BACTERIAL CULTURE METHODS

BASICS

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BACTERIAL NUTRITION AND THE DESIGN OF CULTURE MEDIA

- Based on bacterial metabolism*
- Culture pH
- Culture oxidation- reduction potential.
- Gaseous requirements
  - Oxygen, Carbon dioxide and other gases

CULTURE MEDIA

- Used to grow bacteria
- Can be used to:
  - Enrich the numbers of bacteria
  - Select for certain bacteria and suppress others
  - Differentiate among different kinds of bacteria

OXYGEN CONCENTRATION

- Aerobs
- Anaerobs (do not require oxygen)
- Obligate anaerobs (die in the presence of Ooxygen)
- Facultative anaerobs (E.coli)
- Microaerophilic bacteria

PURPOSE OF CULTURING

- Isolation
- Properties of bacteria
- To create antigens for laboratory use
- Typing with Bacteriophages and Bacteriocins susceptibility
- To test for Antibiotic sensitivity
- Estimate viable counts
- Maintain stock cultures

METHODS OF ISOLATION OF PURE CULTURE WITH ..

- 1. Surface plating
- 2 Enrichment medium
- 3 Selective medium
- 4 Indicator medium

TYPES OF MEDIA USED

General purpose media will support the growth of many microorganisms.

Enriched media are general purpose media supplemented by blood or other special nutrients to encourage the growth of fastidious heterotrophs; (fastidious = having complicated nutritional requirements

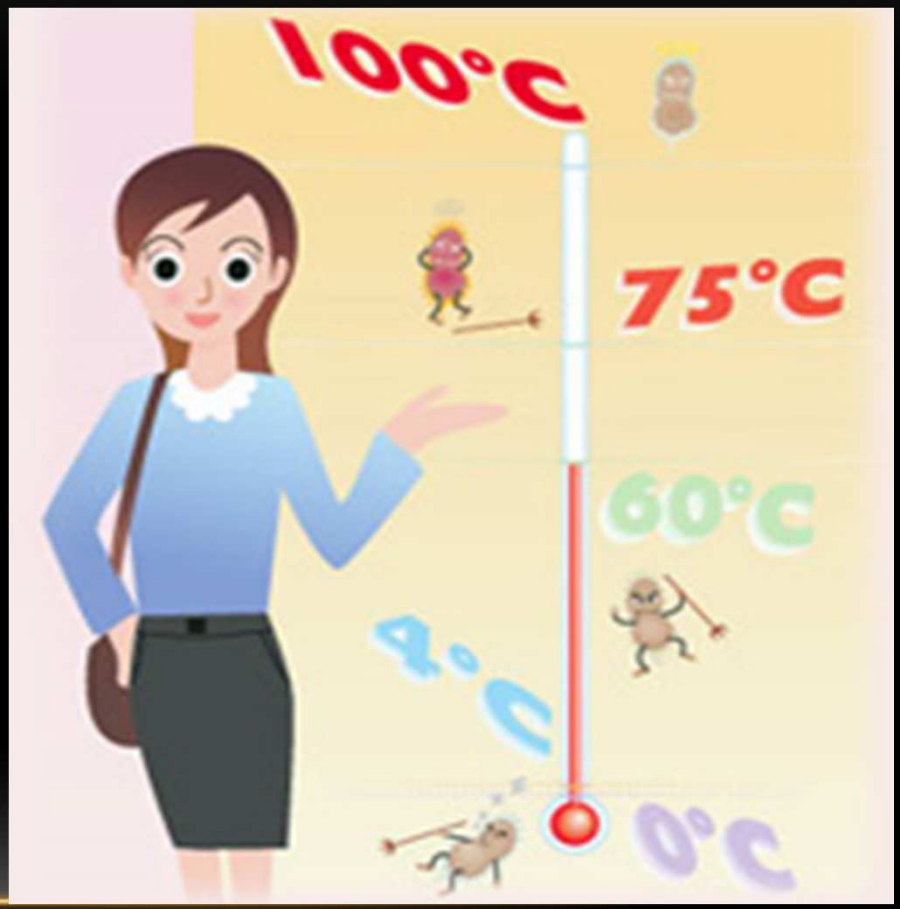
TYPES OF MEDIA USED

Selective media favor the growth of particular microorganisms and inhibits the growth of others.

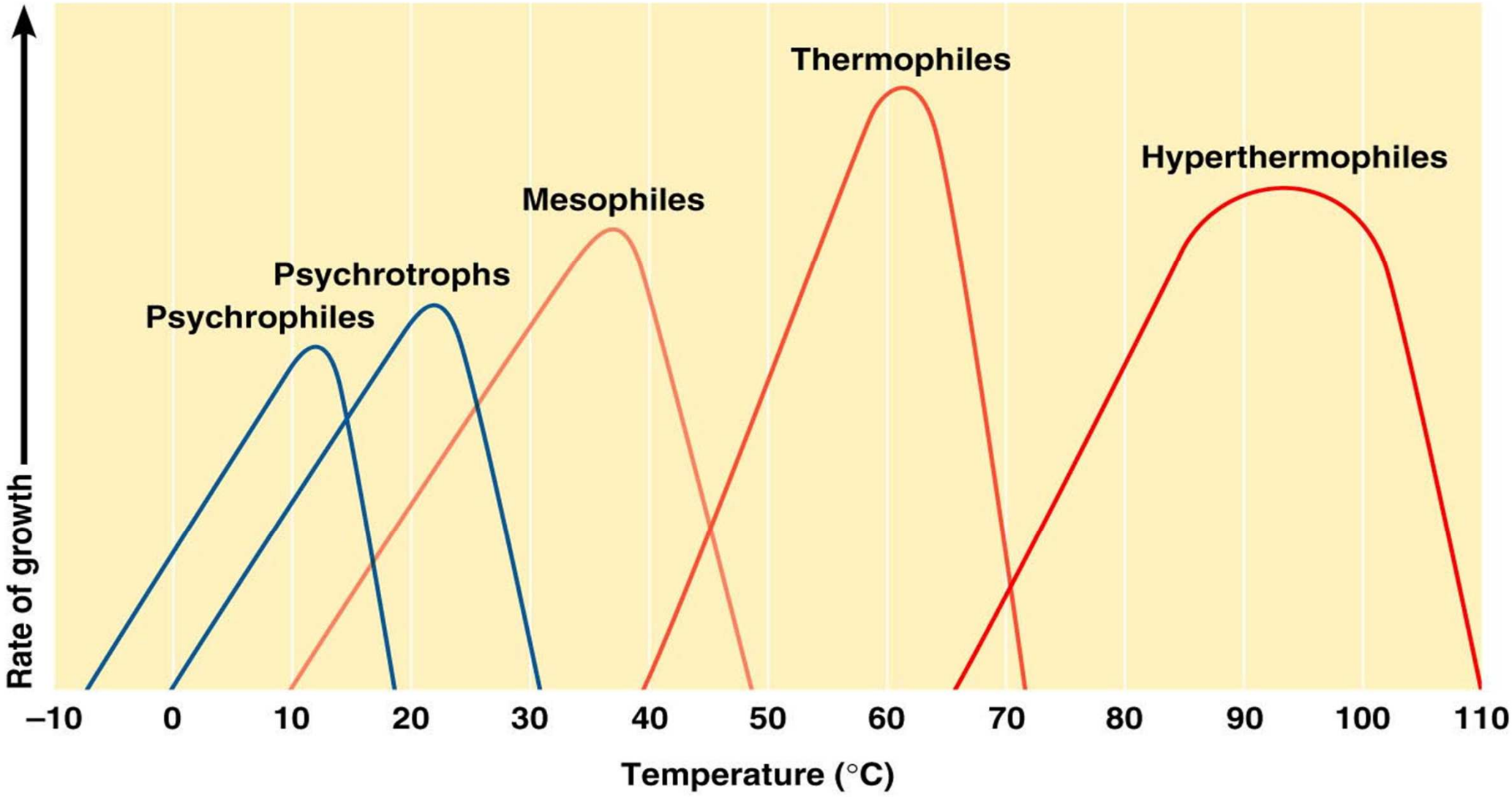
Differential media distinguish between different groups of bacteria on the basis of their biological characteristics; Causes observable change in medium when biochemical reaction occurs

