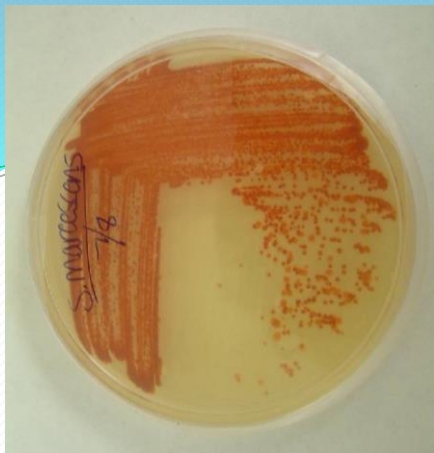


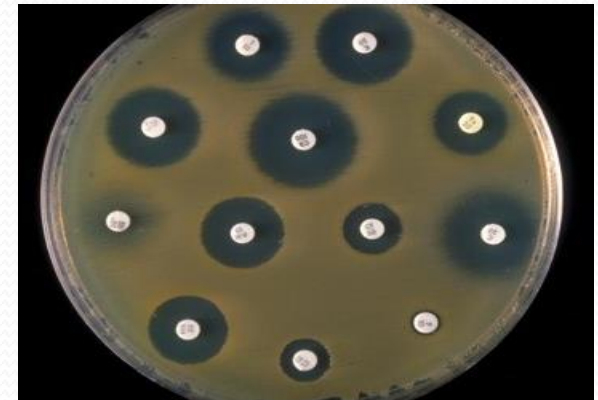
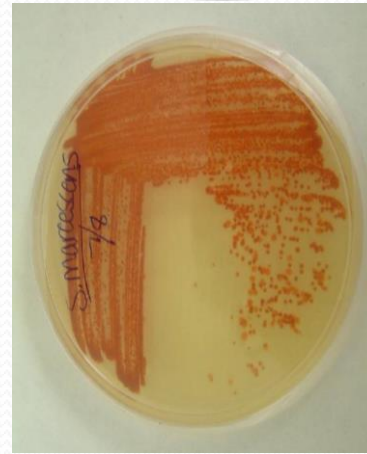


CULTURE METHODS



Culture Methods are needed to:

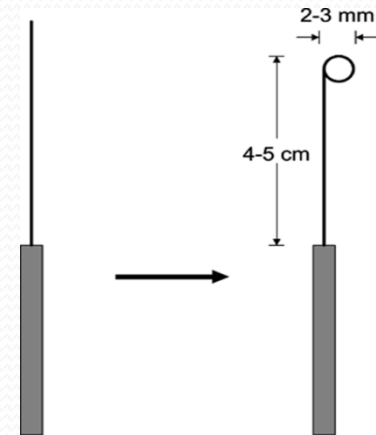
- Isolate the organism in pure form
- Identify the organism.
- See what antibiotics they are susceptible to.
- Make antigens for serological diagnosis.
- To maintain Stock cultures.



