TECHNOCRATS

*Lab Work Book of*

# Pharmaceutical Microbiology

**(BP- 307 P)**

**Department of Pharmacy**

Lab Manual of

# Pharmaceutical Microbiology

**(BP- 307P)**

**Price : ₹ ...../-**

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TECHNOCRATS
PUBLICATIONS

*Lab Work Book*
*of*

# PHARMACEUTICAL MICROBIOLOGY

**(BP-307P)**

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

## Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

## Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

## Mission of the Institute

**M 1:** To inculcate ethical, moral, cultural and professional values in students

**M 2:** To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

**M 3:** State of art learning of professionalism by the faculty and students

**M 4:** To produce well learned, devoted and proficient pharmacists

**M 5:** To make the students competent to meet the professional challenges of future

**M 6:** To develop entrepreneurship qualities and abilities in the students

## PROGRAM OUTCOMES (POs)

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of thelimitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivationissues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well- being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

## PEOs

**PEO 1:** To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

**PEO 2:** To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

**PEO 3:** To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

**PEO 4:** To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

**PEO 5:** To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

## *Course Outcomes (COs):*

**Student will be able to:**

- CO1: Understand various accessories for microbiology practical.
- CO2: Develop basic skill in aseptic techniques.
- CO3: Perform various staining techniques.
- CO4: Isolate and identify microorganism form laboratory sample.
- CO5: Standard protocols in pharmaceutical industry – IP.

# Index

# Experiment No. –1

## OBJECT:

Introduction and study of different equipments and processing e.g. BOD, Incubator, laminar flow, aseptic hood, autoclave, microscopes used in experimental microbiology.

## REFERENCES:

Bhatt D.C, ”Pharmaceutical Microbiology Concepts and Techniques”, Birla Publications; Shahdara, Delhi, Page no-13.

Gaud R.S, Gupta G.D.,”Practical Microbiology”,Nirali Prakashan,fifth edition; Page no-08.

## INSTRUMENTS :

BOD, Incubator, laminar flow, autoclave, microscopes.

## THEORY:

**Hot air oven**

Hot air ovens are electrical devices which use dry heat to sterilize. They were originally developed by Pasteur. Generally, they can be operated from 50 to 300 °C, using a thermostat to control the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminium trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (hertz) used. Temperature sensitive tapes or biological indicators using bacterial spores can be used as controls, to test for the efficacy of the device during use.

## AUTOCLAVE

An autoclave is a pressure chamber used to carry out industrial processes requiring elevated temperature

and pressure different from ambient air pressure. Autoclaves are used in medical applications to perform sterilization and in the chemical industry to cure coatings and vulcanize rubber and for hydrothermal synthesis.Many autoclaves are used to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C (249 °F) for around 15–20 minutes depending on the size of the load and the contents. The autoclave was invented by Charles Chamberland in 1879.

Photograph of Autoclave

## BOD

An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells. The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C (140 to 150 °F), though some can go slightly higher (generally to no more than 100 °C). The most commonly used temperature both for bacteria such as the frequently used E. coli as well as for mammalian cells is approximately 37 °C, as these organisms grow well under such conditions. For other organisms used in biological experiments, such as the budding yeast Saccharomyces cerevisiae, a growth temperature of 30 °C is optimal

## COMPOUND MICROSCOPE

A microscope is an instrument used to see objects that are too small for the naked eye. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope.There are many types of microscopes. The most common (and the first to be invented) is the optical microscope, which uses light to image the sample. Other major types of microscopes are the electron microscope (both the transmission electron microscope and the scanning electron microscope), the ultramicroscope, and the various types of scanning probe microscope.

## STRUCTURAL COMPONENTS

The three basic, structural components of a compound microscope are the head, base and arm.

**Head/Body** houses the optical parts in the upper part of the microscope

**Base** of the microscope supports the microscope and houses the illuminator

**Arm** connects to the base and supports the microscope head. It is also used to carry the microscope.

When carrying a compound microscope always take care to lift it by both the arm and base, simultaneously.


## OPTICAL COMPONENTS

There are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses:

- **Eyepiece or Ocular** is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x-30x.
- **Eyepiece Tube** holds the eyepieces in place above the objective lens. Binocular microscope heads typically incorporate a diopter adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes. The monocular (single eye usage) microscope does not need a diopter. Binocular microscopes also swivel (Interpupillary Adjustment) to allow for different distances between the eyes of different individuals.

