INTRODUCTION

The goal of every laboratory is to expose students to the wide variety of life in the microbial world. Although the study of microbiology includes bacteria, fungi, viruses, algae and protozoa, this MCB 206 session will concentrate primarily on the bacteria, fungi, and protozoa.

MCB 206 is a 4 credit unit course that will explore the central roles of microorganisms in nature and in our daily lives. It demonstrates the standard microbiological techniques that will allow us to investigate the structure and physiology of microorganisms.

The purpose of this manual is to provide students with good techniques in practical microbiology to ensure that investigations proceed safely and achieve the required educational aims successfully.

The objectives of this course are to...

- Become proficient in laboratory skills and safety protocols.
- Learn to follow experimental procedures.
- Begin to gain proficiency in scientific writing (laboratory reports and notebook entries)
- Embark in active learning opportunities in the laboratory.
- Demonstrate good lab citizenry and the ability to work with others.

GENERAL LAB SAFETY RULES

The following are rules that relate to almost every laboratories

1. Before entering the laboratory, remove coats, jackets, and other ornamental objects
2. Before entering laboratory, each student should wear lab coat and appropriate shoes
3. Do not perform activities in the lab until you are given instructions by your laboratory instructor.
4. Be sure to read all fire alarm and safety signs.
5. Make sure you know where your lab's safety equipment's are kept—including first aid kit(s) and fire extinguishers. etc.
6. Make sure you are aware of lab's emergency exits
7. Do not chew gum, drink, or eat while working in the lab.
8. Laboratory glassware should never be utilized as food or beverage containers.
9. Never use lab equipment that you are not approved or trained by your supervisor to operate.
10. If an instrument or equipment fails during use, or isn't operating properly, report the issue to a technician right away. Never try to repair an equipment problem on your own.
11. Do not work alone in the lab.
12. Never smell or taste chemicals.
13. Do not pipette by mouth.
14. Report all injuries, accidents, and broken equipment such as glass to the technician.
15. Decontaminate all affected equipment.

LAB REQUIREMENTS

BASIC PREREQUISITES OF MICROBIOLOGY LABORATORY

i. Correctly setting up and focusing the microscope

ii. Proper handling, cleaning and storage of the microscope

iii. Correct use of all lenses

iv. Recording microscopic observations

GENERAL RULES FOR SAMPLING

Planning ahead is essential when embarking on microorganism investigations. There are five areas for consideration.

- Preparation and sterilization of equipment and culture media.
- Preparation of microbial cultures as stock culture for future investigations and inoculum for the current investigation.
- Inoculation of the media with the prepared culture.
- Incubation of cultures and sampling during growth.
- Sterilization and safe disposal of all cultures and decontamination of all contaminated equipment

LIST OF EQUIPMENTS/APPARATUS USED IN MICROBIOLOGY LABORATORY

1. Anaerobic jar
2. Autoclave
3. Aspirator bottle
4. Bijou bottles
5. Bunsen burner
6. Candle jar
7. Centrifuge
8. Colony counter
9. Culture media
10. Deep Freezer
11. Drying Oven:
12. Forceps
13. Funnel:
14. Heating mantle
15. Homogenizer
16. Hot air oven
17. Hot plate
18. Incubator
19. Laminar Air Flow/ Laminar Hood
20. Magnetic Stirrer
21. Micro plate reader
22. Microscope
23. Microscopic slides
24. Microwave oven
25. Orbital Shaker
26. Petri dishes
27. pH meter
28. PRC Machines
29. Spectrophotometer
30. Spirit lamp
31. Staining solutions
32. Sterile cups
33. Swap stick
34. Syringes
35. Test Tube Holder:
36. Test tubes Racks
37. Test-tubes
38. Thermometer
39. Vortex mixture
40. Volumetric Glass
41. Non Volumetric glass
42. Water baths
43. Water distiller
44. Weighing Balance
45. Wire loop

DESCRIPTION

1. Autoclave

An autoclave is a pressurized chamber used for the sterilization and disinfection of medical and laboratory equipment's. Autoclave is also used for the preparation of culture media used for the isolation and identification of microorganisms. An autoclave combines three factors: time, pressure and steam. The temperature and time used when autoclaving is 121oc for 15 minutes. No any pathogenic bacteria can survive after autoclaving at the stated temperature and time. The figure of a portable autoclave is given in Fig. 1.