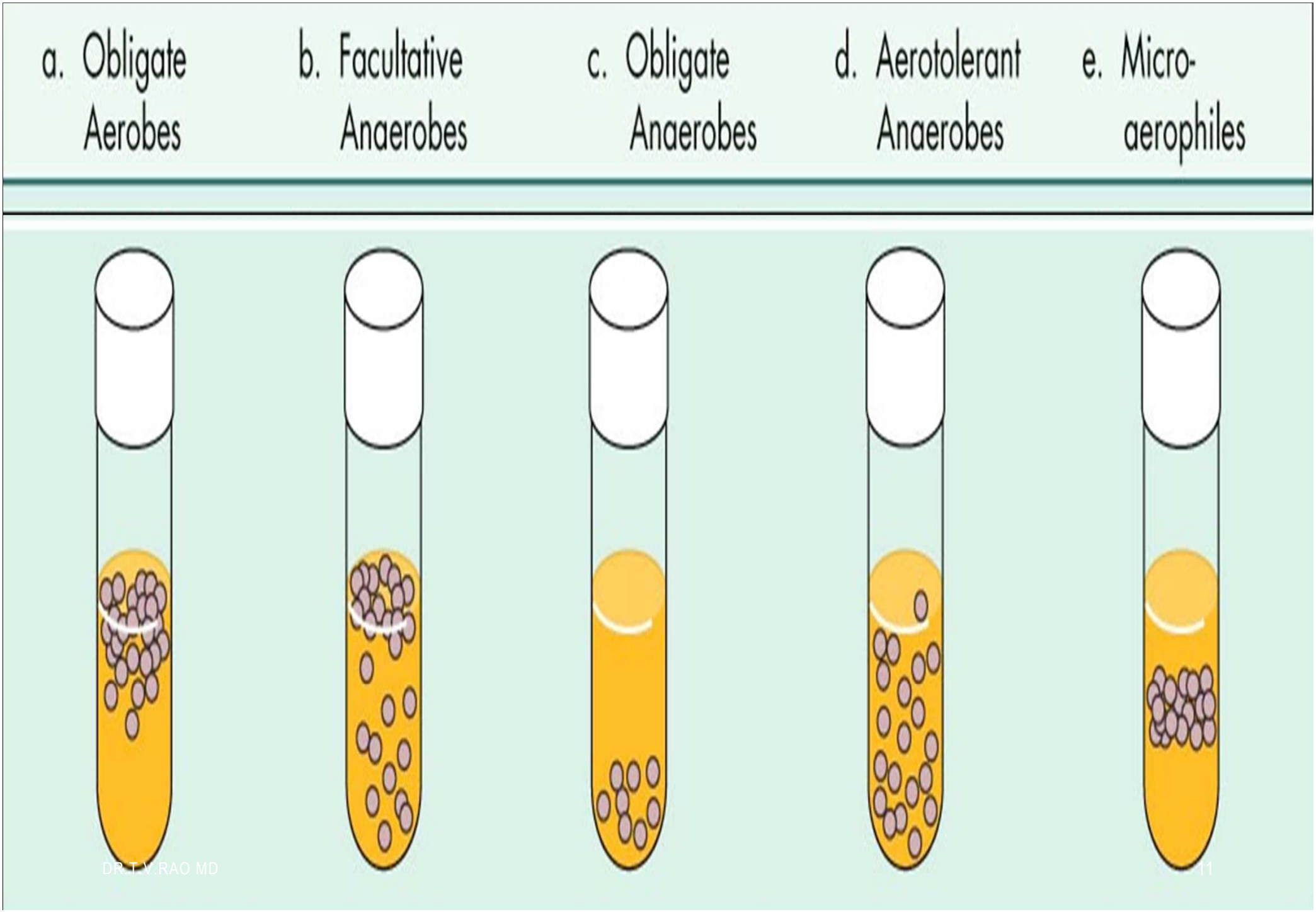
TEMPERATURE (CHARACTERISTIC RANGES)

- Psychrophiles: with optimum growth T around 20 C
- Mesopihles: between 15 and 45 with optimum around 37 C
- Thermophiles: between 30 and 75 with optimum around 55 C
- Hyperthermophiles: T grater than 100C



TEMPERATURE AND BACTERIAL GROWTH




a. Obligate Aerobes
b. Facultative Anaerobes
c. Obligate Anaerobes
d. Aerotolerant Anaerobes
e. Micro-aerophiles

THE REQUIREMENTS FOR GROWTH: PHYSICAL REQUIREMENTS

- **pH**
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophilic grow in acidic environments

CULTURING

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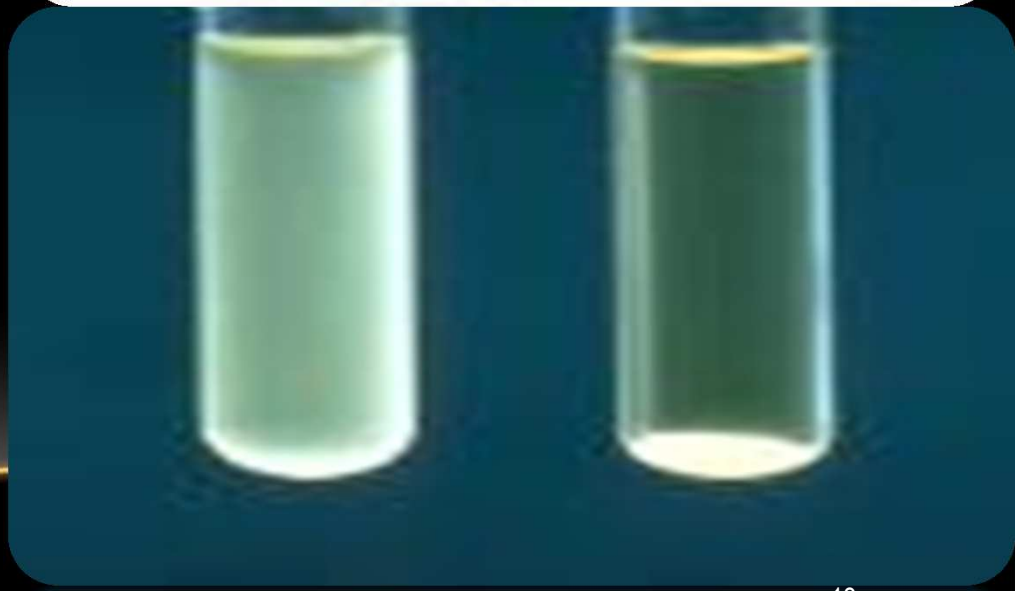
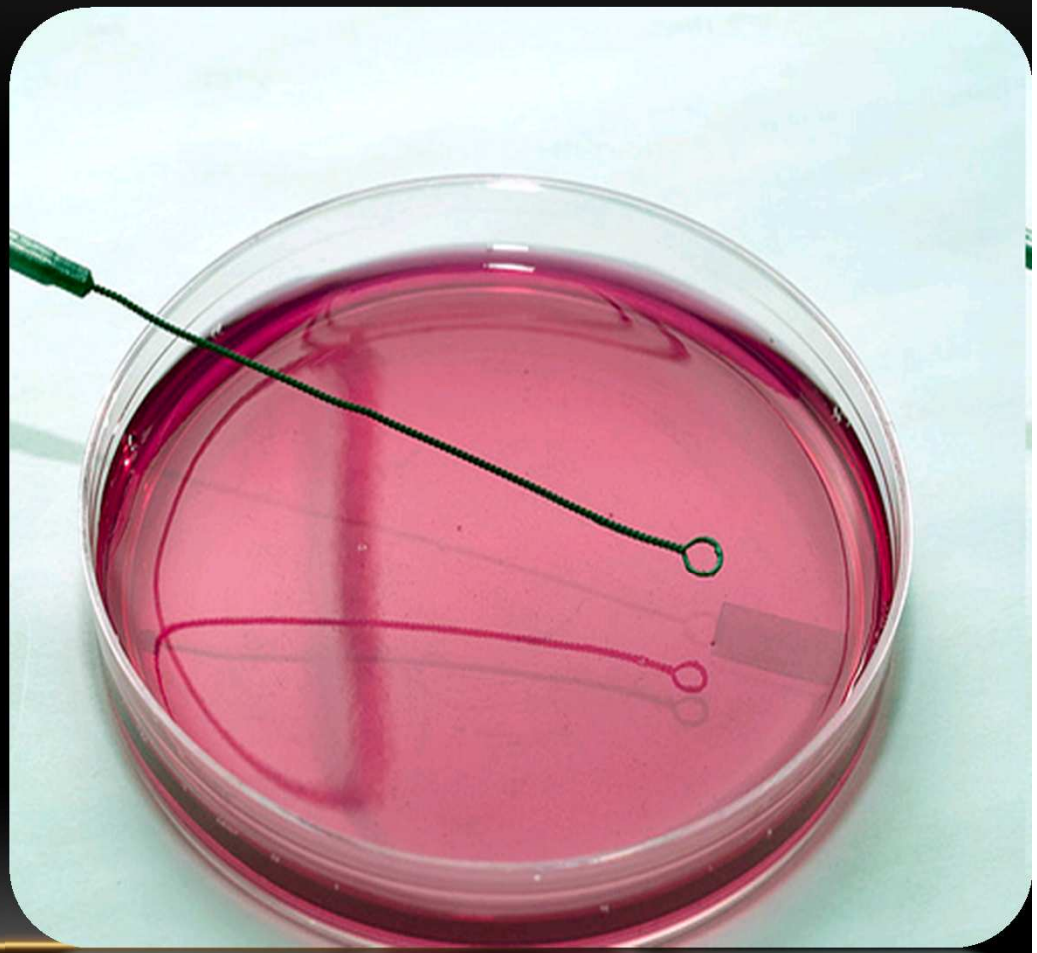