- **Objective Lenses** are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five on lens on most microscopes. Objectives can be forward or rear-facing.
- **Nosepiece** houses the objectives. The objectives are exposed and are mounted on a rotating turret so that different objectives can be conveniently selected. Standard objectives include 4x, 10x, 40x and 100x although different power objectives are available.
- **Coarse and Fine Focus knobs** are used to focus the microscope. Increasingly, they are coaxial knobs - that is to say they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.
- **Stage** is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements of the specimen slide are required.
- **Stage Clips** are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.
- **Aperture** is the hole in the stage through which the base (transmitted) light reaches the stage.
- **Illuminator** is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable lighting control located within the base.
- **Condenser** is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.
- **Iris Diaphragm** controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.
- **Condenser Focus Knob** moves the condenser up or down to control the lighting focus on the specimen.

## LAMINAR FLOW

A laminar flow cabinet or laminar flow closet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive materials. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.

Such hoods exist in both horizontal and vertical configurations, and there are many different types of cabinets with a variety of airflow patterns and acceptable uses.

Laminar flow cabinets may have a UV-C germicidal lamp to sterilize the interior and contents before usage to prevent contamination of experiment. germicidal lamps are usually kept on for 15 minutes to sterilize the interior and no contact is to be made with a laminar flow hood during this time. During this time, scientists normally prepare other materials to maximize efficiency. (It is important to switch this light off during use, to limit exposure to skin and eyes as stray ultraviolet light emissions can cause cancer and cataracts.)


**Result**:-

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# VIVA QUESTIONS

Q.1. Discuss working of Laminar air flow.

Ans- .....

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Q.2. Explain different parts of microscope.

Ans- .....

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Q.3. What is incubator?

Ans- .....

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# Experiment No. –2

## OBJECT:-

To perform Sterilization of glassware.

## REFERENCES:-

Jain N.K, ”Pharmaceutical Microbiology”, Vallabh Prakashion; Pitampura Delhi, Revised second edition; Page no-96.

Gaud R.S, Gupta G.D., B.K.,”Practical Microbiology”,Nirali Prakashan,fifth edition; Page no-51.

## INGREDIENTS:

Hot air oven, Cotton, paper, Glasswares

## THEORY:

Sterilization is a term referring to any process that eliminates (removes) or kills (deactivates) all forms of life and other biological agents (such as prions, as well as viruses which some do not consider to be alive but are biological pathogens nonetheless), including transmissible agents (such as fungi, bacteria, viruses, prions, spore forms, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a specified region, such as a surface, a volume of fluid, medication, or in a compound such as biological culture media. Sterilization can be achieved with one or more of the following: heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization in that sterilization kills, deactivates, or eliminates all forms of life and other biological agents.

## PROCEDURE:

1. Prepare glass material for sterilization ie. flasks should be stoppered with cotton and a piece of paper is tied over the plugged opening. Other pieces of glassware are to be packed in brown paper.

2. Take care for any screw caped bottle to be sterilized by this method, to see whether it contain any material which may be destroyed at temperature to be exposed.

3. All containers should be completely dried before leading. It is better that the containers were oven dried at $100^0$ C prior to sterilization.

4. Closed the door and switch on the heating source.

5. Adjust the heating temperature to $180^0$ C and wait for 20 minutes.

6. Switch off the heating source waiting for some time for temperature to go below $60^0$ C.

## RESULT:-

# VIVA QUESTIONS

Q.1. What is strilization technique?

Ans- .....

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Q.2. How sterilization different from disinfectant?

Ans- .....

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Q.3. State different sterilization methods.

Ans- .....

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# Experiment No. –3

## OBJECT:-

To prepare sub culture of bacteria or fungus using slants or stabs of nutrient media.

## REFERENCES:-

Pelczar J. Michael, Chan E.C.S, Krieg R. Noel, Microbiology, fifth Edition, Tata McGraw-Hill Publishing Company Limited page no-106

Prescott M. Lansing, Harley P. John, Klein A. Donald, Microbiology, sixth edition, McGraw-Hill Higher Education Page no-51.

## INGREDIENTS:

Forceps, Filter paper, Petri dish, U-shaped glass rod, Agar, Stains.

## THEORY:

The fungi are a group of eukaryotic protists that lack chlorophyll. We have seen the velvety blue and green growth on rotting oranges and lemons as well as on stale cheeses, the whitish gray furry outgrowth on bread and jam and the mushrooms in the fields. These are the bodies of various fungi. Thus, fungi have a diversity of morphological appearances, depending on the species. Fungi comprise molds and yeasts. Molds are filamentous and **multi cellular** whereas yeasts are usually **unicellular.**

**Procedure:** A) Slide Culture Preparation

**Aseptically, with a pair of forceps, place a sheet of sterile filter paper in a Petri dish.**

Place a sterile U-shaped glass rod on the filter paper. (Rod can be sterilized by flaming, if held by forceps.)

