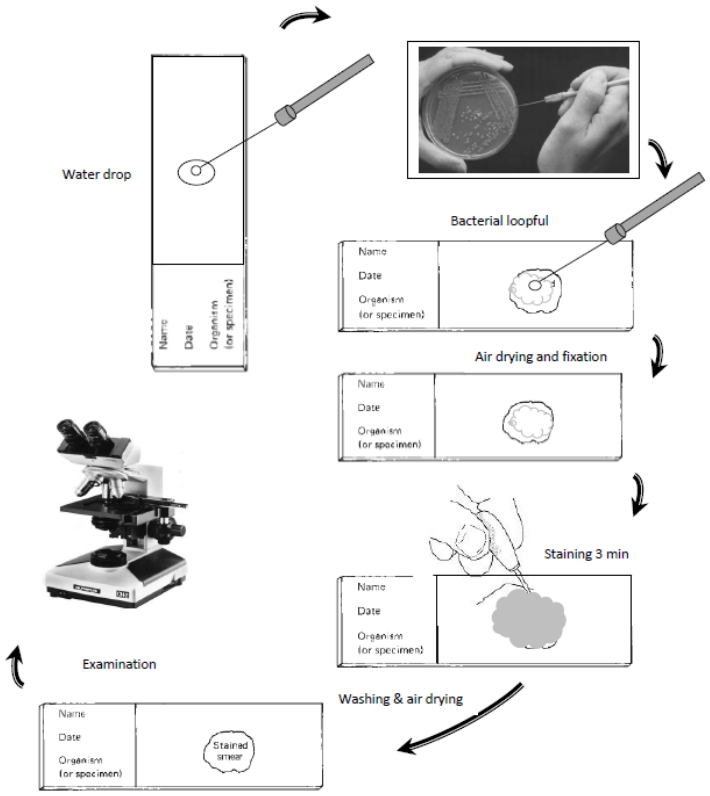
To learn the simple stain technique and its value in studying basic microbial morphology.

Materials:

Alcohol solution.
Glass slides and cover slips.
Bacterial loop.
Sterilized distilled water.
Staining rack
Methylene blue.
Safranin.
Compound microscope

Procedures:

1. Wash appropriate numbers of glass slides by dipping in alcohol and polishing with tissue or soft cloth.
2. Take three clean slides, label them and make a circle (about 1 cm in diameter) in the center of each one using a fine marker.
3. Turn the slides over so that the unmarked side is up and with your inoculating loop, place a loopful of water in the ringed area of the slide.



Simple staining

4. Take loopful of bacterial growth and mixing it (using proper aseptic transfer techniques) in the water and spread it out. Repeat this step until make a smear of bacteria.
5. Allow the smears to air dry until you see a thin white film on each slide. If not, add another loopful of water and more bacteria as in step.
6. Heat-fix the smears by passing the slides rapidly through the Bunsen flame three times so that the smears will not wash off. (Fixing could be done by flooding the slides with absolute alcohol on a staining rack for one minute, then alcohol is drained off and the slides air dries completely).
7. Place the slides on a staining rack and flood them with methylene blue. Leave the stain on for three minutes.
8. Wash each slide gently with distilled water, drain off excess water, place the slides on bibulous paper, and let them dry completely in air.
9. Prepare another set of slides as described above (steps 2-8) but stain them with safranin for three minutes instead of methylene blue.
10. Wash, drain, and dry the slides as in step 8.
11. Examine all slides, microscopically and record the results in lab report.

Gram Stains

Bacteria morphology could be determined using the simple staining, but further staining method is required to distinguish between bacteria of similar morphology. Gram stain (differential staining) could be used in this particular. It stains gram positive bacteria by violet color wile gram negative by red color.

Objective:

To learn the gram stain technique and its value in distinguishing bacterial groups.

Materials:

Alcohol solutions (70-95%).

Glass slides and cover slips.

Bacterial loop.

Sterilized distilled water.

Staining rack

Crystal violet.

Gram's iodine

Safranin.

Bibulous paper

Compound microscope

Procedures:

1. Take for clean slides, mark them and make a circle (about 1 cm in diameter) in the center of each one using a fine marker.
2. Turn the slides over so that the unmarked side is up and with your inoculating loop, place a loopful of water in the ringed area of the slide.
3. Take loopful of bacterial growth and mixing it (using proper aseptic transfer techniques) in the water and spread it out. Repeat this step until make a smear of bacteria.

