A training Report

Submitted to

Atmiya University

In partial fulfillment of the requirements for the degree of

Bachelor of science in Biotechnology

By

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On the first place I would like to record my gratitude to Dr. Jay Parekh– my training guide under whose supervision, guidance and advice I have completed my training in a successful way. She showed interest to teach me and enriched my growth in this field.

Index

Sr.no.	Title of Contents
1.	Instruments use
2.	Requirements of performance Test
3.	Bacteria Morphology
4.	Various types of culture and use
5.	Isolation and identification of Bacteria
6.	Gram Staining
7.	ZN Staining
8.	KOH Staining

Introduction

Sterling Hospital in Rajkot is the largest tertiary care hospital with 208 beds with the largest critical care setup in the regions of Saurashtra & Kutch. Sterling Hospital, the best hospital in Rajkot, has been successful in analyzing and addressing healthcare requirements and existing gaps in services in this region since its beginning in November 2009. This multispecialty hospital in Rajkot has an eminent team of super specialists supported by cutting-edge equipment, technology, and state-of-the-art infrastructure. It has made it possible to set a benchmark of excellence in the healthcare industry.

- Well equipped & highly sophisticated operation theatres with laminar airflow & HEPA filters ensuring maximum sterility, infection control, and desired outcomes in major and minor surgeries
- Two flat-panel Cathlabs with stent boost technology
- The only corporate hospital in Saurashtra that has IVUS and FFR facilities
- 5-well equipped & highly sophisticated operation theatres with laminar airflow & HEPA filters ensuring maximum sterility, infection control, and desired outcomes in major and minor surgeries
- Saurashtra’s largest dedicated ICU Set up with 24*7 hours intensivist, medical officers, and nursing staff available