METHODS TO ISOLATE THE BACTERIA

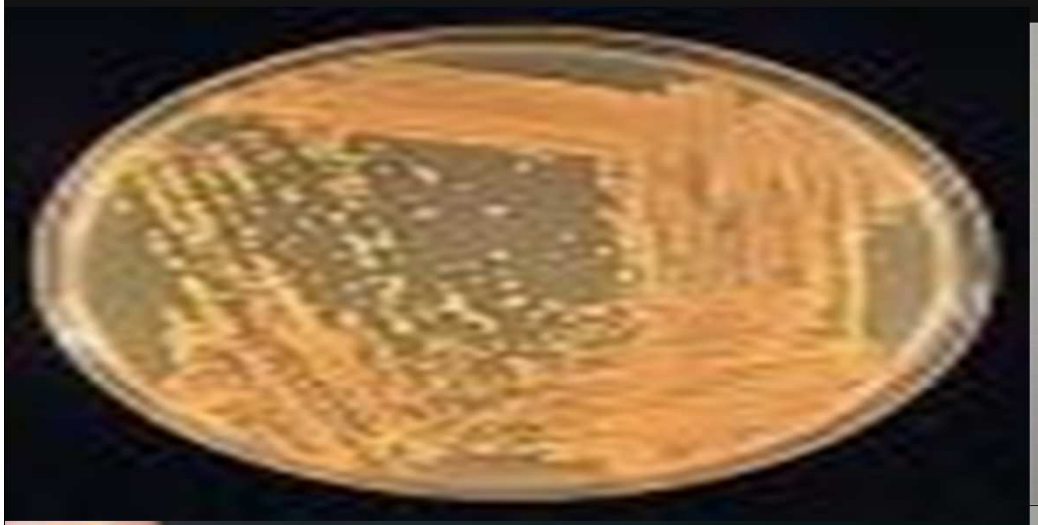
- Streak culture
- Stroke
- Stab
- Pour plate
- Liquid culture
- Special methods for anaerobic cultures

HOW TO INOCULATE A CULTURE PLATE

- Plate: provide large surface for isolation and observation of colonies
- Using a sterile loop or a sterile swab streak your sample on the Petri plate
- **Important let your sterilized loop cool before you pick up your sample**



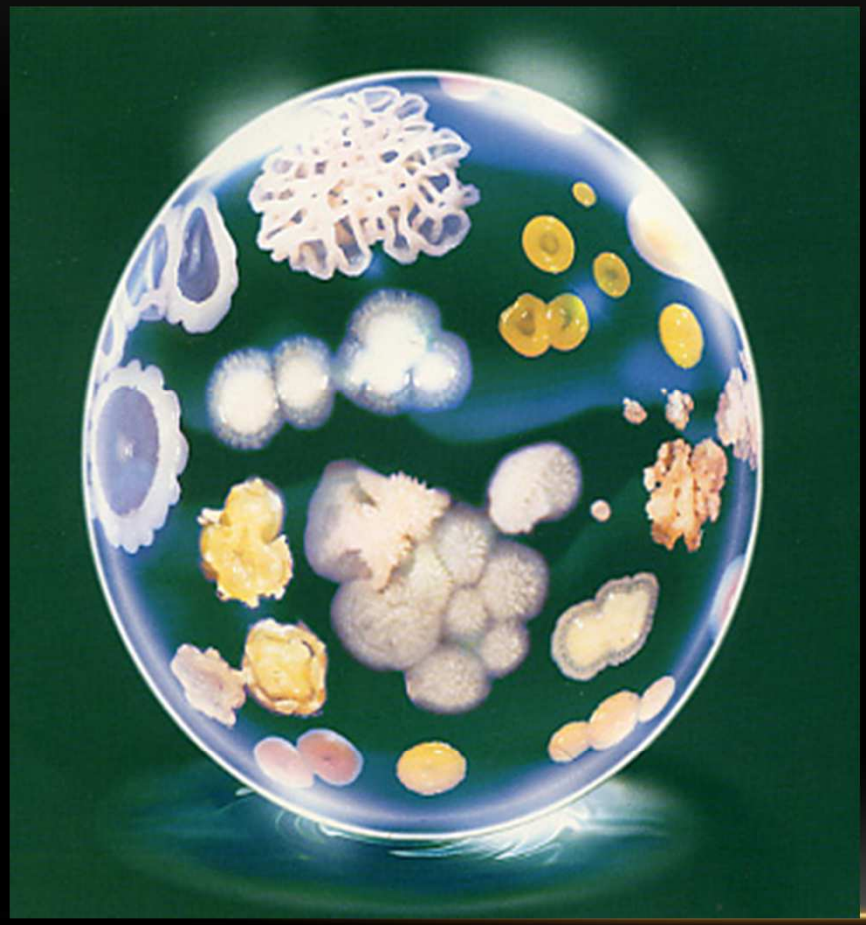
DIFFERENT METHODS OF CULTURING BACTERIA



MACCONKEY AGAR

- **Example:**: MacConkey agar has color indicator that distinguishes presence of acid. Bacteria that ferment a particular sugar (e.g., glucose in culture media) will produce acid wastes on plates, turn pH indicator red.

COLONIES - MAKE A OBSERVATION

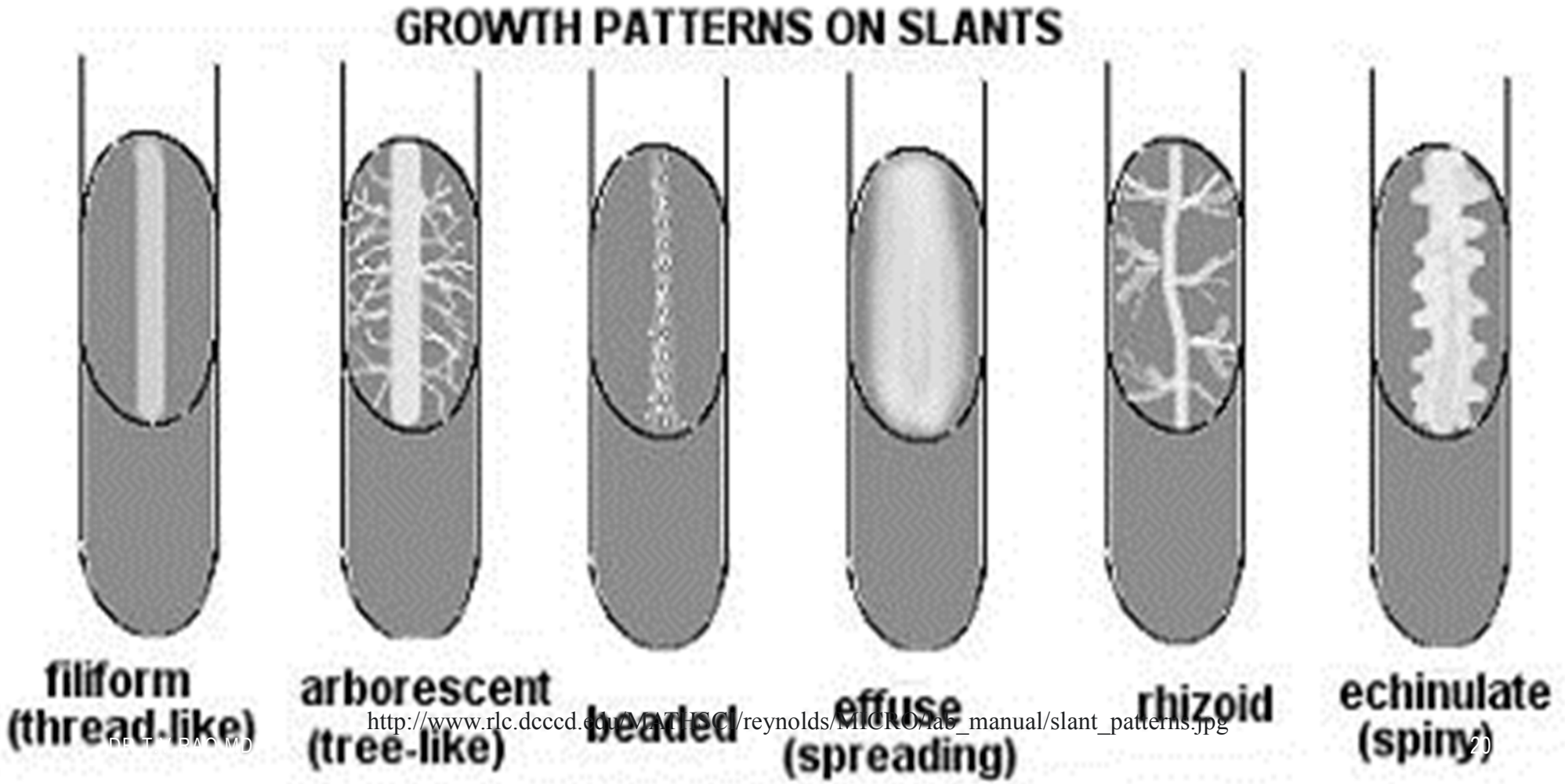


- Shape
- Size
- Elevation
- Edge
- Surface
- Opacity
- Consistency

LIQUID MEDIA

Liquid media: easiest to prepare and use. Good for growing quantities of microbes needed for analysis or experiments. Unless inoculated with pure culture, cannot separate different organisms.

