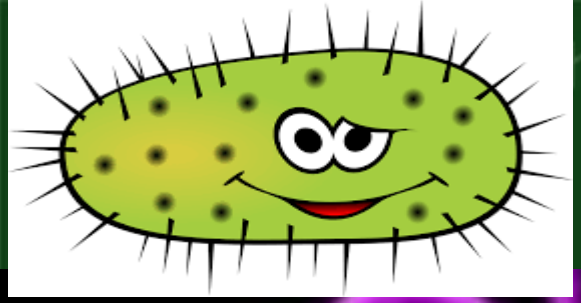

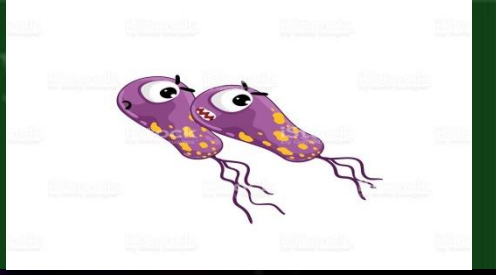

# ENTEROBACTERIACEAE FAMILY

lecture on

E.coli, Klebsiella


Represented by:-

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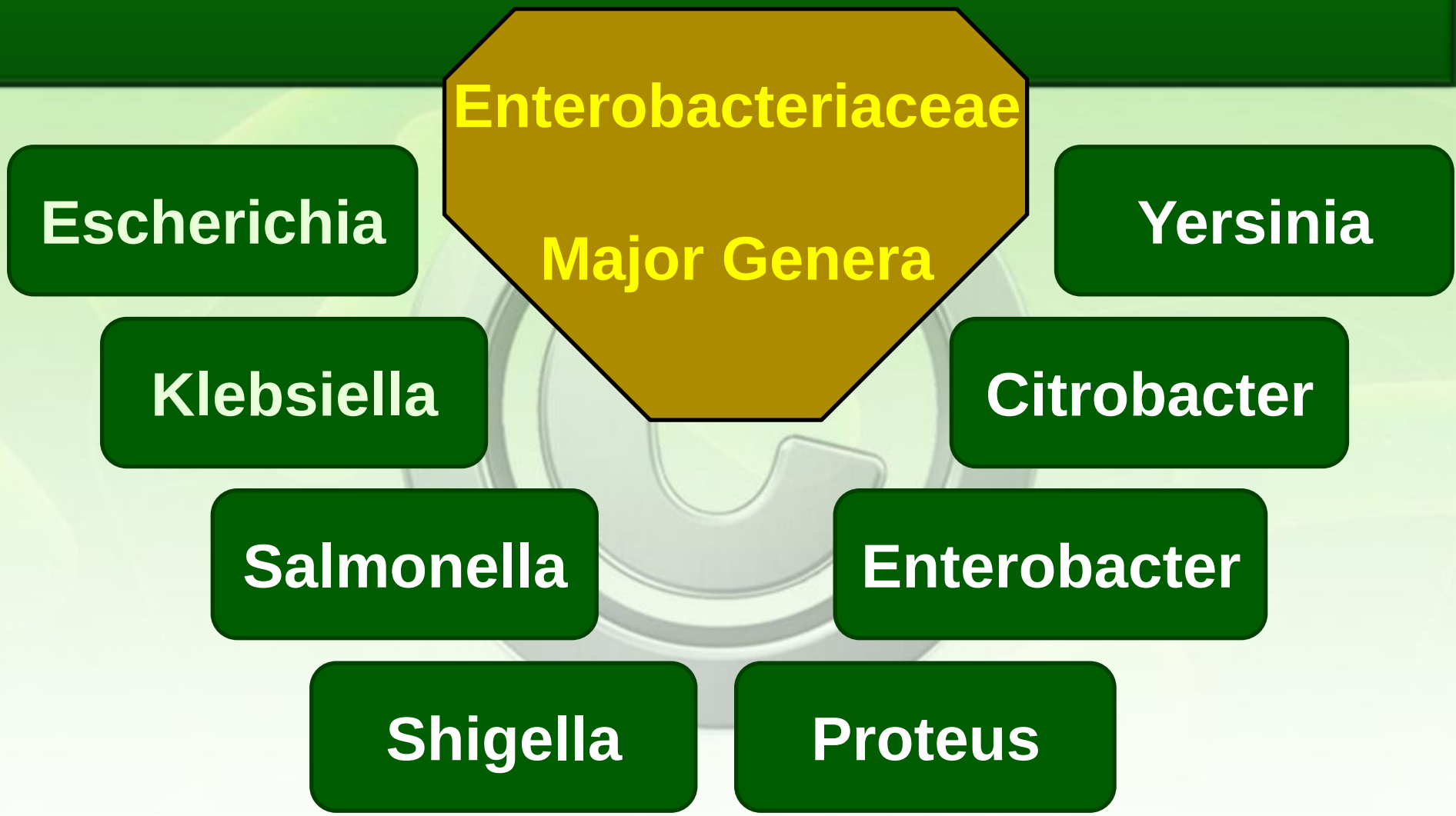
# Enterobacteriaceae