Instruments used in microbiology Lab

1.MICROSCOPE

2.REFRIGERATORS

3.BIOSEFTY CABINET

4.BD BACTEC

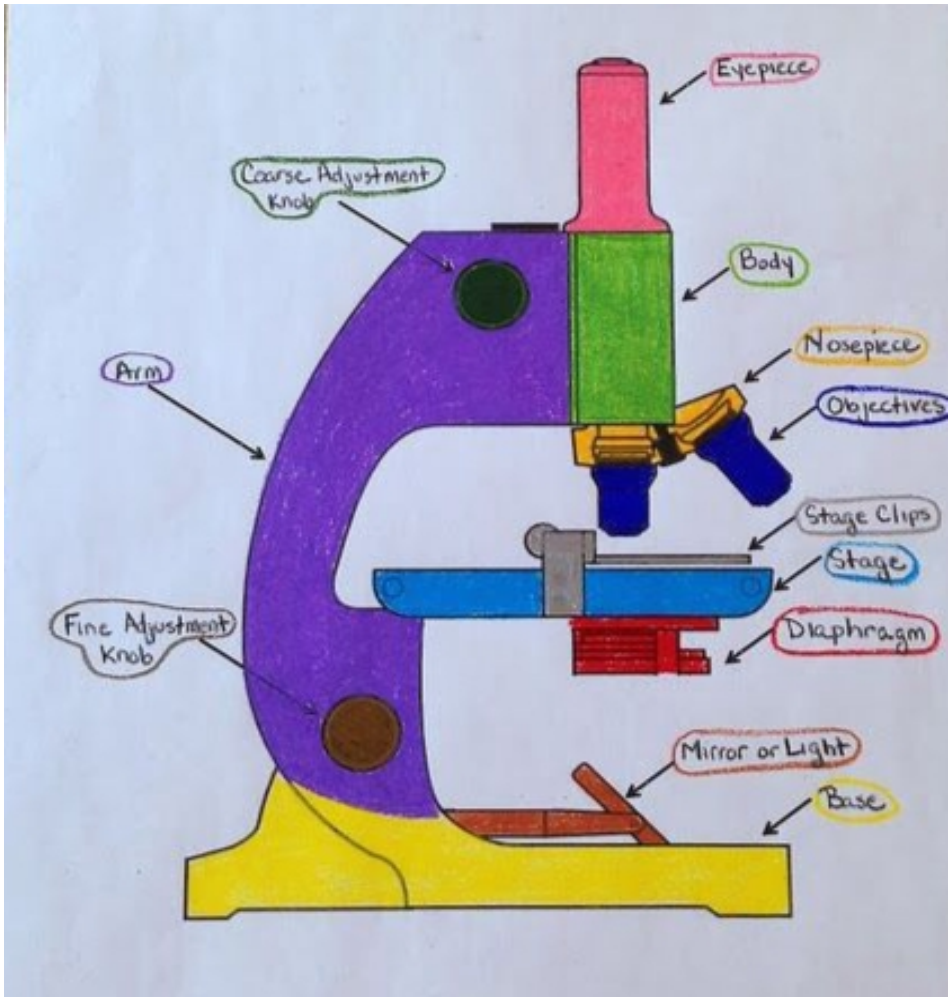
5.INCUBATOR

6.AUTOCLAVE

7.ATTEST AUTOREADER

Microscope

Observing microorganisms and their features. In this field, microscopes are used to study bacteria, cells and many more. This device helps biologists in their study of living organisms and their cell structures.

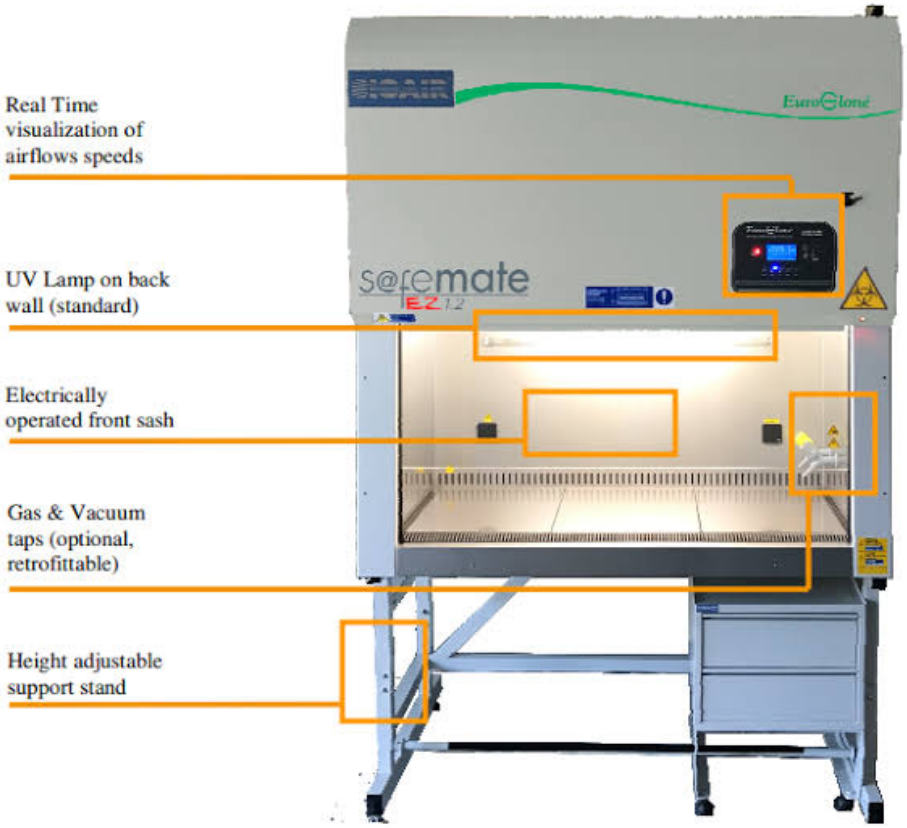


Refrigerator

A laboratory fridge is used by healthcare facilities and professionals to store samples and specimens, as well as vaccines and medicines, at a specific temperature to ensure they do not become spoiled.

Biosafety Cabinet

A biological safety cabinet (BSC) is a primary engineering control used to protect personnel against biohazardous or infectious agents and to help maintain quality control of the material being worked with as it filters both the inflow and exhaust air.



BD Bactec

When microorganisms are present in the cultured vials, they metabolize nutrients in the culture medium, releasing carbon dioxide into the medium.

Detection of aerobes, anaerobes, yeast, fungi and mycobacteria to help improve time to detect and organism recovery2 from both adult and pediatric patients.

Incubator

Incubator, an insulated enclosure in which temperature, humidity, and other environmental conditions can be regulated at levels optimal for growth, hatching, or reproduction.

Autoclave

An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel.temperatures are 121°C (250°F) and 132°C (270°F).

Attest Auto Reader

Auto-readers are designed to incubate and. Read 3M™ Attest™ Biological Indicators, which are used to. Monitor the efficacy of sterilization processes. Auto-readers. (Models 390, 390G, 490, and 490H) come supplied with.

