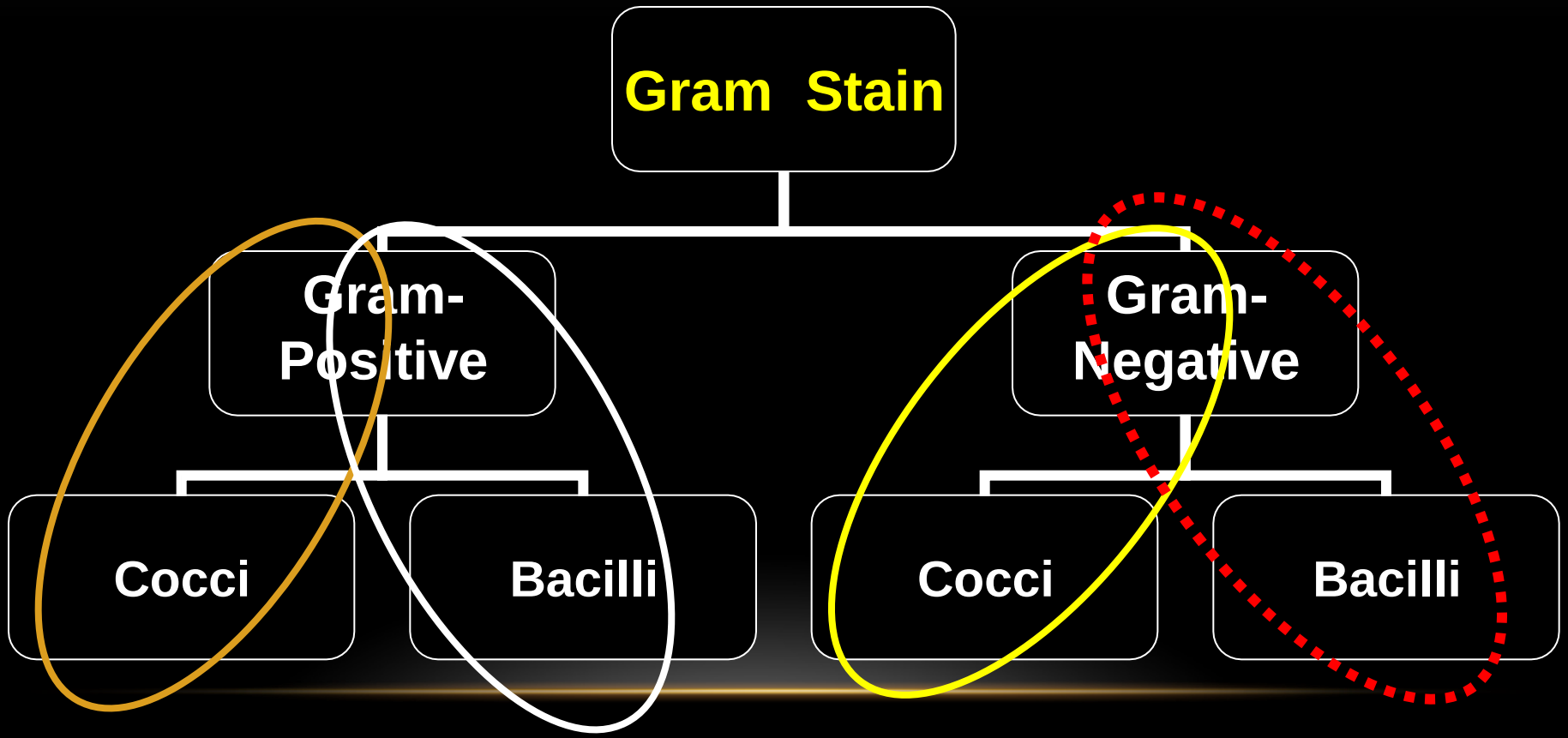
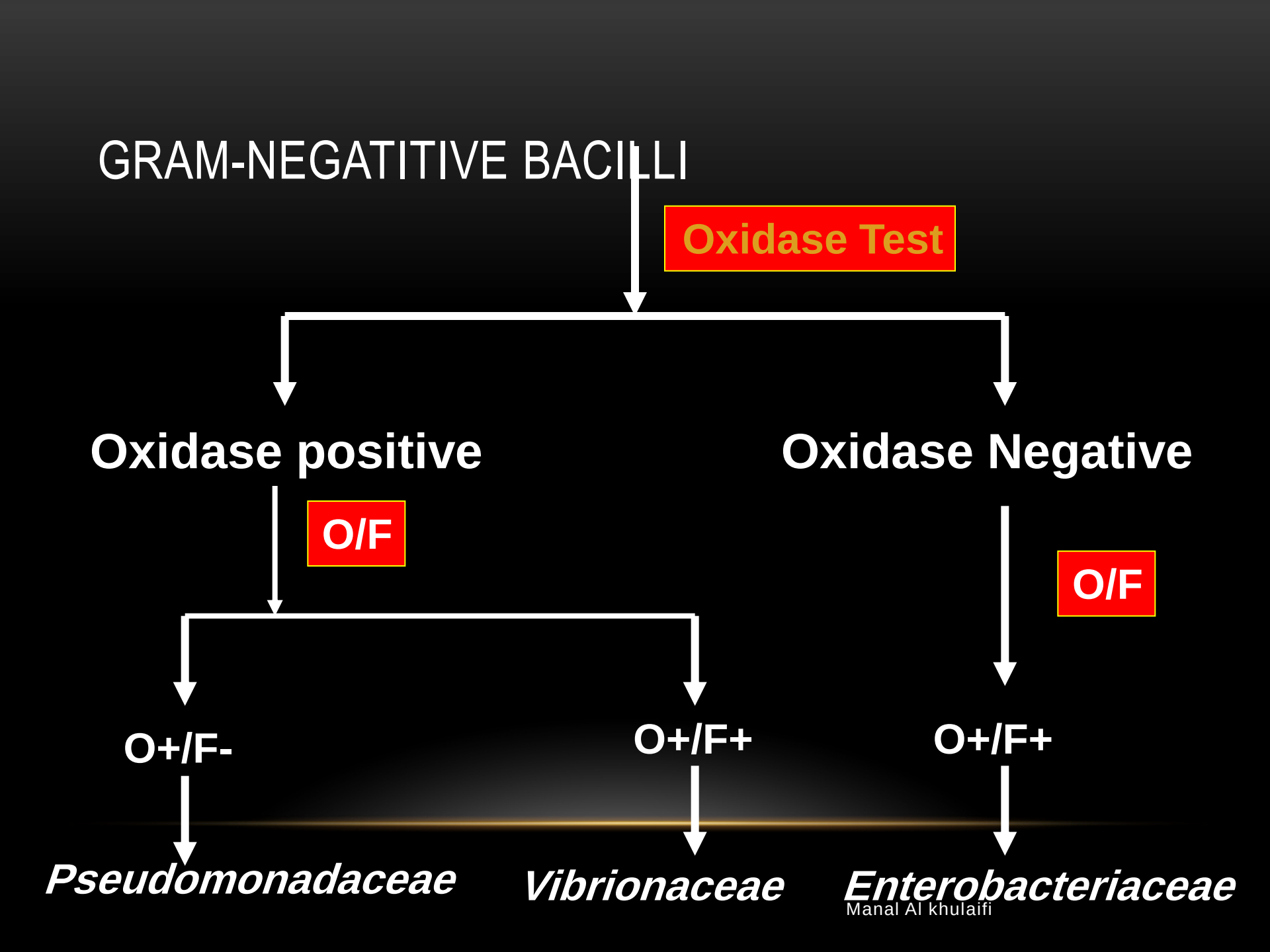
NON-FERMENTATIVE GRAM-NEGATIVE RODS