SLANT OBSERVATION



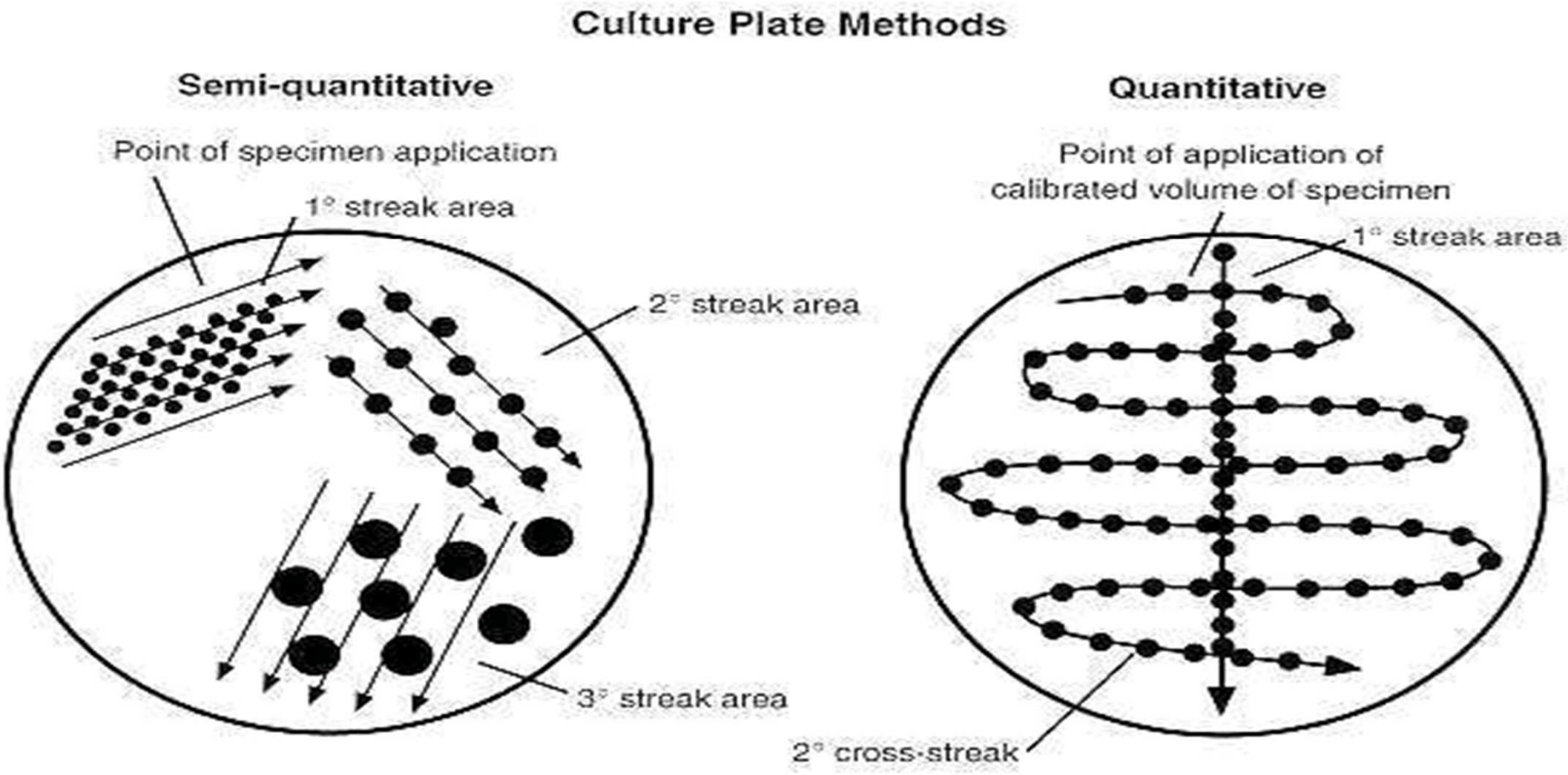
http://www.rlc.dcccd.edu/MATH/CJ/reynolds/MICRO/lab_manual/slant_patterns.jpg

STREAK CULTURE

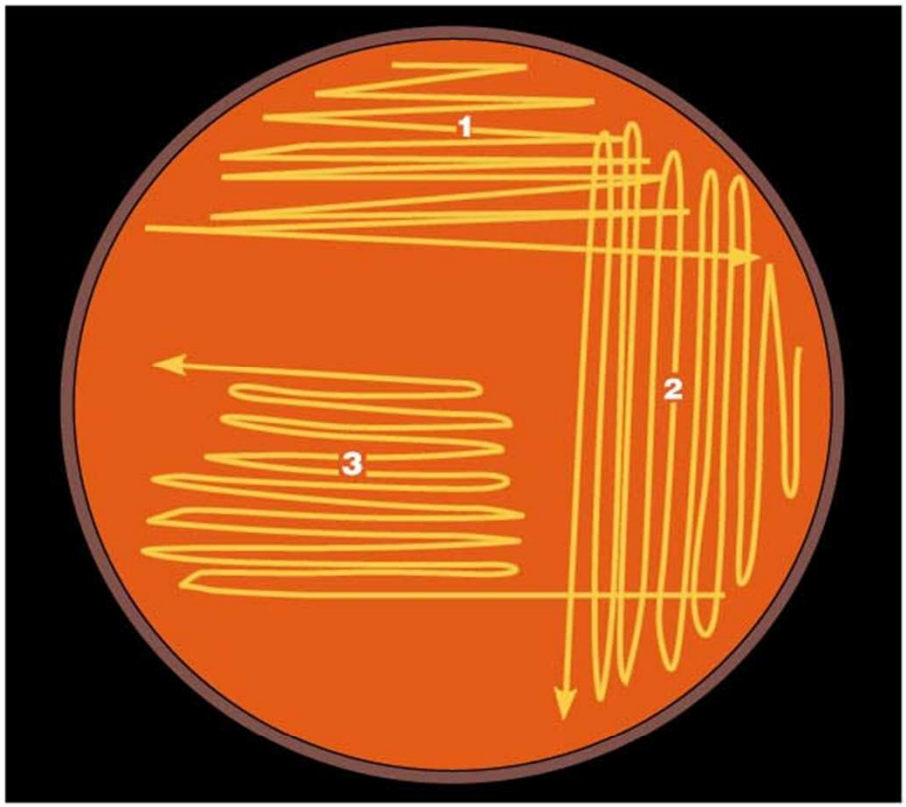
Growing bacteria. Here a sample of bacteria is being streaked onto a petri dish as part of the process of identifying the bacteria in the sample.

- Lawn or carpet culture to create uniform surface of organisms
- Bacteriophages typing
- To obtain large amount of antigens

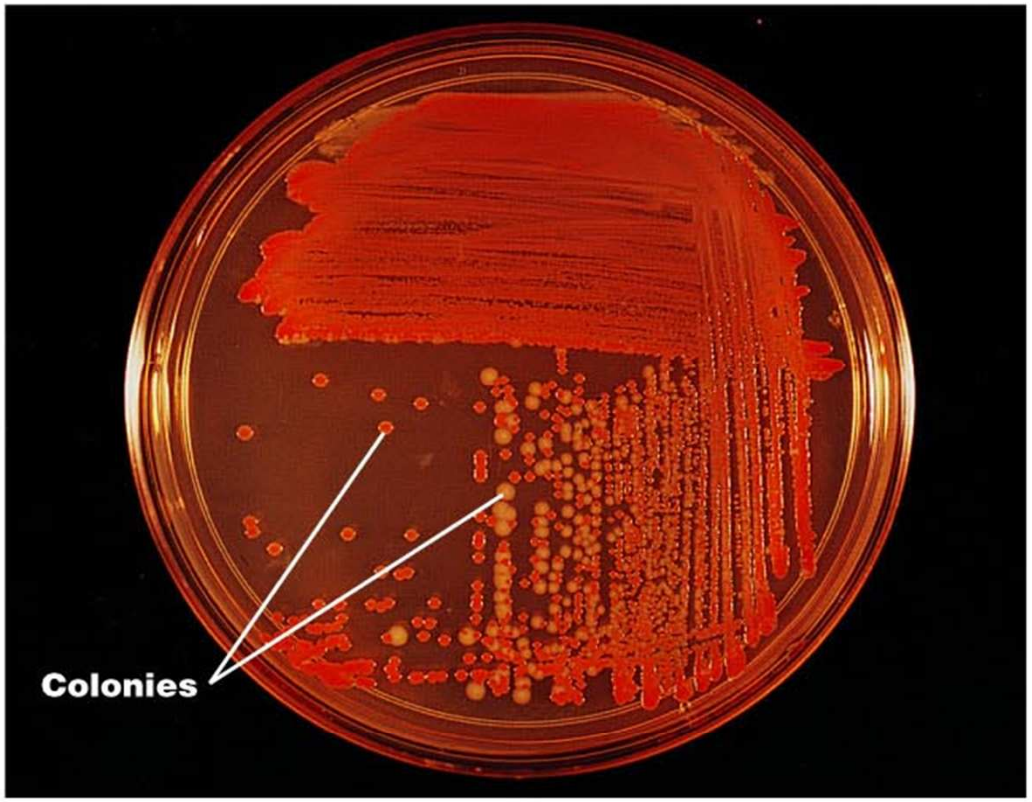
CULTURING THE MICROBES NEEDS SKILLS



STREAK PLATE



(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.



(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.

