PRACTICAL 2 MICROBIOLOGICAL APPARATUS AND EQUIPMENTS

Structure

2.1 INTRODUCTION

Practical 1 focussed on the use and working of a microscope. Practical 2 provides an orientation to the various basic equipments, i.e., instruments, tools, glassware and miscellaneous items used in the laboratory for microscopic examination, isolation, culturing, identification and maintenance of the microorganisms.

Objectives

After undertaking this practical, you will be able to:

- familiarize yourself with the basic equipments, instruments, tools and glassware used in laboratory,
- describe the principle and functioning of these materials, and
- appreciate the usage and care of these material.

2.2 MICROBIOLOGICAL APPARATUS AND EQUIPMENTS: A BASIC INTRODUCTION

Microbiology, as you may know by now, is a science dealing with study of structure, functions and applications of microbes. To study different aspects of microbes, microbiologists require certain equipments in the laboratory for isolation and culturing of these microorganisms in pure form. These equipments can be classified as:

(a) *Instruments required for sterilization:*

- Autoclave
- Oven
- Membrane filter assembly

(b) *Instruments and Tools required for isolation, culturing and maintenance:*

- Bunsen Burner or Spirit Lamp
- Water Bath
- Laminar Flow Safety Hood
- Incubators
- Refrigerators
- Inoculating loop or needle
- Magnetic Stirrer
- Vortex Mixture
- Balances
- Homogenizers`

(c) *Instruments and tools required for microbial observations and assays:*

- Microscope
- Centrifuge
- Spectrophotometer
- Quebec Colony Counter
- Camera Lucida
- Photo Micrographic Camera
- Dissecting Needles and Forcep
- Ocular and Stage Micrometer
- Burette Set Up
- Thermometer

(d) *Glassware:*

- Petri Dishes
- Conical Flasks
- Culture Tubes
- Beakers
- Funnels
- Graduated Cylinders
- Graduated Pipettes
- Dropper Bottle for staining reagents
- Glass Microscopic Slides
- Cover Slips

(e) *Miscellaneous items:*

- Test Tube Rack
- Ingredients for Culture Media and other Chemicals
- Non-absorbent Cotton and Gauze for Cotton Plugs
- Petri Dish and Pipette Cans
- Muslin Cloth
- Brown Paper and Rubber Bands
- Permanent Markers
- Rubber Bulb for Pipettes
- Blotting Paper, Lens Paper, etc.
- Distilled Water
- Discard Container
- Immersion Oil

Principle and working of some of these equipments has been discussed in following sections:

2.3 INSTRUMENTS REQUIRED FOR STERILIZATION

The word sterilization is derived from the Latin word *'Sterilic'* meaning unable to produce offsprings. Sterilization, therefore, is a process of making an object free from all living organisms either by *destroying* or *removing them* from the object. This control of microorganisms is very important in microbiological research, preservation of food, prevention of diseases and in various industries. Sterilization can be carried out either by employing:

(a) Physical Agents, or

(b) Chemical Agents.

These agents being microbicidal (i.e. kill the microorganisms) or microbiostatic (i.e. inhibit microbial growth) control the microorganisms by adversely damaging the essential cell structure and functions. The mode of action of these agents varies. You would realize that there are physical and chemical methods also to control microorganisms. Let us get to learn about these methods, next.

2.3.1 Physical Methods to Control Microorganisms

The physical methods to control microorganisms involve heat, filtration or radiations. Figure 2.1 illustrates these methods.

Figure 2.1: Physical methods to control microorganisms

Temperature influences microorganisms by altering their enzyme systems. Low temperature, you may recall studying in the theory Course, inactivates the enzymes while high temperature destroys them.