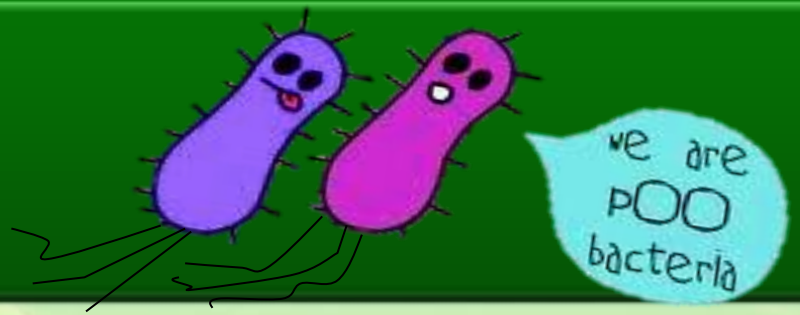
- Family *Enterobacteriaceae* often referred to as "enterics"
- Enterics are ubiquitous in nature
- Except for few, most are present in the intestinal tract of animals and humans as commensal flora; therefore, they are sometimes call "fecal coliforms"
- Some live in water, soil and sewage



# Major Features

- Gram-negative rods
- Non-spore forming
- All except Klebsiella, Shigella and Yersinia are motile
- facultative anaerobes.
- All ferment glucose
- All reduce nitrates ($NO_3$) to nitrites ($NO_2$)
- All are oxidase negative.
- All are Catalase positive.

# Escherichia coli




- E. Coli is a part of commensal flora of human GIT.
- The virulent strains of Escherichia coli predominantly cause gastroenteritis, urinary tract infections, and neonatal meningitis.
- Sporadically this bacterium may cause sepsis, secondary pneumoniae and nosocomial infections.

# strains of Escherichia coli

A-Common strains:
1-Enteropathogenic Escherichia coli (EPEC).
2-Enterotoxigenic Escherichia coli (ETEC).
3-Enterohaemorrhagic Escherichia coli (EHEC)

B-Rare strains of pathogenic Escherichia coli have been isolated from infants and children with diarrhea, including:
1-Enteroinvasive Escherichia coli (EIEC)
2-Enteroaggregative Escherichia coli (EAEC).

# Specimens for isolation

The source of culture material depends on the clinical symptoms.

