

N D E X

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Class CERAMICS Section _____ Roll No. _____ Year 2015

Subject _____

(1) Preparation of Nutrient Agar and Nutrient Broth Media.

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A growth Medium or culture Medium is a solid, liquid or semi-solid chemical composition prepared to support the growth of Micro-organisms. Bacteria and fungi are grown on different types of nutrient Media. The liquid culture Media are termed as Broth and solid cultured Media are termed as Agar-base Media. The selection depends on Microorganisms that one is trying to isolate or identify. As humans, Microorganisms also have some nutrient requirements for their growth. Different types of growth factor is added such as carbohydrate source, organic matter source and proteins i.e peptone and beef extract salt solution i.e NaCl is also added to regulate the osmotic pressure and salt in a Medium. pH of the Media should be maintained at 7.2 as microbes are both Acid-lactic and alkali-lactic.

Nutrient agar is a popular general purpose, nutrient medium used to support the growth of various types of bacteria. Nutrient agar is popular because it is available easily and is a low cost Media, and contains many nutrients for their bacterial growth.

Culture Media Must be initially free from Microorganisms i.e they should be Sterilized, which can be done in an

autoclave at 15 lb/in² pressure for 15 min.

Materials required

at 121°C

(A) Media composition for 300 ml.

(1) Peptone (1%) ~ 3 gm

(2) Beef extract (0.5%) - 1.5 gm

(3) NaCl (0.5%) - 1.5 gm

(4) Agar (2%) - 4 gm

(5) Distilled water - 300 ml.

(B) Glassware and Instruments: Conical flask, petri-dishes, Measuring cylinder, test tubes, cotton, Brown paper, Autoclave, Laminar Air flow.

Media preparation:

(1) Peptone, Beef extract are properly weighed and taken in a flask. For liquid Media, distilled water is added to it to make 300 ml media.

(2) All the contents are mixed and dissolve by proper stirring.

(3) Now for solid Media preparation 200 ml of dissolved mixture is separated in another conical flask.

(4) Now, Agar (2%) is added to it, mixed and dissolved by proper stirring.

(5) Both 100 ml liquid containing conical flask and 200 ml solid Media containing conical flask, petridishes and test tubes are covered in brown papers and put in autoclave at 15 lb/in².

pressure at 121°C for 15mins.

