A

On Job Training Report

On

CLINICAL LABORATORY

Completed at

Shrinidhi Diagnostic Center

'E' Ward, Near Mahalaxmi Pride Apartment,

6th Lane , Rajarampuri ,Kolhapur

By

Miss. Aabida Altaf Mujawar

M.sc Microbiology

Part 1 semester 2

PG Department of Microbiology

Vivekanand College

(An Empowered Autonomous Institute)

Kolhapur,416003

Maharashtra ,India

2025-26

Dissemination of Education for Knowledge, Science and Culture"

-Shikshanmaharshi Dr. Bapuaji Sahakhe

Shri Swami Vivekanand Shikshan Sanstha's

स्थापना (सातारा)

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

PG Department of Microbiology

CERTIFICATE

OF

"ON JOB TRAINING"

This is to certify that Aabida Altaf Mujawar (Roll no.5414) has satisfactorily carried out the required practical work prescribed by the BoS Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for M.Sc. – Part- I Semester II course in On Job Training (Sub code – OJT20MIC21) and this report represents ~~his~~/her Bonafide work in the year 2025- 2026.

Place: Kolhapur

Date:

Examiner

OJT in charge

Head

I/C Head
Department of Microbiology
Vivekanand College Kolhapur
(Empowered Autonomous)

DECLARATION

I hereby declare that I have successfully completed the On Job Training program at Shrinidhi diagnostic center kolhapur. I acknowledge that skills acquired during this raining program are valuable to me and will contribute to my professional development.

I express my gratitude supervisor Mrs . Shilpa Lokapure mam (Owner of Shrinidhi laboratories), At. Shrinidhi laboratories kolhapur and the whole training team for their support and guidance throughout the training.

Date: 23|3|26

Place: Kolhapur

Miss. Aabida Altaf Mujawar

ACKNOWLEDGEMENT

At this juncture where the herculean task is nearing its pinnacle, science deems it a pleasure to look back and acknowledge efforts and support of all kith and kin that helped with zeal to turn a distant dream of an industrial training into reality.

We are extremely thankful to Dr. K. K. Bhise Mam, Assistant Professor, PO Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute), project guide for her valuable guidance and mentorship throughout this project work given to us during the study.

We are indeed grateful to Head Dr. T. C. Gaupale, Coordinator Ms. V. V. Misal, PG Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for their kind co-operation and valuable support and we are also thankful to all the staff members of our department for their direct and indirect support.

We are thankful to Principal Dr. S.P. Thorat sir, for his kind co-operation and valuable support.

Also, we sincerely thank our parents for helping us in all aspects to complete the project. work. Finally, we would like to appreciate our friends, colleagues for their direct and indirect contribution.

Date: 23/3/26

Place: Kolhapur

Miss Aabida Altaf Mujawar

Internship Undertaking

1. Student Name:	Aabida Altaf Mujawar
2. Current Address	Mali colony, Takala Plot no. 9 Imam manzil
3. Residence Address	Mali colony. Takala Plot no 9. Imam manzil
4. Email id	aabidamujawar0603@gmail.com
5. Mobile Nos.	9028363161
6. Aadhar	5287 3830 3650
7. PAN	-
8. Overall GPA	—
9. Mode of Internship	offline
10. Internship Preferences	—

	Location	Core Area	Organization / Institute
Preference-1	Rajarampuri	Rajurampuri	shrinidhi diagnostic centre
Preference-2	—	—	—
Preference-3	—	—	—

I confirm that I agree with the terms, conditions, and requirements of the Internship Policy

Student Signature: ~~A~~ Amujawar

Date 23/3/26

I confirm that the student has attended the internship orientation and has met all paperwork and process requirements to participate in the internship program, and has received approval from ~~his~~/her mentor.

Sign of Department Faculty Coordinator

Date 23/3/26

Student Diary (Log) Recording Format

Week	Task Assigned	Activities Performed	Key Learnings	Additional Remarks
16/12/25 to 22/12/25	Media Prepration Gram staining Antibiotic sensitivity	Performed Media Prepration fo Perimed Gram slaining Antibiotic sensitivity	learned Preparation of different media useing aseptic condition. understood procedure of gram staining of gram +ve	satisfactory
23/12/25 to 29/12/25	Media Prpration AFB staining sample streaking	Performed Media prpration AFB staining sample streaking	understood the procedure of gram staining and differentiation of gram +ve and gram -ve. learned AFB staining	satisfactory
30/12/25 to 31/12/25	Routine Culture Streaking	Performed routeine culture Producers in the lab. Technique For isolation & observation of bactial colonies	learned routine culture technique used in lab. Practical streaking method For isolation of bacterial colonies	satisfactory

Signature of Industry Supervisor

Attendance Sheet

Name & Address of Organization

Shrinidhi Diagnostic Centre
'E' Ward, near mahalaxmi pride
Aparlment, 6th lane, Rajarampuri, Kolhapur.

Name of the Student	Aabida Altaf Mujawar
Roll Number	5414
Name of Course	Microbiology
Date of Commencement of Training	16 Dec 2025
Date of Completion of Training	31 Dec 2025

Month and Year:

Day	Date	Sign of student
1	16/12/25	Amujawar
2	17/12/25	Amujawar
3	18/12/25	Amujawar
4	19/12/25	Amujawar
5	22/12/25	Amujawar
6	23/12/25	Amujawar
7	25/12/25	Amujawar
8	26/12/25	Amujawar
9	27/12/25	Amujawar
10	29/12/25	Amujawar
11	30/12/25	Amujawar
12	31/12/25	Amujawar
13		
14		
15		

- Attendance Sheet should remain affixed in Daily Training Diary. Do not remove or tear it off.
- Holidays should be marked in Red Ink in attendance column. Absent should be marked as A 'in Red Ink.