4. Allow the smears to air dry until you see a thin white film on each slide. If not, add another loopful of water and more bacteria as in step.
5. Heat-fix the smears by passing the slides rapidly through the Bunsen flame three times so that the smears will not wash off. (Fixing could be done by flooding the slides with absolute alcohol on a staining rack for one minute, then alcohol is drained off and the slides air dries completely).
6. Flood the slides with crystal violet (Leave for one minute) then wash off with tap water.
7. Flood with Gram's iodine (Leave for one minute) then wash off with tap water
8. Wash with alcohol (95% for 10–20 seconds) until no more color washes off (avoid over washing) then wash off with tap water.
9. Apply safranin (for one minute) then wash off with tap water.
10. Drain off excess water, place the slides on bibulous paper, let them dry completely in air (just before you examine the preparation microscopically). Label the slides when become dry.
11. Examine all slides under oil with the oil-immersion objective and record your observations in lab report.

Lab 7

Midterm Exam 1

Lab 8

Isolation and purification of fungi from diseased plant parts

Objective:-

Learning how to isolate plant pathogenic fungi from diseased plant parts.

Materials:

Diseased plant sample.
Sterilized filter papers.
Sodium hypochlorite solution 5%.
Water agar medium.
Sterilized Petri plates.
Sterilized distilled water.

Procedures:

The general procedures for isolation of any fungi from diseased plant materials are as follows:-

1. Use running tap water to wash plant materials for at least 30 min.
2. Select the most freshly infected plant parts (from diseased plants), sterilize the selected parts using 5% sodium hypochlorite solution or 70% ethanol for 30 sec to 3 min.
3. Cut each surface sterilized plant part into tissue segments of less than 5 mm.
4. Transfer prepared tissue segments to plated isolation media using sterilized forceps (3-4 segments/9cm. plate).
5. Incubate cultured plates at the appropriate temperature (25-30°C) for 1 to 7 days.
6. Investigate the incubated plates regularly because fungal hyphae may be elongated from the plated tissue segments within a few days.
7. Transfer any appeared hyphal tip to a new agar plate as soon as possible to avoid any contamination.

Lab 9

Fungal staining

Objective:

To learn the simplest preparation technique of stained fungal slides and its value in fungal identification.

Materials

Fungal cultures on Sabouraud dextrose agar (*Aspergillus, Penicillium, Rhizopus & Saccharomyces*).

Clear tape.

Glass slides.

Lactophenol cotton blue (for staining molds).

Light microscope.

Miscellaneous supplies.

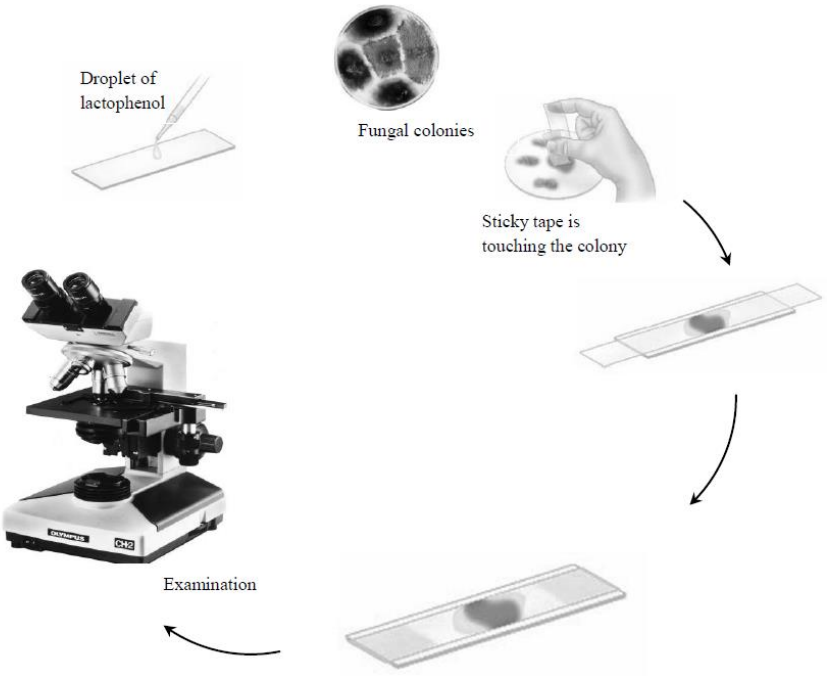
Lens paper.

Procedure:

1. Examine the plated cultures of the four fungi, recording all colony characters that may help recognizing fungal genes.

2. After examining the colonies, make a pressure tape preparation of the 4 fungal cultures as following:

a. Place a drop of lactophenol cotton blue on the center of the slide using a fine dropper.



b. Hold a piece of clear sticky tape in a U-shape, sticky side down as illustrated in the fig.

c. Touch the surface of a fungal colony carefully by the sticky tape.

d. Place the tape sticky side down in a lactophenol cotton blue drop.

e. Fold extra length of tape around edges of slide.

f. Examine this preparation using the light microscope then record your observations.