- Technique of inoculation:-

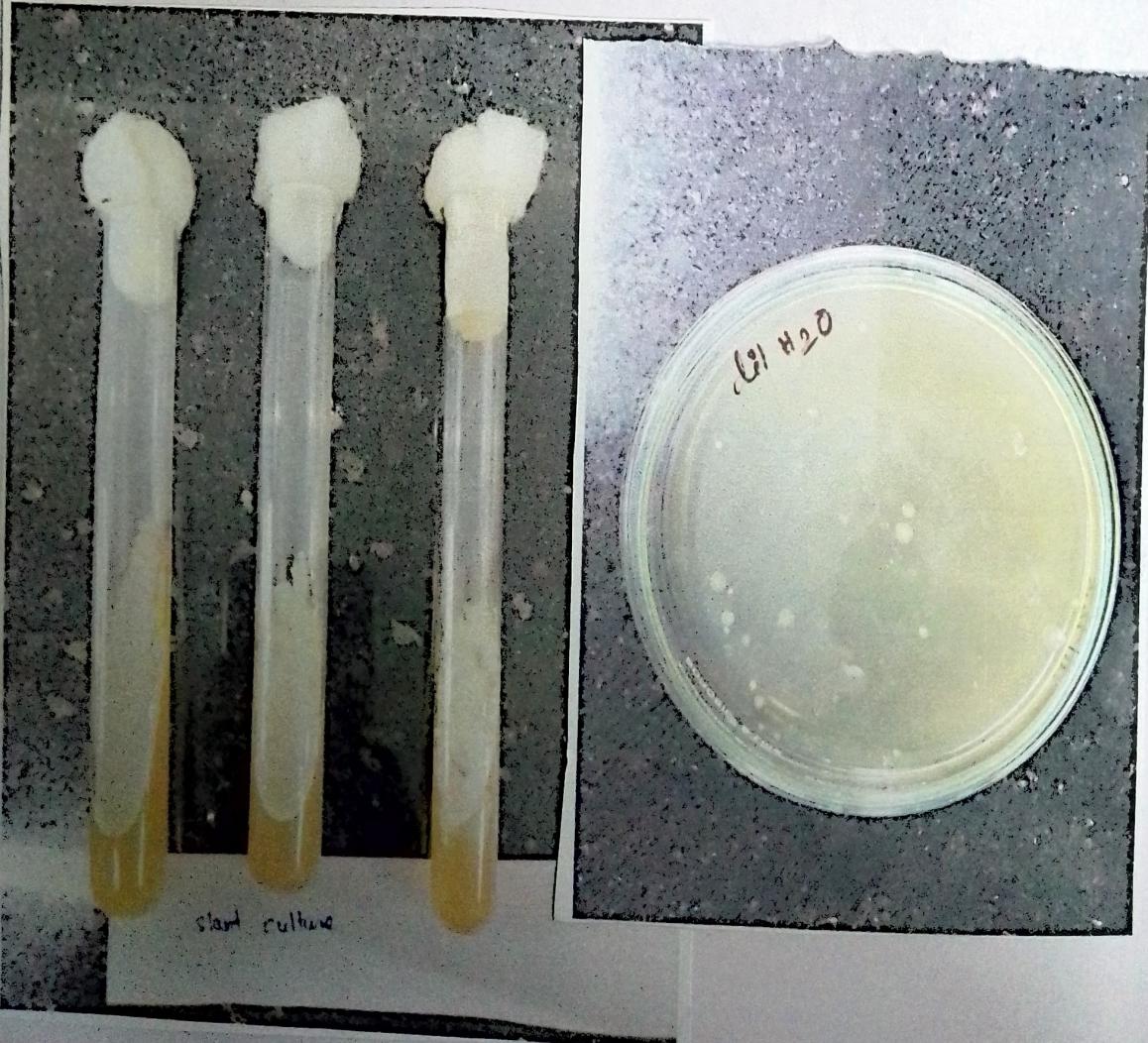
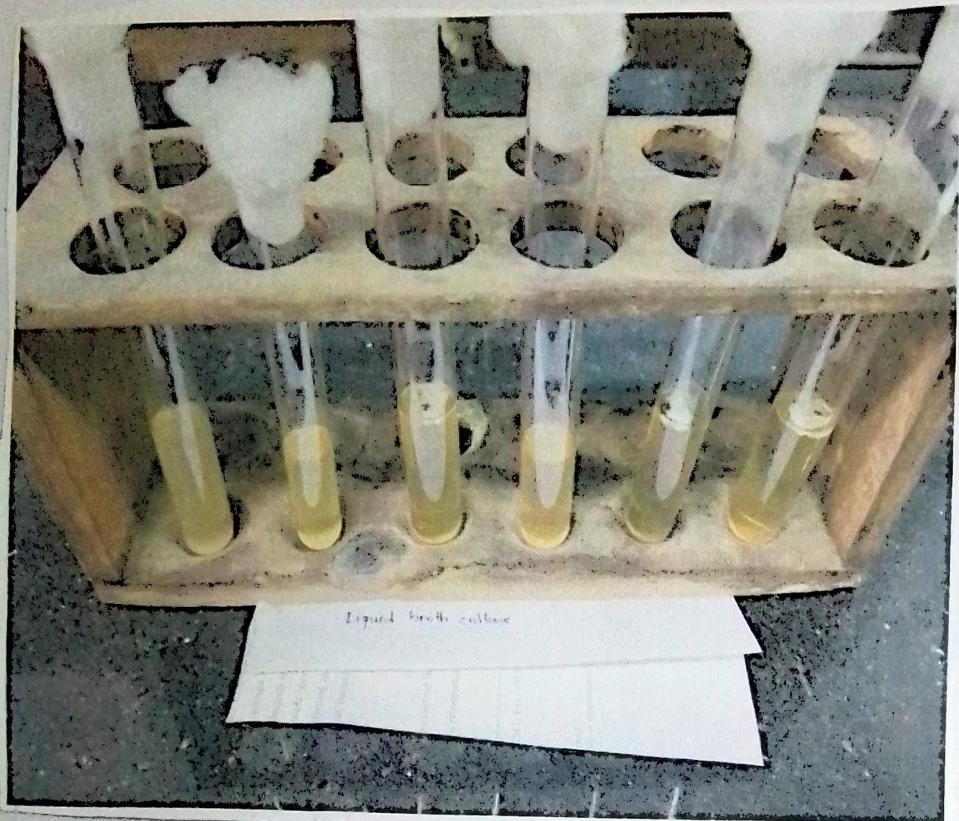
(i) Slant: Commonly inoculated to broth culture, slant culture, plate culture etc. A culture made by inserting an inoculating needle with inoculum down the centre of solid media contained in test tube is called ~~slant~~ culture.