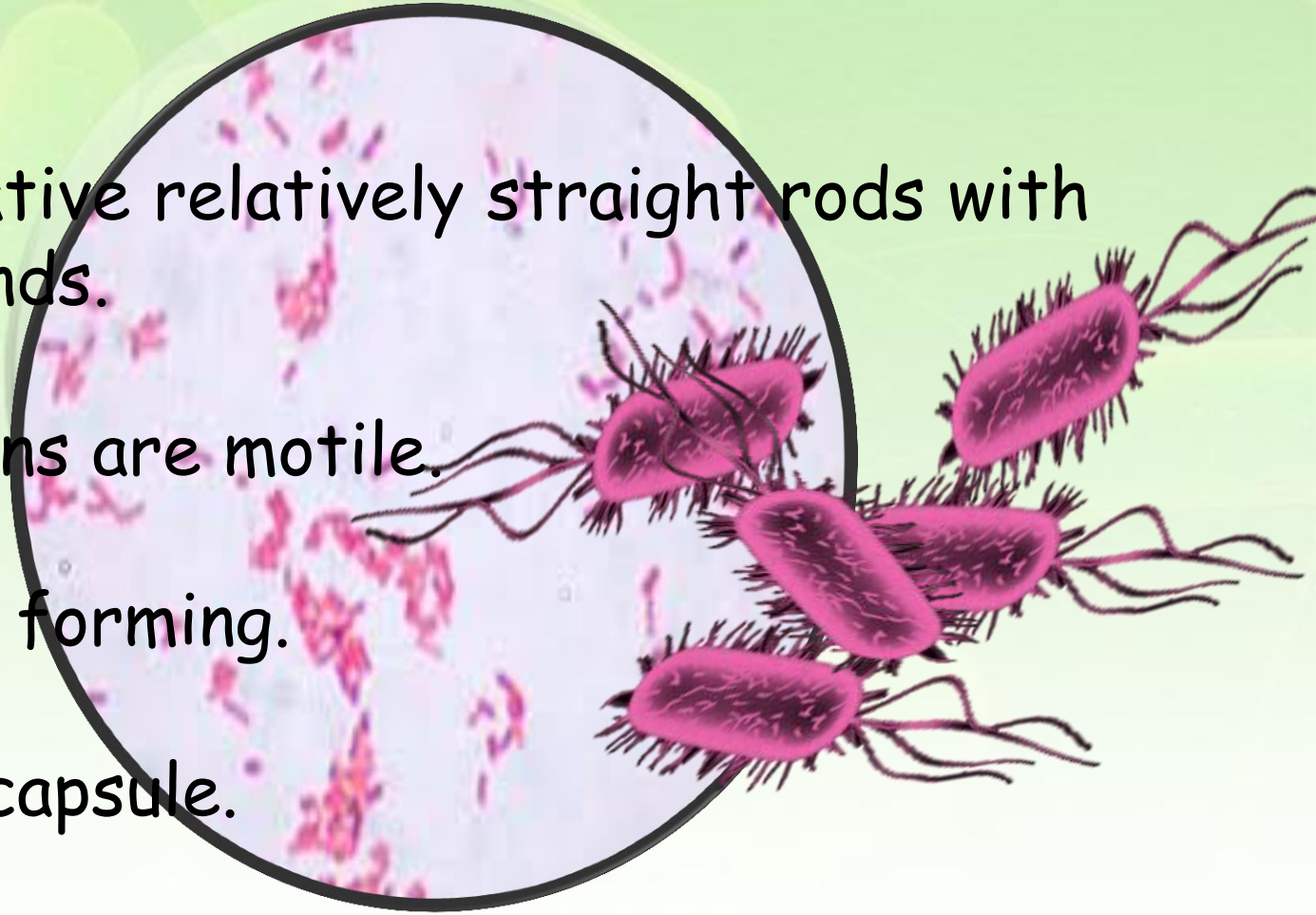
-Faces: when the patient is ill with gastroenteritis.

-Infected tissue: when the bacteria are locally invasive.

-Blood: invasive bacteria (i.e., those causing bacteremia and sepsis).

-Urine: for investigation of urinary tract infection

# Microscopical appearance



- Gram-negative relatively straight rods with rounded ends.
- Most strains are motile.
- Non-spore forming.
- May have capsule.

# Cultural characteres

- ✓ Optimum temperature 37 °C.
- ✓ Aerobic and facultative anaerobes.
- ✓ Colonies on nutrient agar are 2-4 mm in diameter, opaque, smooth, convex with an entire edge.

- ✓ Colonies on eosin methylene blue (EMB) agars are metallic green seen.
- ✓ Most grow on MacConkey agar, which contain lactose and pH indicator. If lactose is fermented, acid will be generated and the colonies appear pink.