Pseudomonas spp

Manal Al khulaifi

Classification of Bacteria




GRAM-NEGATITIVE BACILLI
Oxidase Test
Oxidase positive
Oxidase Negative
O/F
O/F
O+/F-
O+/F+
O+/F+
Pseudomonadaceae
Vibrionaceae
Enterobacteriaceae
Manal Al khulaifi

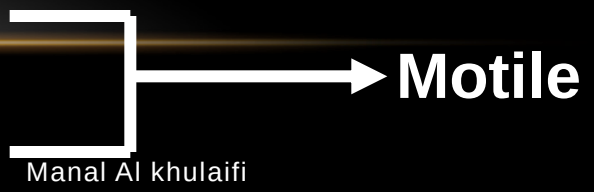
CHARACTERS OF *PSEUDOMONAS*

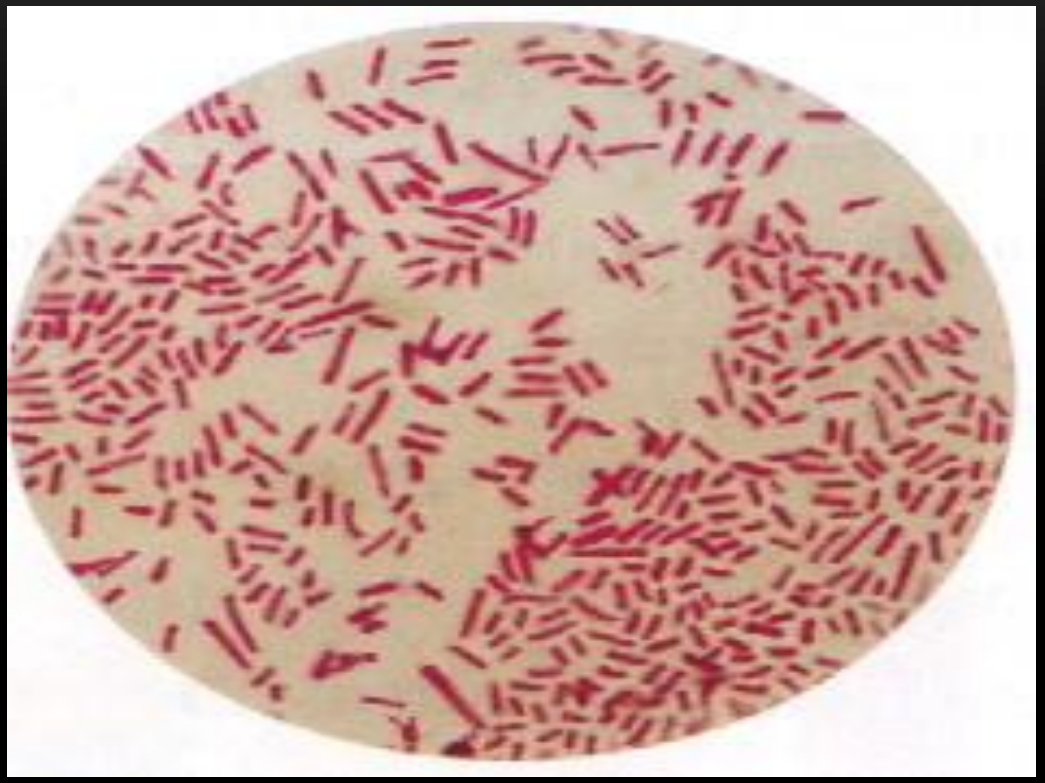
- Gram-negative bacilli belonging to ***Pseudomonadaceae***
- Motile by means of a single polar flagellum.
- Non spore forming
- Capsulated "Polysaccharide capsule"
- Aerobic
- Breakdown glucose by oxidation i.e. Oxidative
- Oxidase and catalase positive
- It has very simple nutritional requirements i.e. non fastidious
- The most important pathogenic organism is *Ps. aeruginosa*
- Optimum temperature is 37 C, and it is able to grow at 42 C
- It is resistant to high concentrations of salts, dyes, weak antiseptics, and many antibiotics
- Common inhabitants of soil, water, GIT

- ***Ps. aeruginosa*** is opportunistic pathogen and associated with a variety of infections including:
  - Urinary tract infections
  - Wound and burn with blue green pus
  - Respiratory system infections (Pneumonia)
  - Eye infection and may lead to blindness
  - Ear infection (external ear or otitis media)
  - Meningitis
  - A variety of systemic infections

IDENTIFICATION OF *PS. AERUGINOSA*

- **Laboratory diagnosis**
  - **Specimen:**
    - Urine, pus, sputum, CSF, blood, skin swap according to the type of infection
  - **Microscopical Examination**
    - **Gram Stain:** Gram-negative rods
    - Motility Test:
      - **Hanging Drop Techniques**
      - **Semisolid agar medium**



