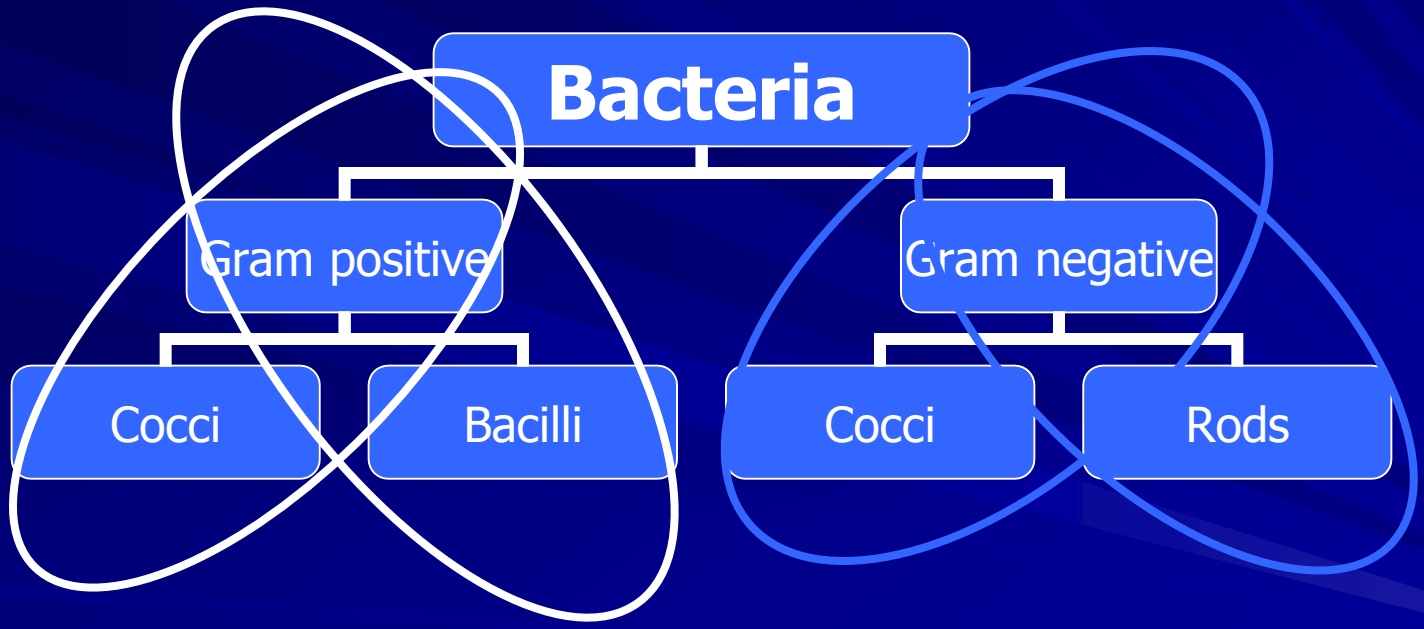
Enterobacteriaceae

Biochemical Reactions

Classification of Bacteria



Characters of *Enterobacteriaceae*

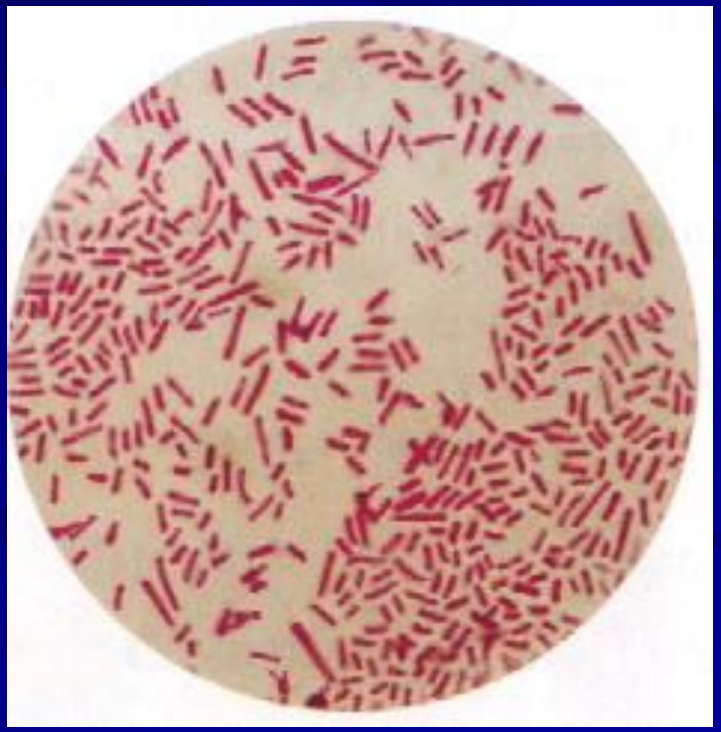
- All *Enterobacteriaciae*
  - Gram-negative rods
  - Reduce nitrates into nitrites
  - Oxidase negative
- Facultative anaerobic
- Motile **except** *Shigella* and *Klebsiella*
- Non-capsulated except *Klebsiella*
- Non-fastidious
- Grow on bile containing media (MacConkey agar)

Enterobacteriaceae

- Some *Enterobacteriaceae* are true pathogens
  - *Salmonella* spp.
  - *Shigella* spp.
  - *Yersinia* spp.
  - Certain strains of *E. coli* (ETEC, EPEC, EIEC, EHEC)
- Most members of the *Enterobacteriaceae* are opportunistic or cause secondary infections of wounds, the urinary and respiratory tracts, and the circulatory system e.g. *E. coli*.
- Enterobacteriaceae divided into TWO main groups according to action on LACTOSE
  - Lactose Fermenters (LF)
    - *E. coli, Citrobacter, Klbesiella, Enterobacter*
  - Lactose Non-Fermenters (LNF)
    - *Salmonella, Shigella, Proteus, Yersinia*

Identification of *Enterobacteriaceae*

- Gram stain
  - All *Enterobacteriaceae* are Gram-negative rods
  - Arranged in single



Identification of *Enterobacteriaceae* Biochemical reactions

- **Oxidase test**
  - All members of *Enterobacteriaceae* are oxidase negative
  - *Pseudomonas* is oxidase positive
- **O/F test**
  - All members of *Enterobacteriaceae* are O+/F+
  - *Pseudomonas* is O+/F-
- See & compare these tests under *Pseudomonas Lab*

Oxidase Test:

Principle:

Tetramethyl p-phenylene diamine
(oxidase reagent)
colourless

Cytochrome oxidase enzyme

Indophenol
(Purple colour)

O/F Test (Oxidation Fermentation Test)

Principle:

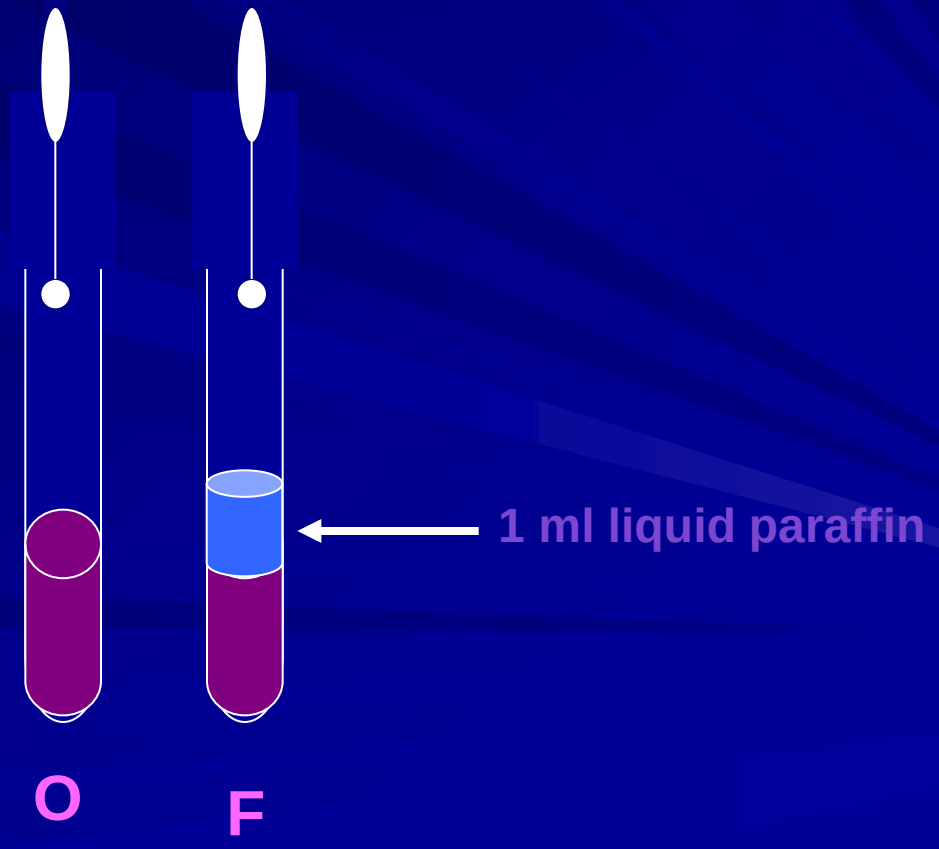
- **Saccharolytic bacteria attack carbohydrates either:**