REQUIREMENTS IN MICROBIOLOGY LEB

- STERILE WIRE LOOPS
- GLOVES
- STERILE CONTAINERS FOR SAMPLE COLLECTION
- STERILE SWAB STICKS FOR SWAB TESTS
- AEROBIC AND ANAEROBIC BLOOD CULTURE BOTTLE
- PETRI PLATES
- SLIDES
- COVERSLIPS
- ETHANOL ,. ETC .

Morphology of Bacteria

MORPHOLOGY OF BACTERIA CAN BE DESCRIBED UNDER THE FOLLOWING HEADINGS :

SIZE : THE SIZE OF BACTERIA IS MEASURED BY MICROMETRY. THE UNIT OF MEASUREMENT USED IS MICROMETER (UM).

1.COCCI : 0.75 UM TO 2 UM

2.BACILLI : 0.7 UM TO 8 UM

3.SPIROCHAETES : 12 UM TO 20 UM

SHAPE : ACCORDING TO SHAPE , BACTERIA HAVE BEEN CLASSIFIED INTO FOLLOWING TYPES :

1.BACILLAR – BACILLI ROD SHAPED CELLS

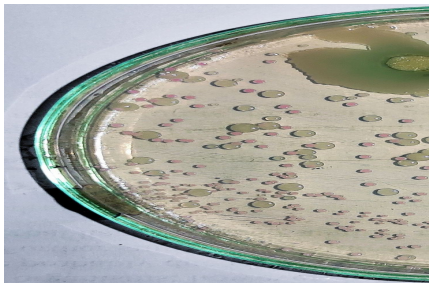
2.SPHERICAL - COCCI ROUND IN SHAPE

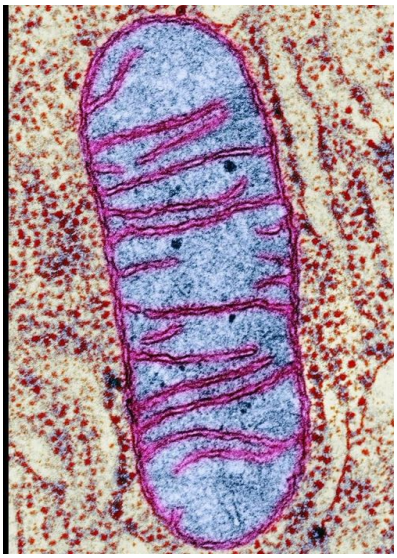
3.COMMA – CURVED APPEARANCE

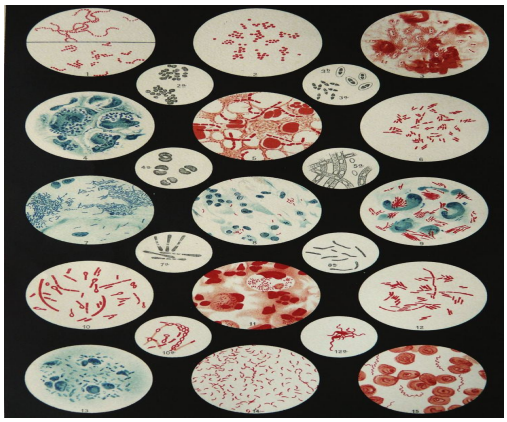
4.SPIRILLA – RIGID SPIRAL FORM

5.SPIROCHETES – FLEXUOUS SPIRAL FORM

6.BRANCHING – FILAMENTOUS FORM E.G, ACTINOMYCETES







Bacteria Arrangements

ARRANGEMENT :

COCCI ARRANGED IN :

CLUSTERS – STAPHYLOCOCCI

CHAINS – STERPTOCOCCI

PAIRS – PNEUMOCOCCI , NEISSERIA

GROUP OF FOUR – TETRADS

PACKETS OF EIGHT – SARCINA

BACILLI ARRANGED IN :