(ii) Streaking: It is a technique to isolate a pure strain from a single species of microorganism, often bacteria. It may be classified as:-

1. Continuous streaking
2. Discontinuous streaking
3. Quadrant streaking

• Distribution of Media:- once everything is autoclaved, they all are taken out from autoclave. The liquid broth (100ml) and solid broth (200ml) containing conical flask have already been sterilized in the autoclave along with petridishes and test tubes are allowed to cool down in front of laminar flow.

The cotton plug of the liquid containing conical flask is removed and the media is transferred to test tube.



in Laminar Air flow. (Laminar Air flow must be cleaned with 70% ethanol before use). The test tube were kept slightly in oblique position by with cotton plugged end. This are Known as Agar-slant and are stored for later use.

Now, the cotton plug of Solid Media containing cotton flask is removed and Media is poured in 10 petridishes - in such a way that it totally covers the bottom of the dish. Then the dishers were covered by the liquid. These are Known as Agar-plate and are stored for further use. This is done in Laminar Air-flow.

• Imoculation of culture:

(a) Agar-slant culture: Nichrome loop was Made free from any Microorganisms by red heating and cooling down. A looped - full organism were taken and streaked in the Agar slant. These tubes were kept for incubation for 24 hr.

(b) Solid-Agar plate: A nichrome loop was made free from any Micro-organism by red heating and cooling down at room temperature. A loop-field Microorganism were taken from pre-culture agar slant and streaked in Zig-Zag function. This Method is 'streak plate' Method. This plate is Kept for incubation for 24 hours.

(c) • Broth culture : (Preparation)

Broth culture Method is used in Microbiology where microorganisms are grown in a liquid nutrient medium consisting - peptone (1%), Beef extract (0.5%), NaCl (0.5%), Distilled water (300ml). Mixed the broth with distilled water and autoclave the broth at 121°C for 15 minutes for sterilization. After that bacterial inoculum was added using inoculation needle. After that the broth was allowed to keep in incubator for 24 hours incubation at 37°C temp.

Broth culture contain peptone, beef extract, NaCl without agar. It's a liquid form of culture Media.

- Observation : After incubation, the cultured media was appeared in turbidity condition which indicate the bacterial growth.