METHODS OF ISOLATION OF PURE CULTURE

- 1. Surface plating
- 2 Enrichment medium
- 3 Selective medium
- 4 Indicator medium

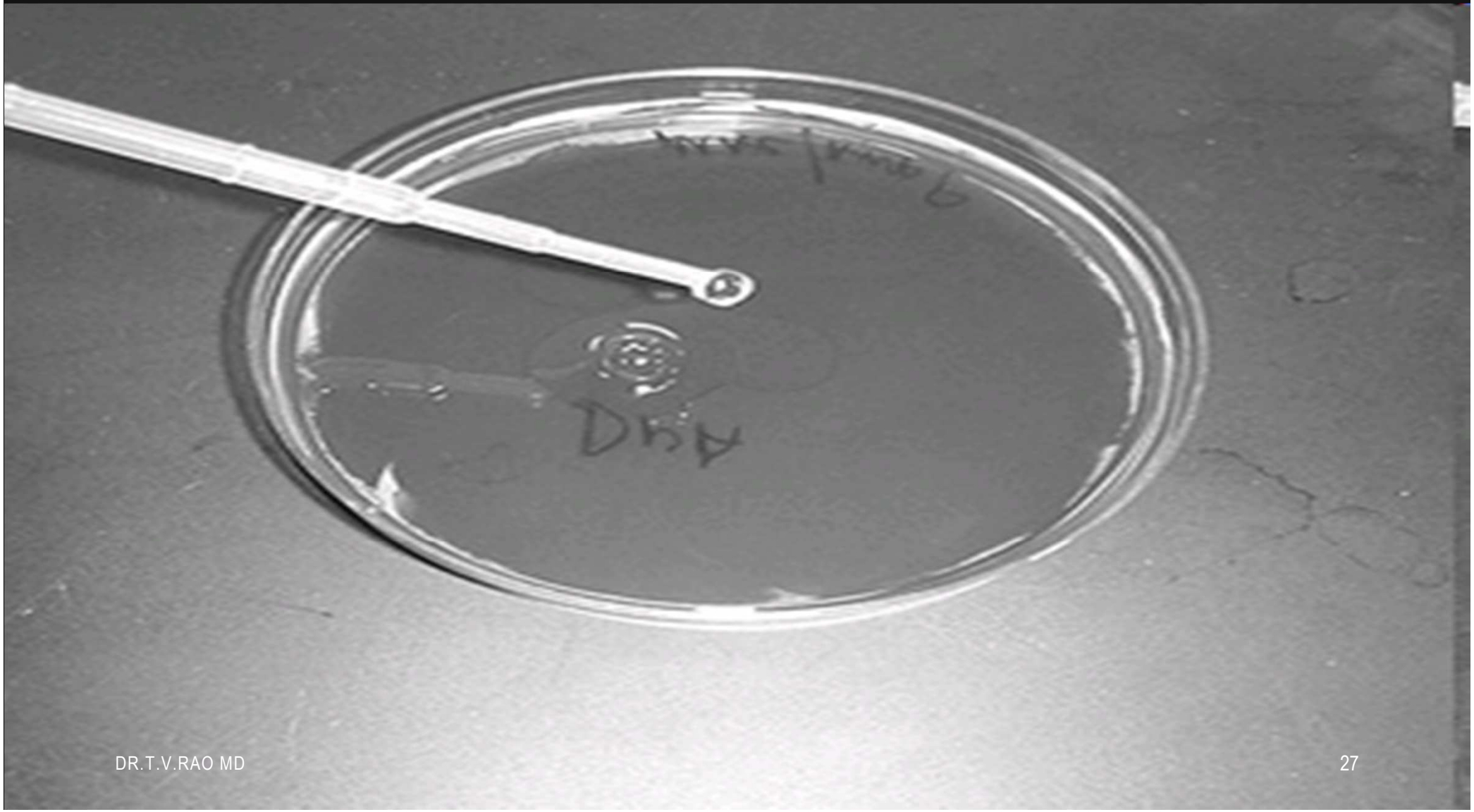
LIQUID CULTURING

- Liquid cultures are done in
- Tubes
- Bottles
- Flasks
- Blood culture
- Water analysis

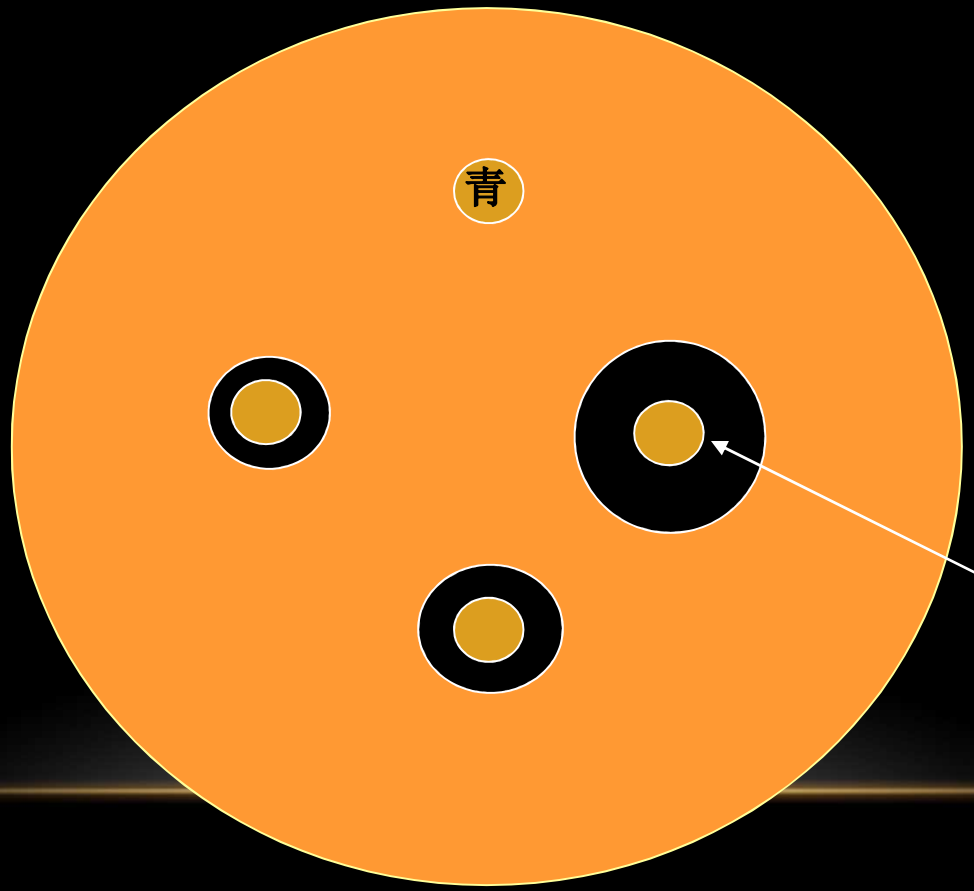
STAB CULTURE

- Puncturing suitable medium such as nutrient agar, gelatin,
- Observe gelatin liquefaction
- Preserving the stock culture.