Lab 11

Physical and chemical factors affecting growth and activities of microorganisms.

Effect of temperature:-

Objectives:

1. Understand how microorganisms are affected by the temperature.

2. Carry out same experiments to differentiate between microorganisms based on temperature sensitivity.

Materials:

Fresh fungal culture.
Fresh bacterial culture.
Agar medium.
Sterilized Petri plates.
Incubators.

Procedure:

1. Prepare appropriate plated agar media for bacteria and another for fungi.

2. Inoculate each plate set with appropriate organism using aseptic technique. Use 3 replicate plates for each organism to be incubated in the same temperature.

3. Label each plate with the name of tested organism, your name, and date.

4. Incubate the inoculated plates for (24 to 48 hours for bacteria and up to one week for fungi) at 4°, 23° to 25°, 60°, 85°, or 100°C.

5. Investigate the incubated plates periodically for the presence of growth. Record your observations; use a + for the presence of growth and a – for the absence of growth.

Effect of pH:-

Objectives:

1. Understand how microorganisms are affected by the pH.

2. Carry out same experiments to differentiate between microorganisms based on pH sensitivity.

Materials:

Fresh fungal culture.
Fresh bacterial culture.
Agar medium.
Sterilized Petri plates.
Incubators.

Procedure:

1. Prepare appropriate agar media for bacteria and another for fungi with pH degrees of pH 3, 5, 7 and 9.
2. Inoculate each plate set with appropriate organism using aseptic technique. Use 3 replicate plates for each organism to be inoculated in the same pH.
3. Label each plate with the name of tested organism, your name, and date.
4. Incubate the inoculated plates for (24 to 48 hours for bacteria and up to one week for fungi) at appropriate temperature.
5. Investigate the incubated plates periodically for the presence of growth. Record your observations; use a + for the presence of growth and a – for the absence of growth.

Effect of chemical disinfectant:-

Objectives:

1. Understand how microorganisms are affected by the chemical disinfectant.

2. Carry out same experiments for different purposes.

Materials:

Fresh fungal culture.
Fresh bacterial culture.
Agar medium.
Sodium hypochlorite solution 5%.
Sterilized Petri plates.
Incubators.

Procedure:

1. Prepare appropriate agar media for bacteria and another for fungi.
2. Inoculate each plate set (4 plates) with appropriate organism using aseptic technique.
3. For bacteria streak the surface of four agar plates with bacterial isolate using a sterile cotton swab. The swab is immersed in the culture tube, and the excess culture is squeezed on the inner side of the test tube. Allow the culture to dry on the plate for 5 to 10 minutes at room temperature with the top in place.
4. For fungi use appropriate concentration of fungal spore suspension to be used either by cotton swab as described above or by transfer droplets of the spore suspension using sterile micropipette and spread these droplets with golf stick shape glass road.
5. Put five (4mm) filter paper disks onto the surface of inoculated plates; gently press the disk with flamed forceps.
6. Saturate each filter paper disk with 25 µl of 5% sodium hypochlorite.
7. Label each plate with the name of tested organism, your name, and date.
8. Incubate the inoculated plates for 24 to 48 hours at appropriate temperature.
9. Investigate the incubated plates periodically recording the inhibition zones.

Lab 11

Microbial enzymatic activities

Some microorganisms capable of hydrolyze large organic molecules due to enzymatic activity and then use the component parts in further metabolic processes.

Amylase activity:

Starch is a polysaccharide that is hydrolyzed by amylase producing microorganisms. When iodine is added to the *intact* starch molecule, a blue-colored complex forms. If starch is hydrolyzed into simple sugars i.e. glucose and maltose, however, no color reaction is seen.

Objective:

To recognize kind of microbe enzymatic activities.

Materials:

Starch agar plates - Fresh culture of *Aspergillius niger*- Gram's iodine solution

Procedures:

1. Prepare starch agar plates. Invert each one, and divided it into two equal sections with marking pencil.
2. Inoculate one section with 6mm plug of *Aspergillius niger* and another section with water agar plug as a control.
3. Label each section of the plate correctly and incubate treated plates for three days at 28°C.
5. When the cultures have grown, drop Gram's iodine solution onto the plate until the entire surface is lightly covered.
6. Record your investigations in lab report.

Results:

Urease activity: -

Some bacteria produce urease enzyme and split urea molecule, releasing carbon dioxide and ammonia. When bacterial cells that produce urease are grown in this medium, urea is degraded, ammonia is released, and the pH becomes alkaline. This pH shift is detected by a change in the Phenol red color from orange-pink to dark pink.