Pour enough sterile water (about 4 ml) on filter paper to completely moisten it.

With forceps, place a sterile slide on the U-shaped rod

Gently flame a scalpel to sterilize, and cut a 5 mm square block of the medium from the plate of Sabouraud's agar or Emmons' medium.

Pick up the block of agar by inserting the scalpel and carefully transfer this block aseptically to the centre of the slide.

Inoculate four sides of the agar square with spores or mycelial fragments of the fungus to be examined. Be sure to flame and cool the loop prior to picking up spores.

Aseptically, place a sterile cover glass on the upper surface of the agar cube.

Place the cover on the Petri dish and incubate at room temperature for 48 hours.

After 48 hours, examine the slide under low power. If growth has occurred there will be growth of hyphae and production of spores. If growth is inadequate and spores are not evident, allow the mold

to grow for another 24 hours before making the stained slides.

B) Application of Stain

Place a drop of lactophenol cotton blue stain on a clean microscope slide.

Remove the cover glass from the slide culture and discard the block of agar.

Add a drop of 95% ethanol to the hyphae on the cover glass. As soon as most of the alcohol has evaporated place the cover glass, mold sidedown, on the drop of lactophenol cotton blue stain on the slide. Examine the slide under microscope.

## RESULT:-

# VIVA QUESTIONS

Q.1. What is stain?

Ans- .....

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Q.2. What are aseptic conditions?

Ans- .....

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Q.3. What are fungus?

Ans- .....

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# Experiment No.–4

## OBJECT:-

To identify the Bacteria by simple staining methods.

## REFERENCES:-

Jain N.K, ”Pharmaceutical Microbiology”, Vallabh Prakashion; Pitampura Delhi, Revised second edition; Page no-30.

Gaud R.S, Gupta G.D., B.K.,”Practical Microbiology”,Nirali Prakashan,fifth edition; Page no-21.

## INGREDIENTS:

Microscope Broth culture, inoculating loop, stains, Bunsen burner etc.

## THEORY:

A stain is a discoloration that can be clearly distinguished from the surface, material, or medium it is found upon. They are caused by the chemical or physical interaction of two dissimilar materials. Staining is used for biochemical research, The primary method of stain formation is surface stains, where the staining substance is spilled out onto the surface or material and is trapped in the fibers, pores, indentations, or other capillary structures on the surface. The material that is trapped coats the underlying material, and the stain reflects back light according to its own color. Applying paint, spilled food, and wood stains are of this nature.

A secondary method of stain involves a chemical or molecular reaction between the material and the staining material. Many types of natural stains fall into this category.

Finally, there can also be molecular attraction between the material and the staining material, involving being held in a covalent bond and showing the color of the bound substance.

## PROCEDURE:

Place the three fixed smears on a staining loop or rack over a sink or other suitable receptacle.

Stain one slide with alkaline methylene blue for 30-40 seconds; one slide with carbol fuchsin for 5 to 10 seconds; and one slide with crystal violet for 20 to 30 seconds.

Wash stain off slide with water for a few seconds.

Blot slide dry with blotting paper.

Be careful not to rub the smear when drying the slide because this will remove the stained

bacteria.

Examine under the oil immersion lens.

You may want to treat smears of the same bacterium with all three stains in order to compare

them more directly. It is also instructive to cover bacterial smears for varying lengths of time with a given stain in order to get a feel for how reactive they are and the results of overstaining or understaining a slide preparation. For examples of bacteria stained with crystal violet.

**RESULT:-**

# VIVA QUESTIONS

Q.1. Why staining is done?

Ans- .....

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Q.2. What is over staining?

Ans- .....

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Q.3. What is slide fixing?

Ans- .....

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# Experiment No. –5

## OBJECT:-

Isolation of pure culture of micro organisms by multiple streak plate technique

## REFERENCES:-

Gaud R.S, Gupta G.D., B.K.,"Practical Microbiology",Nirali Prakashan,fifth edition; Page no-81.

Bhatt D.C, "Pharmaceutical Microbiology Concepts and Techniques", Birla Publications; Shahdara, Delhi, Page no-75.