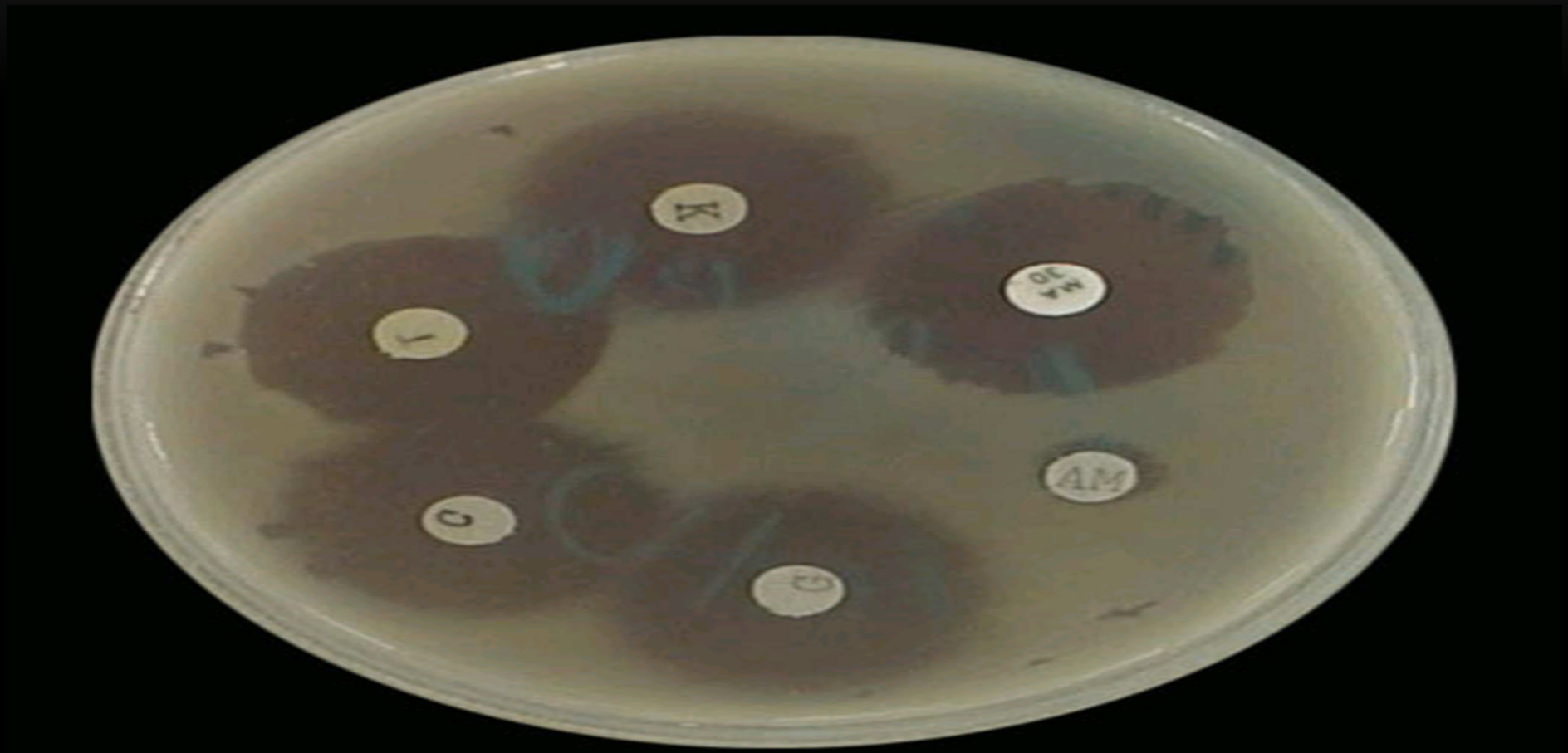
SWEEP PLATE METHOD



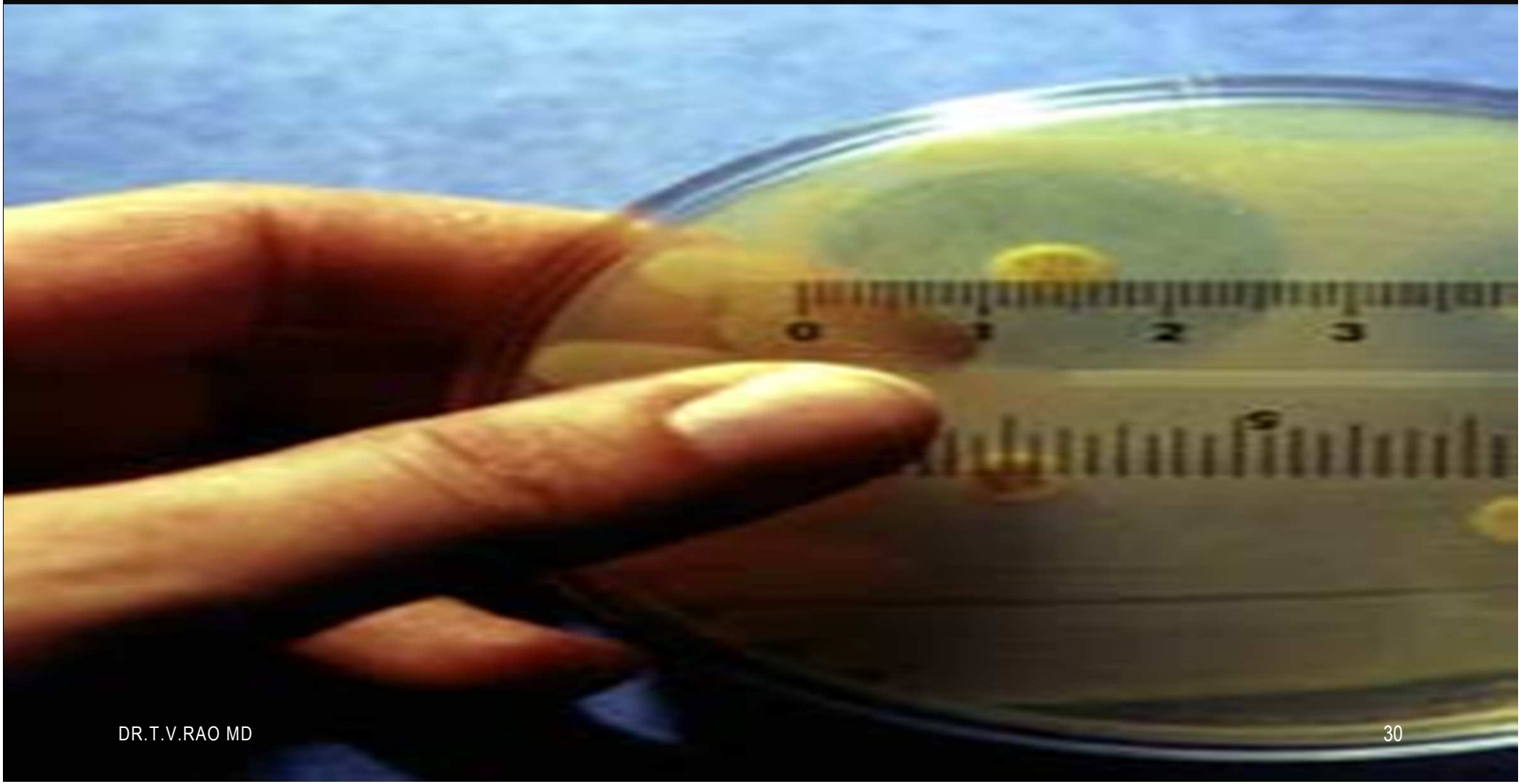
MICROBIAL ANTIBIOTIC SUSCEPTIBILITY TEST
（THE AGAR DIFFUSION TEST ）



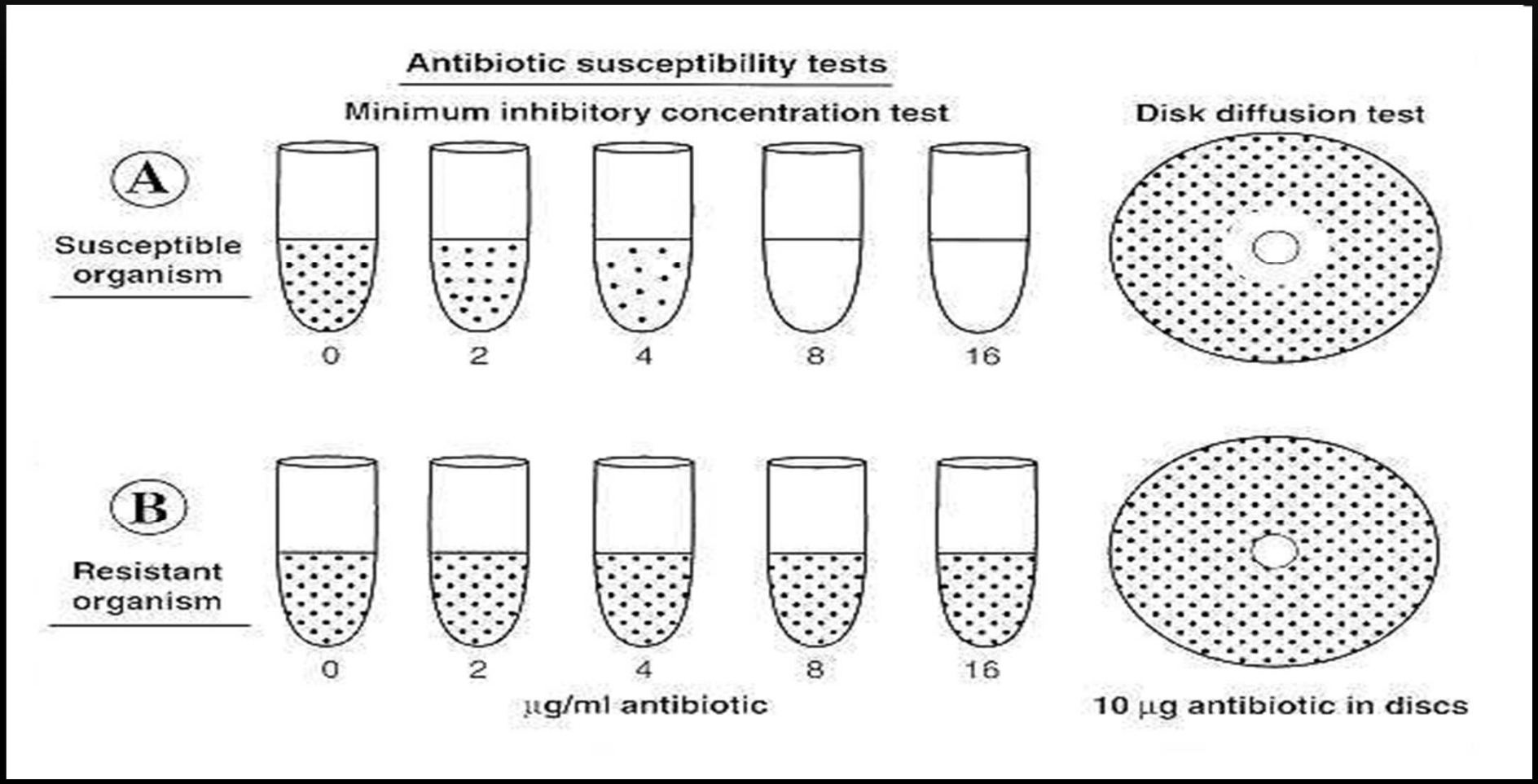
MULLER HINTON AGAR FOR ANTIBIOTIC TESTING