- **fermentatively (in absence of oxygen) to yield relatively strong acids, or**

- **oxidatively (in presence of oxygen) to yield weak acids.**

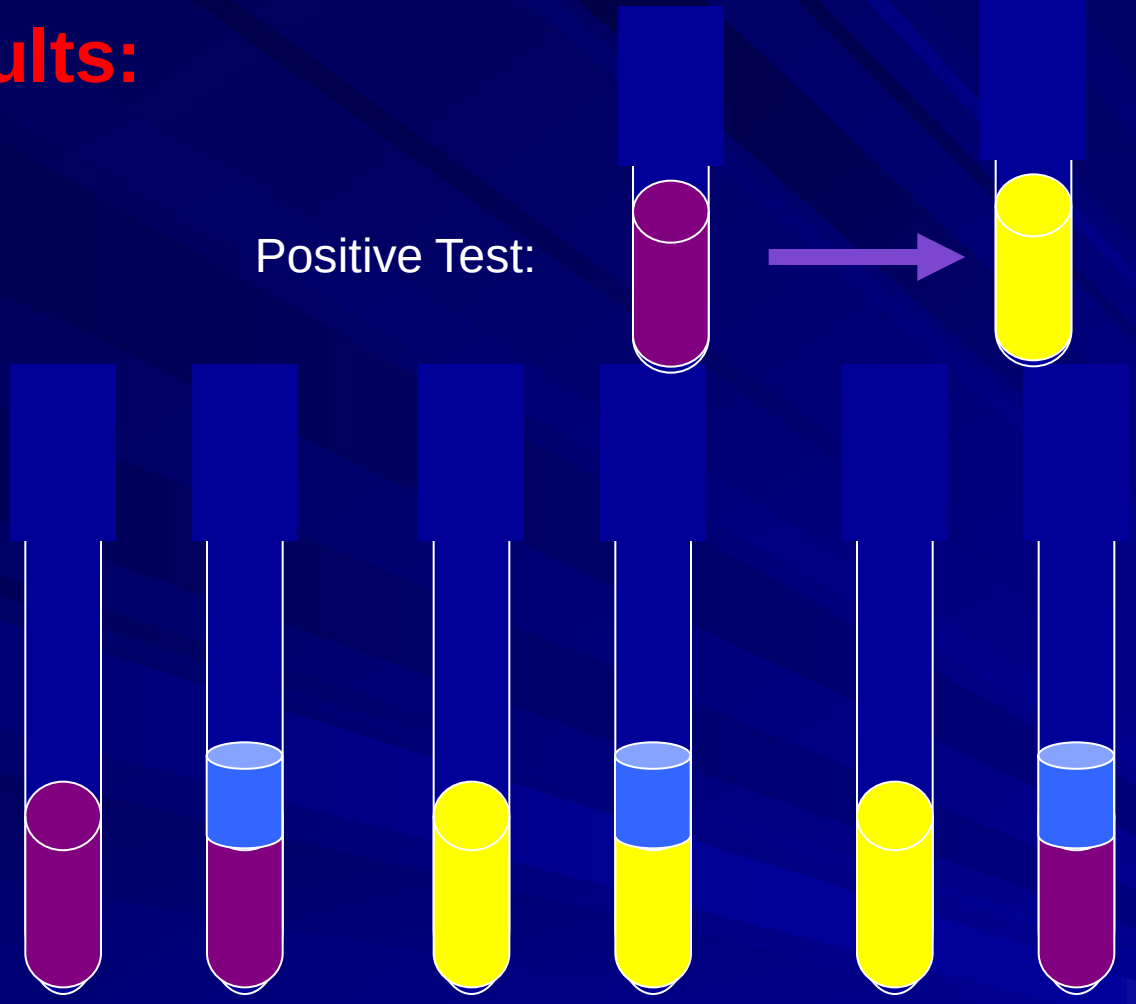
- **Oxidation process is much more easier than Fermentation process.**

Procedure:



Results:

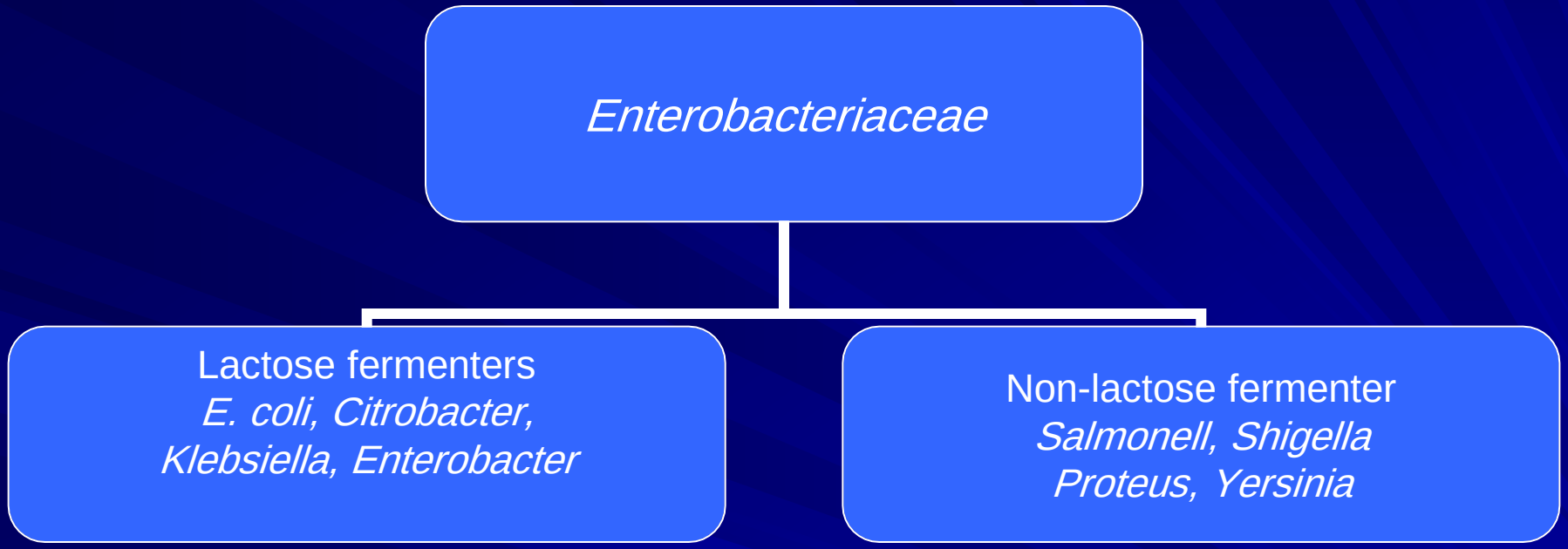
Positive Test:



O^-/F^- O^+/F^+ O^+/F^-

Non Saccharolytic **Fermentative** **Oxidative**

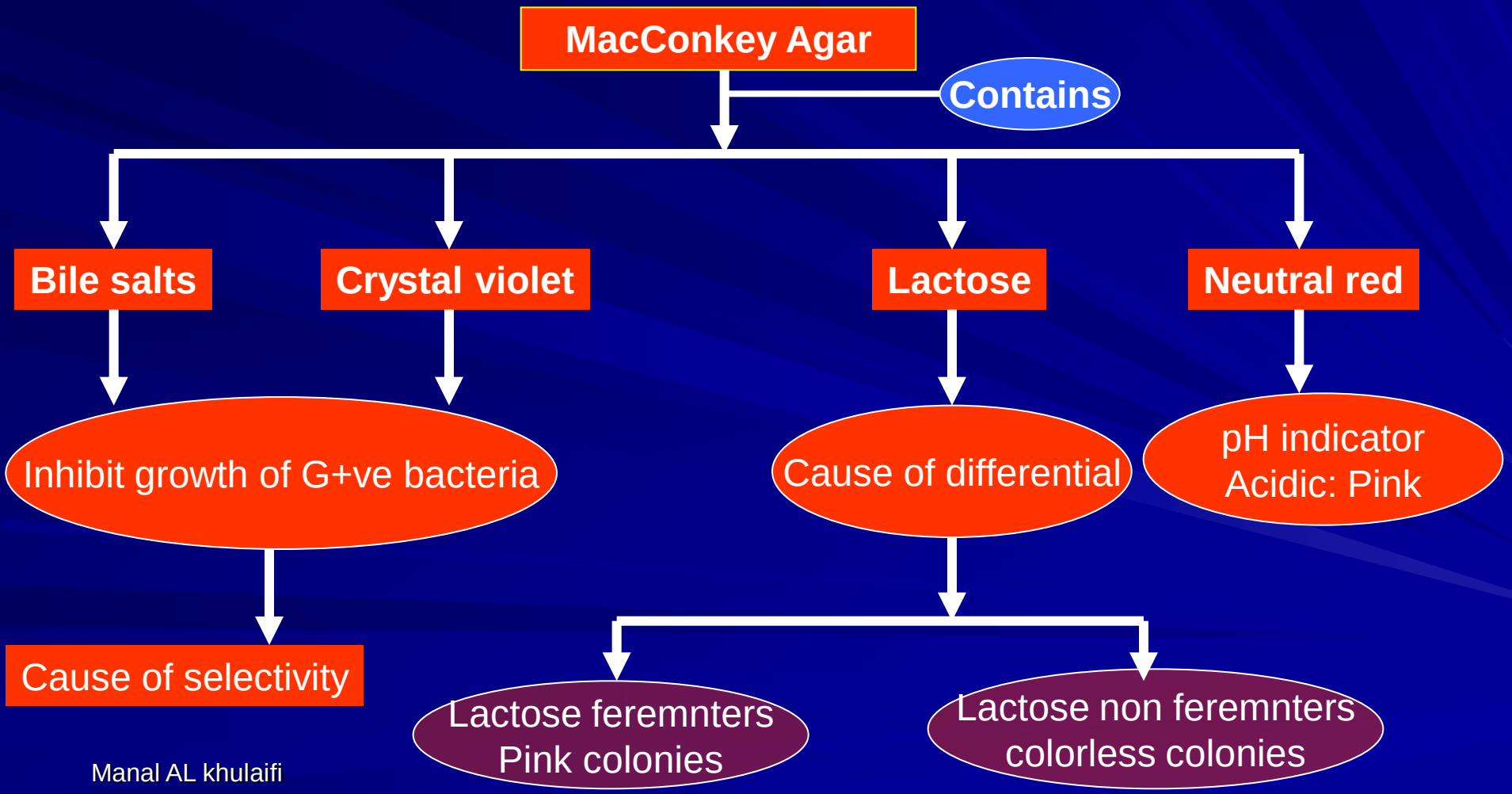
Classification of *Enterobacteriaceae*



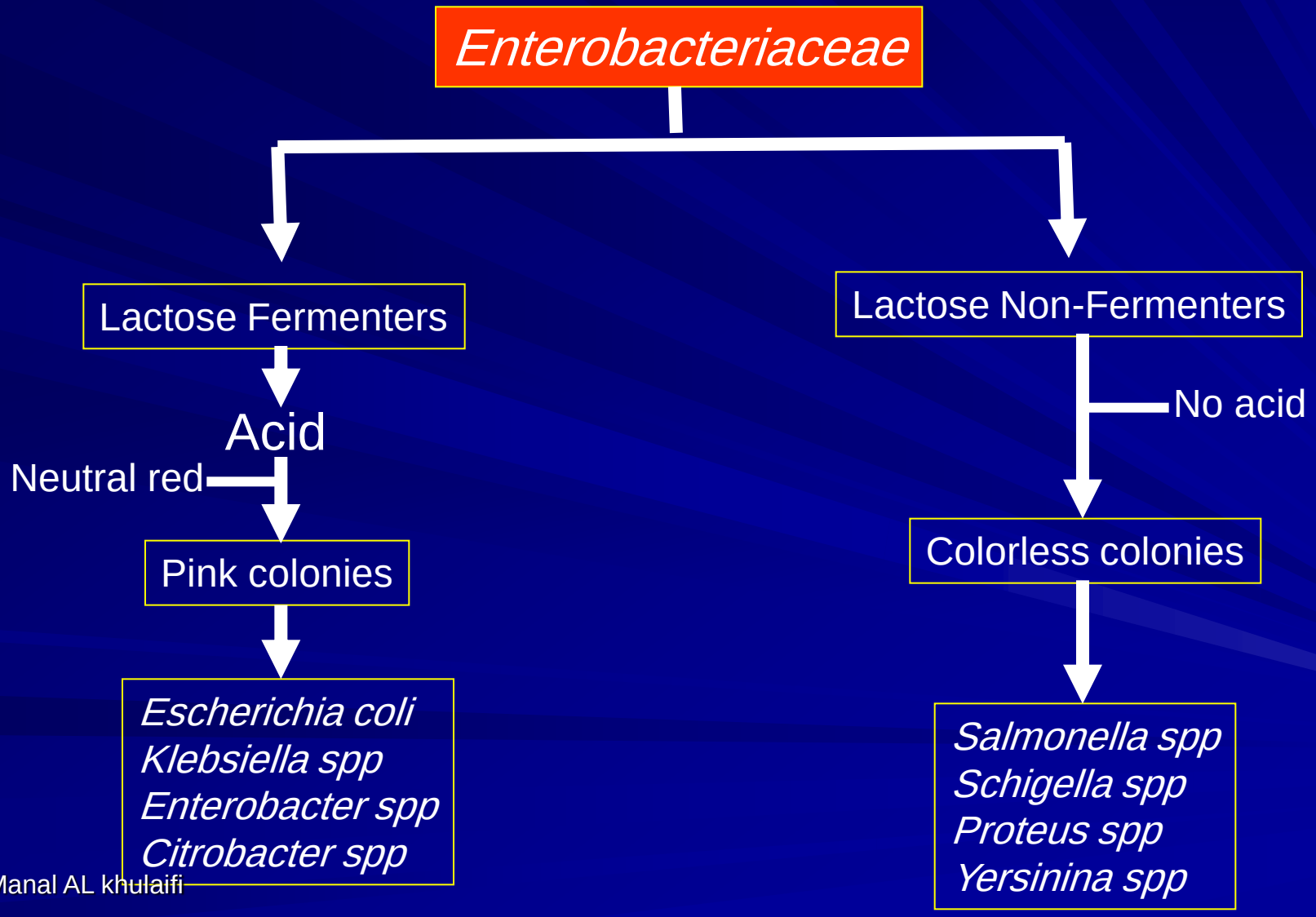
- There are several selective and differential media used to isolate distinguishes between LF & LNF
- The most important media are:
  - MacConkey agar
  - Eosin Methylene Blue (EMB) agar
  - Salmonella Shigella (SS) agar
  - In addition to Triple Sugar Iron (TSI) agar

Differentiation between LF & NLF by Growth on MacConkey agar

- **MacConkey agar is selective & differential medium for *Enterobacteriaceae***

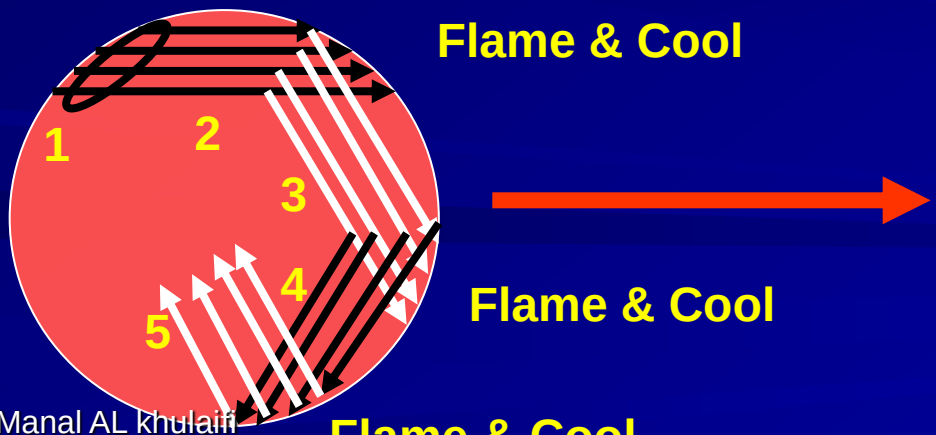


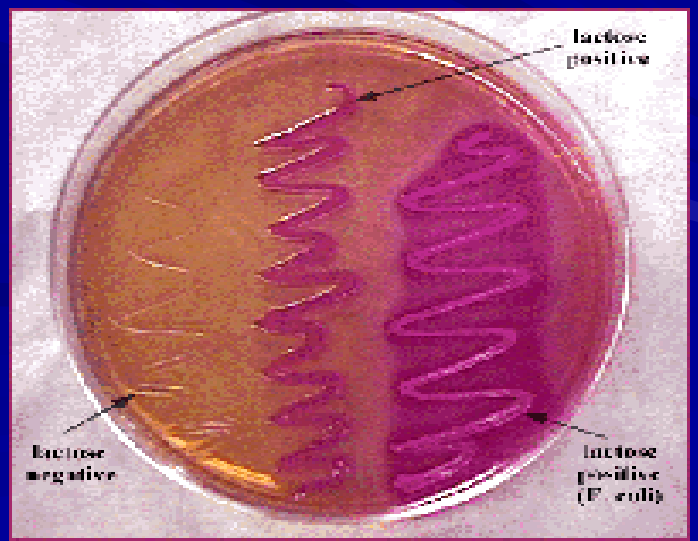
Classification of *Enterobacteriaceae* according to lactose fermentation (growth on MacConkey Agar)



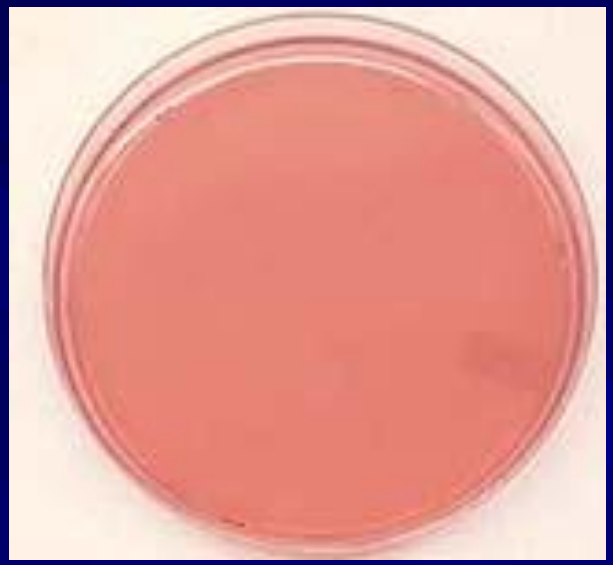
Identification of *Enterobacteriaceae*
Differentiation between LF & NLF by Growth on MacConkey agar

- **Method:**
  - MacConkey agar is inoculated with tested organism using streak plate technique
  - Incubate the plate in incubator at 37 C/24 hrs
- **Results:**
  - LF organism appears as pink colonies (e.g. *E. coli*)
  - NLF organism appears as colorless colonies (e.g. *Shigella*)