Heat is commonly employed for controlling microorganisms. Both moist heat and dry heat can be used for this purpose. Moist heat results in coagulation of proteins and degrades nucleic acid and may even disrupt cell membrane. Moist heat has more penetrability than dry heat and kills the cells more rapidly that too at a lower temperature. Microbes exhibit difference in their resistance to moist heat. Generally, bacterial spores require temperature above 100^{o}C for destruction. Moist heat can either sterilize or disinfect the object depending upon the temperature employed. Moist heat can be used either (1) at temperature <100^{o}C (pasteurization-used to remove pathogens), (2) as free flowing steam at 100^{o}C (by boiling or tyndallisation) or (3) by saturated steam under pressure (autoclaving).

Let us get to know about autoclaving, which uses moist heat method for sterilization.

1. *Autoclave:* For sterilization, steam under pressure is generally employed using an instrument called autoclave. Figure 2.2 illustrates the autoclave. Autoclave was developed by *Chamberland* in 1884. As you may have noticed in Figure 2.2, autoclave is a double walled cylindrical metal vessel made of stainless steel or copper. Autoclave lid is provided with the pressure gauze for monitoring the pressure, exhaust valve to remove the air and safety valve to avoid explosion during operation. The articles are kept loosely in the autoclave chamber and then water is boiled and steam is released into the autoclave's chamber. The exhaust valve is kept open till the air present in the chamber is out. The exhaust valve is then closed and pressure of the steam in the chamber is allowed to reach to the desired value. The temperature of the steam inside the chamber depends on the pressure in autoclave. Relation of autoclave pressure with the temperature of steam is presented in the Table 2.1.

Figure 2.2: The autoclave

Table 2.1: Relation of Autoclave pressure with the temperature of steam

Pressure (Pounds/square inch-psi)	Temperature (^{o}C)	Time (minutes)
0	100	-
5	108	> 20
10	115	20
15	121	15
20	126	10
30	134	3

More the pressure, higher is the temperature and less is the time used for autoclaving. The autoclave cycle presented in Figure 2.3 illustrates this concept. Generally, pressure of 15 pounds with temperature at 121^{o}C is employed for 15-20 minutes for autoclaving.

Figure 2.3: The autoclave cycle

Saturated steam heats an object about 2500 times more efficiently than dry heat at the same temperature. Steam condenses on the cooler surface of the object and transfer its heat energy to the object and sterilize it.

Autoclave can be used for sterilizing culture media, scalpel and other heat resistance instruments, glasswares, etc. but not for oils, powders and plastics.

Where autoclave is not available, pressure cooker can be used. It works on the same principle as of autoclave.

Note: Following points should be kept in mind while working with autoclave.

1. Autoclave should not be packed tightly otherwise steam won't be able to come in contact with every object in the autoclave.

2. The air initially present in autoclave chamber should be removed before closing exhaust valve, otherwise temperature won't reach to 121°C though the pressure would be 15 pounds.

3. For larger sample of liquid, autoclave time should be increased so that centre of the liquid should reach to 121°C.

4. After autoclaving, steam should be released slowly otherwise liquid media would come out.

Besides autoclave, hot air ovens are also used for sterilization. Let us get to know about hot air ovens.

2. *Hot Air Oven* : Hot air oven sterilizes the object by hot dry air. It kills the microorganisms by oxidizing cellular constituents. Dry Heat is less effective in killing microorganisms than moist heat. Higher temperature for a longer time period is used with dry air because it-

(i) has less penetration power, and

(ii) removes moisture from microorganisms and thus interferes with coagulation of microbial proteins.

Hot air oven can be used at different temperatures. Operating time depends on the temperatures used as highlighted in Table 2.2.

Table 2.2: Relation between temperature of hot air oven and the time period of sterilization

Temperature (°C)	Time (Hours)
140	3
150	2½
160	2
170	1

You would realize that the most commonly used temperature is 160°C for 2 hours.