Name and Signature with date of Internship Supervisor Rucep (Dr. Rashmi S. Pawar)
29/12/2025

Supervisor Evaluation of Intern

Student Name: Aabida Altaf Mujawar Date: 29/12/26

Work Supervisor: Shilpa Lokapure Title: Owner

Organization: Shrinidhi Diagnostic Centre

Internship Address: 'E' Ward, Near Mahalaxmi pride Apartment 6th lane

Dates of Internship: From 16/12/25 To 31/12/25

Please evaluate intern by indicating the frequency with which you observed the following behaviors:

Parameters	Needs Improvement	Satisfactory	Good	Excellent
Behaviors			✓	
Performs in a dependable manner			✓	
Cooperates with co-workers and supervisors			✓	
Shows interest in work			✓	
Learns quickly				✓
Shows initiative			✓	
Produces high quality work			✓	
Accepts responsibility			✓	
Accepts criticism			✓	
Demonstrates organizational skills			✓	
Uses technical knowledge and expertise			✓	
Shows good judgment			✓	
Demonstrates creativity/originality			✓	
Analyzes problems effectively			✓	

Is self-reliant			✓	
Communicates well			✓	
Writes effectively			✓	
Has a professional attitude			✓	
Gives a professional appearance			✓	
Is punctual			✓	
Uses time effectively			✓	

Overall performance of student intern (circle one):

(Needs improvement / Satisfactory / Good / Excellent)

Additional comments, if any:

Signature of Industry supervisor Rashmi (Dr. Rashmi S. Pawar)

HR Manager _____

Student Feedback of Internship

(To be filled by Students after Internship completion)

Student Name: Aabida Altaf Mujawar Date: 23/3/26

Industrial Supervisor: Shilpa Lokapure Title: Owner of laboratory

Supervisor Email: – Internship is: ____ Paid – Unpaid ✓

Organization: Shrinidhi diagnostic center

Internship Address: 'E' Ward, near mahalaxmi pride Apartment 6th lane rajarampuri

Faculty Coordinator: - Department: –

Dates of Internship: From 16 Dec 2025 To 31 Dec 2025

Give a brief description of your internship work (title and tasks for which you were responsible):

Was your internship experience related to your major area of study?

- Yes, to a large degree
- Yes, to a slight degree
- No, not related at all

Indicate the degree to which you agree or disagree with the following statements.

This experience has:	Strongly Agree	Agree	No opinion	Disagree	Strongly Disagree
Given me the opportunity to explore a career field	✓				
Allowed me to apply classroom theory to practice	✓				
Helped me develop my decision-making and problem-solving skills	✓				
Expanded my knowledge about the work world prior to permanent employment	✓				
Helped me develop my written and oral communication skills	✓				
Provided a chance to use leadership skills (influence others, develop ideas with others, stimulate decision-making and action)	✓				

Expanded my sensitivity to the ethical implications of the work involved	✓				
Made it possible for me to be more confident in new situations	✓				
Given me a chance to improve my interpersonal skills	✓				
Helped me learn to handle responsibility and use my time wisely	✓				
Helped me discover new aspects of myself that I didn't know existed before	✓				
Helped me develop new interests and abilities	✓				
Helped me clarify my career goals	✓				
Provided me with contacts which may lead to future employment	✓				
Allowed me to acquire information and/ or use equipment not available at my Institute	✓				

- In the Institute internship program, faculty members are expected to be mentors for students. Do you feel that your faculty coordinator served such a function? Why or why not?

  Yes, the Faculty coordinator guided and supported me through out the internship.

- How well were you able to accomplish the initial goals, tasks and new skills that were set down in your learning contract? In what ways were you able to take a new direction or expand beyond your contract? Why were some goals not accomplished adequately?

  I was able to acheive most of the goals and learned several new practical skills.

- In what areas did you most develop and improve?

  I improved my laboratory techniques, communication and practical knowledge.

- What has been the most significant accomplishment or satisfying moment of your internship?

  successfully performing laboratory experiments independently.

- What did you dislike about the internship?

  limited time to explore more advanced techniques.

- Considering your overall experience, how would you rate this internship? (Circle one).

  -Satisfactory/(Good)/ Excellent

  Good

- Give suggestions as to how your internship experience could have been improved. (Could you have handled added responsibility? Would you have liked more discussions with your professor concerning your internship? Was closer supervision needed? Was more of an orientation required?)

  More hands on training and longer internship duration would improve the experience.

Signature of Student: ~~A~~ Mujawar

Name: Aabida Altaf Mujawar

Roll number: 5414

Date: 23/3/26

SHRINIDHI DIAGNOSTIC CENTRE

Where We Grow Your Bugs

INTERNSHIP CERTIFICATE

Date: 20 /01 /2026

This is to certify that Ms. Aabida Altaf Mujawar, a student of M.Sc. part 1 Microbiology, Vivekanand College, Kolhapur, has successfuly completed an internship in the Microbiology Department at Shrinidhi Diagnostic center Laboratory from 16 December to 31 December 2025.

During the internship period, she was exposed to routine microbiology laboratory procedures, sample handling, staining techniques, culture methods, and basic diagnostic practices. Her performance was found to be sincere, disciplined, and satisfactory throughout the training period.

We wish her all the best for her future academic and professional endeavors.

Name : Dr Rashmi Powar

Designation : MD Microbiology

Shrinidhi Diagnostic Centre
2019, 'E' Ward Near Mahalaxmi
Pride Appa[illegible]t, 6th Lane,
Rajaram[illegible] Kolhapur

shrindhidiagnostic@gmail.com
9096619090
2019, 'E' Ward, Near Mahalaxmi Pride Apartment,
6th Lane, Rajarampuri, Kolhapur.