## INGREDIENTS:

Petriplate, Inoculation loop, mixed culture sample, Bunsen burner etc..

## THEORY:

The streak plate method is a rapid qualitative isolation method. The techniques commonly used for isolation of discrete colonies initially require that the number of organisms in the inoculums be reduced. It is essentially a dilution technique that involves spreading a loopful of culture over the surface of an agar plate. The resulting diminution of the population size ensures that, following inoculation, individual cells will be sufficiently far apart on the surface of the agar medium to effect a separation of the different species present. Although many type of procedures are performed, the four ways or quadrant streak is mostly done. In the streaking procedure, a sterile loop or swab is used to obtain an uncontaminated microbial culture. The process is called "picking colonies" when it is done from an agar plate with isolated colonies and is transferred to a new agar or gelatin plate using a sterile loop or needle. The inoculating loop or needle is then streaked over an agar surface. On the initial region of the streak, many microorganisms are deposited resulting in confluent growth or the growth of culture over the entire surface of the streaked area. The loop is sterilized by heating the loop in the blue flame of the Bunsen burner, between streaking different sections, or zones and thus lesser microorganisms are deposited as the streaking progresses. The streaking process will dilutes out the sample that was placed in the initial region of the agar surface. There are two most commonly used streak patterns, a three sector "T streak " and a four quadrant streak methods.

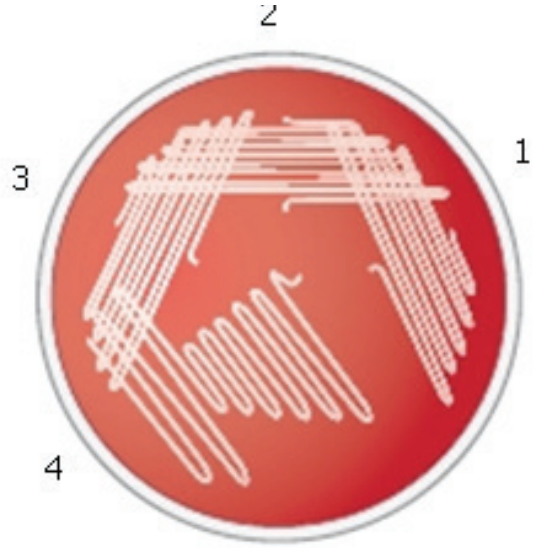
## PROCEDURE:

**Four Quadrant Streak method:**

Loosen the cap of the bottle containing the inoculum.

1. Hold an inoculation loop in your right hand.
2. Flame the loop and allow it to cool.
3. Lift the test tube containing the inoculum with your left hand.
4. Remove the cap/ cotton wool plug of the test tube with the little finger of your right hand.
5. Flame the neck of the test tube.

6. Insert the loop into the culture broth and withdraw. At all times hold the loop as still as possible.
7. Flame the neck of the test tube again.
8. Replace the cap/ cotton wool plug of the test tube using the little finger of your right hand. Place the test tube in a rack. For a liquid culture, dip the loop into the broth, or for solid media, lightly touch a colony with the loop.
9. Partially lift the lid of the Petri dish containing the solid medium.
10. Place a loopful of the culture on the agar surface on the area 1. Flame the loop and cool it for 5 seconds by touching an unused part of the agar surface close to the periphery of the plate, and then drag it rapidly several times across the surface of area1.
11. Remove the loop and close the Petri dish.
12. Reflame and cool the loop, and turn the petri dish 90°C then touch the loop to a corner of the culture in area1 and drag it several times across the agar in area 2, hitting the original streak a few times. The loop should never enter area 1 again.
13. Remove the loop and close the Petri dish.
14. Reflame and cool the loop and again turn the dish 90°C anticlockwise. streak area 3 in the same manner as area 2, hitting last area several times.
15. Remove the loop and close the Petri dish.
16. Flame the loop, again turn the dish 90°C and then drag the culture from a corner of a area3 across area 4, contacting area 3 several times and drag out the culture as illustrated. Using a wider streak. Do not let the loop touch any of the previously streaked areas. The flaming of the loop at the points indicated is to effect the dilution of the culture so that fewer organisms are streaked in each area, resulting in the final desired separation.
17. Remove the loop and close the Petri dish.
18. Tape the plate closed and incubates the plate in an inverted position in an incubator for 24-48 hours.
19. Flame the loop before putting it aside.




**RESULT:-**

# VIVA QUESTIONS

Q.1. What is Streak plate method?

Ans- .....

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Q.2. How many types of streaking are there?