Q) Simple Staining of Microbes :-

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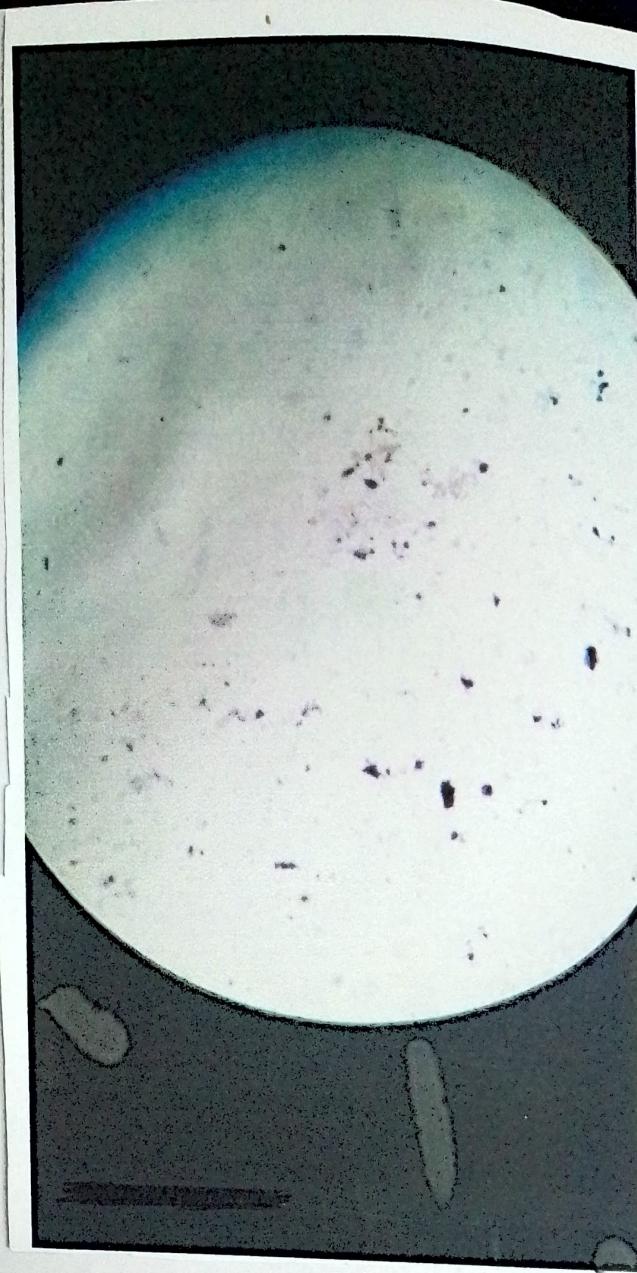
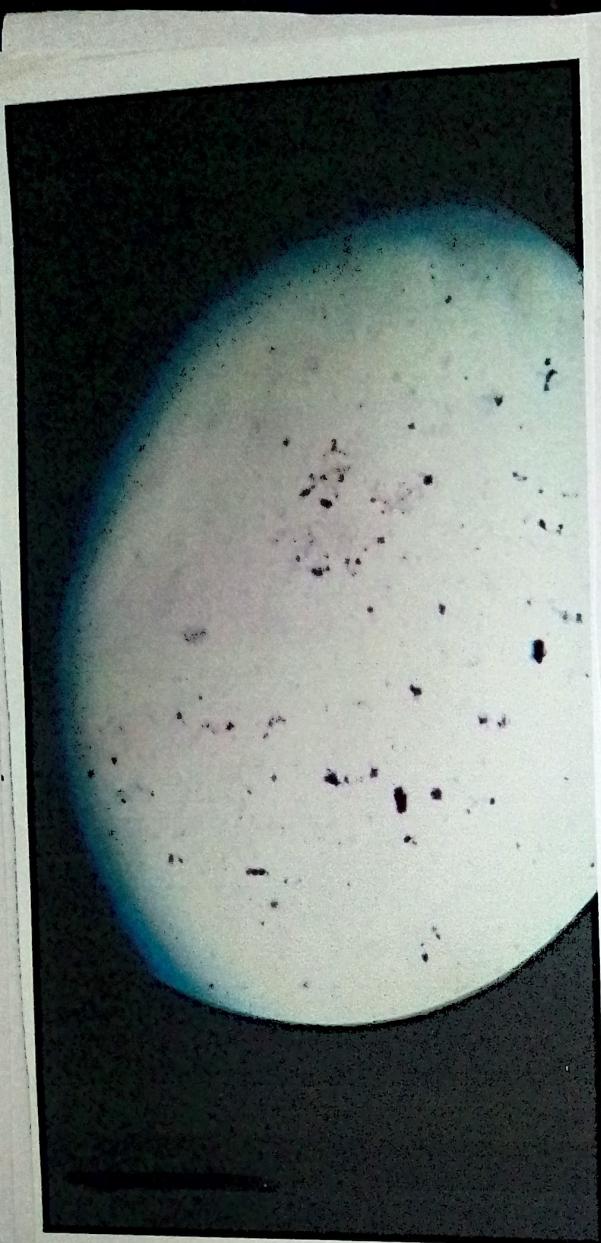
• Theory :- The simple stain can be used as a quick and easy way to determine cell shape, size and arrangement of bacteria. The simple stain is a very simple staining procedure involving single solution of stain. Any basic dye such as Methylene blue, crystal violet can be used to colour the bacterial cells. So, the bacterial cells are generally stained with a dye which makes them contrast with colour with their surroundings and so easily visible, these stains will readily give up an hydroxide ion or accept a hydrogen ion. So, the dyes are generally salts in which one of the ion is coloured. If the colour is in positive ion the dye is basic dye and if the colour is in the negative ion the dye is acidic.

Bacterial cells have a slight negative charge when pH is near neutrality and so easily combines with basic dyes.

• Procedure :-

(a) Preparation of smear :- Using a sterilized inoculation loop, we transferred loopfull of liquid suspension containing bacteria to a slide, on transferring of an isolated from a cultural plate to a slide with water, we allowed the smear to dry thoroughly. Now we heat fixed the smear by passing the underside of the slide through the burner flame two or three times. It

Teacher's Signature.....



fixed the cell in the slide. We should not overheat the slide as it will deposit in the bacterial cell.