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ABOUT LABORATORY

Shrinidhi diagnostic centre is situated in Kolhapur. As compare to other this laboratory in full of all equipment, and higher facilities. This laboratory provides all type of services at reasonable rate. The results of this laboratory Accurate. All type of tests conducted in this laboratory. The stop of this Laboratory is punctual & highly qualified with great experience in their field; go this lab is very useful for the people.

This lab offers various opportunities for students to think creatively, develop techniques & explore their interests. Therefore, Pathology lab is essential for students to learn & explain pathological facts & theories.

A medical laboratory or clinical laboratory is a laboratory where test are conducted out on clinical specimens of patient to aid in diagnosis, treatment & prevention of disease.

INSTRUMRNTS

1) INCUBATOR:

Fig no.1: Incubator

Lab Incubators are essential equipment in the laboratory that provides a temperature controlled environment to support growth of microbial cultures. A laboratory incubator is heated, insulated box used to grow and maintain microbiological or cell cultures. The incubator maintains optimal temperatures, humidity and gaseous content of the atmosphere inside.

Working of Incubator:-

An Incubator is based on the principle that organism require a particular set of parameters for their growth and development with the optimal condition (under artificial conditions) of temperature, humidity, oxygen & con levels.

1. Cabinet:- The cabinet is the main body of the incubator consisting of the double walled cuboidal enclosure with capacity ranging from 20 to 800 L.

2. Door: - A door is present in all incubators to close the insulated cabinet. A Handle is present on the side of the door to help with the manoeuvring of the door.

3. Control Panel: - Control panel also has a switch to control the thermostat of the device.

4. Thermostat :-Thermostat is used to get the desired temperature of the incubator.

5. Perforated Shelves:-The perforations on the shelves allow the movement of hot air throughout inside of the incubator.

6. L-shaped thermometer:-A thermometer is placed on the top part of the outer wall of the incubator.

7. HEP A Filter:-Some advanced incubators are also provided with HEP A filters to lower the possible contamination created due to airflow.

8. Humidity & gas control:-The con incubators are provided with a reservoir underneath the chamber that contains water.

Uses of Incubator:-

Some of the uses of incubators are-

1. Incubators also provide controlled condition for sample storage before they can be processed in the laboratories

2. Laboratory incubators provide a controlled, contaminant free environment for safe reliable work with cell & tissue cultures under artificial conditions.

3. Microbiological incubators are used for the growth & Storage bacterial culture Colonies & the determination of biochemical oxygen demand

4. Laboratory Incubators are essential for cell & tissue culture, biochemical &hematological studies) pharmaceutical work & food analysis.

2) CENTRIFUGE:

Fig no. 2: Centrifuge

Centrifugation play an important role in both biological sectors as well as industrial sectors Medical laboratories use centrifuges to separate Plasma from heavier blood cells. Modem centrifuges can even separate mixtures of different sized molecules of microscopic particles such as parts of cells.

Uses:

- The centrifuge used to separate solids suspended in a liquid by sedimentation.
- The rotational movements allow forces much greater than gravity to be generated in controlled periods of time.
- In the laboratory, centrifuge, can used to separate blood components: red cells , white cells, platelets to carry out further analysis tests & treatments.
- There is a wide range of centrifuges capable of serving specific. Industry & research.

3) FREEZE:

Fig no. 3: Freeze

Laboratory freezers are suitable for storing and maintaining the temperature of certain materials like cool samples of specimens for Preservation. They include Refrigeration units for storing blood Plasma & other blood Products, as well as Vaccines & other medical or pharmaceutical supplies. A Freezer is a crucial component of a laboratory as it is often necessary for the long-term storage of biological materials, like vaccines, bacteria samples, tissue samples & certain chemicals.

4) ANALYZER:

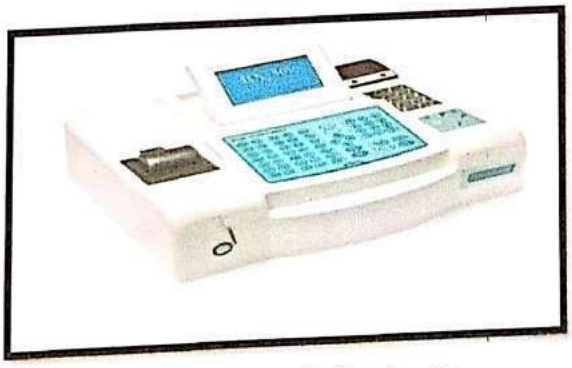


Fig no 4. Analyzer

Analyzer is medical laboratory device used to calculate the concentration of certain substances within samples of serum, plasma, urine and other bodyfluids. Substances analyzed through these instruments include certain metabolites, electrolytes, proteins and /or drugs.

Benefits of analyzer:

- It allows timely and accurate sample analysis and quality laboratory testing.
- It speeds up the analysis of complex biochemical reactions.
- It helps to streamline routine laboratory activities.
- It provides the best quality, reliable diagnostic information by providing test reports as soon as possible.

5) BACTEC:

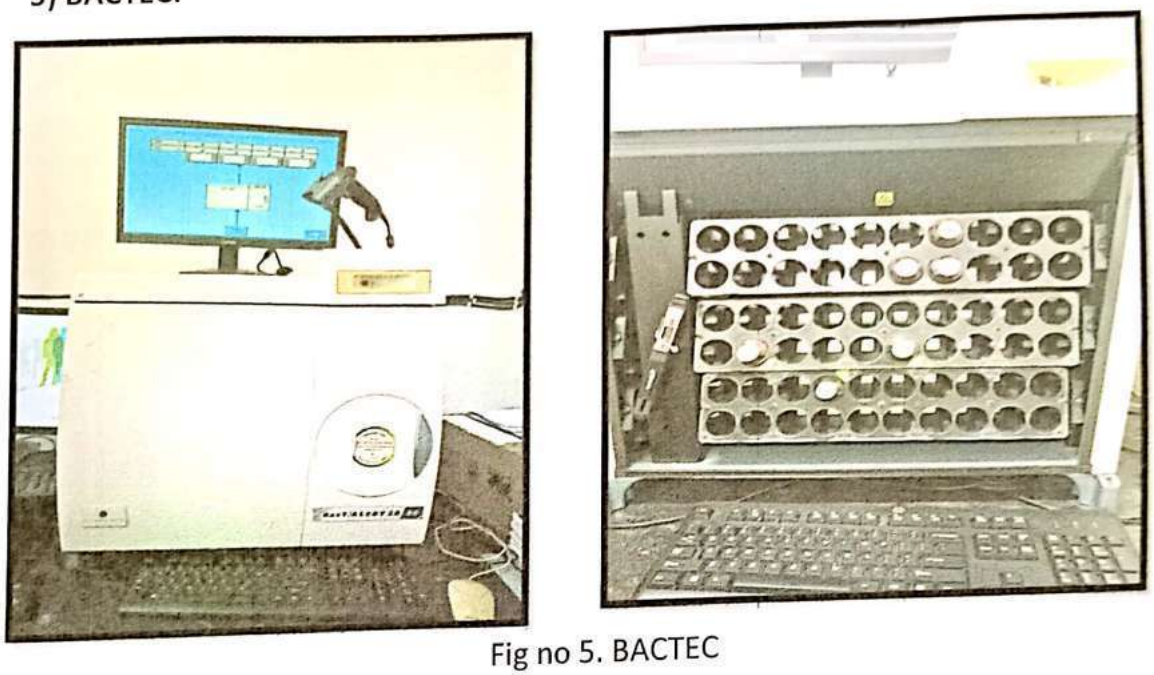


Fig no 5. BACTEC

BACTEC is an automated blood culture system developed by BD (Becton Dickinson), widely used in clinical microbiology laboratories to detect microorganisms such as bacteria and fungi in blood samples. It helps diagnose bloodstream infections (e.g., bacteria or sepsis) by incubating and monitoring blood cultures for microbial growth. This system is non invasive and continuous-monitoring, making it efficient for high-volume labs

Primary Application: Rapid detection of pathogens in blood for diagnosing infections, especially in cases like prosthetic joint infections or catheter-related bloodstream infections.

Specialized Uses: Effective for slow-growing organisms (e.g., Propionibacteria) without needing prolonged cultures. Also used for fungal and mycobacterial detection with specific bottles like BACTEC Myco/F Lytic.

Lab Integration: Instruments like BACTEC FX40 fit small spaces and integrate with lab workflows for efficiency. It's used in hospitals, reference labs, and research settings.

Advantages

- **Speed and Sensitivity**: Faster detection (often within hours) and higher recovery rates than manual methods, reducing false negatives and time to targeted therapy.
- **Efficiency**: Reduces contamination risks and simplifies procedures, with features like differential time-to-positivity for identifying catheter infections.
- **Verification**: Labs must verify performance per guidelines (e.g., CLSI M47) using seeded samples.

6) VITEK

Fig no. 6 VITEK

VITEK is a series of automated microbiology analyzers developed by bioMérieux for microbial identification (ID) and antibiotic susceptibility testing (AST) in laboratories. It includes systems like VITEK 2 (for bacterial and yeast ID/AST using fluorescence technology) and VITEK MS (for rapid ID via MALDI-TOF mass spectrometry). These tools are essential in clinical, pharmaceutical, and industrial labs for detecting bacteria, yeasts, and fungi,

Primary Application: Rapid ID of bacteria/yeasts from clinical samples (e.g., blood for sepsis) and AST to guide antibiotic therapy.

Specialized Uses: In pharma/food industries for contaminant detection; epidemiological studies; and testing slow-growing organisms. VITEK MS suits industrial microbiology for quick environmental monitoring.

Lab Integration: Used in hospitals for routine diagnostics, with models like VITEK 2 Compact for smaller labs.

Advantages

- **Speed and Accuracy**: Results in hours/minutes, higher sensitivity than manual methods, reducing treatment delays.
- **Efficiency**: Minimal training, standardized workflow, and broad database for diverse microbes.

7) BIOSAFETY CABINET:

Fig no.7: Biosafety cabinet

Biosafety Cabinet (BSC) is an enclosed, ventilated laboratory workspace designed to protect personnel, the environment, and products from harmful aerosols and contamination when handling infectious microorganisms. Utilizing HEPA-filtered laminar airflow, they are essential for safe, aseptic manipulation in research, clinical, and pharmaceutical settings.

Key Features and Principles

Protection Type: Provides triple protection—personnel (inward airflow), product (HEPA-filtered down flow), and environmental (HEPA-filtered exhaust).

Airflow: Uses a negative pressure design to prevent contaminants from escaping, ensuring air is cleaned before leaving the cabinet.

HEPA Filtration: HEPA filters are used to clean the air going into the work area and out to the environment, removing aerosols and airborne particles.

Uses and Applications

1. **Handling Pathogens:** Used for work with bacteria, viruses, and fungi, particularly in studies involving COVID-19, Ebola, or tuberculosis.
2. **Cell Culture:** Provides an aseptic, clean environment for growing and maintaining cells
3. **Diagnostic Procedures:** Used in labs to prevent exposure to infectious aerosols during patient sample analysis.
4. **Pharmaceutical Compounding:** Ensures products are safe and free from contamination.
5. **Chemical/Biological Work:** Class II Type B2 cabinets allow for work with hazardous biologicals in combination with toxic chemicals, as they provide 100% total exhaustion.

Types of Biosafety Cabinets

1. **Class I:** Protects personnel and the environment, but not the product.
2. **Class II:** The most common type, providing personnel, environmental, and product protection via vertical laminar airflow.
3. **Class III:** Totally enclosed and gas-tight, designed for high-risk (BSL-4) agents.