SINGLY – E.COLI

CHINESE LETTER – C. DIPTHERIA

CHAINS – STRPTOBACILLI

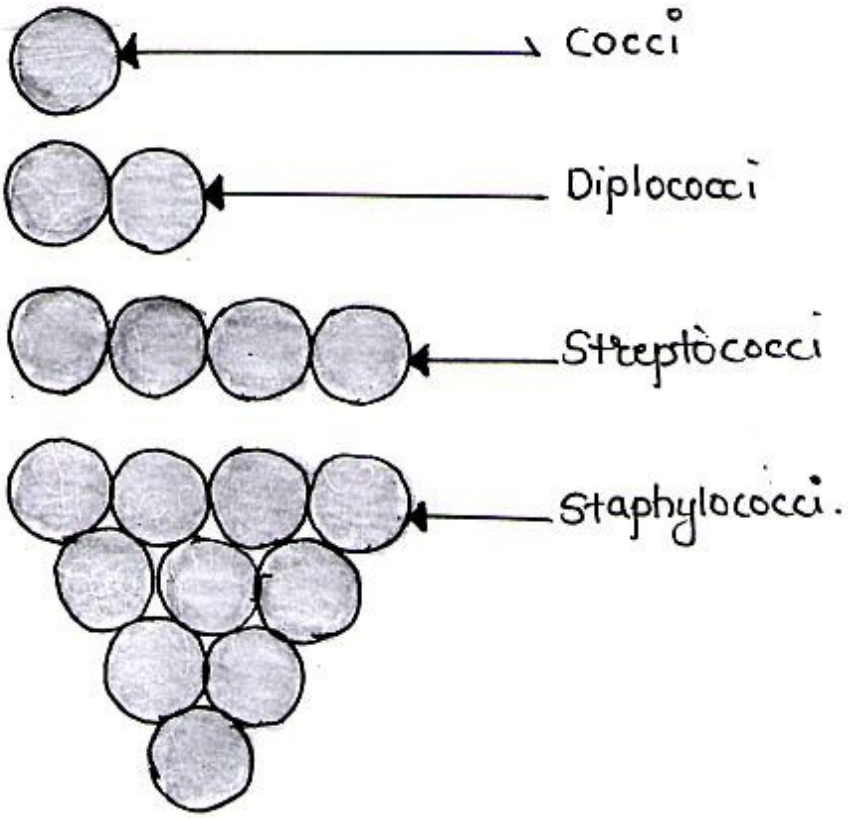


Figure:- Arrangement of Cocci Shape bacteria.

Media Classification

TYPES OF MEDIA =

- **SOLID MEDIA**
- **LIQUID MEDIA**
- **SEMISOLID MEDIA**

Medium	Composition	Uses
Nutrient agar	Nutrient broth, agar 2%	Routine culture
MacConkey medium	Peptone, lactose, sodium taurocholate, agar, neutral red	Culture of Gram-negative bacteria, such as *Escherichia coli*
Blood agar	Nutrient agar, 5% sheep or human blood	Routine culture, culture of fastidious organisms, such as *Streptococcus* spp.
Chocolate agar	Heated blood agar	Culture of *Haemophilus influenzae* and *Neisseria*
Deoxycholate citrate agar	Nutrient agar, sodium deoxycholate, sodium citrate, lactose, neutral red, etc.	Culture of *Shigella* spp. and *Salmonella* spp.
Thiosulfate citrate bile salt sucrose agar	Thiosulfate, citrate, bile salt, sucrose, bromothymol blue, thymol blue	Culture of *Vibrio cholerae*
Loeffler's serum slope	Nutrient broth, glucose, horse serum	Culture of *Corynebacterium diphtheriae*
Lowenstein–Jensen medium	Coagulated hen's egg, mineral salt solution, asparagine, malachite green	Culture of *Mycobacterium tuberculosis*

Isolation of Bacteria

Bacterial isolation is defined as the technique of separating one species of bacteria from the bacteria's mixed culture by different plating methods like pouring, spreading, streaking, and serial dilution.

There are two main ways to isolate organisms.

- **Streaking for isolation on an agar plate.**

- **The pour plate meth**

Gram Staining

WHAT IS GRAM STAINING ?