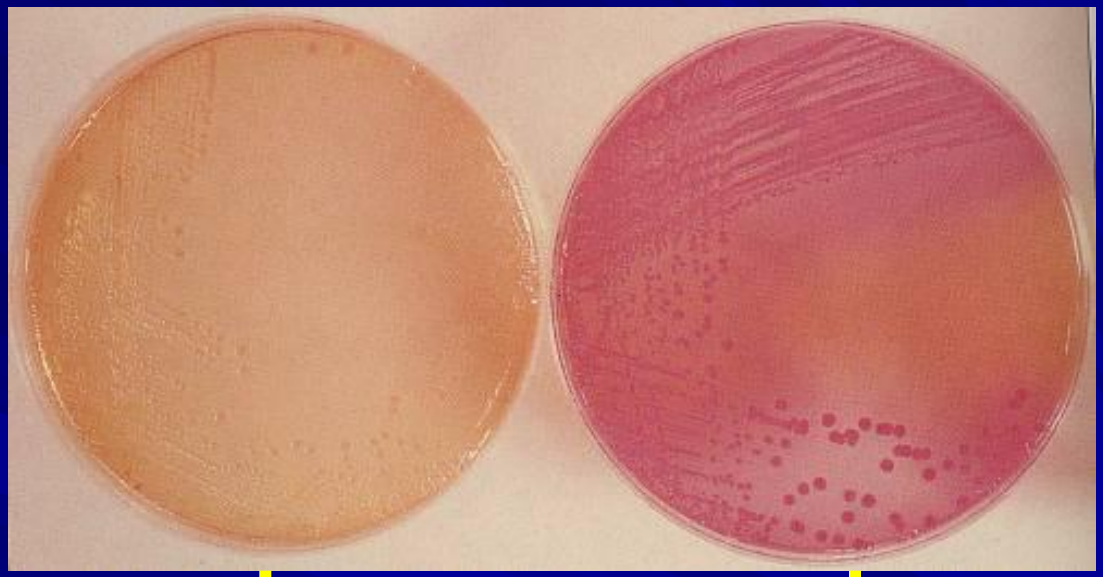




Growth of *Enterobacteriaceae* on MacConkey agar



Uninoculated plate



Colorless colonies

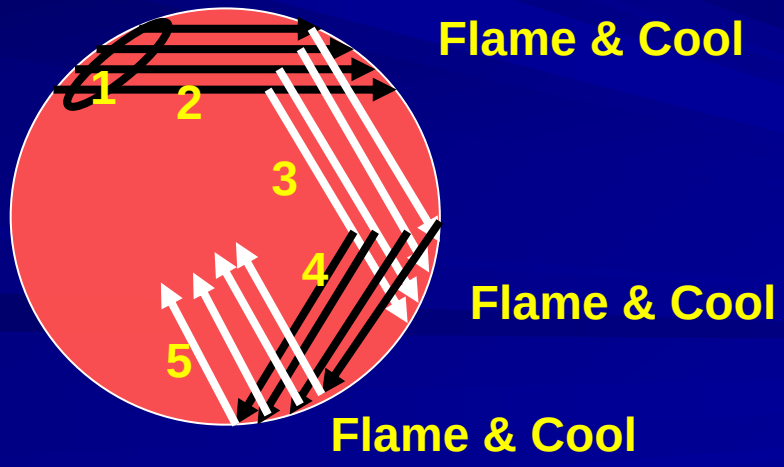
Lactose non feremters
Salmonella, Shigella, Proteus

Pink colonies

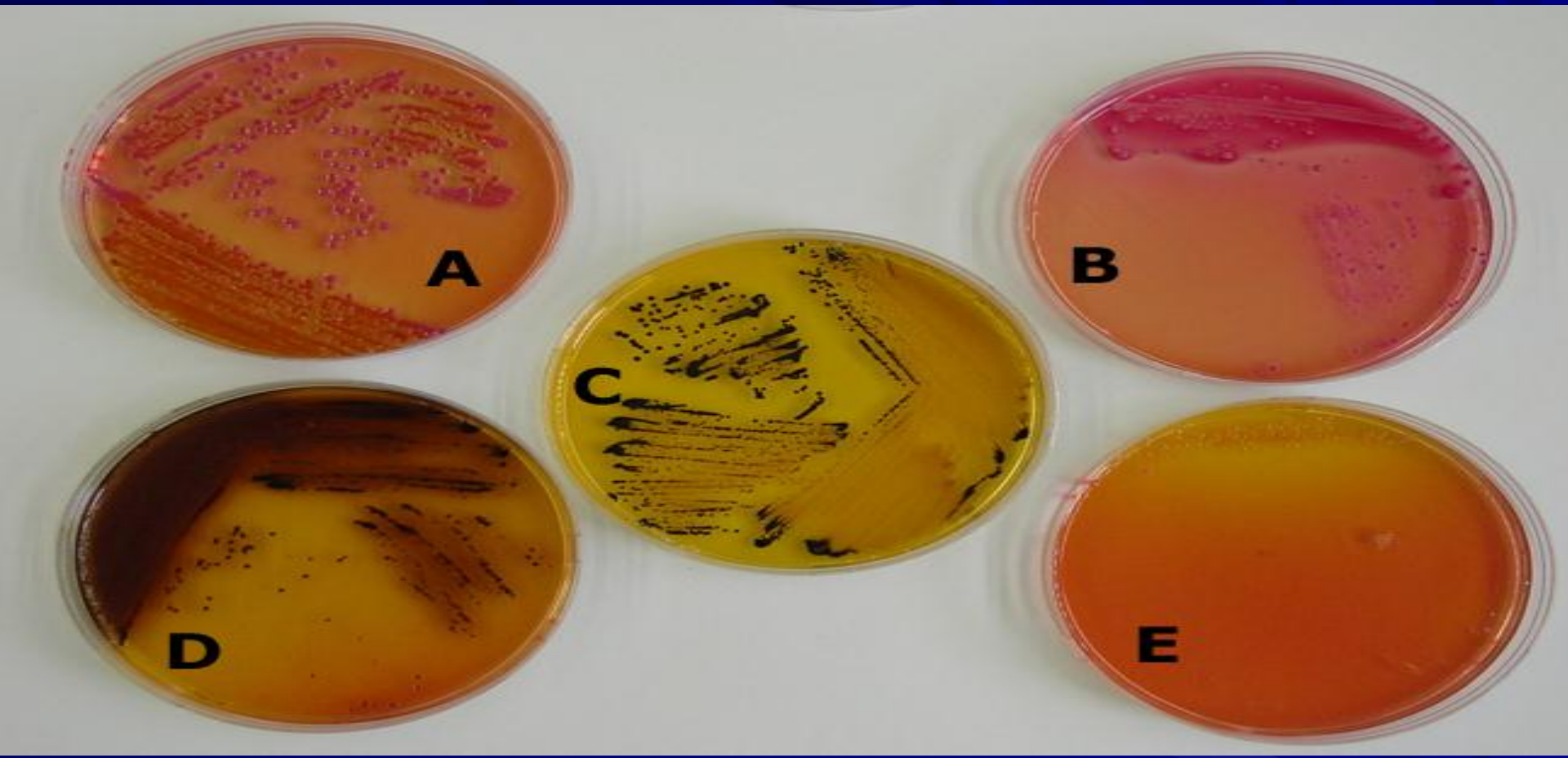
Lactose feremters
E. coli, Citrobacter Klebsiella, Enterobacter

Identification of *Enterobacteriaceae*
Differentiation between LF & NLF by Growth on SS agar

- **Method:**
  - SS agar is inoculated with tested organism using streak plate technique
  - Incubate the plate in incubator at 37 C/24 hrs



Growth of *Enterobacteriaceae* on SS agar



A .*Klebsiella pneumoniae*
B .*Escherichia coli*
C :*Salmonella sp.*
D :*Proteus mirabilis*
E :*Ps. aeruginosa*

→ *Both* are lactose fermenters

→ Both *Salmonella sp. & Proteus* product H2S

→ *Pseudomonas* colonies are nearly colorless

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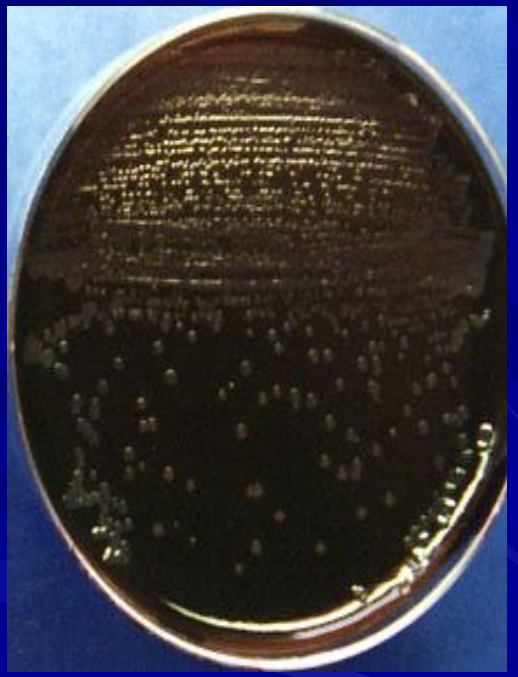
Growth of *Enterobacteriaceae* on EMB agar



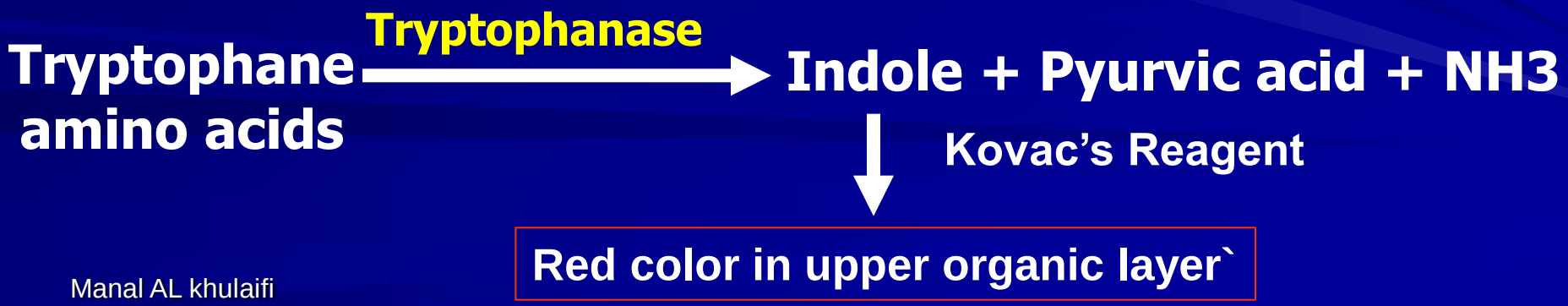
Coli-type colonies are very dark, almost black e.g. *E. coli*

IMViC Test

- **I**ndole, **M**ethyl Red, **V**oges-Prosakaur, **C**itrate (IMViC) Tests:
  - The following four tests comprise a series of important determinations that are collectively called the IMViC series of reactions
  - The IMViC series of reactions allows for the differentiation of the various members of *Enterobacteriaceae*.