(b) Staining:- We covered the smear with 2-3 drops of crystal violet solution as per requirement. Then we hold the slide under ~~running~~ tap water and then the slide was dried in air.

Now we placed the stained smear on the microscopic stage, smear side up using 10X object and also captured using the polar achromat 40X obj.

Observation

The bacterial cells usually stain uniformly and the colour of cell depends on the type of dye used. As here crystal violet solution is used, some granules in interior of bacterial cells may appear more deeply stained than the rest of the cells, which is due to presence of different chemical substances when the stained smear was placed on the microscopic stage ~~at~~ smear side up and smear was focused using the different magnification 40X, 100X.

- 40X:- Bacteria having crystal violet stain was observed
- 100X:- Bacteria having crystal violet stain and shape of bacteria observation.

Conclusion: By observation of the bacteria, it is easy to understand that the bacteria is spherical shaped and rod shaped.

3) Environmental testing Using Agar Plate

Method:-

Objective:- To ~~detect~~ detect and compare Microbial contamination in different environments (Laminar Air flow, Pharmacy lab, outside and toilet) using Agar plate Method by exposing nutrient agar plates to air and counting colony-forming units (CFUs) after incubation.

Principle:- The agar plate Method is based on a passive air sampling technique, where air borne microorganisms naturally settle onto the surface of a sterile nutrient agar plate. When the plate is left uncovered in a specific environment for a fixed duration, from the surrounding air such as bacteria, fungal spores and other microorganisms were captured onto the plate. These microbes utilize the nutrients in the agar, and upon incubation at an optimal temperature (usually 37°C), begin to multiply. After 24 to 48 hours, they form visible colonies that can be counted and recorded as Colony-forming units (CFUs).

This Method is highly valued because it is simple, cost-effective, and requires minimal equipment, making it accessible for routine microbial monitoring in a wide variety of settings. It is especially useful in environments where sterility is critical, such as in pharmaceutical manufacturing units, microbiology laboratories, hospitals and food processing units.

Since this method does not require complex instruments it allows the detection of microbial hotspots, help track contamination sources, and ensures Good Manufacturing practices (GMP).

In this experiment, we used nutrient agar plates, which support the growth of a broad spectrum of non-fastidious bacteria. The plates were exposed in three different environments laminar air flow, pharmacy lab, outside and toilet for 15 and 30 minutes. After incubation, the colonies were counted and expressed as CFUs, providing a comparative view of microbial contamination in each area. Microorganisms that are air-borne for extended periods, too small to settle, or require specialized nutrients or anaerobic conditions may not be detected. Despite these limitations, the agar plate method remains a reliable and widely used technique for environmental surveillance and is effective for trend analysis and routine microbial assessments.

Theory:-

Microorganisms are ubiquitous and present in virtually every environment, including soil, water, surfaces, and even the air we breathe. Though invisible to the naked eye, the airborne microbes such as bacteria,

fungal spores, and viruses continuously flow in the air and settle on exposed surfaces. When these microorganisms were cultured on a nutrient-rich medium under appropriate environmental conditions typically 37°C for bacterial growth, they multiply and form visible colonies. This natural process helps us to estimate microbial presence using agar plates.