Fig. 1.

2. Anaerobic jar

An anaerobic jar is an equipment used in Microbiology laboratory which provide anaerobic environment for the cultivation of obligates anaerobes such as *Clostridium* spp. The figure of different anaerobic jar is given in Fig. 2 and 3.

Fig. 2

Fig. 3

3. Aspirator bottles

Aspirator bottle is a reservoir used for dispensing distilled water and for a large scale fermentation. Figure 4 shows a typical Aspirator bottle.

Fig. 4

4. Bunsen burner

A Bunsen burner named after Robert Bunsen is a standard tool used in microbiology laboratories for processes like sterilization of wire loop during inoculation, heat-fixing of smears and creating a sterile zone for aseptic operation. A Bunsen burner is shown in Fig. 5

Fig. 5

5. Bijou bottles

Bijou bottles are a glass containers used for storing microbial isolates as stock culture for future investigation. Figure 6 shows different size of Bijou bottles.

Fig. 6

6. Culture media

A culture media is any medium that contain nutrients which supports the growth of microorganisms. It can either be general purpose media such as Nutrient agar, selective media such as *Salmonella and Shigella* agar, differential media such as mannitol agar and enrichment media such as blood agar. Figure 7, 8 and 9 shows solid and liquid culture media, Nutrient broth and agar.

Solid Culture Media

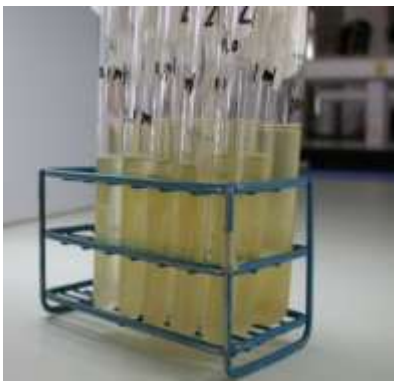

Fig. 7

Liquid Culture Media

Fig. 8

Nutrient Broth and Agar

Fig. 9

7. Candle Jar

A Candle jar is an instrument used for the growing of microaerophilic bacteria that require low concentration of oxygen to survive. The figure of candle Jar is given in Fig. 10 below.

Fig. 10

8. Centrifuge

A centrifuge is a device that allows the rotation of a liquid sample. It is mostly used in microbiology laboratory for separation of serum from blood. The figures of different Centrifuge machines are illustrated in Fig. 11 and 12.

Fig. 11

Fig. 12

9. Colony counter

A Colony counter is an equipment used to estimate or count the number of bacterial colonies on an agar plates. Figure 13 shows a typical Colony counter.

Fig. 13

10. Deep Freezer

Deep freezers are used in microbiology laboratories to storing and preserving medical equipment's, cultures, media and blood samples for a very long period of time. A very common Deep freezer is given in Fig. 14.

Fig. 14

11. Drying Oven:

Drying oven is a device used in microbiology laboratory for the drying of glassware's, after proper cleaning and rinsing with distilled water at 100°C till the glassware's dry up completely. The figure of Drying oven is given in Fig. 15

Fig. 15

12. **Funnel**:

Funnels are instrument used in microbiology laboratory for filtration. The figure of funnels oven is given in Fig. 16

Fig. 16

13. **Forceps**

Forceps are often used for holding or removing tissues that are too small for hand or fingers. Figure 17 shows a typical Forcep.