# Klebsiella spp.

It cause a variety of opportunistic infections in debilitated patients. Common klebsiellae infections in humans include (1)pneumonia, (2)UTI, (3)nosocomial infection (4)septicaemia , (5)soft tissue infection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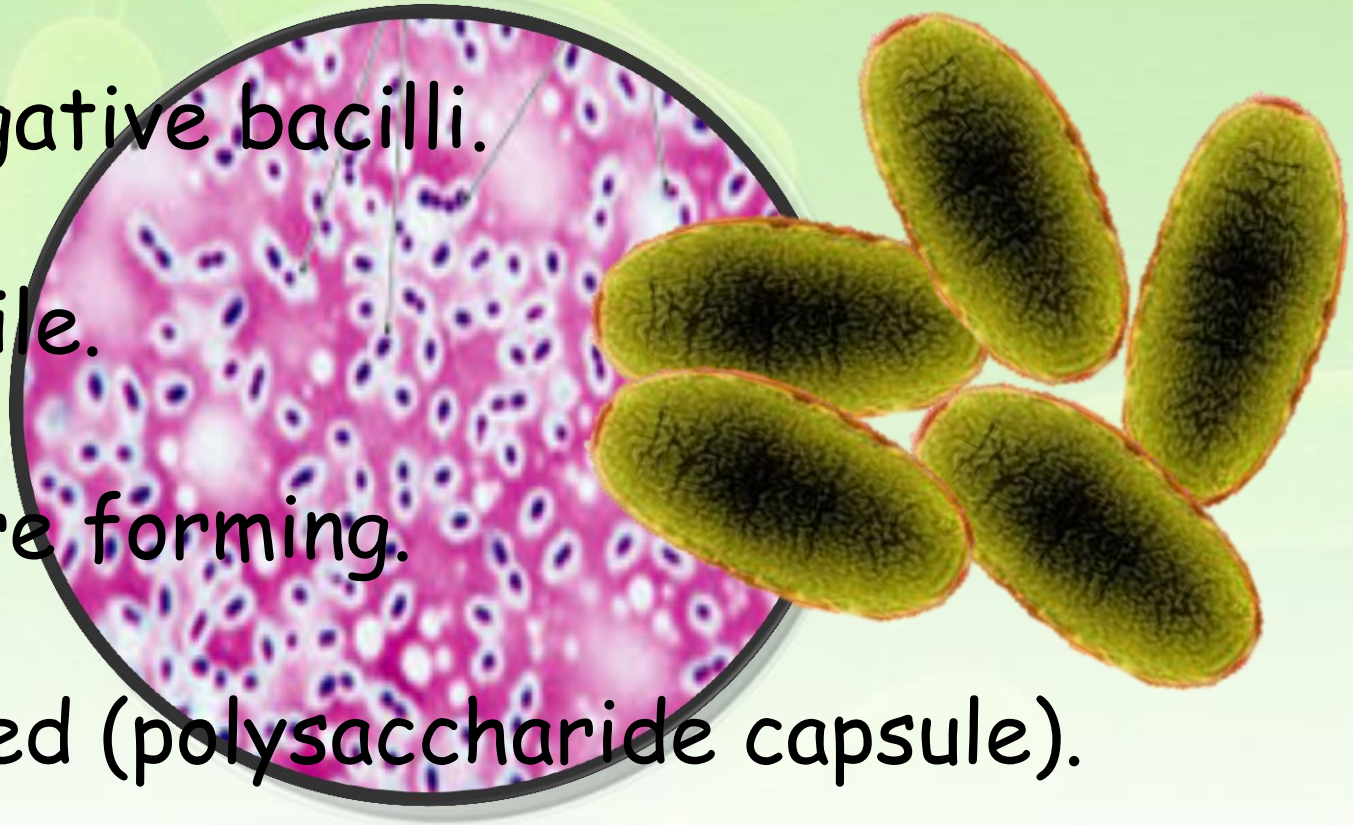
# Microscopical appearance

- Gram negative bacilli.
- Non-motile.
- Non-spore forming.
- Capsulated (polysaccharide capsule).