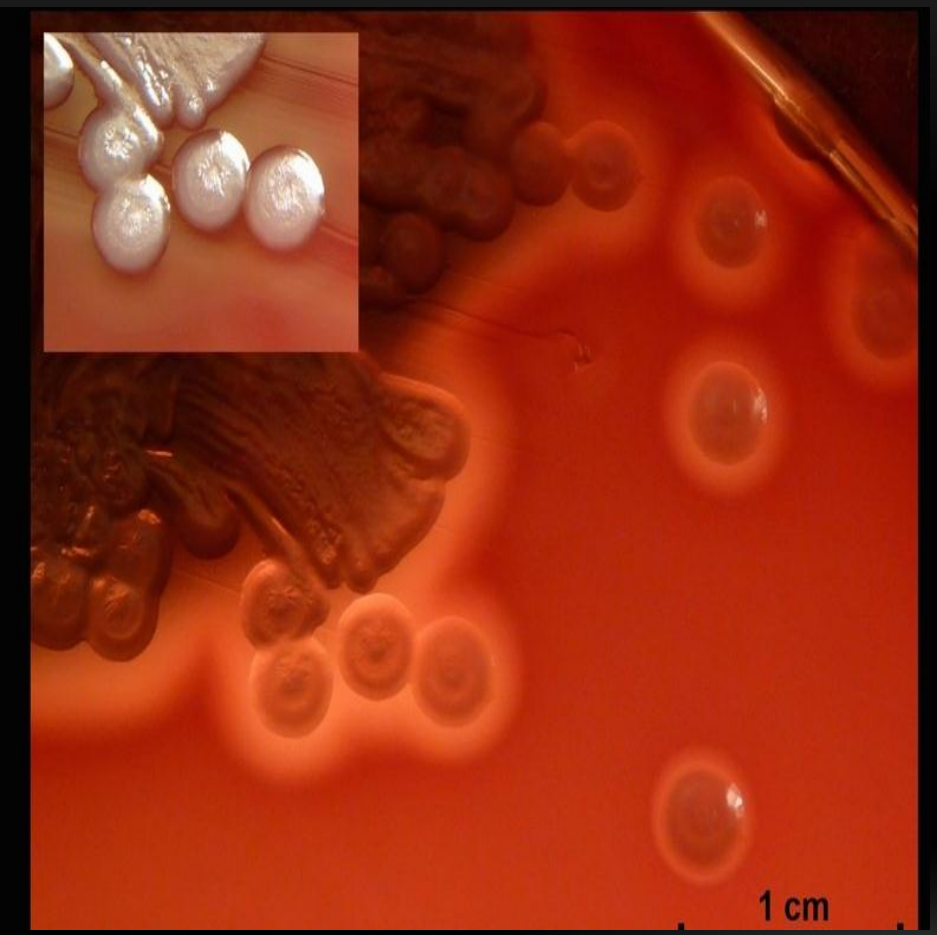


Gram Stain of *Pseudomonas*

CULTURAL CHARACTERISTICS

- **On Nutrient agar:**
  - Colonies are surrounded by bluish green coloration
- **On selective media "Cetermide"**
  - Pigments are more obvious
- **On Blood agar**
  - β-hemolytic colonies
- **On MacConkey agar**
  - Pale yellow colonies i.e. non lactose fermenters
- *Ps. aeruginosa* able to grow at 42 C for 3 days

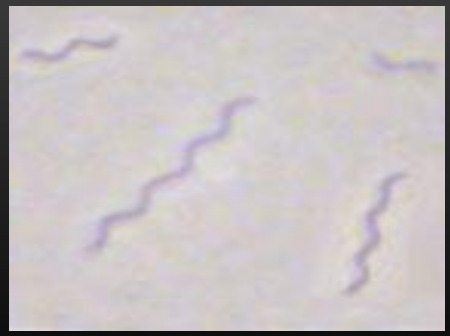
CULTURAL CHARACTERISTICS



Pseudomonas on blood agar

Ps. aeruginosa on cetrimide agar

Ps. aeruginosa on Nutrient agar

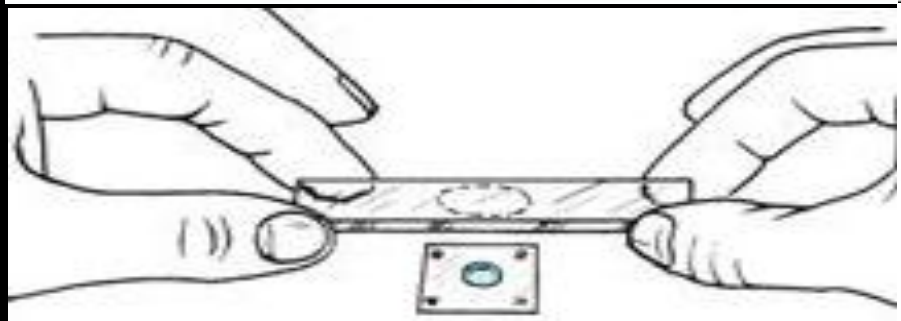


MOTILITY TEST :

direct microscopic observation (hanging drop technique)

1 A small amount of Vaseline is placed near each corner of the cover glass with a toothpick.

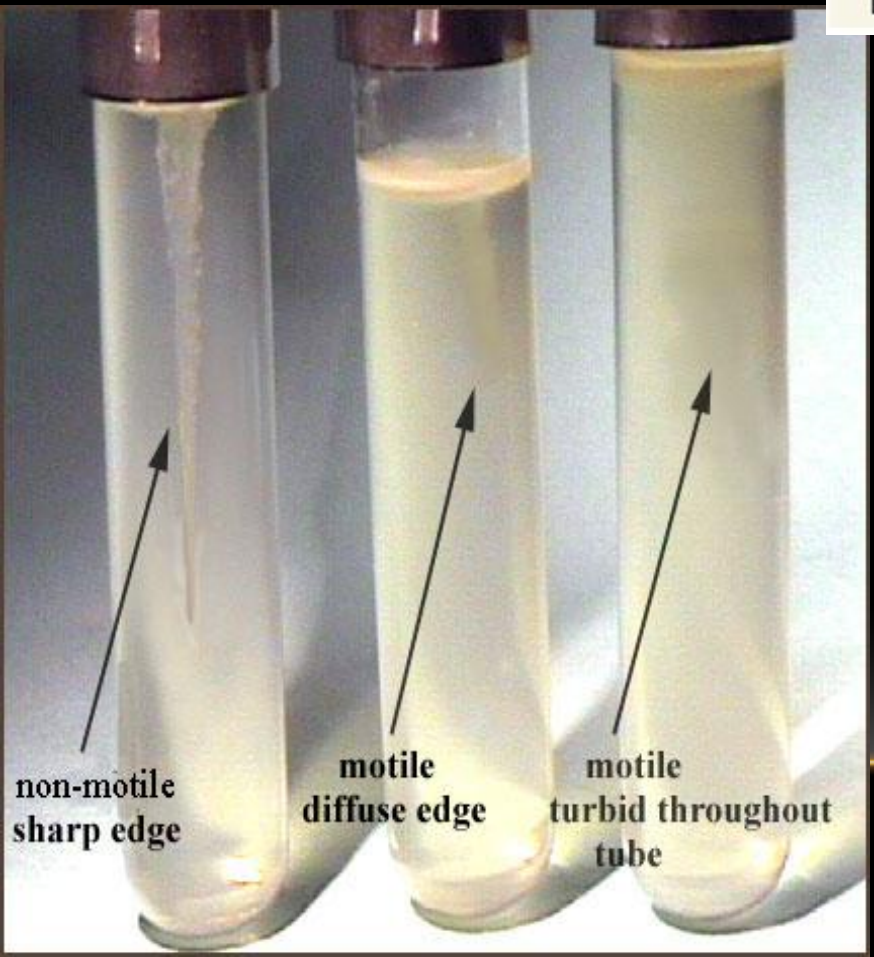
2 Two loopfuls of organisms are placed in center of cover glass.