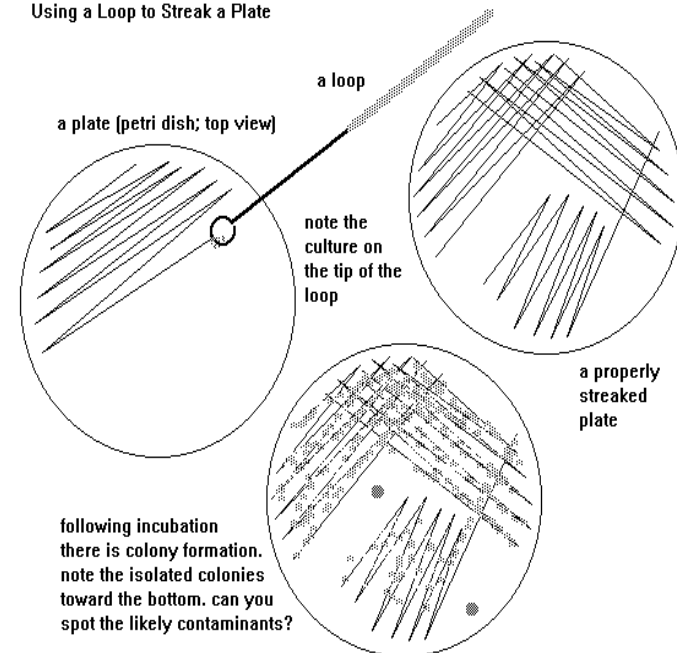
AEROBIC CULTURE

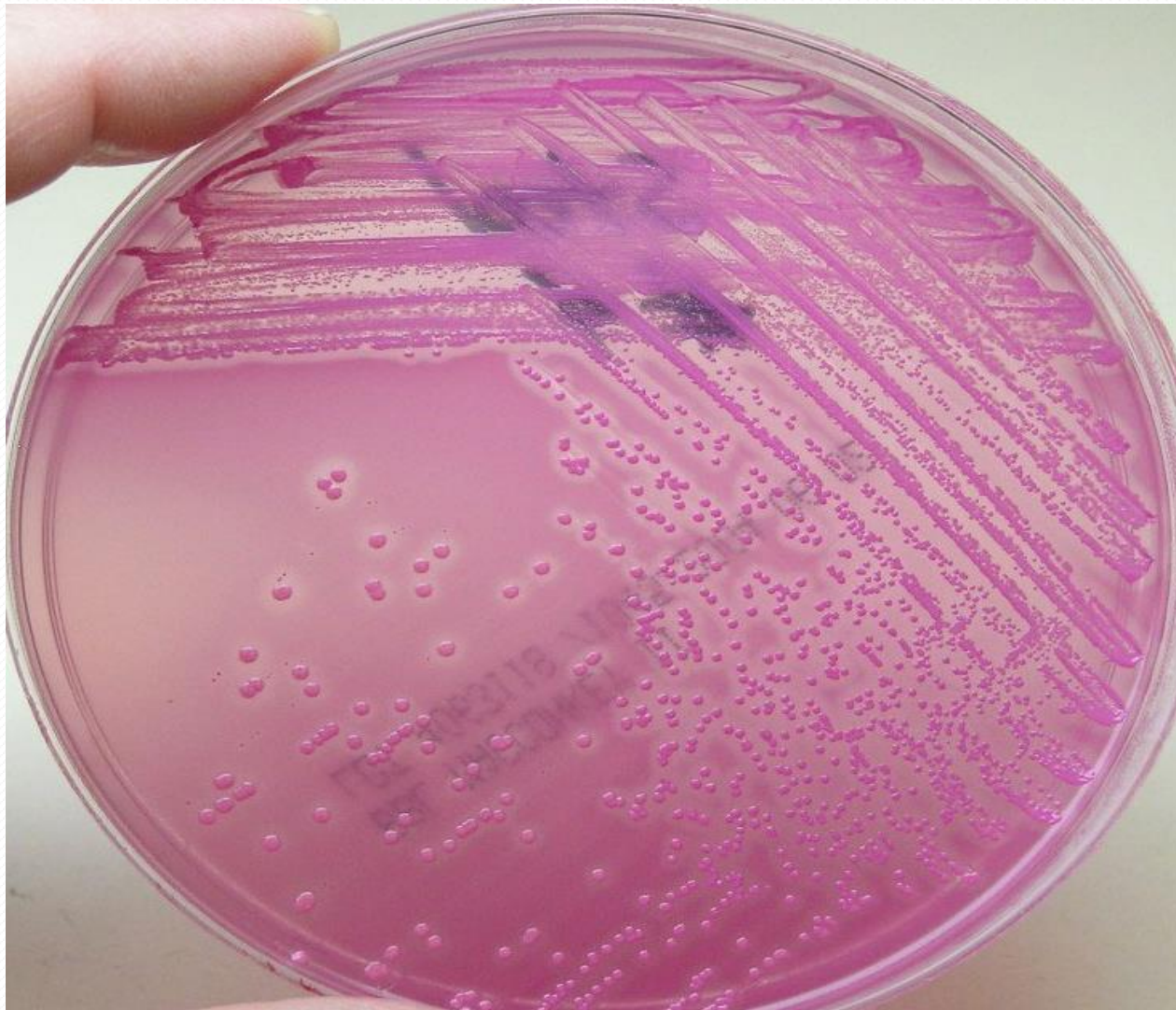
Methods of inoculation SURFACE PLATING

- Used for the isolation of bacteria in pure culture from clinical specimens.
- Platinum wire or Nichrome wire is used.
- One loopful of the specimen is transferred onto the surface of a well dried plate.
- Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks



Using a Loop to Streak a Plate





LAWN CULTURE

- Provides a uniform surface growth of the bacterium.
- Uses
 - Antibiotic sensitivity
 - Bacteriophage typing.
 - In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.



STROKE CULTURE

- Stroke culture is made in tubes containing agar slope / slant.
- Uses
 - Provide a pure growth of bacterium for biochemical tests and for slide agglutination.



STAB CULTURE

- Prepared a long, straight, charged wire.
- Uses
 - Demonstration of motility.
 - Oxygen requirements of the bacterium under study.
 - Maintenance of stock cultures.