IMViC: Indole test

- **Principle**
  - Certain microorganisms can metabolize tryptophan by tryptophanase
  - The enzymatic degradation leads to the formation of pyruvic acid, indole and ammonia
  - The presence of indole is detected by addition of Kovac's reagent.



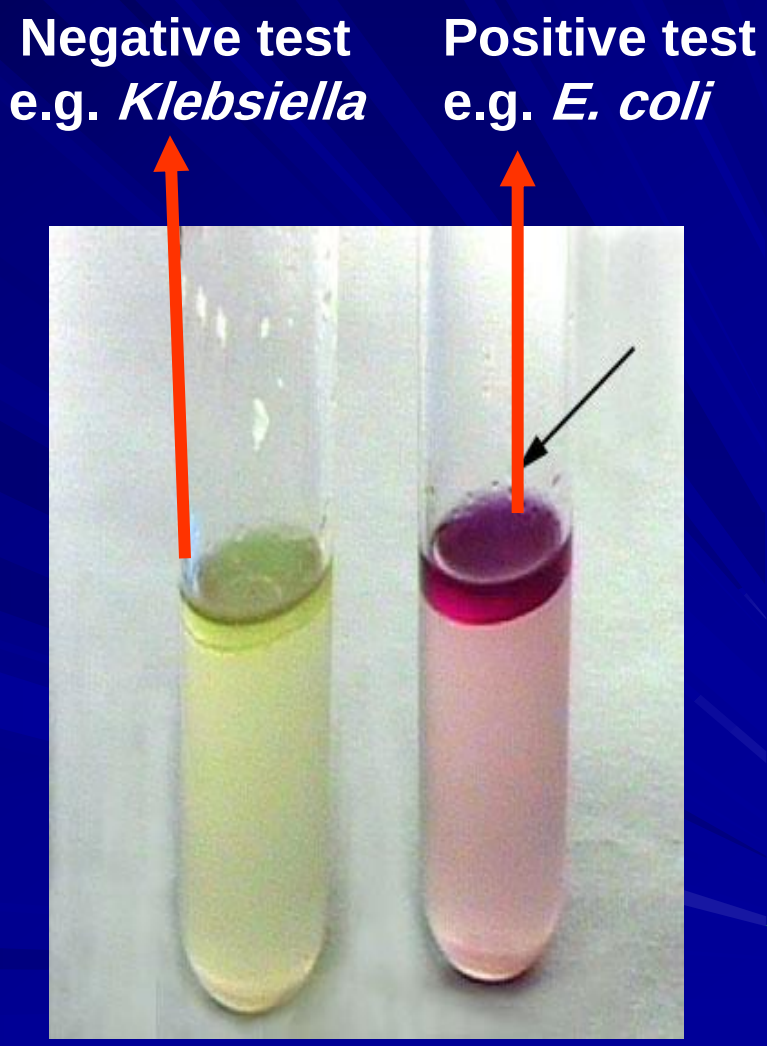
IMViC: Indole test

- **Method:**
  - **Inoculate tryptone water with the tested microorganism**
  - **Incubate at 37°C for 24 hours**
  - **After incubation interval, add 1 ml Kovacs reagent, and read immediately**

IMViC: Indole test

- **Result:**
  - A bright pink color in the top layer indicates the presence of indole
  - The absence of color means that indole was not produced i.e. indole is negative

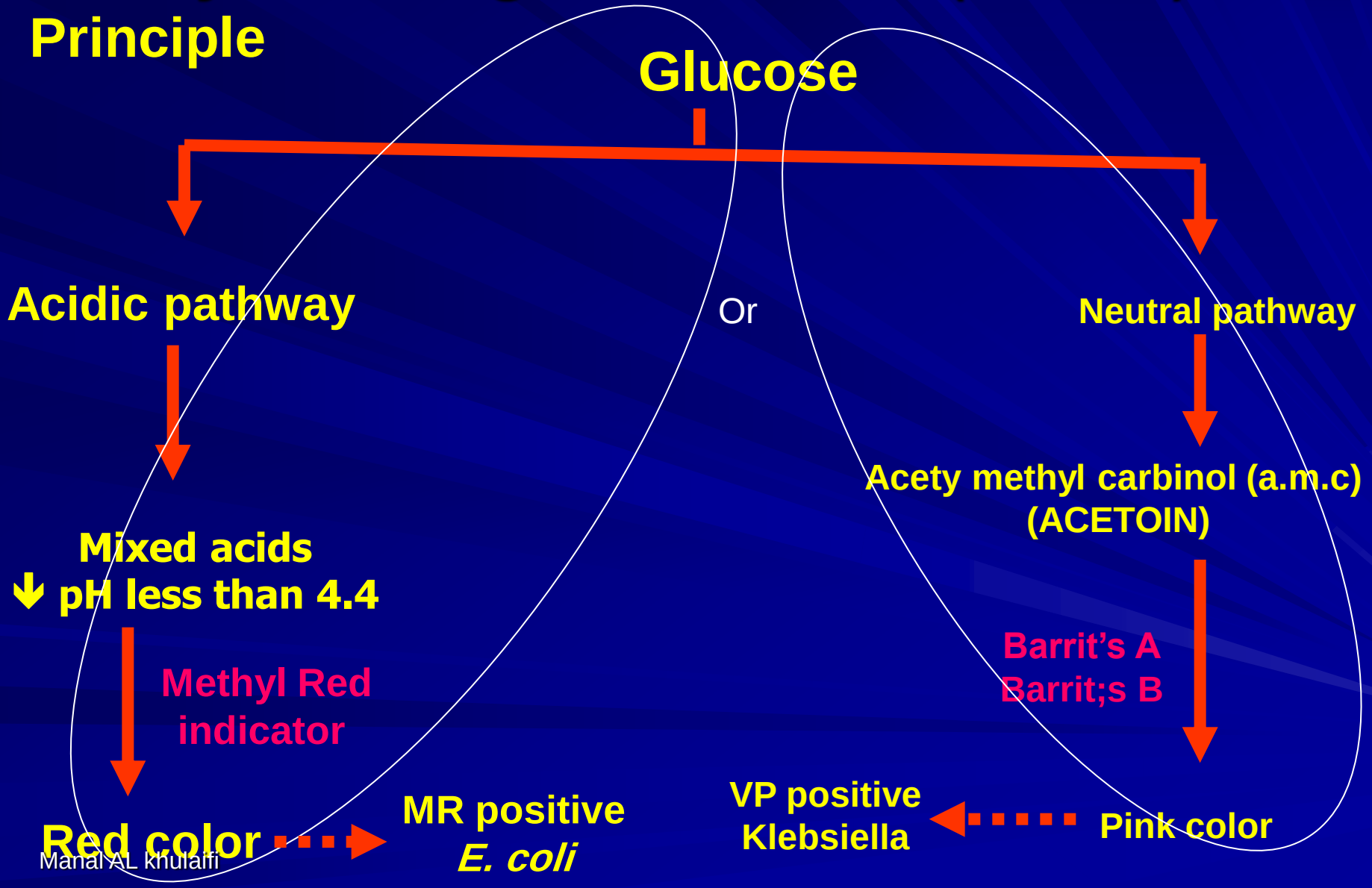
- **Special Features:**
  - Used in the differentiation of genera and species. e.g. *E. coli* (+) from *Klebsiella* (-).



IMViC test

Methyl Red-Voges Proskauer (MR-VP) Tests

Principle



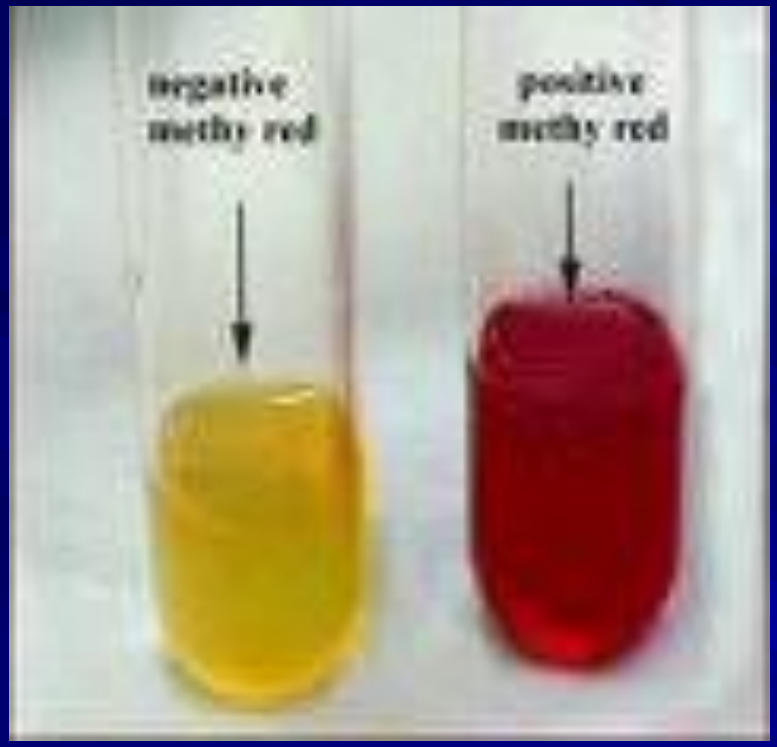
IMViC test: MRVP test

❖Method

- Inoculate the tested organism into **two tubes** of MRVP broth
- Incubate the tubes at 37°C for 24 hours
  - For methyl red tube : Add 6-8 drops of methyl red reagent.
  - For Voges-Proskauer tube : Add 12 drops of Barritt's A (α-naphthol), 4 drops of Barritt's B (40% KOH), mix
  - Let sit, **undisturbed**, for at least 1hour