We are all familiar with hot air ovens. Hot air oven contains an insulated cabinet kept at constant temperature by electric heating mechanism and thermostat. Look at Figure 2.4, which illustrates the hot air oven. Air keeps on circulating within the cabinet through a fan. For proper circulation of hot air, the shelves are perforated. An oven is used to sterilize glassware, corrodeable metal instruments, powder, oil etc. which can tolerate prolonged heat exposure but get spoiled by moist heat. However, it is not suitable for heat sensitive materials, like plastic and rubber items.

Figure 2.4: Hot air oven

Having learnt about the physical methods, next let us get to look at the chemical methods for control of microorganisms.

2.3.2 Chemical Methods for Control of Microorganisms

Different chemicals can be used which can act as disinfectant, antiseptics or even chemical sterilants. These are:

Ethylene Oxide	-	For plastic dishes and pipettes
Alcohols, e.g., Ethanol and Isopropanol	-	For disinfecting thermometer and small instruments
Halogens, e.g., Iodine and chlorine	-	For preoperative skin degerming, as disinfectant in hospitals, in dairy and food industry
Heavy metals, e.g., Silver, Copper	-	in lakes, swimming pools, in burns etc.
Phenols	-	Used in hospital nurseries
β-Propiolactone	-	For living tissues

With a knowledge of physical and chemical methods for control of microorganisms, we end our discussion about instruments/methods used for sterilization. Next, we shall get to know about instruments needed for isolation, cultivation and maintenance of microbes.

2.4 INSTRUMENTS NEEDED FOR ISOLATION, CULTIVATION AND MAINTENANCE OF MICROBES

A wide variety of instruments are available for isolation, cultivation and maintenance of microbes. These are illustrated and described in this section. We begin with bunsen burner.

1. *Bunsen Burner* - It is a type of gas burner that gives very hot flame by allowing air to enter at the base and mix with the gas. It is used –

   (a) for sterilizing inoculating loop/needle/forcep etc.

   (b) for sterilizing mouth of the flask, test tubes and other glass apparatus.

   (c) at the time of culturing or transferring the microorganisms to avoid contamination.

Here the sterilization is done with dry heat. If bunsen burner is not available, spirit lamp can be used. Figure 2.5 illustrates the bunsen burner and spirit lamp.

Figure 2.5: The bunsen burner and the spirit lamp

2. *Laminar Flow Hood (biological safety cabinet)* - Look at the Figure 2.6. It illustrates the laminar flow hood. As you can see, this biological safety cabinet contains HEPA (high efficiency particulate air) filters which remove, 99.97% of the particles having size more than 0.3 μm. Air is forced through these HEPA filters and a vertical column of sterilize air gets formed across the cabinet opening. It prevents the contamination of room and the workers from microorganisms. The cabinet also has UV light, which is switched on about 15-20 minutes earlier before and after the work is finished to make the working surface sterile. Laminar flow hood is employed in the research labs and in industries, for conducting assays, preparing media and culturing microbes.

Figure 2.6: Laminar flow hood

Note : (i) Never switch on the UV light while working in the laminar chamber.

(ii) Air flow should be on, at the time of working in the chamber.

3. *Incubator* - The growth of the microorganisms is altered by the chemical and physical nature of its surrounding. Most important environmental features altering microbial growth are pH, water activity, oxygen level and temperature.

Each microorganism has specific requirement of temperature for its growth. The cardinal temperatures, i.e., minimum, optimum and maximum growth temperatures, varies greatly with the type of microorganisms. Therefore, for cultivation of microorganisms, prime requirement is to provide the optimum temperature for growth. This can be achieved by using incubators and shaking water bath as illustrated in the Figure 2.7 and Figure 2.8 respectively.

An incubator is an insulated cabinet fitted with heating element at the bottom. It has perforated shelves and is provided with double door. Inner door is of glass to view the content of incubator. Like an oven, most of the incubators use dry heat for temperature control. Moist environment can be provided by keeping a beaker of water inside the cabinet. Incubator is used for culturing the organism at its optimum temperature. Here temperature can be adjusted according to organism's requirement and then maintained at a desired level thermostatically.