Ans- .....

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Q.3. Explain multiple streaking method.

Ans- .....

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# Experiment No.–6

## OBJECT :

To perform microbial assay of antibiotics by cup plate method**.**

## REFERENCES:-

Bhatt D.C, ”Pharmaceutical Microbiology Concepts and Techniques”, Birla Publications; Shahdara, Delhi, Page no-122.

Gaud R.S, Gupta G.D., B.K.,”Practical Microbiology”,Nirali Prakashan,fifth edition; Page no-111.

## INGREDIENTS:

Microbial Inoculum, Culture Medium, Petri-dish, Measuring Cylinder, Needle, Innoculating Loop, Forcep, Incubator etc.

## THEORY:

Microbial assay measures the activity of antibiotics (Extent of ability to inhibit the growth of micro organism) or vitamins and amino acids (Extent to support the growth of micro organism) where as chemical assays of such substances estimate only their potency i.e concentration or amount. Cup plate method depends on diffusion of the antibiotic through a solidified agar layer in a Petri dish or plate. The growth of the specific microorganisms inoculated into the agar is prevented in a circular area or zone around the cylinder containing the solution of the antibiotic.

## PROCEDURE:

1. Sterilize the glass ware, media, antibiotic solution (standard) and plant samples.
2. Prepare the inoculated plates by adding 2 % of microbial suspension into the medium plate i.e., 0.5 ml of suspension per 25 ml of nutrient agar medium.
3. Allow the petri plates for solidification for about 10 min.
4. By using sterile glass (Pyrex) bores, make cups by maintaining approximate distance between cups (cup diameter: 6X8 mm2).
5. Label the cups properly to enable the introduction of the test sample, standard and control precisely.
6. Introduce the concerned samples into appropriate wells with the help of micropipette.
7. Fill all the cups with equal volumes of sample.
8. To minimize the effect of variants allow the petriplates to store at room temperature for 1-4 hrs, and allow the plates to incubate for a time period of 18-24 hrs.
9. Examine and measure the zone of inhibition with the help of antibiotic zone reader.

**Result**:-

# VIVA QUESTIONS

Q.1. What is Zone of Inhibition?

Ans- .....

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Q.2. How the activity of antibiotics can be measured?

Ans- .....

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Q.3. Discuss two methods for Microbial assay.

Ans- .....

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# Experiment No. –7

## OBJECT :

To determine motility by hanging drop method.

## REFERENCES :

Jain N.K, ”Pharmaceutical Microbiology”, Vallabh Prakashion; Pitampura Delhi, Revised second edition; Page no-26.

Bhatt D.C, ”Pharmaceutical Microbiology Concepts and Techniques”, Birla Publications; Shahdara, Delhi, Page no-66.

## INGREDIENTS :

Tooth pick , Microscope, coverslip, Slide, Stains, Bacterias.

## THEORY :

Many bacteria show no motion and are termed nonmotile. However, in an aqueous environment, these same bacteria appear to be moving erratically. This erratic movement is due to Brownian movement. Brownian movement results from the random motion of the water molecules bombarding the bacteria and causing them to move. True motility (self-propulsion) has been recognized in other bacteria and involves several different mechanisms. Bacteria that possess flagella exhibit flagellar motion. Helical-shaped spirochetes have axial fibrils (modified flagella that wrap around the bacterium) that form axial filaments. These spirochetes move in a corkscrew- and bending-type motion. Other bacteria simply slide over moist surfaces in a form of gliding motion.The above types of motility or nonmotility can be observed over a long period in a hanging drop slide. Hanging drop slides are also useful in observing the general shape of living bacteria and the arrangement of bacterial cells when they associate together.

## PROCEDURE :

1. With a toothpick, spread a small ring of Vaseline around the concavity of a depression slide. Do not use too much Vaseline.
2. After thoroughly mixing one of the cultures, use the inoculating loop to aseptically place a small
3. drop of one of the bacterial suspensions in the center of a coverslip.
4. Lower the depression slide, with the concavity facing down, onto the coverslip so that the drop
5. protrudes into the center of the concavity of the slide (figure 2.1c). Press gently to form a seal.
6. Turn the hanging drop slide over and place on the stage of the microscope so that the drop is over the light hole.
7. Examine the drop by first locating its edge under low power and focusing on the drop. Switch to the high-dry objective and then, using immersion oil, to the 90 to 100× objective. In order to see the bacteria clearly, close the diaphragm as much as possible for increased contrast. Note bacterial shape, size, arrangement, and motility. Be careful to distinguish between motility and

Brownian movement.