IMViC test: MR/VP test

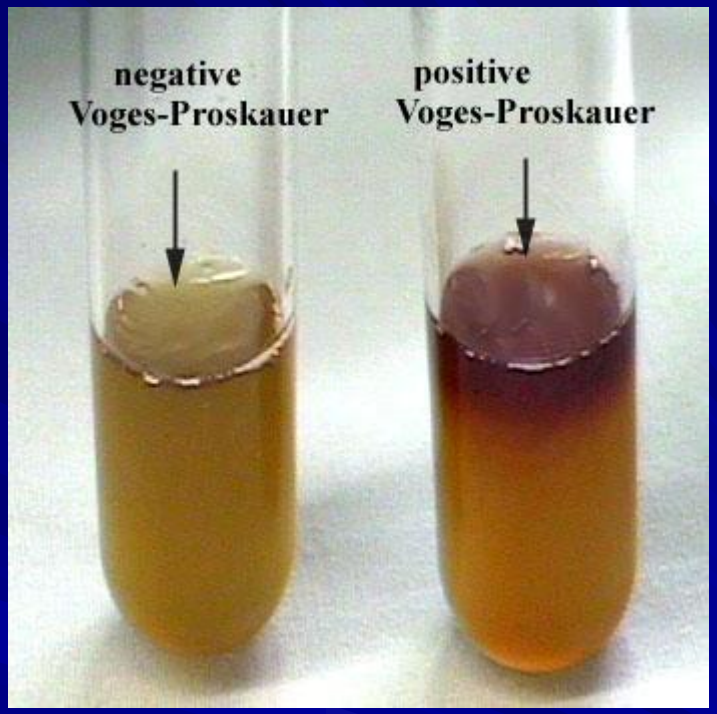
❖Results



Methyl Red test

✓Red: Positive MR (*E. coli*)

✓Yellow or orange: Negative MR (Klebsiella)



Voges-Proskauer test

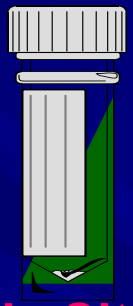
✓Pink: Positive VP (*Klebsiella*)

✓No pink: Negative VP (*E. coli*)

Citrate Utilization Test

Principle:

Citrate → Pyruvate → $CO_2 + Na + H_2O$ → Na_2CO_3



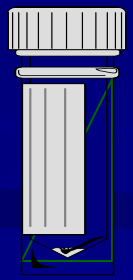
Simmone's Citrate media

Contains Citrate as a sole of C source

Alkaline, ↑pH

Bromothymol blue

Positive test



Blue colour

- **Positive test: Klebsiella, Enterobacter, Citrobacter**
- **Negative test: *E. coli***

Citrate Utilization Test

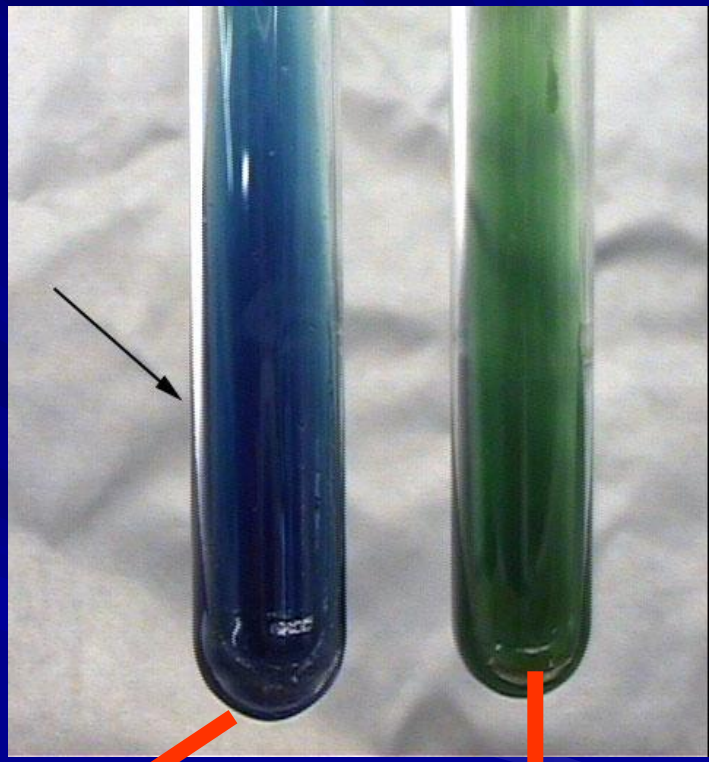
❖Method

- Streak a **Simmon's Citrate agar** slant with the organism
- Incubate at 37°C for 24 hours.

Citrate Utilization Test

❖Result

- Examine for growth (+)
- Growth on the medium is accompanied by a rise in pH to change the medium from its initial green color to deep blue



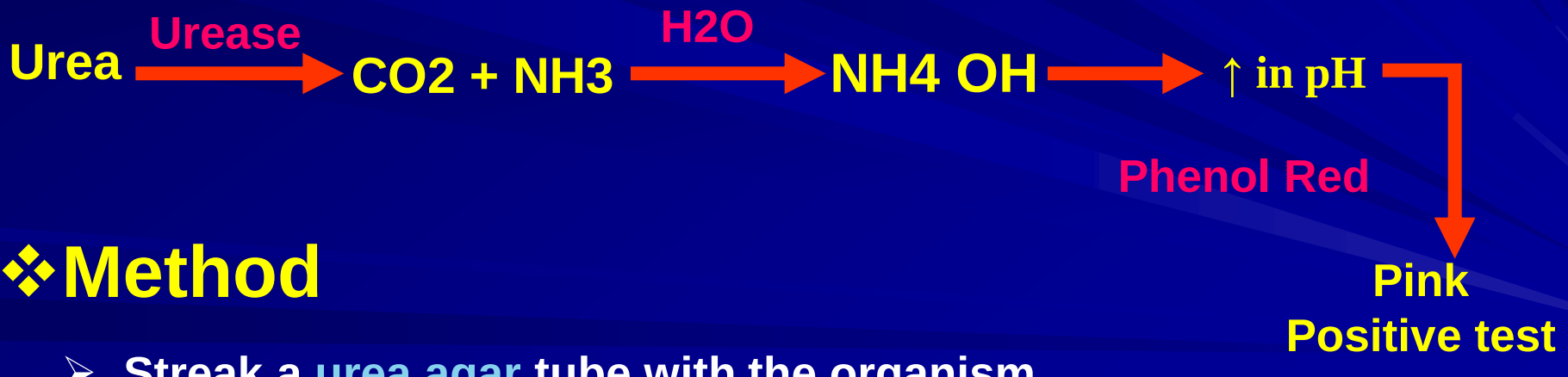
Positive
Klebsiella, Enterobacter

Negative
E. coli

Urease Test

❖Principle

- Urea agar contains urea and phenol red
- Urease is an enzyme that catalyzes the conversion of urea to CO2 and NH3
- Ammonia combines with water to produce ammonium hydroxide, a strong base which ↑ pH of the medium.
- ↑ in the pH causes phenol red to turn a deep pink. This is indicative of a positive reaction for urease



❖Method

- **Streak a urea agar tube with the organism**
- **incubate at 37°C for 24 h**

Urease Test

❖Result

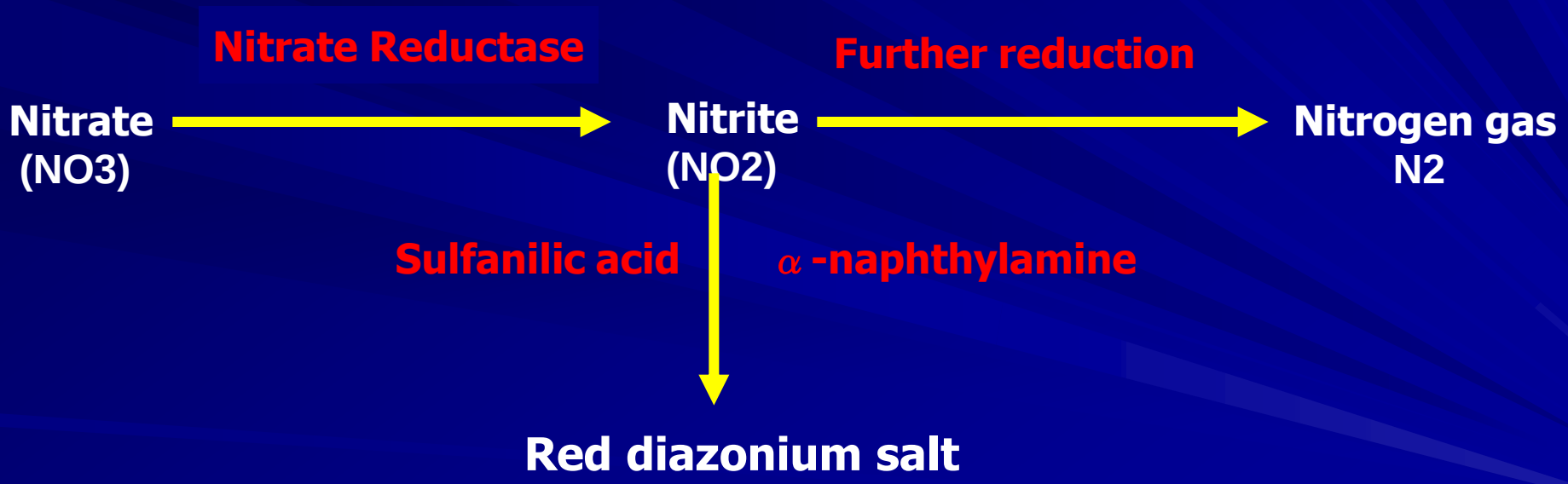
- If color of medium turns from yellow to pink indicates positive test.
- *Proteus* give positive reaction after 4 h while *Kelebsiella* and *Enterobacter* gave positive results after 24 h