Figure 2.7: The incubator

Shaking water baths as illustrated in the Figure 2.8 can also be used for microbe's cultivation. Here also temperature is maintained thermostatically. However, it can be used only for liquid culture. The advantages of using shaking water baths are:

(a) Uniform and rapid transfer of heat to the culture can be obtained, and

(b) Enhanced aeration is possible because of agitation.

Figure 2.8: The water bath

4. *Magnetic Stirrer* – It can be used for mixing ingredients at the time of media or reagents preparation. Mixing happens with spinning of a teflon coated magnetic bar inside the container under a magnetic field created by magnetic stirrer. Figure 2.9 illustrates the magnetic stirrer.

Figure 2.9: The magnetic stirrer

5. *Vortex Mixture* - It is also used to mix the reagents during microbiological assay by vortex effect. Figure 2.10 illustrates the vortex mixture.

Figure 2.10: Vortex mixture

6. *Quebec Colony Counter* – It is used for counting the number of colonies in a plate. Culture plate is kept on the screen which is illuminated from beneath. Counting is done by using a magnifying glass (magnification 1.5X) above the plate as shown in Figure 2.11. Counting can also be done manually.

Figure 2.11: Quebec colony counter

7. *Centrifuge* – Centrifuge is used for separating the substance in liquid or concentrating the microorganisms on the basis of size or mass under centrifugal force (noted as rpm). Depending upon the speed limit, centrifuges may be –

   (a) Low Speed (Maximum Speed 5000 rpm)

   (b) High Speed (18000 rpm)

   (c) Ultra Centrifuges (20000 to 60000 rpm)

   The centrifuge consists of a head or rotor (of various sizes), which hold the tubes containing the material to be separated. The rotor is revolved by an upright motor. After completion of centrifugation, particulate matter settles down at the bottom of tubes and can be separated.

8. *pH meter* – pH is a negative logarithm of H^+ ion concentration. Its value remains between 0 and 14. Pure water has a pH of 7 (neutral). pH value less than 7 is acidic and more than 7 is basic. The measurement

of pH can be done by using pH meter. pH meter is used to measure and set the pH of culture media or reagents used for microbiological and biochemical assays. It is important as different microbes have different pH requirements for growth. Most of the bacteria, in general, have optimum pH for growth between 6.5 to 7.5, though there are certain exceptions. Some bacteria are acidophiles (grow at acidic pH) or alkalophiles (needs high pH in alkaline range for growth). Most fungi have pH optima around 4 to 6 and yeasts need pH around 3 to 5.

The pH meter has a glass electrode for measuring the pH. During determination of pH, first the instrument is calibrated with standard buffers of pH 4, 7 and 9. Then the pH of the sample solution is determined by dipping the glass electrode in the solution and pH is read directly from the pH meter scale.

9. *Refrigerator* – Refrigerator is a basic requirement in microbiological laboratory. It is used for maintaining microbial cultures, keeping media to prevent dehydration, for storing thermolabile solutions, antibiotics, serum and biochemical agents.

10. *Spectrophotometer* or *Colorimeter* – These can be used to measure the microbial growth or can be used in various microbial and biochemical assays. Figure 2.12 illustrates the spectrophotometer.

Figure 2.12: The spectrophotometer

The working principle of spectrophotometer is graphically presented in Figure 2.13.