- **THIS IS A STAINING METHOD USED FOR IDENTIFYING GRAM POSITIVE AND GRAM NEGATIVE BACTERIA .**

- **The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content, whereas gram-negative organisms have higher lipid content.**

List of Gram positive and Gram negative Bacteria

- Gram positive(+ve)
  - Staphylococcus aureus
  - Staphylococcus Haemolytycus
  - Staphylococcus Hom hominis
  - Streptococcus pyogens
  - Streptococcus pneumonide
  - Enterococcus faecium
  - Enterococcus faecalis

- Gram Negative(-ve)
  - E.coli
  - Pseudomonas aeruginosa
  - Proteus mirabilis
  - Proteus valgeris
  - Salmonella typhi
  - Salmonella para Typhi
  - Bracella

Gram Stainning:

- Prepare smear

↓ Heat fix

- Crystal Violet (1 min)

↓ Tap water

- Iodine (3 min)

↓ Tap water

- Decolorization (2-3 sec)

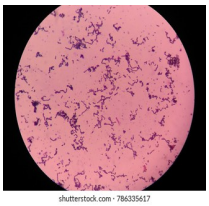
↓ Tap water

- Saffranin (1 min)

↓ Tap water

- Oil drop and observe under 100x

Results:
Gram positive : purple
Gram negative : pink in color
Yeast : violet



ZN Staining

The Ziehl-Neelsen method uses a carbol fuchsin stain, acid alcohol decolorizer, and methylene blue counterstain. Acid-fast organisms stain red, while the background of debris stains blue. The ZN stain confirms the acid-fast property of mycobacteria.

ZN Staining

- Prepare smear

↓ Heat fix

- Carbol fusion (5 min)

↓ 3 times heat fix till fumes observe

- Acid fast decolorization (1 min)

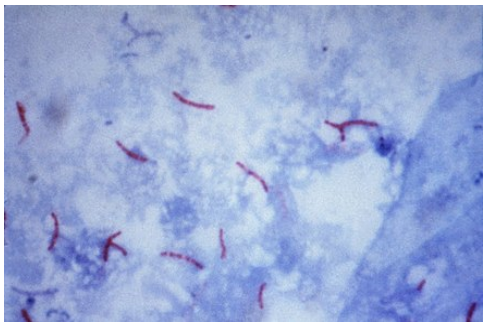
↓ Tap water

- Methyl blue (1 min)

↓ Tap water

- Air dry and observe under 100x

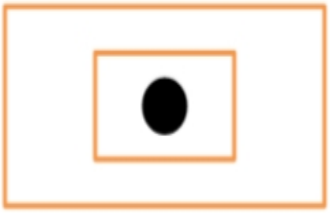
Results:
Acid fast bacilli : rod pink



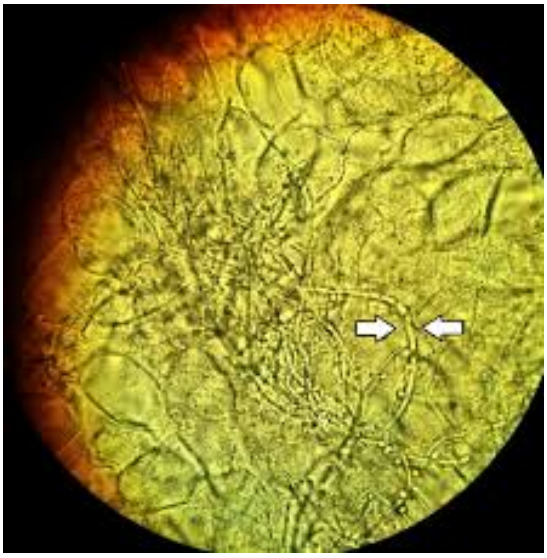
KOH staining

Principle of Potassium hydroxide (KOH) Test
KOH separates the fungal elements from intact cells as it digests the protein debris and dissolves cement substances that hold the keratinized cells together surrounding the fungi so that the hyphae and conidia (spores) of fungi can be seen under the microscope.

KOH Stain:



- Sample 1-2 drop
- 10% KOH 2-3 drop
- Mix well
- Put cove slip
- Observe under 40x



Skills learned and the Experiences

- I have learned about Staining
- Using different instruments in lab
- Learned about Different types of culture media used in microbiology lab
- I have learned Phlebotomy
- Handling microorganisms

Conclusion

During work in this laboratory we experienced that how to work with doctors, lab technicians and staff.

Also learned that how to operate some automated machines, how to work with this machine, how to communicate with patients.

Overall, I found the laboratory internship experience to be positive, and I am sure I would be able to use the skills I learned in my career later.

Future Aspects

The scope of microbiology is vast and extends to many areas of science. Over the years, the development of microbiology has been seen in the fields of medicine, pharmacy, clinical research, dairy industry agriculture, water industry, and Chemical Technology.

The Future aspects after completing an internship Course with At. Sterling Accuris Hospital is to establish mastering technical skills, knowledge on different applications.