8) MICROSCOPY:

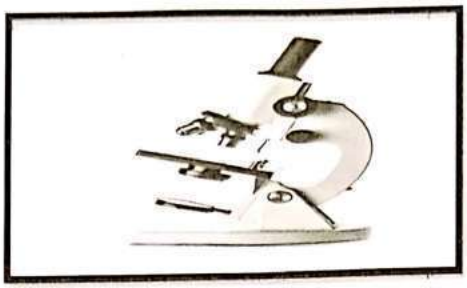


Fig no 8. Microscopy

The human vision has limitation and unable to see microscopic objects. Microscopy deals with enlargement of microscopic objects in order to make them visible to the naked or unaided eyes. The instruments used for enlargement of such microscopic objects is called microscope.

Light Microscope:

It uses natural or artificial light to illuminate the object and system of glass lens for magnification light microscopes are extremely versatile instruments .

Principle

As mentioned earlier, light microscopes visualize an image by using a glass lens, and magnification is determined by the lens's ability to bend light and focus on the specimen, which forms an image. When a ray of light passes through one medium into another, the ray binds at the interface causing refraction. The bending of light is determined by the refractive index, which is a measure of how great a substance slows the speed of light. The direction and magnitude of the bending of the light are determined by the refractive index of the two medium that form the interface.

Body Parts

Base it is horseshow or U shaped. It provides the fem and statin base

Pillar: is a pair of a short pillar like structure raised from the base of the microscope. The pillar provides the Finn and movable attachment site for body of the microscope

Arm: it is a backbone of the microscope in a curved structure like a letter 'C'. The microscope is assembled on the arm. At the base, the arm is joined ti pillars in such a say that it can incline

Stage and mechanical stage: The stage is plane, horitzontal platfom with the hole at the center (2 to 3 inches in diameter) the stage provides the space for mounting a slide. The opening at the center allows the passage of light rays to illuminate the object on the slide. The mechanical stage is the assemble on the stage and works on rack.

Application of light microscope:

- It is used to study of bacteria, fungi, protozoa, etc.
- It is used to carry microscopic analysis of clinical sample in pathological laboratories.
- It is used to study tissue section of plants and animals.
- It is used to trace evidence in forensic laboratories.

BIOCHEMICAL TEST

1) Indole test:

Indole test is a biochemical test conducted on bacterial species to detect their ability to produce indole from tryptophan in the presence of a group of enzymes called 'tryptophanase'.It is a qualitative test that tests the conversion of tryptophan into indole. The test is performed as a part of the IMViC test that is used to differentiate the members of the Enterobacteriaceae family

A) Principle:

Certain bacteria possess the enzyme tryptophanase, which breaks down the amino acid tryptophan into indole, pyruvicacid, and ammonia. When Kovac's reagent (containing paradimethylaminobenzaldehyde) is added to the culture, it reacts with indole to form a red-colored dye (rosindole dye) that forms a ring at the top of the medium.

B) Media use:

Tryptophan: Provides the essential substrate (tryptophan) for the enzymatic reaction.

Water: The solvent for the medium.

Sodium Chloride: Maintains osmotic balance

C) Procedure:

- Inoculation: Inoculate the medium (tryptone broth/peptone water) with the test organism.
- Incubation: Incubate at 37°C for 24–48 hours.
- Reagent Addition: Add 0.5 mL of Kovac's reagent to the broth culture.
- Observation: Gently shake the tube and observe for a color change at the surface.

D) Result :

i. Red coloured ring : Indole Positive

ii. No colour change (yellow) : Indole Negative

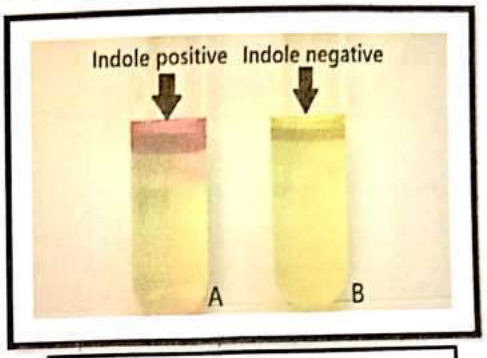


Fig no.9 Indole test

E) Result and interpretation: Table No. 1 Result of Indole

Result	Colour	Example of organism
Positive	Red coloured ring	*Escherichia coli, Proteus vulgaris*
Negative	No colour (yellow)	*Klebsiella pneumonia Salmonella species*

2) voges proskeur (VP) test-

Voges-Proskauer (VP) test is a biochemical test used to detect the production of acetoin (acetyl methyl carbinol) from glucose fermentation.

A) Principle-

Some bacteria ferment glucose by the butylene glycol pathway and produce acetoin as an intermediate product.When a-naphthol and 40% potassium hydroxide (KOH) are added to the culture, acetoin is oxidized to diacetyl, which reacts with peptone in the medium to give a pink/red color.

B) Media use-

MR-VP broth (Glucose phosphate broth)-

Contains

i) Peptone(nitrogen source)

ii) Dextrose (glucose)

iii) Dipotassium phosphate (K_2HPO_4)

iv) Distilled water

C) Procedure-

i) Inoculate MR-VP broth with test organism.

ii) Incubate at 37°C for 24-48 hours.

iii) After incubation add 5 drops of a-naphthol and KOH.

iv) Shake well and keep for 15-30 minutes.

v) Observe for color change.

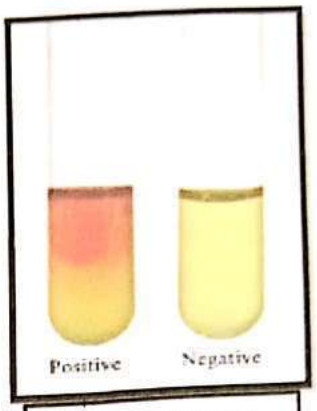


Fig no.10 VP test

D) Result-

i) Red colour → VP positive.

ii) No color change (yellow) → VP negative.