8. Discard your coverslips and any contaminated slides in a container with disinfectant solution.
9. Complete the report.

## RESULT:-

# VIVA QUESTIONS

Q.1. Why are unstained bacteria more difficult to observe than stained bacteria?

Ans- .....

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Q.2. What are some reasons for making a hanging drop slide?

Ans- .....

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Q.3. Why do you have to reduce the amount of light with the diaphragm in order to see bacteria in a hanging drop slide?

Ans- .....

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# Experiment No. – 8

## OBJECT:-

To perform bacteriological analysis of water

## REFERENCES:-

Bhatt D.C, ”Pharmaceutical Microbiology Concepts and Techniques”, Birla Publications; Shahdara, Delhi, Page no-92.

The South East Asian Medical information centre/ International Medical Foundation of Japan Manual for the laboratory diagnosis of bacterial food poisoning and the assessment of sanitary quality of food. Japan, Technocrat Division Fuji Marketing, 1978, p. 70.

## INGREDIENTS :

Nutrient Agar, Ringers solution, petridishes, Digital colony counter.

## THEORY:

Water, of adequate quantity and quality, is essential for healthy life. The associations between sanitation, water and health are well known. Many diseases are associated with contaminated water and water shortages. The most important factor to take into account is t

hat, in most communities, the principal risk to human health derives from faecal contamination. In some countries there may also be hazards associated with specific chemical contaminants such as fluoride or arsenic, but the levels of these substances are unlikely to change significantly with time. Thus, if a full range of chemical analyses is undertaken on new water sources and repeated thereafter at fairly long intervals, chemical contaminants are unlikely to present an unrecognized hazard. In contrast, the potential for faecal contamination inuntreated or inadequately treated community supplies is always present. The minimum level of analysis should therefore

include testing for indicators of faecal pollution (thermotolerant (faecal) coliforms), turbidity, and chlorine residual and pH (if the water is disinfected with chlorine).

Bacteriological water analysis is a method of analysing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and recreational waters are safe to use.

The interpretation and the action trigger levels for different waters vary depending on the use made of the water. Very stringent levels applying to drinking water whilst more relaxed levels apply to marine bathing waters, where much lower volumes of water are expected to be ingested by users.

## PROCEDURE:

**Standard Plate Count (SPC):**
The standard plate count is to be done by pour plate technique using 10 fold dilutions (upto 10-6) in ringers solution.

1. Pour one ml of each dilution (duplicates) in empty, sterilized petridishes.

2. Add about 12 to 15 ml of plate count agar (kept at 45°C in a waterbath) in each plate.

3. Incubate plates after solidification at 37°C for 24 to 48 hours.

4. Count plates showing 30—300 colonies to determine the SPC per ml of sample tested.

## RESULT:-

# VIVA QUESTIONS

Q.1. Why microbiological examination of water is needed?

Ans- .....

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Q.2. What is SPC?

Ans- .....

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Q.3. How many colonies of microbes are to be determined?

Ans- .....

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# Experiment No. –9

## OBJECT:

To perform Biochemical tests of microbiology by IMViC method.

## REFERENCES:

Bhatt D.C, ”Pharmaceutical Microbiology Concepts and Techniques”, Birla Publications; Shahdara, Delhi, Page no-99.

## INGREDIENTS:

Bacterial culture, Trytophan broth, Kovac’s reagent, Simmons citrate agar, Methyle Red, Barritt’s reagents.

## THEORY:

Each of the letters in “IMViC” stands for one of these tests. “I” is for indole; “M” is for methyl red; “V” is for Voges-Proskauer, and “C” is for citrate, lowercase “i” is added for ease of pronunciation. IMViC is an acronym that stands for four different tests

Indole test

Methyl red test

Voges-Proskauer test

Citrate utilization test

To obtain the results of these four tests, three test tubes are inoculated: tryptone broth (indole test), methyl red – Voges Proskauer broth (MR-VP broth), and citrate. IMViC tests are employed in the identification/ differentiation of members of family enterobacteriaceae.