Positive test **Negative test**

Nitrate Reductase Test

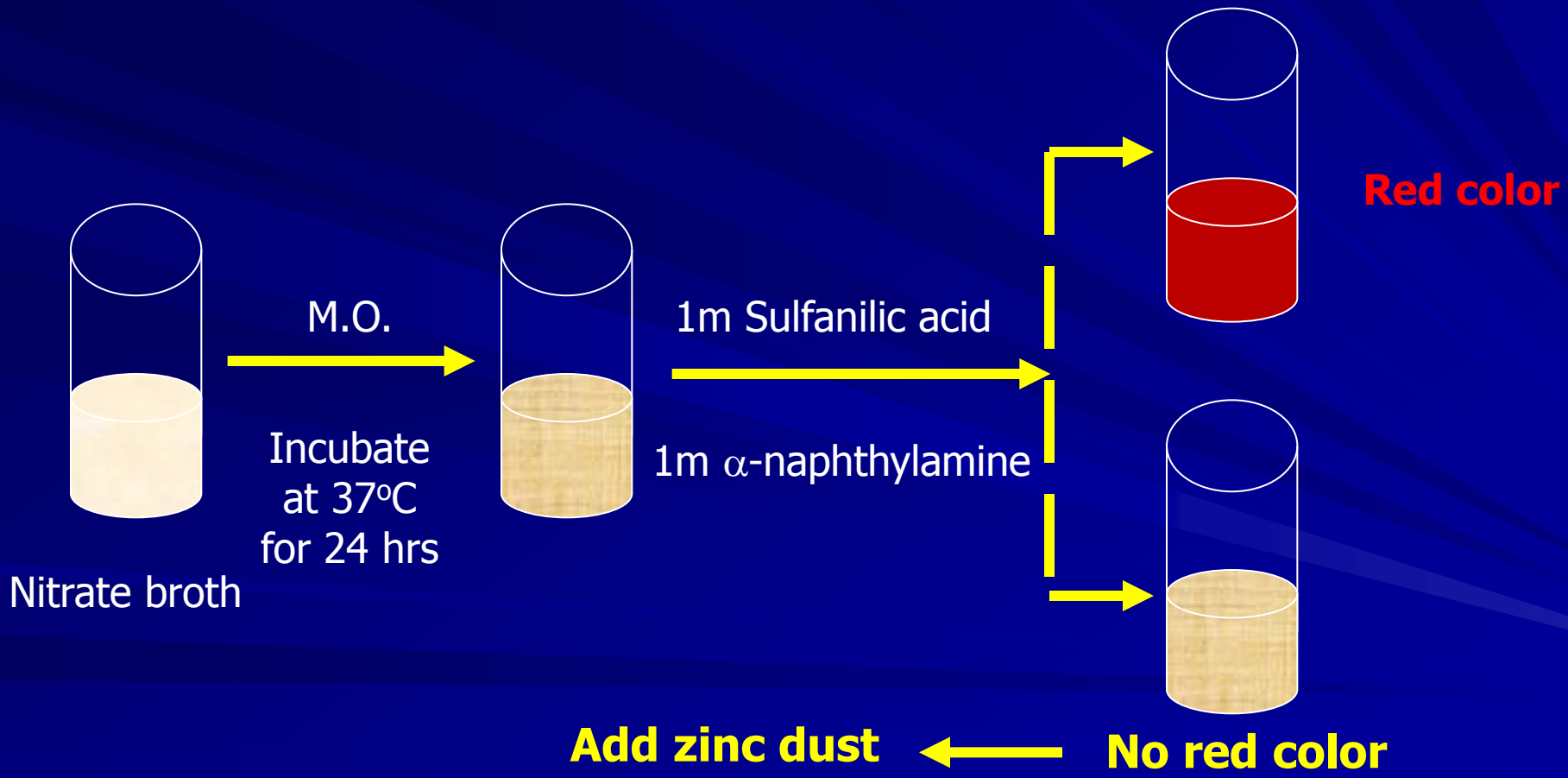
- **Principle**
  - To determine the ability of an organism to reduce nitrate to nitrites or free nitrogen gas
- **Method**
  - Inoculate a nitrate broth with tested M.O.
  - incubate for 24 hrs at 37°C.
  - After incubation, add 1 ml of sulphonilic acid and 1 ml of α-naphtylamine to nitrate broth tube
- **Result**
  - The production of a red color occurs in the presence of nitrite indicates the ability of the organism to reduce nitrate to nitrite.
  - To broths showing a negative reaction add a few particles of zinc. The appearance of a red color indicates that nitrate is still present and hence has not been reduced by the organism. If the solution does not change color the organism has reduced the nitrate through nitrite to nitrogen gas.

Nitrate Reductase Test: Principal

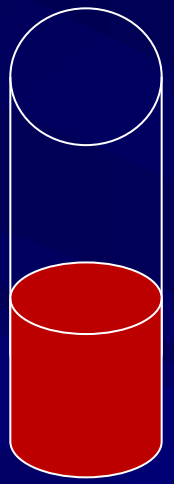


If no red color! → Add zinc dust (reducing agent)

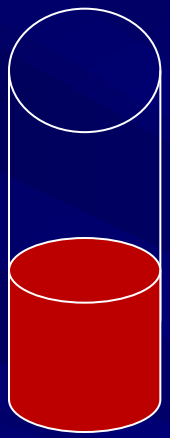
Nitrate Reductase Test: Procedure



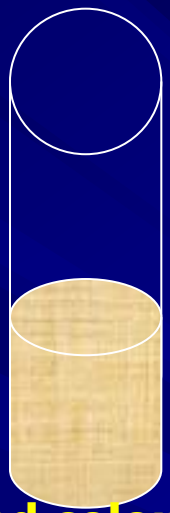
Nitrate Reductase Test: Results



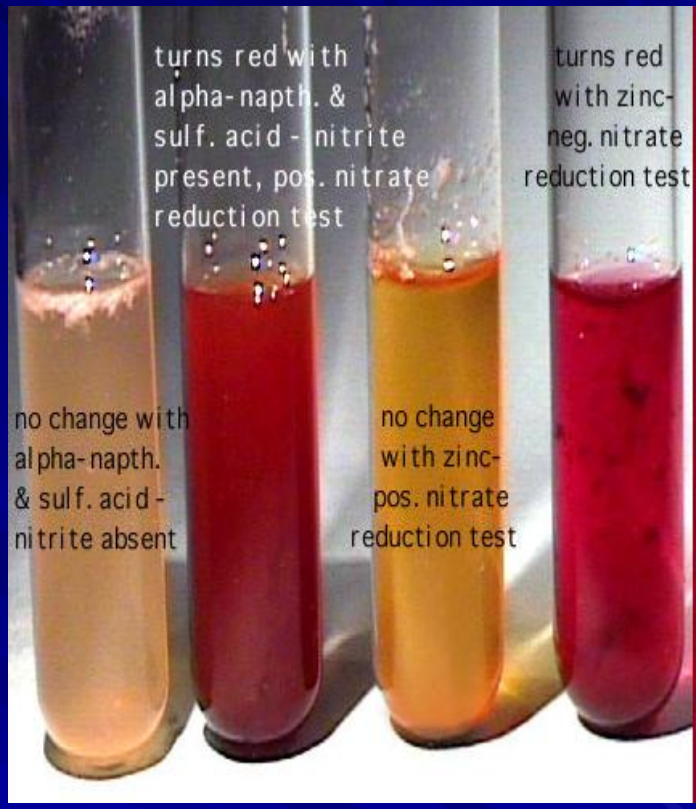
Red color after addition of sulfanilic acid & α-naphtylamine



Red color after addition of zinc dust



No red color after addition of zinc dust



Reduction of Nitrate to nitrite

-ve reduction Nitrate unreduced

Nitrate reduced into nitrite and further reduction to Nitrogen

Reaction on Triple Sugar Iron (TSI) Agar

- TSI contains
  - Three different types of sugars
    - Glucose
    - Lactose
    - Sucrose
    - Phenol red (acidic: Yellow)
- **Principle**
  - To determine the ability of an organism to attack a specific carbohydrate incorporated into a basal growth medium, with or without the production of gas, along with the determination of possible hydrogen sulphide production.

Reaction on TSI

- Method:
  - Inoculate TSI medium with an organism by inoculating needle by stabbing the butt and streaking the slant
  - Incubate at 37°C for 24 hours

2- Triple Sugar Iron (TSI) agar:

Principle:

(glucose 0.1%, lactose 1%, sucrose 1%).