E) Result and interpretation: Table No. 2 Result of VP

Result	Colour	Example of organism
Positive	Red	*K.pneumoniae, Enterobacter*
Negative	No colour(yellow)	*E.coli*

3) Methyle red (MR) test –

Methyl Red (MR) test determines if bacteria perform mixed-acid fermentation, metabolizing glucose into stable acid end-products that lower the pH to 4.4 or less

A) Principle:

The MR test detects the ability of an organism to produce and maintain high concentrations of acid (pH 4.4) from glucose fermentation. Organisms that use the mixed acid pathway produce stable, strong acids that overcome phosphate buffers in the medium, unlike organisms that produce neutral end products.

B) Media Used:

- MR-VP Broth (Methyl Red- Voges Proskauer) broth : contains peptone (source of protein)
- Glucose (carbohydrate source)
- Dipotassium phosphate (buffer)

C) Procedure:

1. Inoculation: Inoculate a tube of sterile MR-VP broth with a pure culture of the bacteria.
2. Incubation: Incubate the broth at for 48 hours to 5 days.
3. Testing: Add 5 drops of Methyl Red indicator to 5 mL of broth.
4. Observation: Observe for an immediate color change

D) Result:

i. Red colour – MR positive

ii. No colour (yellow) – MR negative

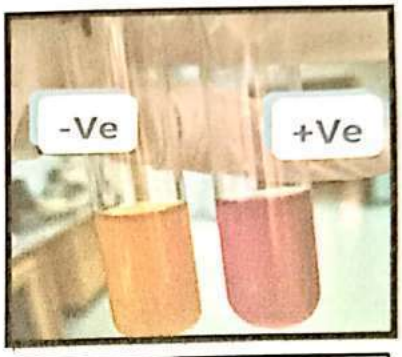


Fig no.11 MR test

E) Result and interpretation: Table No. 3 Result of MR

Result	Colour	Example of organism
Positive	Red	*E.coli*
Negative	No colour(yellow)	*K.pneumoniae*

4) Urease test :

The urease test detects the enzyme urease, which hydrolyzes urea into ammonia and carbon dioxide .The produced ammonia creates an alkaline environment, changing the phenol red indicator from yellow/orange to a bright pink, indicating a positive result.

A) Principle :

Enzyme Activity: Microorganisms that produce urease hydrolyze urea into ammonia and carbon dioxide Phenol red, a pH indicator, changes color from yellow/orange (pH 6.8) to intense magenta/bright pink (pH 8.1 or higher) in an alkaline environment.

B) Media Used: Christensen's Urea Agar

Urea Agar Base is a specialized medium that contains:

Urea: Substrate.

Phenol Red: pH indicator.

Agar & Peptone: Solidifying agent and nutrients.

C) Procedure:

(Urea Agar Slant)

1. Inoculation: Streak the entire surface of a sterile Christensen's urea agar slant with a loopful of a pure culture.
2. Incubate at 370 c for 24 hrs .
3. Observe for colour change

Fig no.12 Urease test

D) Result :

Bright pink colour on the slant - Urease positive

No colour on slant – Urease negative

E) Result and interpretation: Table No. 3 Result of Urease

Result	Colour	Example of organism
Positive	Bright pink	*Proteus* spp., *Helicobacter pylori*
Negative	No change	*Escherichia coli, Salmonella* spp

Antibiotics Sensitivity Test

1) Antibiotic sensitivity :

An antibiotic sensitivity test (or susceptibility test) identifies which antibiotics effectively kill specific bacteria or fungi causing an infection. It helps clinicians choose the right treatment to avoid antibiotic resistance and ensure faster recovery. The process involves sampling the infection, culturing microbes, and testing them in a laboratory

A) Principle:

Disk Diffusion (Kirby-Bauer): Paper discs impregnated with specific antibiotic concentrations are placed on agar inoculated with a test organism. The antibiotic diffuses from the disc into the agar, forming a concentration gradient. If the bacteria are susceptible, a clear area of "no growth" (zone of inhibition) forms around the disc.

Minimal Inhibitory Concentration (MIC): The lowest concentration of an antibiotic that prevents visible growth of the organism

B) Media used:

Mueller Hinton Agar(MHA media) : This is the standard medium used for testing most fastidious bacteria.

Contain : Acid Hydrolysate of Casein

Beef Extract

Starch

Agar Agar

Distilled water

C) Procedure:

1. Inoculum Preparation: A bacterial suspension was prepared
2. Inoculation: A sterile cotton swab is dipped into the suspension and used to streak the entire surface of a Mueller-Hinton agar (MHA) plate three times (rotating the plate 60^0 each time) to ensure confluent growth.
3. Application of Discs: Antibiotic-impregnated disks are applied to the surface of the agar using sterile forceps or a dispenser
4. Diffusion :Keep the plate in freeze for 10min for diffusion
5. Incubation: Plates are incubated at 37^0c for 24 hours.

6. Measurement: The diameters of the zones of inhibition are measured in millimeters, including the diameter of the disc.

Fig no. 13 Antibiotic sensitivity

D) Result:

Sensitive (S): The antibiotic is effective; the zone is large.

Intermediate (I): The antibiotic may be effective at higher doses.