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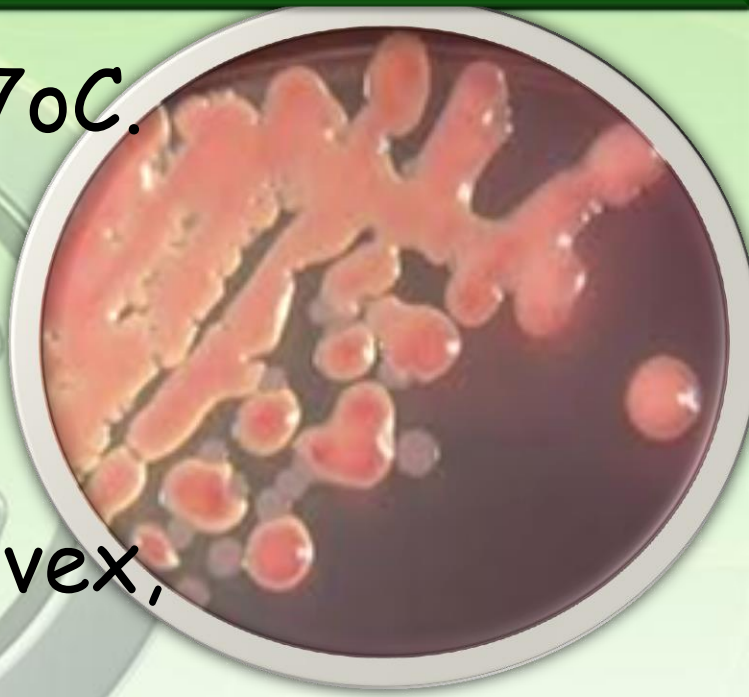
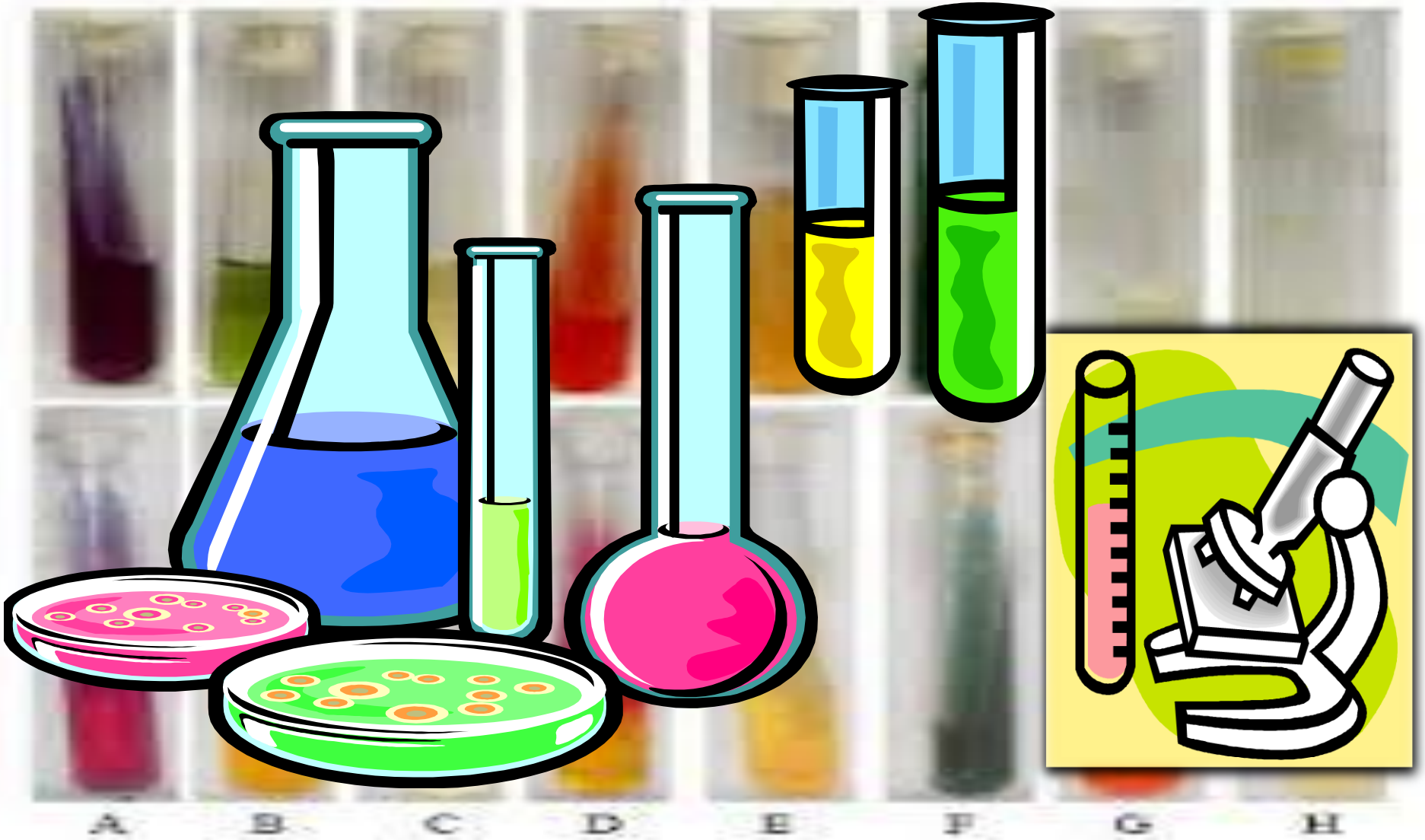
# Cultural characteristics



- Optimum temperature is 37oC.
- Aerobic and facultatively anaerobic.
- Colonies are large, high convex, mucoid and tend to coalesce.
- On MacConkey's agar the majority of strains give pink colonies due to lactose fermentation.