Non fermentation

glucose Fermentation:

Butt: acidic (yellow) Slant: alkaline (red)

glucose Lactose/ sucrose Fermentation:

Butt: acidic (yellow) Slant: acidic (yellow)

Results:

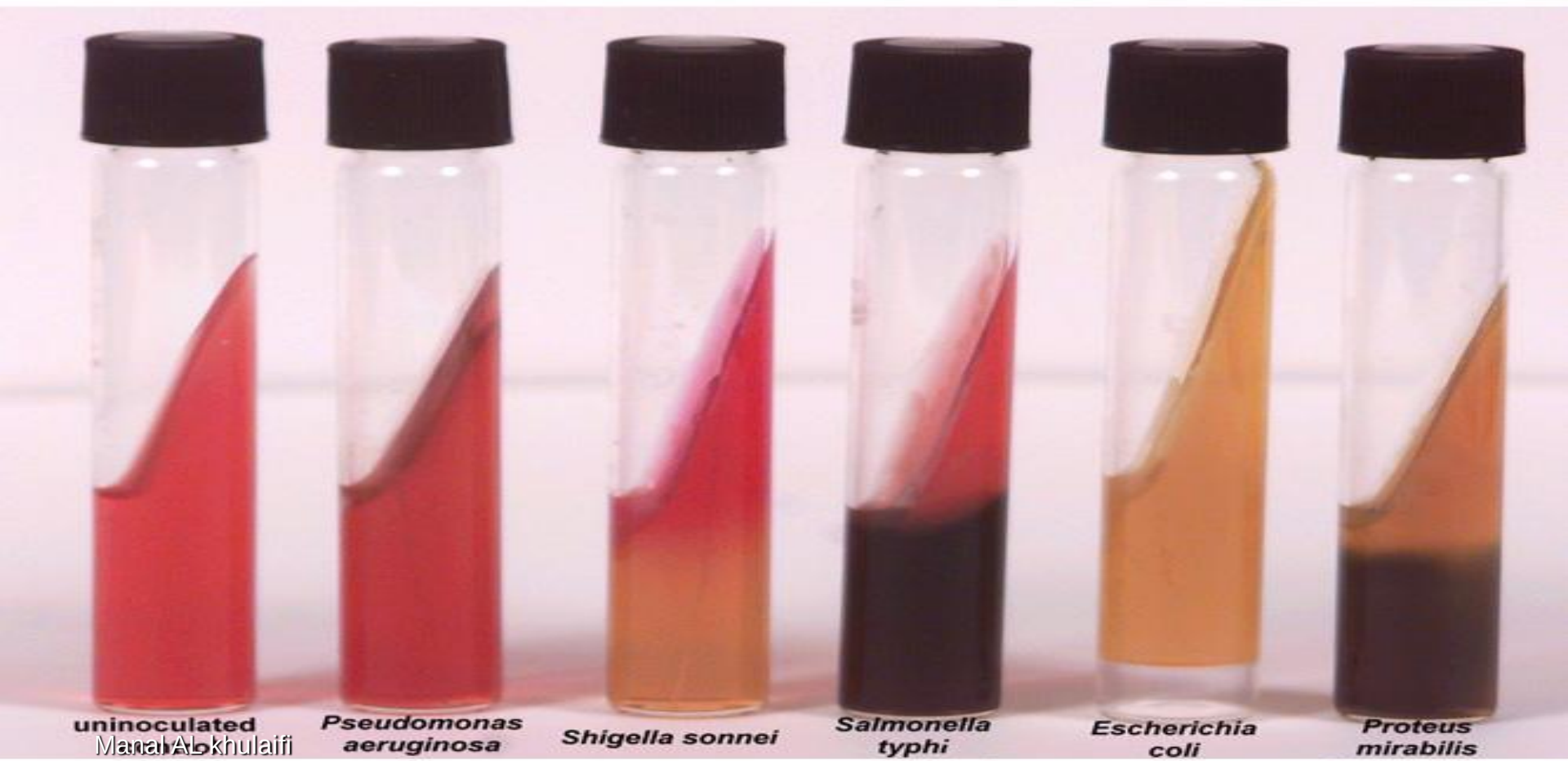
Butt:	acidic (yellow)	acidic (yellow)	acidic (yellow)
Slant:	acidic (yellow)	alkaline (red)	alkaline (red)
H_2S Production:	-ve	-ve	+ve

Result

- This medium contains also ferrous sulfate as an indicator for H2S production.

$$H_2S + FeSo_4 \longrightarrow FeS$$

Black color of ferrous sulfide



Reaction on TSI			Result	Example
Butt color	Slant color	H_2S		
Yellow	Red	Negative	A/Alk/- (Glucose fermented)	LNF e.g. *Shigella*
Yellow	Red	Positive black in butt	A/Alk/+ (Glucose fermented with H_2S)	LNF e.g. *Salmonella* & *Proteus*
Yellow	Yellow	Negative	A/A/- (three sugars are fermented)	LF e.g. *E. coli, Klebsiella,* *Enterobacter*
Red	Red	Negative	Alk/Alk/- (No action on sugars)	Non fermenter e.g. *Pseudomonas*

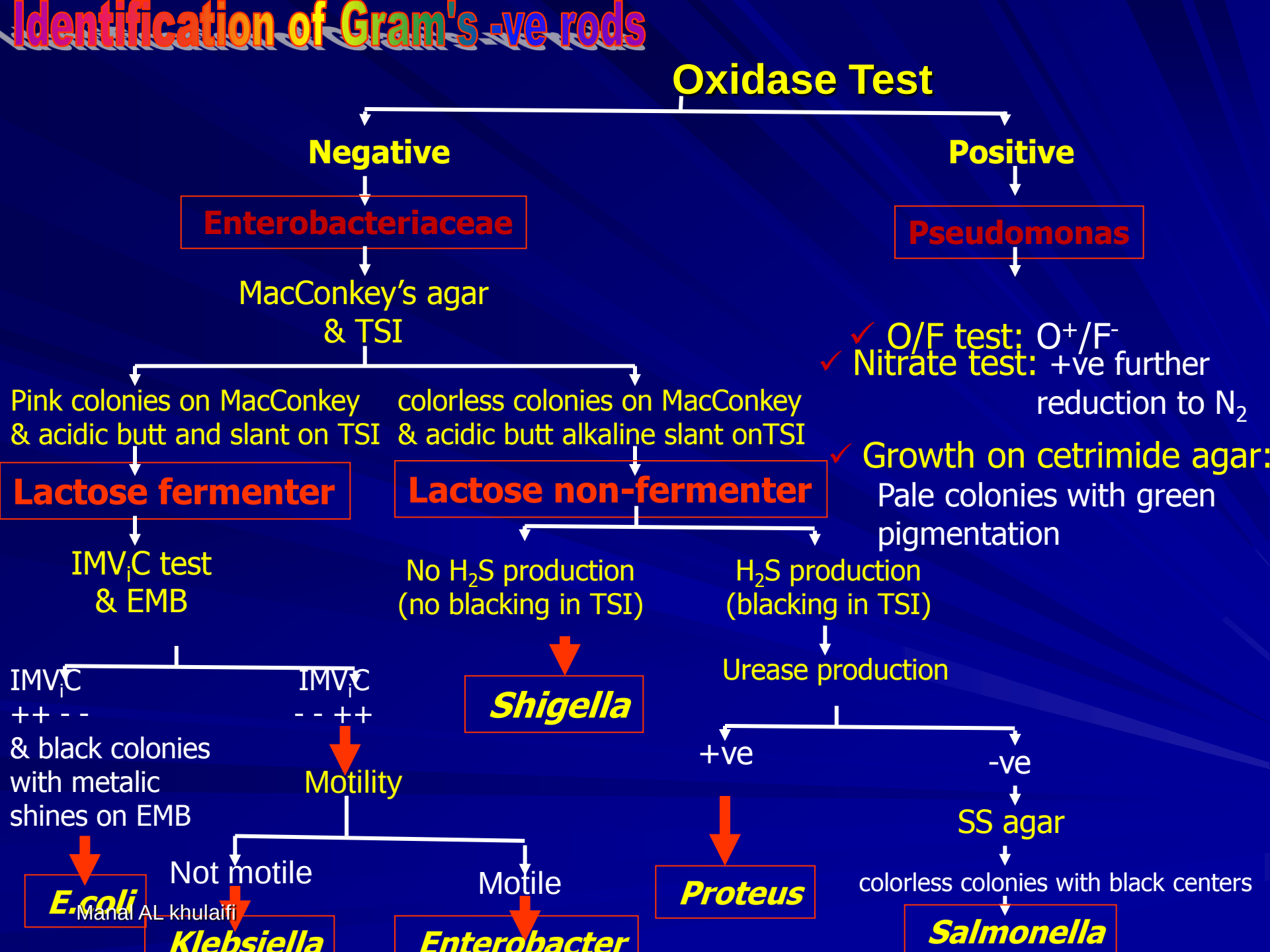
Summary of morphology, cultural characteristics, and biochemical reactions of *Enterobacteriaceae*

	Gram stain	Oxidase	Nitrate reductase	O/F	MacCon key	SS	EMB
E. coli	-ve rod	-ve	+ve	O+/F+	LF	LF	Metallic sheen
Citrobacter	-ve rods	-ve	+ve	O+/F+	LF	LF	Dark
Klebsiella	-ve rods	-ve	+ve	O+/F+	LF	LF	Dark
Enterobacter	-ve rods	-ve	+ve	O+/F+	LF	LF	Dark
Salmonella	-ve rods	-ve	+ve	O+/F+	NLF	NLF/ H2S	Colorless
Shigella	-ve rods	-ve	+ve	O+/F+	NLF	NLF	Colorless
Proteus	-ve rods	-ve	+ve	O+/F+	NLF	NLF/ H2S	Colorless

Summary of morphology, cultural characteristics, and biochemical reactions of *Enterobacteriaceae*

	TSI	Indole	MR	VP	Citrate	Urease	Motility
E. coli	A/A/-	+ve	+ve	-ve	-ve	-ve	Motile
Citrobacter freundii	A/A/-	+ve	+ve	-ve	+ve	-ve	Motile
Klebsiella pneumoniae	A/A/-	-ve	-ve	+ve	+ve	+ve	Non motile
Enterobacter cloacae	A/A/-	-ve	-ve	+ve	+ve	+ve	Motile
Salmonella typhi	A/Alk/+	-ve	+ve	-ve	+ve	-ve	Motile
Shigella boydii	A/Alk/-	-ve	+ve	-ve	-ve	-ve	Non motile
Proteus mirabilis	A/Alk/+	-ve	+ve	-ve	+ve	+ve	Motile Swarwing

Identification of Gram's -ve rods



Practicle Work

- Gram stain
- Oxidase test
- Growth on MacConkey's agar
- Growth on EMB agar
- Growth on SS agar
- Reaction on TSI
- Indole Test
- MR test
- VP test
- Citrate Utilization test
- Urease test