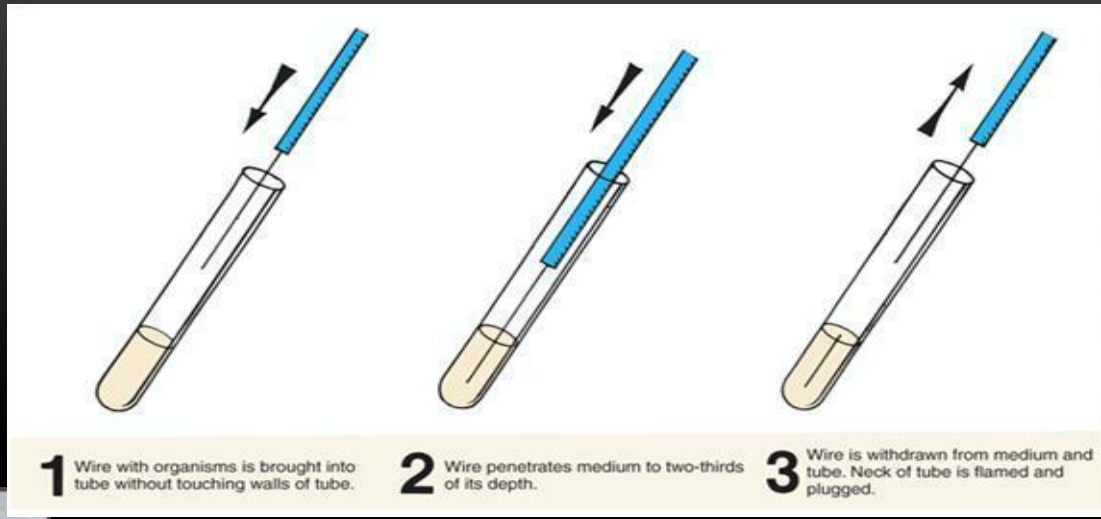


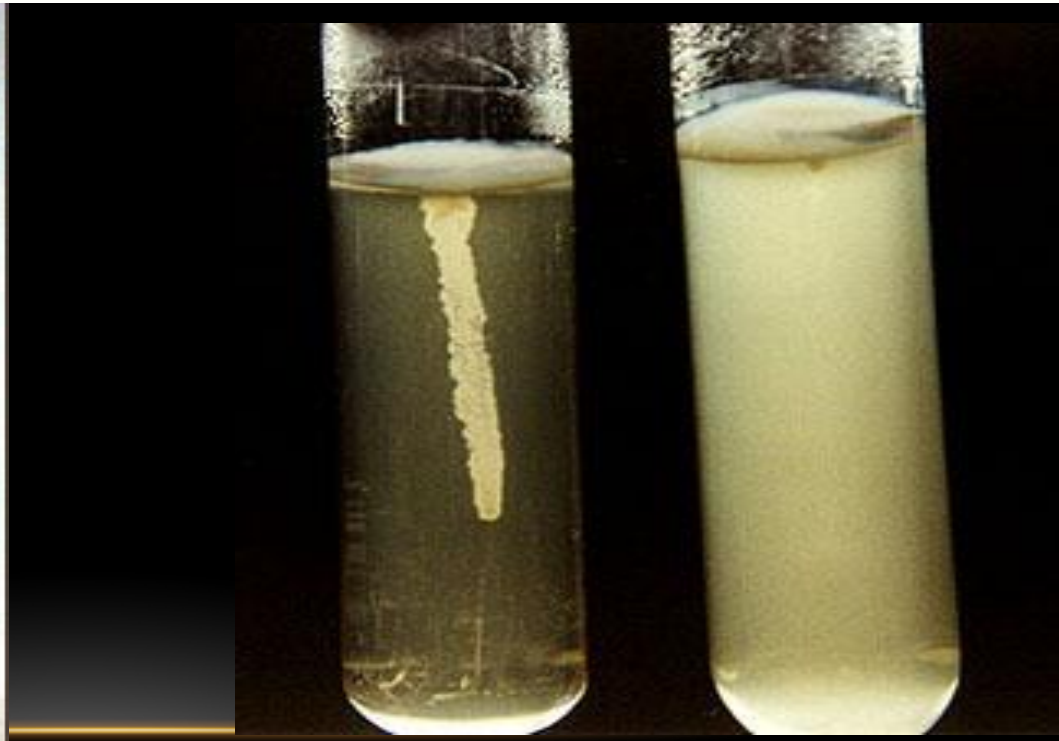
3 Depression slide is pressed against Vaseline on cover glass and quickly inverted.

4 The completed preparation can be examined under oil immersion.

MOTILITY TEST







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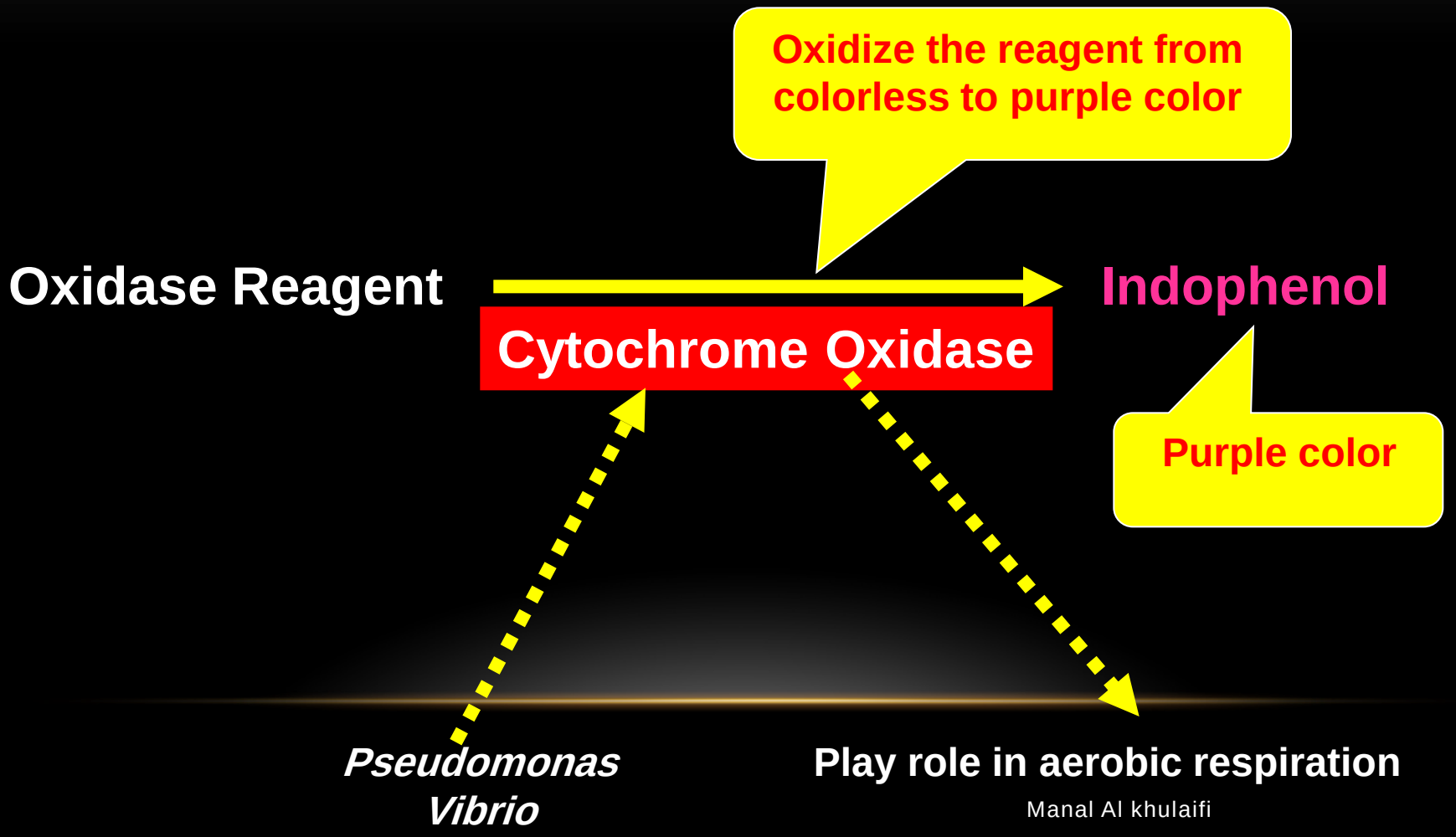
Semi solid agar