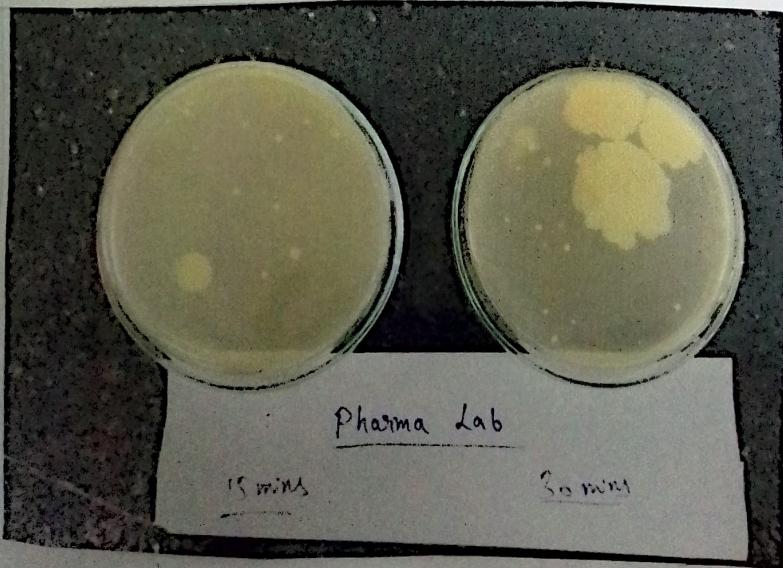
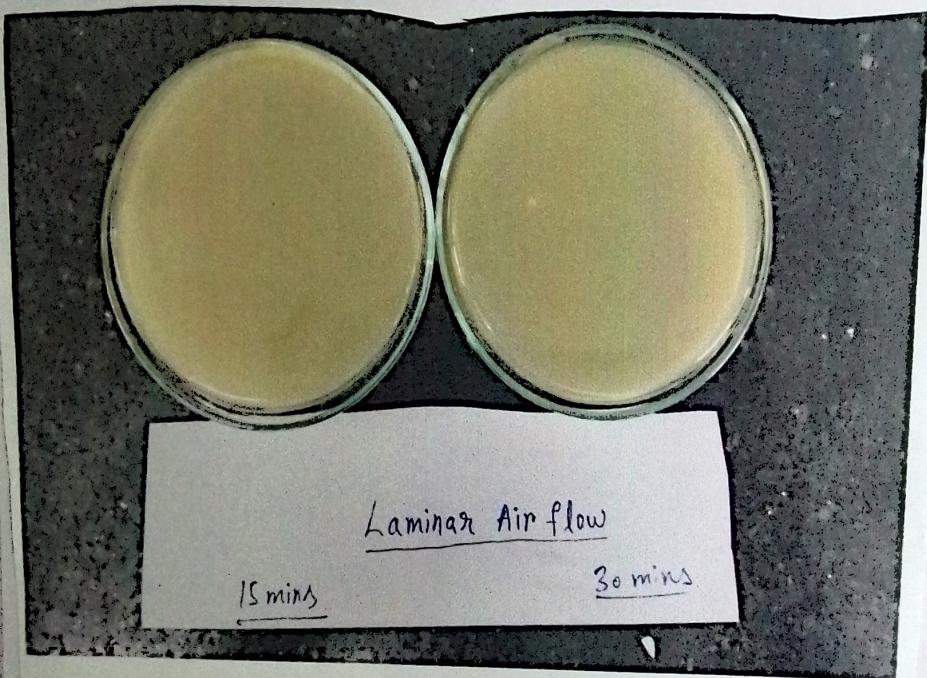
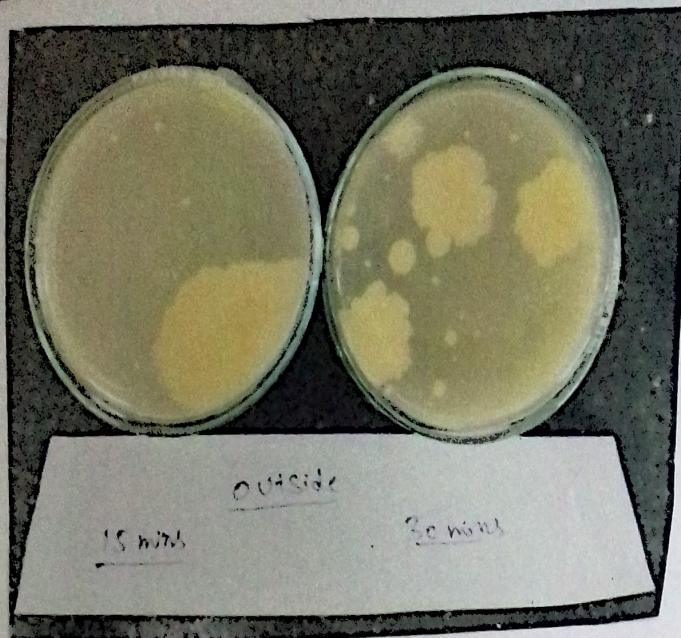
This experiment helped us to understand how airborne microbial load can be detected and quantified using the agar-plate method. By exposing sterile nutrient agar-plates for duration (15 and 30 minutes), airborne microbes were allowed to settle and incubated to promote colony development. The (CFUs) indicate the level of microbial contamination in each environment. High CFUs counts in areas like toilets suggest poor air quality, while absence of CFUs in laminar air flow after incubation period implied that the laminar air flow is contamination free confirm effectiveness of HEPA (High-Efficiency Particulate Air) filters and UV light sterilization. This technique is widely used in pharmaceutical industries, cleanrooms, hospitals, and laboratories etc.