# Biochemical Tests



# IMViC Test

- **IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae.**

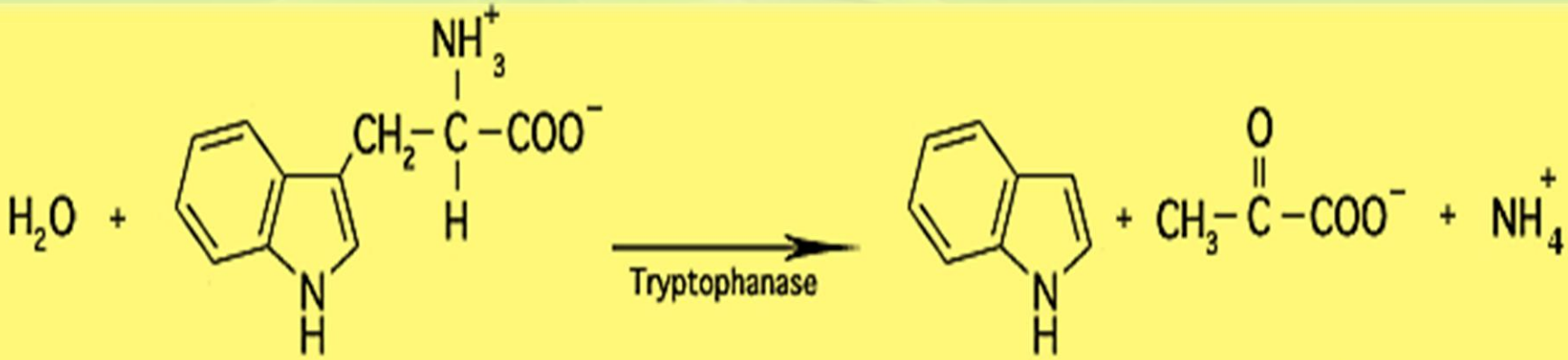
- The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test.

# IMViC: Indole test

## Principle

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

# Chemical equitation




**Tryptophan amino acids** —(Tryptophanase)→ **Indole + Pyurvic acid + NH4**

↓ Kovac's Reagent

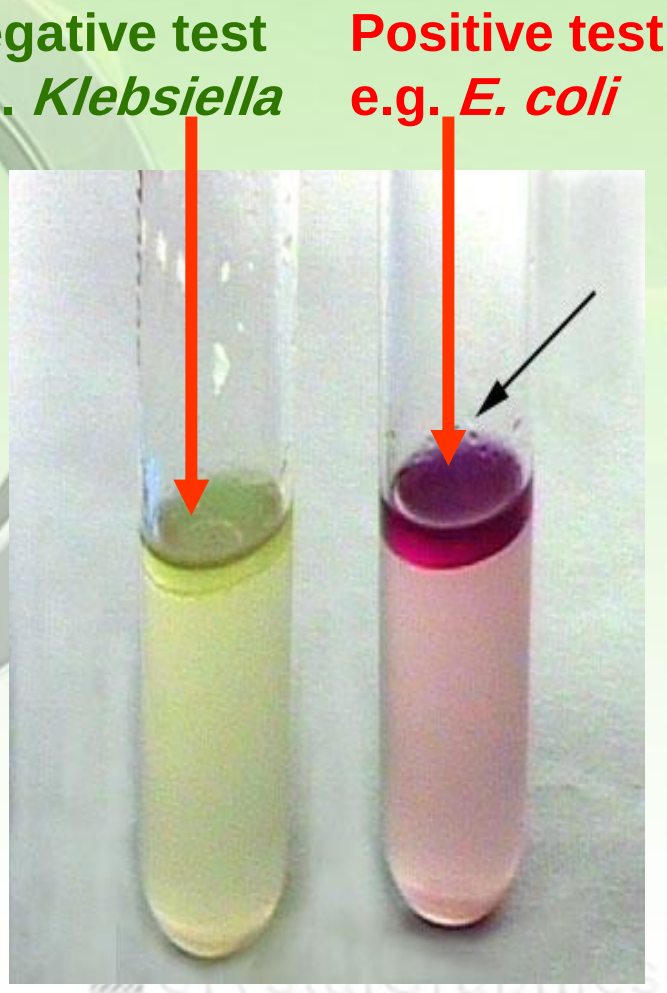
**Red color ring in upper layer**

# Method:

- Inoculate peptone water with the tested microorganism
- Incubate at 37°C for 24 hours
- After incubation interval, add 1 ml Kovacs reagent, shake the tube gently and read immediately