BIOCHEMICAL REACTIONS

- Oxidase positive
- Gelatinase positive
- Nitrate test
- O/F test

OXIDASE TEST: PRINCIPAL

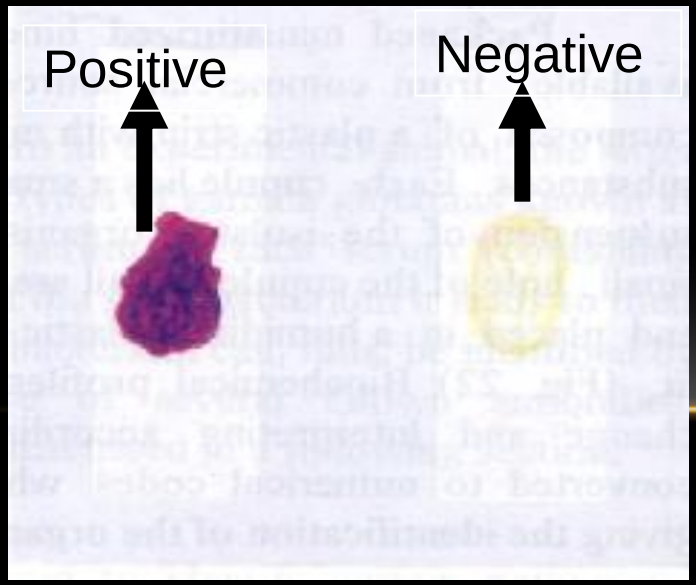


Method:

- hold a piece of the oxidase test paper with forceps and touch onto an area of heavy growth
- Use loop or wood stick