POUR PLATE CULTURE

- Agar medium is melted (15 ml) and cooled to 45°C.
- 1 ml of the inoculum is added to the molten agar.
- Mix well and pour to a sterile petri dish.
- Allow it to set.
- Incubate at 37°C, colonies will be distributed throughout the depth of the medium.
- Uses
 - Gives an estimate of the viable bacterial count in a suspension.
 - For the quantitative urine cultures.

LIQUID CULTURES

- Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.
- **Uses**
 - Blood culture
- **Disadvantage**
 - It does not provide a pure culture from mixed inocula.
- **Advantage:**
 - Inhibitory substances are diluted
 - Useful as enrichment media.



INCUBATION

- Most of pathogenic bacteria grow best at 37°C or body temperature, hence the media after inoculation are kept in the incubator.
- Incubator is a double jacket chamber having glass door separators, heated electrically and having thermostat which maintains inside temp. at 37°C constantly, and automatically.



ANEROBIC CULTURE

- Certain bacteria cannot grow in presence of oxygen. These are called as anaerobic bacteria. It is necessary to remove atmospheric oxygen during growth of these bacteria.

Methods-

- Production of vacuum
- Displacement of oxygen with other gases
- Chemical methods
- Biological methods
- Reduction of medium

Production of vacuum:

- Incubate the cultures in a vacuum desiccator.

Disadvantages:

1. Detachment of media from the plate
2. No 100% anaerobiosis.

Displacement of oxygen with other gases

- Displacement of oxygen with hydrogen, nitrogen, helium or CO₂.
- Eg: Candle jar



Chemical method

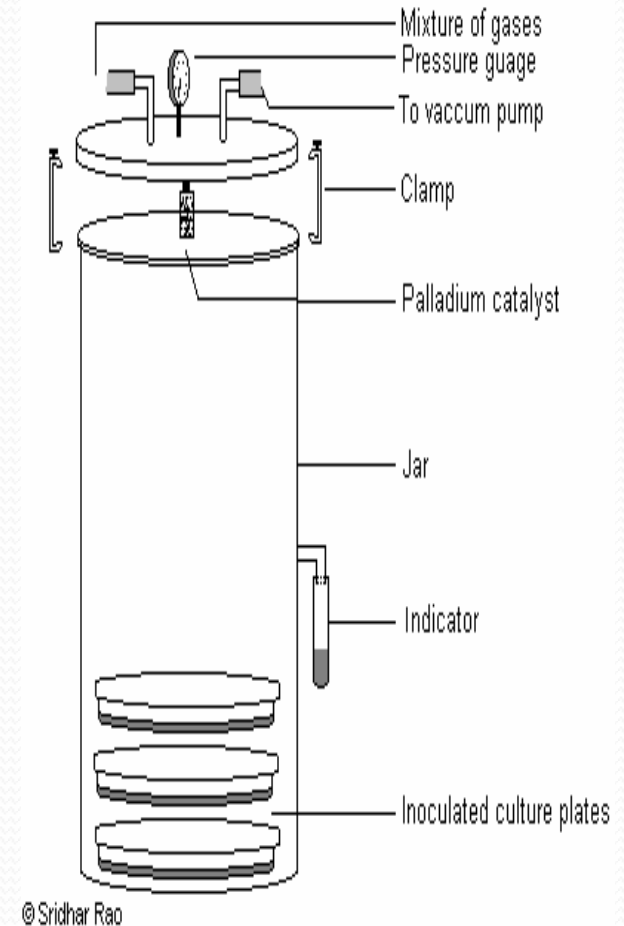
- Alkaline pyrogallol + sod.hydroxide
- Chromium & sulphuric acid (Rosenthal method)

Disadvantage:

- Many organisms are inhibited

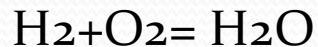
McIntosh – Fildes' anaerobic jar

- Consists of a metal jar or glass jar with a metal lid which can be clamped air tight.
- The lid has 2 tubes – gas inlet and gas outlet
- The lid has two terminals – connected to electrical supply.
- Under the lid – small grooved porcelain spool, wrapped with a layer of palladinised asbestos.