Figure 2.13: Working principle of spectrophotometer

Spectrophotometer measures either the amount of light transmitted (% transmission) or the amount of light absorbed (A) by a sample, as you may have noticed from Figure 2.13. Here monochromatic light (beam of light at a single wavelength) is passed through a liquid sample. Depending upon the cells suspended in the culture, light is scattered. The scattering of light is directly proportional to the cell mass or indirectly to the cell number. More the number of cells, more would be scattering of light. The unscattered light is measured by a photoelectric cell and is recorded as 0% to 100% transmission (T). However, in practice, cell density is represented in form of optical density or absorbance because it

is directly proportional to the cell concentration while transmission is inversely related to cell density. Population growth can be easily measured spectrophotometrically as long as the population is high enough to give detectable turbidity. At very high concentration of cells, light scattered by one cell may be rescattered by another cell and it may appear as unscattered. This results in the loss of linearity between cell number and turbidity at very high cell concentration. Commonly used wavelengths for bacterial turbidity measurements are 540 nm (green), 600 (orange) or 660 nm (red). Estimation of cell number is based on turbidity measurement. Turbidity increases as the cell number increases.

The discussion so far focussed on the study of the apparatus/equipments required for the sterilization, isolation or cultivation of microbes. Next, we will review, the tools we would need for inoculating and culturing microorganisms in the laboratory.

2.5 TOOLS NEEDED IN MICROBIOLOGY LABORATORY FOR INOCULATION AND CULTURING

We begin our study on this topic by first defining the terms inoculation and culturing.

What is Inoculation and Culturing?

In microbiology, *inoculation* is the process where a microbe of interest is introduced into a previously sterilized growth medium for the purpose of growing a microbe in the laboratory and getting a pure culture of it.

Culturing is a process of growing microorganisms in a culture medium for study. We shall study about the culture media in the next practical.

Isolation and culturing of microorganisms, you would realize, requires aseptic transfer of the culture and use of inoculating loops, inoculating needles and pipettes. Let us get to know these tools.

Inoculating Loops and Needles – These are most commonly used tools for inoculation. The inoculating loop consists of insulating handle at the end of which inoculating wire is present. Inoculating wire is about 3 inches long and made of nichrome or platinum. It is a straight wire in case of inoculating needle but in inoculating loop end of the wire is bent round to form loop. Figure 2.14 illustrates the inoculating needle and loop.

Figure 2.14: Inoculating needle and loop

During inoculation, inoculating wire is sterilized by heating in blue (hottest) portion of the flame till it becomes red hot followed by cooling either by touching to petri dish cover or inner surface of culture tube to avoid killing of microbial cells. Only a small amount of culture is used for inoculation. This can be accomplished by touching a single colony on agar surface by inoculating needle or by taking a loopful of culture from broth medium. After inoculation, inoculating wire is again sterilized to destroy remaining microorganisms.

Caution:

1. Heat the inoculating wire till it becomes red hot.
2. Never use hot inoculating wire for culturing otherwise cells would be killed.
3. Don't dig/drag the agar surface for taking the inoculum.

Pipettes - Sterile glass pipettes or disposable pipettes can be used for transferring the known volume of liquid or culture aseptically. Sterilization of pipettes can be done by autoclaving after keeping them in canisters or by hot air oven after wrapping in brown paper. Look at Figure 2.15, which illustrates the pipette. Pipettes can be fixed volume (to-deliver) pipettes or blow out pipettes. These can carry different volumes.

Caution:

(1) Never do pipetting with mouth.

(2) For culturing, sterilized pipettes should be used.

(3) Never keep pipettes on working surfaces.

Figure 2.15: Pipette

Having studied about the apparatuses and equipments required in the laboratory to carry out the microbiology practicals, we still need to know about the glassware we would require. The next section focuses on the glassware used for culturing.

2.6 GLASSWARE USED FOR CULTURING

Glass test tubes, petri plates and conical flasks are used for microbial cultivation. Disposable petri plates can also be used. These glasswares are described herewith.

Glass Test Tubes – Sterile glass tubes are used for culturing microorganisms in liquid or solid media. Sterility in tubes is maintained by using cotton plugs developed by *Schroeder & Von Dusch* or by heat resistant plastic and metal caps, as shown in the Figure 2.16 (a). Metal caps have the advantage over the cotton plugs as: (i) these save labour, and (ii) slip on and off the test tubes easily.