Fig. 17

14. **Homogenizer**

A Homogenizer is a device used in laboratories for extraction and purification of different macromolecules like proteins, nucleic acids, and lipids. Figure 18 shows a typical Homogenizer popularly in Microbiology laboratory.

Fig. 18

15. Hot plate

A Hot plate is an equipment used in microbiology laboratories for heating media, chemicals or reagents in a conical flask or beaker. Figure 19 and 20 shows different Hot plates.

Fig. 19

Fig. 20

16. Hot air oven

Hot air oven is used in microbiology laboratory for sterilization of medical equipment's and laboratory glass wares such as test tubes, pipettes and glass petri dishes using dry heat sterilization at 180°C for 3 hours. The figure of Hot air oven is given in Fig. 21.

Fig. 21

17. Heating mantle

Heating mantle is a device used in microbiology laboratory for heating or melting samples and reagents in a beaker. The figure of Heating mantle is given in Fig. 22

Fig. 22

18. Incubator

An incubator is an equipment used in the microbiology laboratories for the growth and maintenance of microorganisms and cultures. Incubator provides an optimal temperature, pressure and moisture required for the growth of microorganisms. Figure 23 shows an Incubator popularly in Microbiology laboratory.

Fig. 23

19. Laminar Air Flow/ Laminar Hood

It is a device used in microbiology laboratory that allows the flow of a sterile air. It used during inoculation and pouring of prepared culture media in to Petri dishes in other to avoid microbial contamination. The figure of a Laminar air flow is shown in Fig. 24.

Fig. 24

20. Microscope

Microscopes are devices that are used to view microorganisms that cannot be seen with naked eye. There are many different types of microscopes, these are

a. **light microscope** also called a compound microscope because it contains two types of lenses that function to magnify an object.
b. **Fluorescent** microscope uses ultraviolet light as its light source
c. **Electron** microscopy which uses beam of electrons.
d. **Scanning** electron microscope
e. **Transmission** electron microscope

Figure 25 shows a typical Light or Compound microscope.

Fig. 25

21. Magnetic Stirrer

A Magnetic Stirrer is a device with a heating system commonly used in microbiology laboratories for the purpose of mixing various components of liquids. The figure of Magnetic Stirrer is given in Fig. 26

Fig. 26

22. Microscopic slides

Microscopic slides are used to prepare specimen for microscopic examination. Figure 27 shows a bunch of Microscopic slides.

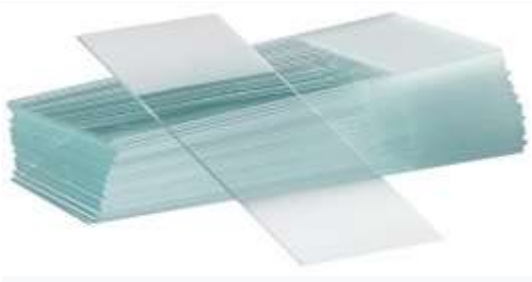

Fig. 27

23. Micro plate reader

A microplate reader is used to detects light signals produced by samples which have been pipetted into a microplate. Figure 28 shows a typical Micro plate reader.

Fig. 28

24. Microwave oven

Microwave is an equipment used in microbiology laboratory for heating samples, preparing solutions, drying and heating samples or products. Figure 29 shows a typical Microwave oven.


Fig. 29

25. Petri dishes

Petri dishes are used for culturing and inoculation of the culture of microorganisms. Figure 30 shows a set of Petri dishes.


Fig. 30

26. Orbital Shaker

An orbital shaker is an instrument used in microbiology laboratory for mixing and blending of substances in a tubes or flasks by shaking them. The figure of orbital shaker is shown in Fig. 31


Fig. 31

27. pH meter

A pH meter is an equipment used to measure the acidity or alkalinity of a solution. Figure 32, 33 and 34 shows different pH meter.