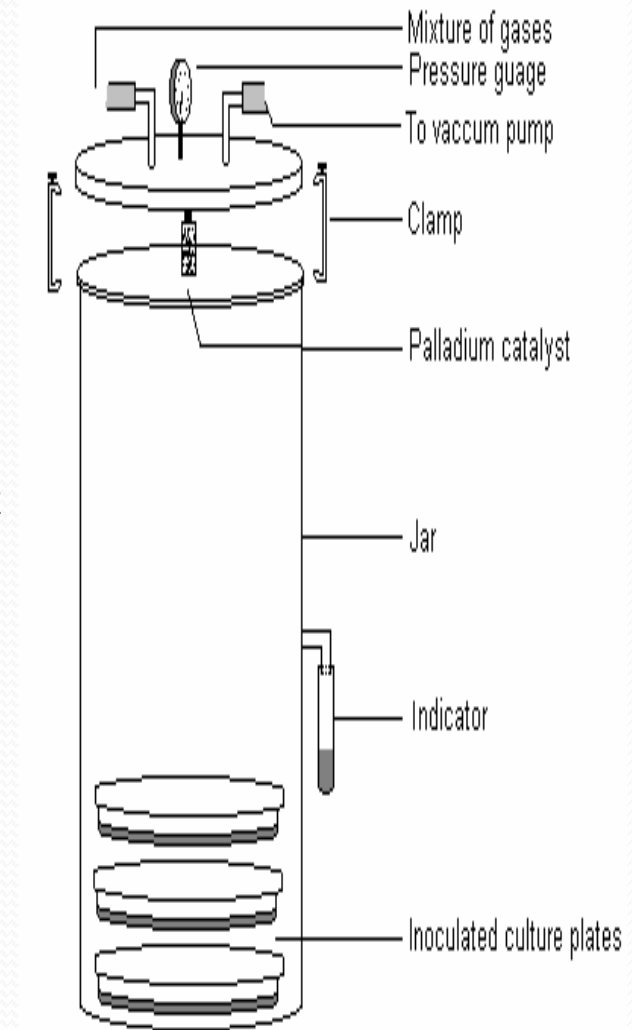
Working:

- Inoculated plates are placed inside the jar and the lid clamped air tight.
- The outlet tube is connected to a vacuum pump and the air inside is evacuated.
- The outlet tap is then closed and the inlet tube is connected to a hydrogen supply.
- After the jar is filled with hydrogen, the electric terminals are connected to a current supply, so that the palladinised asbestos is heated.
- Act as a catalyst for the combination of hydrogen with residual oxygen.



Indicator- Mixture of NaOH, methylene blue and glucose is used.

- It is blue in colour on contact with oxygen.
- Becomes colourless on exclusion of oxygen.



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Gas pack

- Air tight jar, made of thick plastic .Inoculated plated are placed in the jar.
- A packet containing sodium borohydride , cobalt chloride, citric acid and sodium bicarbonate is cut open and small amount of water is added.
- The packet is placed in the jar and lid is tightly close.
- On addition of water these chemicals generate hydrogen and carbon dioxide inside jar.
- Hydrogen combines with oxygen in presence of a catalyst (allumin pallets coated with palladium), kept on under surface of the lid.
- Indicator to be used - reduced methylene blue
 - Colourless – anaerobically
 - Blue colour – on exposure to oxygen



Biological method

- Absorption of oxygen by incubation with aerobic bacteria, germinating seeds or chopped vegetables.

Reduction of oxygen

- By using reducing agents – 1% glucose, 0.1% Thioglycolate broth, 0.1 % Ascorbic acid and 0.05% cysteine

Example- Robertson's cooked meat medium

ROBERTSON'S COOKED MEDIUM

- Contains nutrient broth and pieces of fat free minced cooked meat of ox heart.
- Unsaturated fatty acids present in meat utilise oxygen for auto oxidation, action catalysed by haematin in meat.
- Some reducing substances in meat e.g glutathione and cysteine also utilise oxygen, thus creating anaerobiasis.
- After inoculation of RCM , by clinical material , the broth is covered by 1cm layer of sterile liquid paraffin, to avoid contact with air. RCM is incubated for minimum 48hrs before oil removed and broth examined for anaerobes.



Glove Box

Pre-reduced media and an anaerobic chamber

- Air tight, glass fronted cabinet filled with inert gas ($H_2+N_2+CO_2$)
- Entry lock for introduction or removal of materials
- Gloves for hands.



Important Questions:

- **Short Answer questions:**

1. Enumerate different aerobic culture methods with one use of each.
2. Enumerate various Anaerobic culture methods.
3. Write a note on Mc Intosh Fielde's Jar. Explain with the help of a neat labeled diagram.
4. Write a note on Robertson cooked meat media.

Internal Assessment Calculation

Theory	15 Mks
Theory Performance in 1 st Term, 2 nd Term and Prelim	10 Mks
Day to Day assessment (Attendance + 3 MCQ tests at the end of each term- 20 Mks Each	5 Mks
Practicals:	15 Mks
Practical Performance in 1 st Term, 2 nd Term and Prelim	10 Mks
Day to Day Assessment (Attendance +Seminars)	5 Mks