Figure 2.16: Culture tubes

Solid media in test tubes can be used to make agar slants or deep tubes, as shown in the Figure 2.16 (b). Agar slants are prepared by allowing liquefied solid media to harden in the slanted position. These provide more surface area for culturing than deep tubes and are used for maintaining the pure microbial cultures. Agar slants have an advantage over the petri plate as: (i) it is easy to store and transport, and (ii) less readily contaminated.

Agar deep tubes are prepared by allowing liquified solid media to harden in the upright position as shown in Figure 2.16 (b). These are used to (i) study the gaseous requirement of microorganism, or (ii) preparing agar plates after liquefecation.

Petri Plates – Petri plates were discovered by *Richard Petri* in Ninteenth century (1887). It consists of two dishes. Look at the Figure 2.17 which illustrates the two dishes and petri plate can.

Figure 2.17: Petri plate and petri plate can

Bottom dish (base) is smaller and carry 20-25 ml of the culture medium. Upper plate (cover) is bigger and provides a loose cover to bottom dish to avoid contamination. Petri plates are available in different sizes but for routine purpose, generally, plate of 9-10 cm diameter is used. Liquefied molten solid culture medium is poured into the bottom plate and is allowed to solidify. As petri plates provide larger surface area, these are useful for isolation and cultivation of microorganisms.

Note: Storage and incubation of petri plates should be done in an inverted position to prevent dropping down of water droplets formed by condensation during solidification of media on to the surface of the hardened agar surface. This can spread the microorganisms on the agar surface, resulting in confluent growth instead of discrete colonies.

Conical Flasks – Conical flasks of different volumes can be used for media preparation and also for culturing large amount of microorganisms in liquid media.

With the study of the different apparatus, equipments and glassware required in the laboratory, we are ready to get started with the microbiology practicals.

Let us begin by first testing our knowledge about the equipment/apparatus/tools we have just studied about. Answer the questions given in the Review Questions section next. Subsequently, attempt the exercise given herewith as a part of this practical.

2.7 REVIEW QUESTIONS

1. What is sterilization?

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2. Name the methods used for sterilization.

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3. What is the principle of autoclaving and dry heating?

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4. At what temperature generally the sterilization is done using autoclave and hot air oven?

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5. What precautions should be followed while performing autoclaving?

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6. How does the laminar flow safety hood work?

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7. Why do we need incubators?

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8. What is the use of quebec colony counter?

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9. Write the principle of Spectrophotometer.

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10. Why O.D. is used for assessing the cell number?

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11. How inoculating loop and needle can be sterilized?

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12. What is the difference between agar slants and deep agar? Where these are used?

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13. Why petri plates are incubated in inverted position?

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14. What precautions should be taken while using pipettes?

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Now go ahead carry out the exercises.

EXERCISE 1

FUNCTIONING AND USE OF VARIOUS MICROBIOLOGICAL LABORATORY EQUIPMENTS

Date : .....

Aim : To study the functioning and use of various microbiological laboratory equipments.

Requirement:

List the different Equipments/Instruments/Tools/Glassware you would require for this exercise in the space provided herewith.

Procedure:

Now carry out this activity following the steps given herewith:

1. Observe each instrument (as mentioned above) carefully.
2. Learn its principle and see its working and use.
3. Try to sterilize and use inoculating loops and needles.
4. Prepare cotton plugs and try to sterilize different glasswares by autoclaving and using hot air ovens

Precautions:

1. Follow the instructions given with various instruments.
2. Place all the equipments and chemicals to their respective locations.

Observation and Results:

Hints:

1. Comment on the principle and functioning of the equipments/apparatuses.
2. Comment on sterilization and use of inoculating needles/loop.
3. Comment on sterilizing glassware in terms of time required etc. in the space provided herewith.

Submit the exercise for evaluation.

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Counsellor Signature