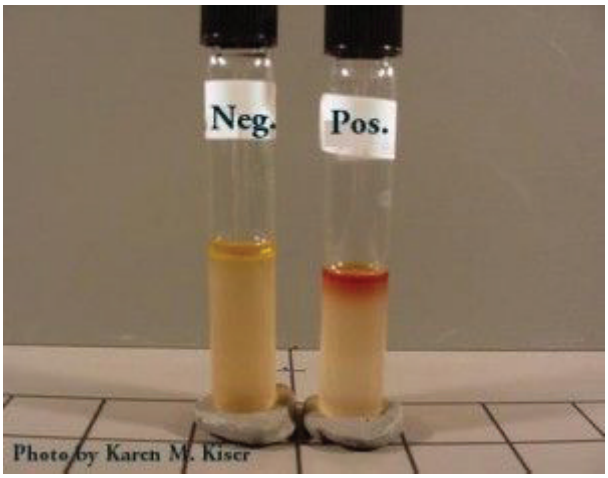
## PROCEDURE :

Cultures of any members of enterobacteriaceae have to grow for 24 to 48 hours at 37°C and the respective tests can be performed:

Indole test

It is performed on sulfide-indole-motility (SIM) medium or in tryptophan broth. Result is read after adding Kovac’s reagent.

1. The positive result is indicated by the red layer at the top of the tube after the addition of Kovács reagent.
2. A negative result is indicated by the lack of color change at the top of the tube after the addition of Kovács reagent.




Indole Test Results: Positive-development of Red-ring

Methyl red test and Voges-Proskauer test both are done in methyl red–Voges-Proskauer (MR-VP) broth, but the reagents that we add differs

## METHYL RED (MR)TEST:

- Positive methyl red test are indicated by the development of red color after the addition of methyl red reagent.
- A negative methyl red test is indicated by no color change after the addition of methyl red reagent

## VOGES-PROSKAUER (VP) TEST:

- Negative test is indicated by lack of color change after the addition of Barritt's A and Barritt's B reagents.
- A positive Voges-Proskauer test is indicated by the development of red-brown color after the addition of Barritt's A and Barritt's B reagents.

## CITRATE UTILIZATION TEST

The test is performed on Simmons citrate agar:

- Negative citrate utilization test is indicated by the lack of growth and color change in the tube
- A positive citrate result as indicated by growth and a blue color change.

## RESULT:- IMVIC TESTS OF ESCHERICHIA COLI

1. Indole: Positive
2. Methyl-Red: Positive
3. Voges-Proskauer test: Negative
4. Citrate test: Negative

# VIVA QUESTIONS

Q.1. What is IMViC stands for?

Ans- .....

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Q.2. What are biochemical study of microorganisms?

Ans- .....

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Q.3. Why biochemical tests are performed?

Ans- .....

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# Experiment No. –10

## OBJECT:-

To identify the given sample of micro organism by Grams staining method.

## REFERENCES:-

Prescott M. Lansing, Harley P. John, Klein A. Donald, Microbiology, sixth edition, McGraw-Hill Higher Education

Ananthanarayanan R, Text book of microbiology, sixth edition, Longman Private Limited

## REQUIRMENTS:

Inoculating loop, Bunsen burner, Bibulous paper, Microscope, Lens paper and lens cleaner, Immersion oil, Distilled water.

## THEORY:

Gram staining, also called Gram's method, is a method of staining used to differentiate bacterial species into two large groups (gram-positive and gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of gram-positive bacteria.[1] Gram-positive bacteria retain the crystal violet dye, and thus are stained violet, while the gram-negative bacteria do not; after washing, a counterstain is added (commonly safranin or fuchsine) that will stain these gram-negative bacteria a pink color.

The Gram stain is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to *gram-variable* and *gram-indeterminate* groups.

## PROCEDURE:

1. Place slide with heat fixed smear on staining tray.
2. Gently flood smear with crystal violet and let stand for 1 minute.
3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram's iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
6. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
7. Immediately rinse with water.G

8. Gently flood with safranin to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.
11. View the smear using a light-microscope under oil-immersion.

## RESULT:-

# VIVA QUESTIONS

Q.1. How many types of staining are there?

Ans- .....

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Q.2. What is Gram Staining?

Ans- .....

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Q.3. Why wasing of slide is done with water?

Ans- .....

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# Experiment No. –11

## OBJECT:-

To identify the bacteria by  acid fast staining.

## REFERENCES:-

Jain N.K, ”Pharmaceutical Microbiology”, Vallabh Prakashion; Pitampura Delhi, Revised second edition; Page no-96.

Gaud R.S, Gupta G.D., B.K.,”Practical Microbiology”,Nirali Prakashan,fifth edition; Page no-51.

## INGREDIENTS:

Carbol fuchsin stain, Clean slide, HCL, Alcohol.

## THEORY:

It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called Ziehl-Neelsen staining techniques. Neelsen in 1883 used Ziehl’s carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed.