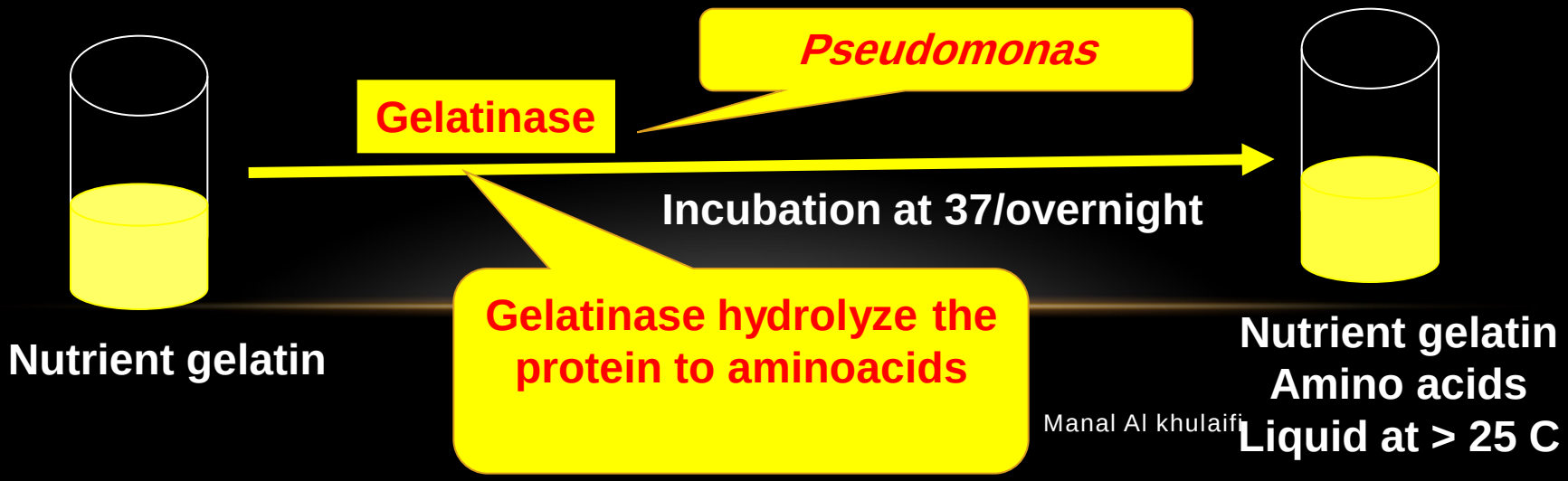
Results

- Color change to purple

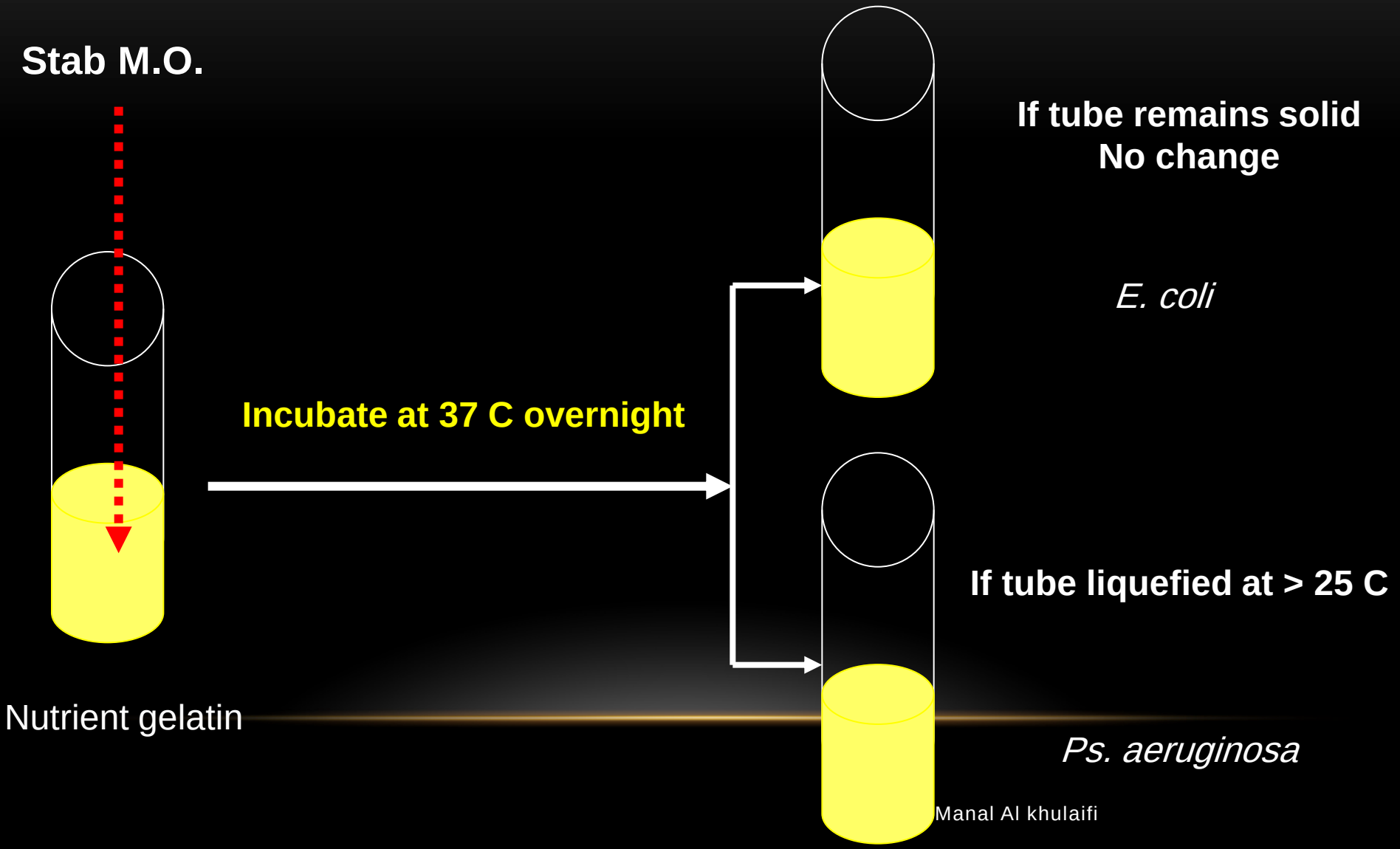


GELATIN LIQUIFACTION TEST: PRINCIPLE

- Certain bacteria are capable of producing a proteolytic exoenzyme called gelatinase
- Gelatinase hydrolyze the protein (solid) to amino acids (liquid)
- At temperature below 25°C, gelatin will remain a gel, but if the temperature rises about 25°C, the gelatin will be liquid.
- Gelatin hydrolysis has been correlated with pathogenicity of some microorganisms
- Pathogenic bacteria may breakdown tissue & spread to adjacent tissues

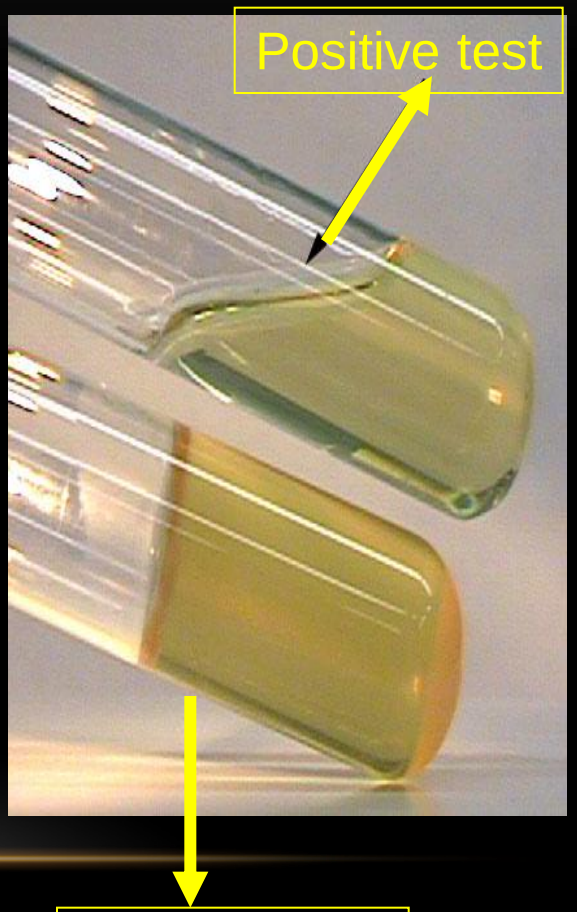


GELATINASE TEST:
PROCEDURE



GELATIN LIQUIFACTION TEST

- **Method**
  - Stab a nutrient gelatin tube with inoculums of the tested organism
  - Inoculated nutrient gelatin tube is incubated at 37°C for 24 h
- **Result**
  - If a tube of gelatin liquefy indicates positive test (*Ps. aeruginosa*)
  - If a tube of gelatin remains solid indicates negative test (*E. coli*)