MEASURING THE ZONE OF INHIBITION



MINIMUM INHIBITORY CONCENTRATION DETECTS ANTIBIOTIC SENSITIVITY PATTERNS



- Anaerobic Bacterial Isolation and Identification Needs specified conditions

DESICCATOR

- In Desiccator some oxygen is left

Not suitable for fluid culture

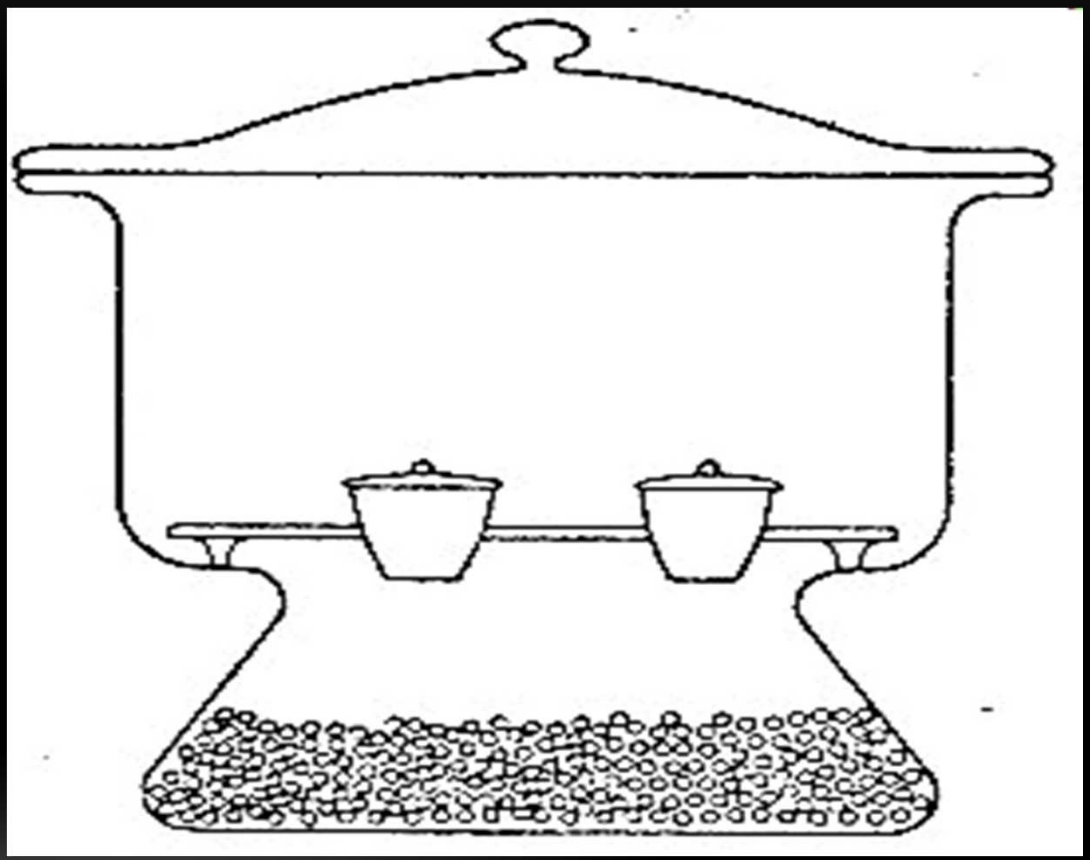
Displacement of oxygen is done with

Hydrogen

Nitrogen

Helium

Co_2



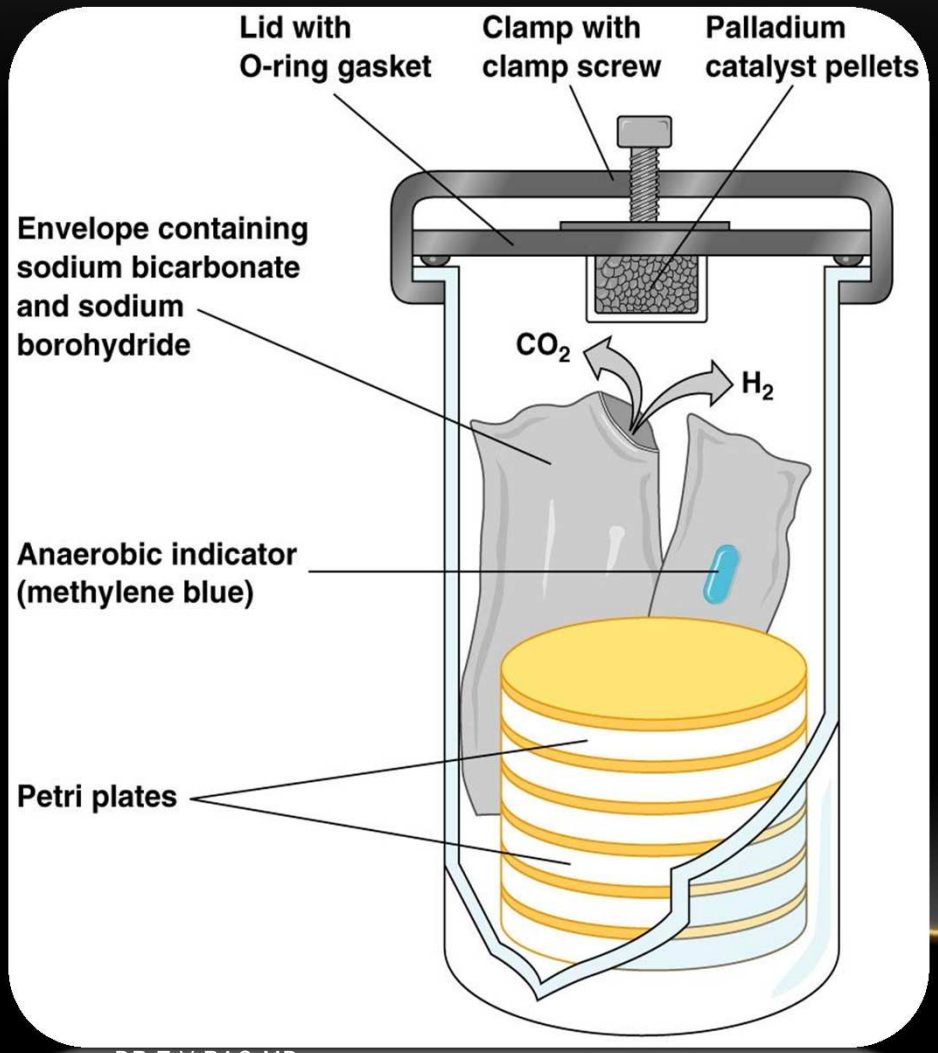
CANDLE JAR

- Inoculated plates are kept
- Burning candle use up all oxygen
- But a little o_2 is left
- But presence of Co_2 stimulates the most bacterium

MAC IN TOSH FILDES ANAEROBIC JAR

- Contain inlet and outlet
- Electrical supply
- Inoculated culture plates
- When electrified palladinised asbestos heating acts as catalyst for combination of hydrogen with residual oxygen causes complete anaerobiasis

GAS PACK



- A disposable envelop contains chemicals which generate hydrogen and carbon dioxide on addition of water
- Inoculated plates are kept in jar
- Water is added hydrogen and carbon dioxide are liberated
- Presence of cold catalyst in the envelop permits the combination of Hydrogen and oxygen to produce anaerobic environment
- Indicator is methylene blue
- Colorless when anaerobic environment.