Resistant (R): The antibiotic is ineffective; no zone or a very small zone is observed

2) Vitek :

The VITEK system is a widely used, fully automated microbiology platform designed for the rapid identification (ID) of microorganisms and antimicrobial susceptibility testing (AST). It uses fluorescent and turbidimetric technology to provide results much faster than conventional manual methods

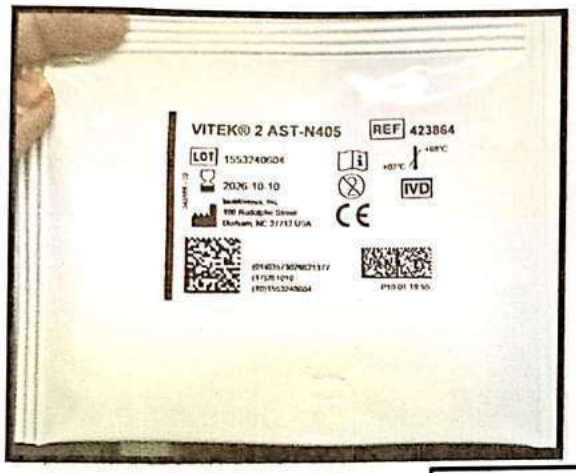
A) Purpose:

- Rapid Identification: Identifies a broad range of clinically significant bacteria (Gram-negative and Gram-positive), yeasts, and fastidious organisms.
- Antimicrobial Susceptibility Testing (AST): Determines the Minimum Inhibitory Concentration (MIC) of antibiotics, assisting in selecting appropriate targeted therapy.

B) Procedure:

1. Take 2 vitek tube label it as ID and AST
2. Put that tubes into cassette

3. Add 3ml if saline in both the tube
4. Clean the tube with filter paper and check the Densi with Densichecker and adjust it to "0" .
5. Take a fresh colony to be tested and rub on side wall of the tube and mix the saline with help of micropipette (145microliter for GNB and 280 microliter for GPC) mix properly .
6. Clean the tube and check the Densi with Densichecker take 3 reading of each tube and adjust it to 0.63/cell
7. Insert the appropriate card to that tube (If checking ID inset card into ID ,if checking AST inset card into AST)
8. Insert the cassette into vitek machine
9. Observe the result after 8-10 hrs.



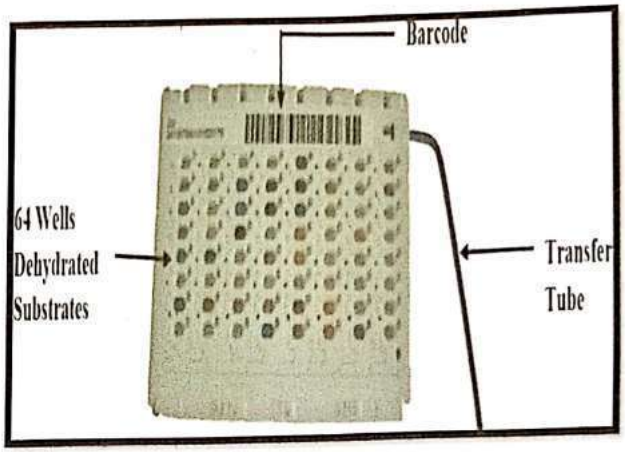


Fig no. 14 Vitek card

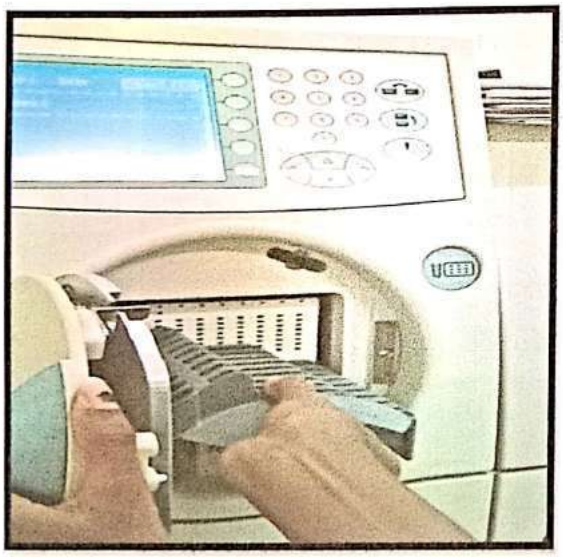
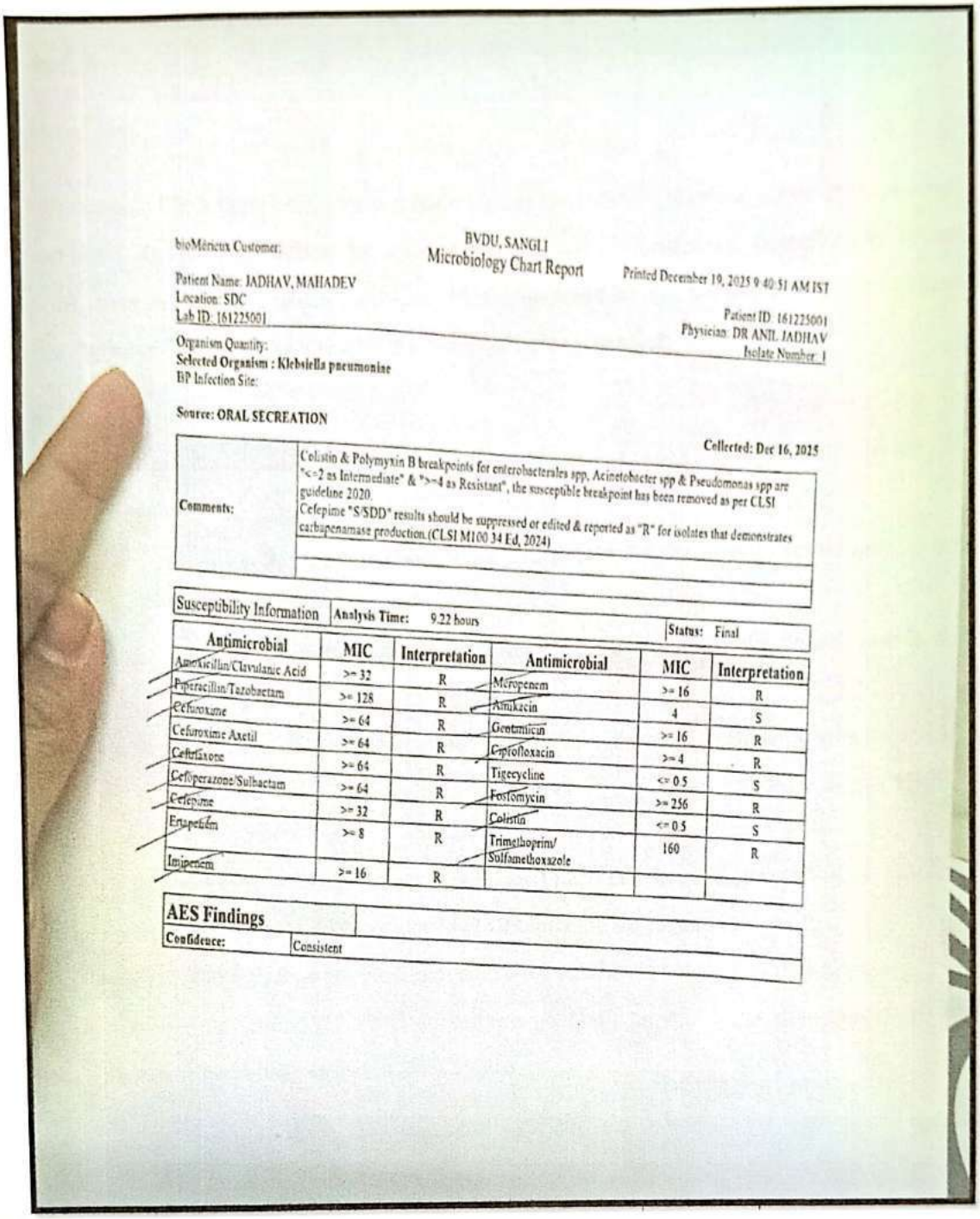