Fig. 32

Fig. 33

Fig. 34

28. PCR Machines

A PCR machine are used in microbiology laboratory to detect and identify trace bits of DNA, from a virus or bacteria, diagnose an infection, or for forensic examination. The figure of PCR machine is shown in Fig. 35

Fig. 35

29. Spectrophotometer

A Spectrophotometer is a device used in a microbiology laboratory for the measurement of concentration of protein, nucleic acids, bacterial growth, and enzymatic reactions. Figure 36 and 37 shows different version of Spectrophotometer.

Fig. 36

Fig. 37

30. Spirit lamp

Spirit lamp is similar to Bunsen burner but it is portable. It is used to sterilized wire loop during inoculation The lamp must be covered with a lid when not in use to prevent loss of spirit. The figure of Spirit lamb is given in Fig. 38.


Fig. 38

31. Syringes

Syringes are used in microbiology for collection of blood samples from patients and for making serial dilutions. The figure of Syringes is shown in Fig. 39.


Fig. 39

32. Swap stick

Swap stick are used for collection of specimen for microbiological analysis. The figure of Swap sticks is illustrated in Fig. 40.


Fig. 40

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33. Sterile cups

Sterile cups are containers used for holding and transporting of patient samples such as urine and sputum for microbiological analysis. The figure of a Sterile cup is demonstrated in Fig. 41.

Fig. 41

34. Staining solutions

Staining solutions are reagent used to differentiate microorganism either gram positive or gram negative bacteria and so on. E.g gram staining reagents, zhiel-nilsen staining reagents, and endo spores staining reagents. The figure of Staining solutions reagent is illustrated in Fig. 42.


Fig. 42

35. Test-tubes

Test-tubes are used in microbiology laboratories for either collection of sample, heating of chemicals or growing of microorganisms. The figure of Test-tubes is given in Fig. 43.


Fig. 43

36. Test tubes Racks

Test Tubes Racks are used in microbiology laboratory for holding of test tube in the upright position. They are made of metal or plastics. Figure 44 shows a typical Test tubes racks made of wood.

Fig. 44

37. Test Tube Holder:

They are used for holding the test tubes during heating. Figure 45 shows Test tubes holder.

Fig. 45

38. Thermometer

Thermometer is an instrument used in microbiology laboratory for checking the temperature of a sample. Figure 46 shows a typical Thermometer.

Fig. 46

39. Volumetric Glassware:

Volumetric Glassware includes cylinders, pipettes, burette & volumetric flasks. They are used in measuring accurate volume of a liquid. The figures of Volumetric glass wares are given in Fig. 47, 48, 49 and 50.

Fig. 47

Fig. 48

Fig. 49

Fig. 50

40. Non-Volumetric Glassware

These includes Beakers and Conical flasks. They are used for heating liquid and for preparing reagent solution. Figures 51 and 52 shows Volumetric Glass wares.

Fig. 51

Fig. 52

41. Vortex mixture

A vortex mixture is a device used in microbiology laboratory for the mixing of fluid samples in a glass tubes or flasks. The figure of a typical vortex mixture is given in Fig. 53.

Fig. 53

42. Weighing Balance

Weighing Balance is an instrument used in microbiology laboratory for weighing of media and other chemicals precisely and quickly. Figure 54 shows an electrical Weighing Balance.

Fig. 54

43. Water bath

Water baths is a device used primarily in microbiology laboratory for heating samples under a controlled temperature. Figure 55 and 56 shows different water bath.

Fig. 55

Fig. 56

44. Water distiller

A water distiller is a device used in microbiology laboratories for the purification of water by a process of distillation. It is used to obtain distilled water required for the preparation of culture media. Figure 57 and 58 shows different Water distiller.

Fig. 57

Fig. 58

45. Wire loop

A wire loop is an instruments used in microbiology laboratory for picking colony from a culture of microorganisms growing on a media plates or a liquid media. It is also used for the cultivation of microbes on plates by streaking method. Figure 59 shows a typical Wire loop.

Fig. 59