The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups.

This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid-fast staining.

When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appears blue while acid-fast cells retain the red color.

## PROCEDURE:

Prepare bacterial smear on clean and grease free slide, using sterile technique.

1. Allow smear to air dry and then heat fix.
   *Alcohol-fixation: This is recommended when the smear has not been prepared from sodium hypochlorite (bleach) treated sputum and will not be stained immediately. M. tuberculosis is killed by bleach and during the staining process. Heat-fixation of untreated sputum will not kill M. tuberculosis whereas alcohol-fixation is bactericidal.*

2. *Cover the smear with carbol fuchsin stain.*

3. *Heat the stain until vapour just begins to rise (i.e. about 60 C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes.*
   *Heating the stain: Great care must be taken when heating the carbol fuchsin especially if staining is carried out over a tray or other container in which highly flammable chemicals have collected from previous staining. Only a small flame should be applied under the slides using an ignited swab previously dampened with a few drops of acid alcohol or 70% v/v ethanol or methanol. Do not use a large ethanol soaked swab because this is a fire risk.*

4. *Wash off the stain with clean water.*
   *Note: When the tap water is not clean, wash the smear with filtered water or clean boiled rainwater.*

5. *Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink.*
   *Caution: Acid alcohol is flammable, therefore use it with care well away from an open flame.*

6. *Wash well with clean water.*

7. *Cover the smear with malachite green stain for 1–2 minutes, using the longer time when the smear is thin.*

8. *Wash off the stain with clean water.*

9. *Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).*

10. *Examine the smear microscopically, using the 100 X oil immersion objective.*

## RESULT:-

# VIVA QUESTIONS

Q.1. What is Ziehl-Neelsen staining technique?

Ans- .....

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Q.2. Why heating of stain is done?

Ans- .....

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Q.3. What is Alcohol-fixation?

Ans- .....

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# Experiment No.–12

## OBJECT:

To prepare and sterilize media.

## REFERENCES:

Cappuccino G .James, Sherman Natalie, Microbiology A laboratory manual, seventh edition, Pearson Education; Page no-52.

Brown E. Alfred, Benson's Microbiological Applications, ninth edition, McGraw Hill; Page no-10.

## INGREDIENTS:

Peptone, beef extract, agar, Tryptone, Soytone, Sodium chloride,

## THEORY:

Bacteria display a wide variety of nutritional and physical requirements for their growth. This includes water, a source of energy, sources of carbon, sulfur, nitrogen phosphorus, minerals such as Ca2+, Mg2+, Na+, and other vitamins and growth factors. Nutrient agar is a complex medium as it contains ingredients with unknown amounts or types of nutrients. Nutrient agar typically contains 0.5 % peptone, 0.3 % beef extract, 1.5 % agar in water (pH adjusted to neutral). Beef extract is the commercially prepared dehydrated form of autolysed beef and is provided in the form of a paste. Peptone source is casein (milk protein) that has been digested with the action of the enzyme pepsin. Peptone is dehydrated and supplied in the medium as powdered form. Peptone and beef Extract is a mixture of amino acids and peptides. Beef Extract also contains digest products which are water soluble, other macromolecules such as nucleic acids, fats, polysaccharides as well as vitamins and trace minerals (cannot be chemically defined). There are many ingredients in the media which are complex, which includes yeast extract, tryptone, and others. The importance of complex media is that they will support the growth of a wide range of micro organisms. Agar is obtained from red algae in which it is a supplement of polysaccharide (polygalacturonic acid) in their cell walls. Agar is not a nutritional component, but a better solidification agent as it dissolves at or near the boiling temperature (100°C) but solidifies at 45°C. So molten (liquid) agar is prepared at 45°C, cells are mixed with it, and then allowed to solidify thereby trapping living cells.

## PROCEDURE:

**Weigh accurately:**

1. 15 g of Tryptone(Pancreatic Digest of Casein )
2. 5 g of Soytone (Papaic Digest of Soybean Meal )
3. 5 g of Sodium Chloride
4. 15 g of Agar.
5. Suspend 40 grams in 1 liter distilled or deionised water.

6. Heat to boiling to dissolve completely.
7. Autoclave at 121°C for 15 minutes.
8. Cool to room temperature.

## RESULT:-

# VIVA QUESTIONS

Q.1. What is Nutrient agar?

Ans- .....

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Q.2. Why agar is used?

Ans- .....

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Q.3. Why deionised water is used?

Ans- .....

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