OTHER REDUCING AGENTS

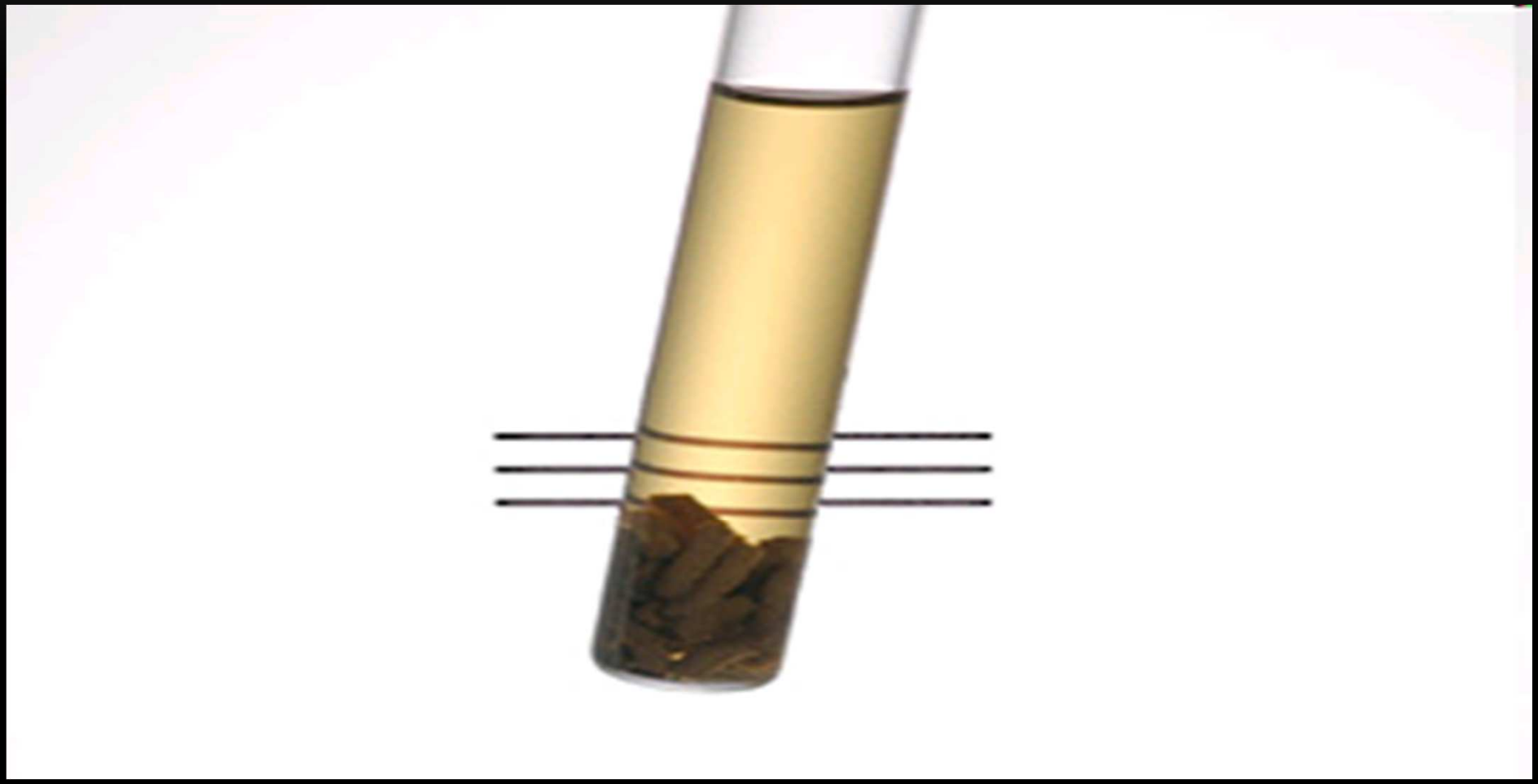
- Reducing agents

  O.1% Thiglyclolate

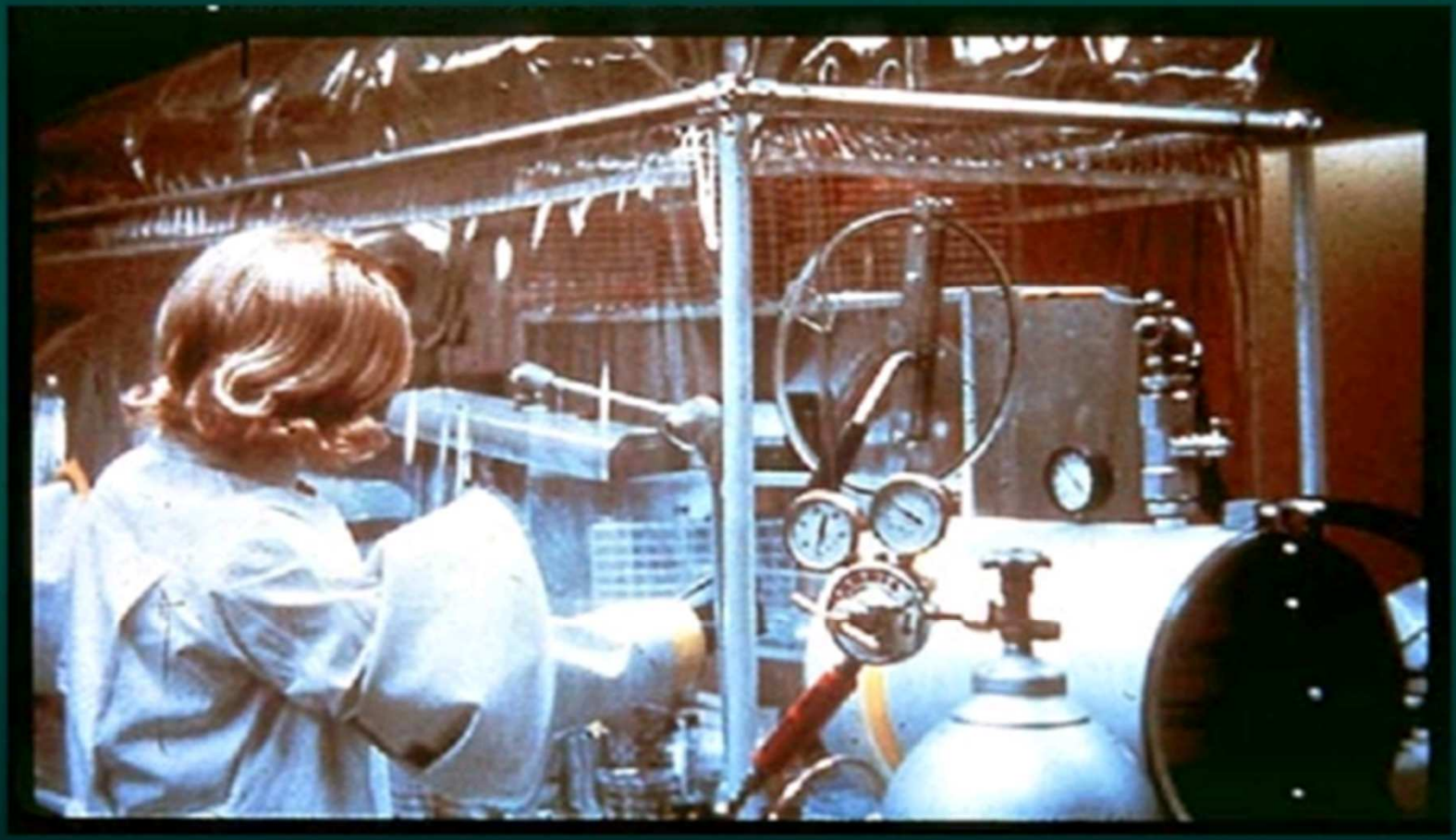
  0.1% Ascorbic acid

  0.05 % cysteine

ROBERTSON COOKED MEAT MEDIUM ROUTINELY USED IN ANAEROBIC SPORE BEARING BACTERIA

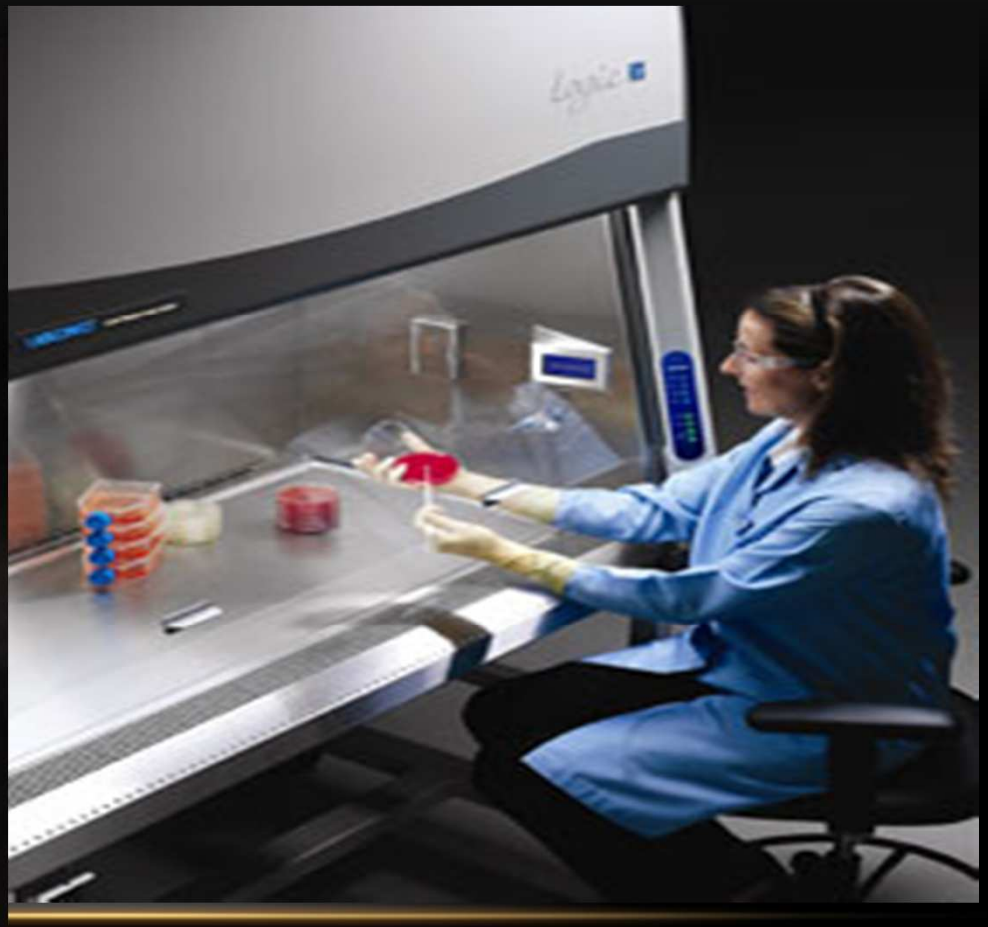


An Anaerobic Culture Chamber



LOWENSTEIN JENSEN MEDIUM - CULTIVATION OF MYCOBACTERIUM TUBERCULOSIS

WORKING WITH MYCOBACTERIUM NEEDS BIOSAFTEY CONCERNS



- Created by Dr.T.V.Rao MD for Basic learning on Culturing Bacteria for Medical and Paramedical students in Microbiology

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