PRACTICAL WORK

- Gram stain
- Oxidase test
- Gelatinase test
- Motility test

GRAM NEGATIVE RODS

Vibrionaceae

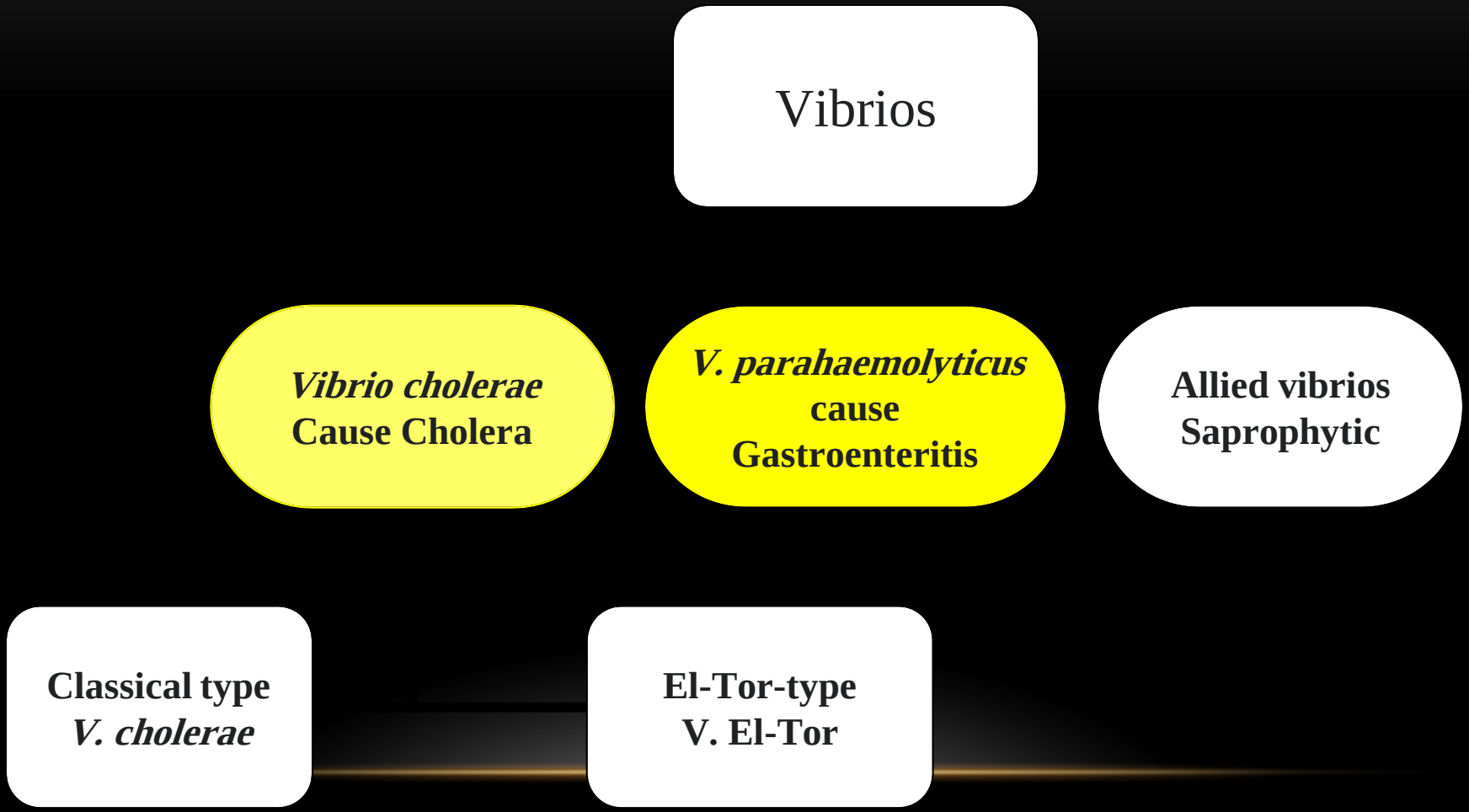
Vibrio

Manal Al khulaifi

GENERAL CHARCTERS OF *VIBRIONACEAE*

- Gram negative, curved, comma shaped bacilli
- Motile by single polar flagella
- Non spore forming
- Non capsulated
- grow well in alkaline pH
- Facultative anaerobes
- Vibrios are capable of both respiratory & fermentative metabolism i.e. O+/F+.
- Oxidase and catalase positive
- Natural inhabitants of aquatic environment

SPECIES OF *VIBRIO*



SPECIES OF *VIBRIO*

- ***V. cholerae*** is the causative agent of cholera
  - *V. cholerae* divided serologically into 6 groups based on somatic O-antigens
- ***Vibrio parahaemolyticus*** is the cause of acute gastroenteritis following ingestion of contaminated sea-food such as raw fish
- *V. cholerae* & *V. parahaemolyticus, are* pathogens of human, produce diarrhea

IDENTIFICATION OF *V. CHOLERAE*

- **Specimen and microscopical examination:**
  - **Rice watery stool or rectal swap collected in acute stage of disease**
  - **Dark-field microscopy** of stool specimen from patients with cholera reveal large numbers of *Vibrio* (short, curved rods) with a characteristic motility that gives the appearance of shooting stars
- **Culture:**
  - **Inoculation** of rice water stool in enrichment media (alkaline peptone water, pH8.5), in which the organisms multiply rapidly and tend to form pellicle at the surface of the medium after 6-8 h at 37 C.
  - **Subculture** is made into Thiosulphate Citrate Bile Sucrose (TCBS) agar.

Identification of *V. cholerae* Growth on TCBS

❖Principle

➢TCBS medium is selective because

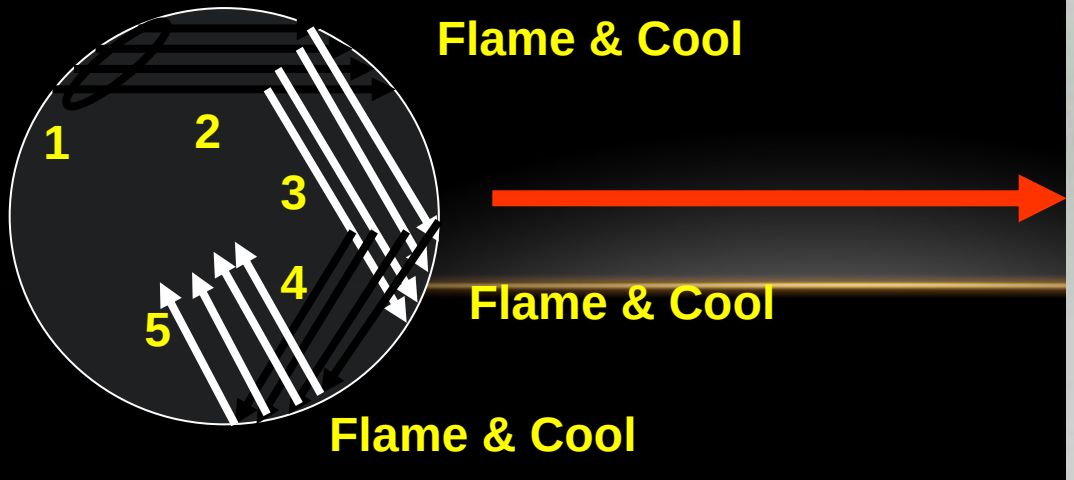
- High conc. of thiosulfate and citrate & strong alkalinity of this medium (pH9)
  - Also, contains bile salts kills most intestinal commensals

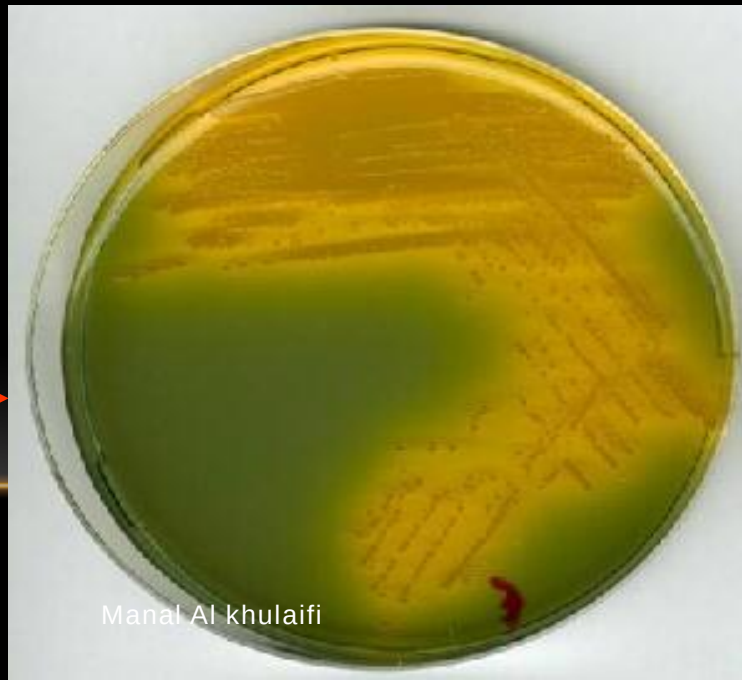
➢TCBS medium is differential because

- It contains sucrose
- It contains bromothymol blue
  - Alkaline pH: blue
  - Neutral pH: green
  - Acidic pH: yellow
- Some species ferment sucrose & others not ferment
- Sucrose fermenting *Vibrio spp* (*V. cholerae*) appears as yellow colonies
- Sucrose non fermenting *Vibrio spp (V. parahemolyticus)* appears as blue to green colonies
- Sucrose fermentation on TCBS is the gold standard in its identification