Objective:

To observe the activity of urease and to distinguish bacteria that produce it from those that does not.

Materials:

Urea broth or urea agar medium and *Proteus vulgaris* culture.

Procedures

1. Inoculate a tube of urea broth or agar with the *Proteus* culture. Include un-inoculated treatment.
2. Incubate the tubes at 35°C for 24 hours.

5. When the cultures have grown, drop Phenol red solution in the broth or onto the plate until the entire surface is lightly covered.
6. Record your investigations in lab report.

Results:

Lab 12

Midterm Exam 2

Lab 13

Antibiotic production

Production of antibiotic by *Acremonium chrysogenum*

Objective: learning the simplest method of antibiotic production by fungi.

Materials:

Inoculum medium*:* composed of (g/l): soluble starch 15.0, yeast extract 4.0, dipotassium hydrogen phosphate 1.0, magnesium sulphate 1.0 and pH 7.0.

Substrate*:* Sugarcane bagasse, dried in the open air sunlight for 2 days and grounded to a fine particles.

Salt solution: composed of was (g/l): dipotassium hydrogen orthophosphate 0.5, magnesium sulphate 0.5, ferrous sulphate 0.01 and sodium chloride 0.5.

a. Inoculation

1. Transfer 5 ml of suspension; prepared from a 72-hour-old *Acremonium chrysogenum* slant culture to 250 ml Erlenmeyer flasks containing 45 ml sterile inoculum medium.

2. Incubate the flasks on a rotary shaker at 220 rpm /30^{O}C for 2 days.

b. Solid state fermentation

1. Place 10 g of substrate in 250 ml Erlenmeyer conical flasks with 1 ml of salt solution, then add distilled water to adjust the final moisture content to 60 %, then mixe thoroughly and autoclave at 121 ^{O}C for 15 min.

2. Inoculate the flasks, after cooling to room temperature, with 5% inoculum level (108 spores/ml).

3. The contents should mix thoroughly and incubate at 30 ^{O}C for one week in a slanting position to provide maximum surface area (Adinarayana *et al.* 2003).

Antibiotic extraction

1. Treat the biomass (at the end of fermentation) with 50 ml of distilled water and agitate it thoroughly on a magnetic stirrer for 30 min.
2. Filter the whole contents through muslin cloth.
3. Treat the residue again with another 50 ml of distilled water, in the same way and filter it.
4. Pool the filtrate, centrifuge and use the clear supernatant as the antibiotic source.

Antibiotic assay

5. Seed nutrient agar plates with *S. aureus*, then put five (4mm) filter paper disks onto the surface of seeded plates; gently press the disk with flamed forceps.
6. Saturate each filter paper disk with 25 µl of the clear supernatant (antibiotic source).
7. Label each plate with the name of tested organism, your name, and date.
8. Incubate the inoculated plates for 24 to 48 hours at 35 ^{o}C.
9. Investigate incubated plates and record notifications.

Lab 14

Isolation of microorganisms from the Human Body

Objectives:

1. Isolate pure cultures of bacteria from throat, skin, and rectal area

2. Learn the techniques of isolation of the human body microbe.

Materials:

Blood agar plates.
Wax pencil.
Bunsen burner.
Inoculating loop.
35°C incubator.
Gram-stain reagents.
Disinfectant for swabs and.
Tongue depressors test-tube rack or can.

Throat Culture:

1. Use a sterile tongue depressor to keep the tongue from interfering with the swab.

2. Using a sterile cotton swab, take a swab from the throat around the tonsillar area (not the roof of the mouth).

3. Roll the swab back and forth near one edge of a blood agar plate, then streak from this area with a sterile loop.

4. Label the plate using the wax pencil, with your name, body site, date, and type of medium.

5. Incubate the plate, inverted, at 35°C for 24 to 72 hours.

6. Investigate the incubated plate for the various types of colonies. Characterize the colonies.

7. Select a number of well-isolated colonies. Stain each colony you select with the Gram stain.

8. Record your notification.

Skin Culture:

Materials:

Tryptic soy agar (TSA) plates.

Sterile swabs tube of 0.85% saline (NaCl).

Wax pencil.

Bunsen burner.

Disinfectant for swabs and.

Procedure:

1. Take a swab, moisten it in 0.85% saline (squeeze out the excess fluid by pressing the swab against the side of the tube) and roll it over your arm.
2. Streak a TSA plate, label the inoculated plates.
3. Keep the plates inverted, at room temperature for 24 to 72 hours.
4. Investigate the incubated plate for microorganisms that have been isolated from the skin. If no colonies grew after 24 hours, incubate for an additional 24 to 48 hours.
5. Select isolated colonies, describe their appearance, do a Gram stain and record your notification.