Materials used

- Sterile nutrient agar plates
- Marker for labeling
- Timer or Stopwatch
- Incubator (set to 37°C)
- Sterile gloves
- Lab coat and safety gear.

Procedure :-

- We started by preparing and labeling the sterile nutrient agar plates with the location name (laminar bench, pharmacy lab, toilet) exposure time (15 to 30 min) and date.
- We took the plates to each selected environment and place them on a flat surface. The lid was removed and the plate was left open to the air for the mentioned exposure time either 15 minutes or 30.
- After exposure, I carefully closed each plate to avoid contamination and brought them back to the lab for incubation.
- We incubated all the exposed plates in an incubator set at 37°C for 24 hours. This allowed the airborne microorganisms that had landed on the agar to grow into visible colonies.
- After incubation, we observed and counted the colonies (CFUs) on each plate. These values were recorded for comparison on a table chart.



- We cleaned the workspace and ensured the used plates were disposed of in a biohazard waste bin according to lab safety rules.

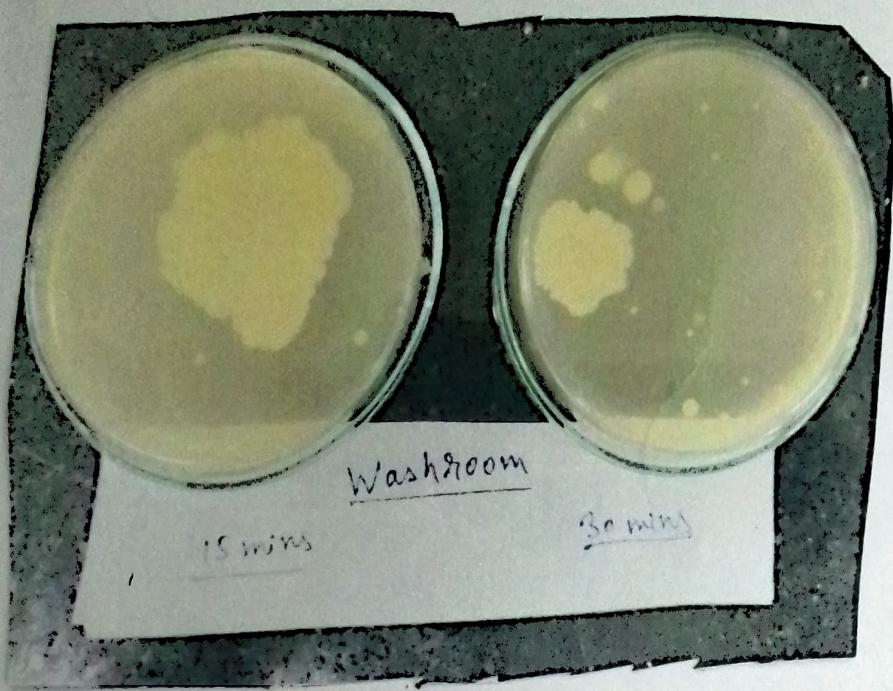
Results:

Environment	Exposure time (minutes)	CFU count
Laminar Air Flow	15	0
Laminar Air Flow	30	0
Pharmacy Lab	15	13
Pharmacy Lab	30	22
Toilet	15	5
Toilet	30	25
Outside	15	10
Outside	30	20

The results clearly indicate that the toilet had the highest microbial load after 30 minutes of exposure which suggest poor air quality, followed by moderate contamination in the pharmacy lab, and no CFU counts on the laminar air flow.

This experiment also demonstrated that longer exposure time significantly increased CFU counts across all environments.

The toilet showed the highest Microbial load with the maximum (CFUs) due to frequent human use, poor ventilation, lack of air filtration. The pharmacy



lab had a moderate no. of colonies indicating some level of cleanliness but still lacking effective air purification system. On the other hand, in Laminar Air flow, CFU was not found, that indicate a conclusion regarding contamination free and maintaining a sterile condition through HEPA filter and UV light.

30 min exposed plates were contained more CFU rather 15 mins exposed plates which indicates that the exposure time impacts on microbial deposition

Conclusion:-

The agar plate Method effectively revealed varying microbial loads, with the toilet showing highest contamination whereas contamination free laminar air flow, confirming the importance of environmental hygiene and proper air filtration system.