IDENTIFICATION OF *VIBRIO*
DIFFERENTIATION BETWEEN SF & NSF BY GROWTH ON TCBS

- **Method:**
  - TCBS agar is inoculated with tested organism recovered from alkaline peptone water using streak plate technique
  - Incubate the plate in incubator at 37 C/24 hrs
- **Results:**
  - SF organism appears as yellow colonies (V. cholerae)
  - NSF organism appears as blue to green colonies (*V. parahaemolyticus*)

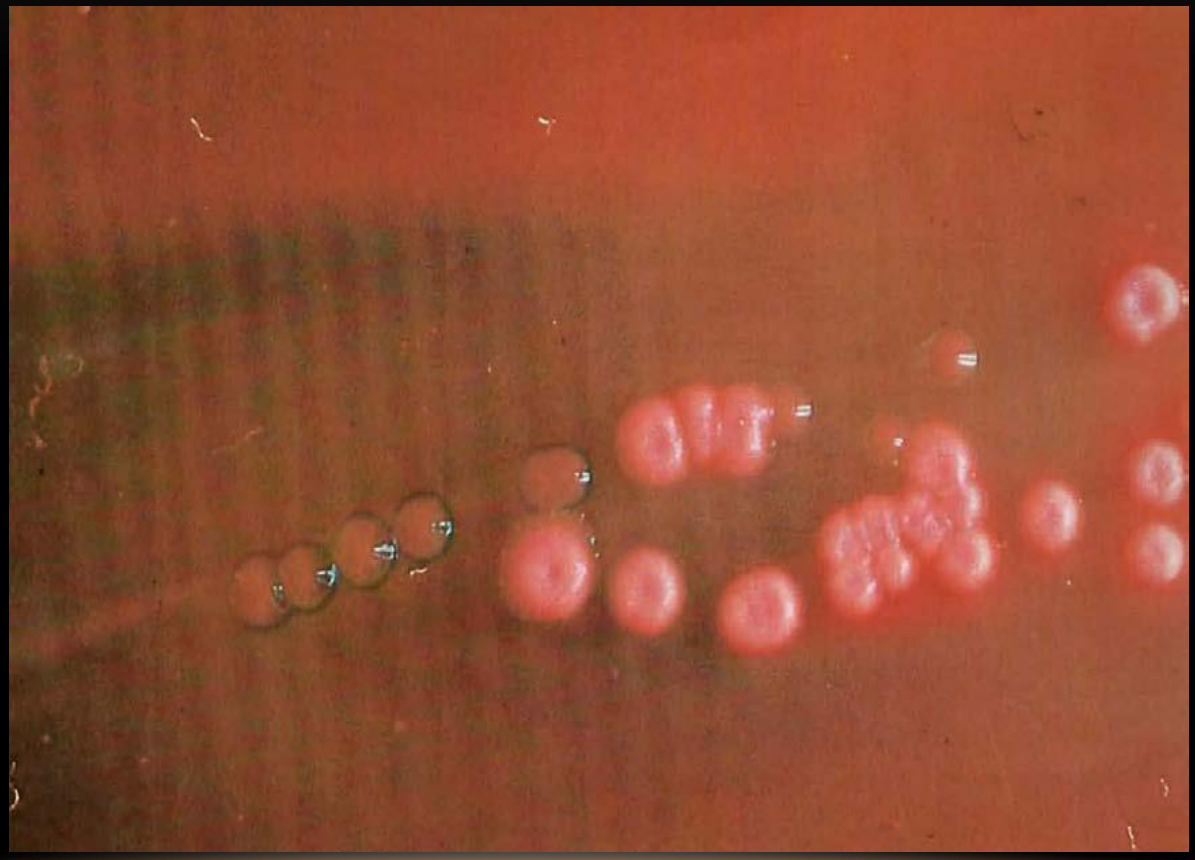




REACTION ON TCBS

Yellow colonies of *V. cholorae* due sucrose fermentation and green colonies of *V. parahaemolyticus* on TCBS

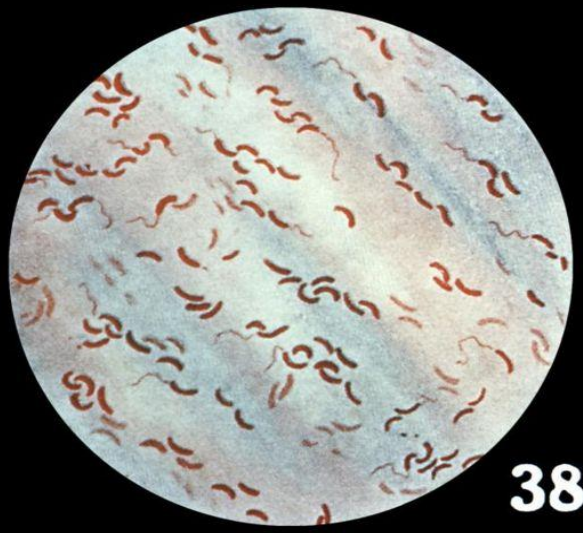
ON MACKONCEY MEDIA



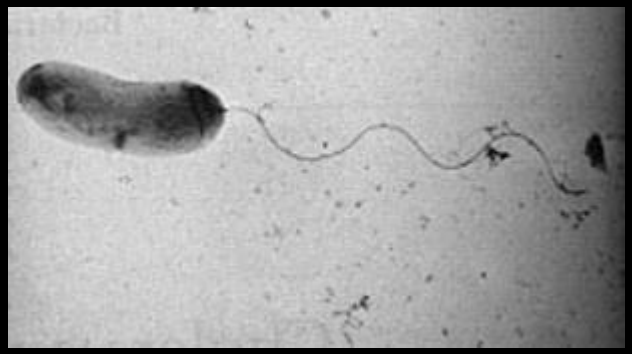
Noo lactose fermented

IDENTIFICATION OF *VIBRIO CHOLREAE*

- **Gram stain:**
- **Gram negative short rods, comma shaped, motil**



Gram stain of *Vibrio cholorae*



Electron Micrograph of *V cholerae*
Rods with single polar flagella

- **Serology**