Fig no. 16 Insertion of card

Fig no. 15 Cassette



bioMérieux Customer: BVDU, SANGLI

Microbiology Chart Report

Printed December 19, 2025 9:40:51 AM IST

Patient Name: JADHAV, MAHADEV
Location: SDC
Lab ID: 161225001

Patient ID: 161225001
Physician: DR ANIL JADHAV
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae
BP Infection Site:

Source: ORAL SECREATION

Collected: Dec 16, 2025

Comments:	Colistin & Polymyxin B breakpoints for enterobacterales spp, Acinetobacter spp & Pseudomonas spp are "<=2 as Intermediate" & ">=4 as Resistant", the susceptible breakpoint has been removed as per CLSI guideline 2020. Cefepime "S/SDD" results should be suppressed or edited & reported as "R" for isolates that demonstrates carbapenamase production.(CLSI M100 34 Ed, 2024)

Susceptibility Information | **Analysis Time:** 9.22 hours | **Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Amoxicillin/Clavulanic Acid	>= 32	R	Meropenem	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Amikacin	4	S
Cefuroxime	>= 64	R	Gentamicin	>= 16	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin	>= 4	R
Ceftriaxone	>= 64	R	Tigecycline	<= 0.5	S
Cefoperazone/Sulbactam	>= 64	R	Fosfomycin	>= 256	R
Cefepime	>= 32	R	Colistin	<= 0.5	S
Ertapenem	>= 8	R	Trimethoprim/ Sulfamethoxazole	160	R
Imipenem	>= 16	R			

AES Findings

Confidence:	Consistent

Fig no .17 Report of Vitek

3) BACTEC:

The BD BACTEC system is an automated blood culture instrument designed for the rapid detection of bacteria, fungi, and mycobacteria in blood or sterile fluid samples

A) Principle :

CO_2 Sensing: The system uses special culture bottles containing a chemical sensor that responds to increased CO_2 levels generated by microbial metabolism.**Fluorescence Detection:** As microbes grow, they release CO_2, which increases the fluorescence of the sensor, which is continuously monitored (every 10–60 minutes) by the machine's photodetector.

B) Procedure:

Collection: Blood is collected aseptically from the patient and inoculated into specific BD BACTEC™ culture bottles (Aerobic, Anaerobic).

Incubation: Bottles are loaded into the BACTEC instrument, which provides continuous agitation and incubation at 37^0C

Monitoring: The instrument automatically monitors fluorescence to detect growth over a 5-day period.

Positive Alert: When the CO_2threshold is met, the system triggers an alert, identifying the bottle as positive.

C) Results:

- Positive Result: A positive alert indicates microbial growth, triggering a subculture (streaking on agar) and Gram staining to identify the pathogen.
- Negative Result: If no growth is detected after 5 days, the bottle is marked negative.
- Time to Detection (TTD): BACTEC systems generally provide faster detection times, with many cultures flagging within 24–72 hours

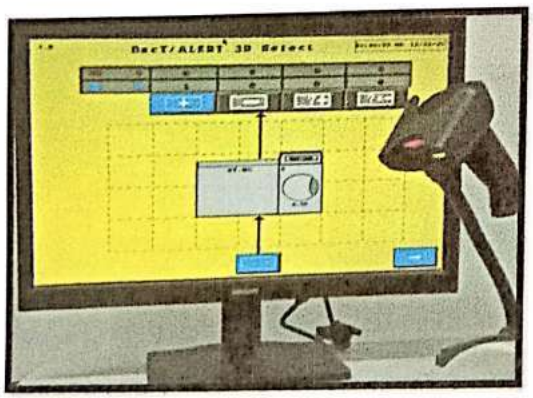


Fig no. 18 screen showing +ve result

Fig no. 19 Blood collection bottle