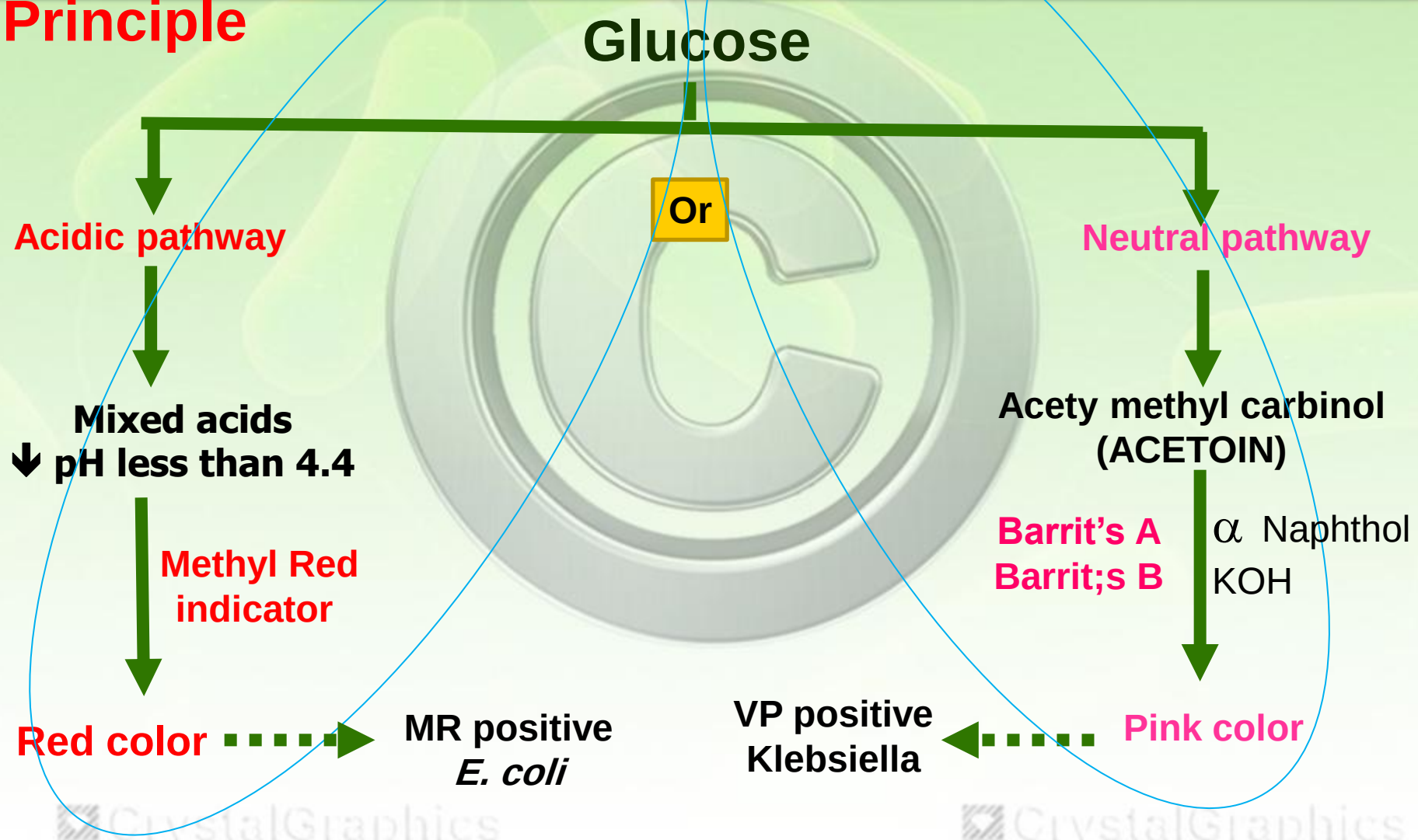
# Result

- A bright pink color in the top layer indicates the presence of indole
- The absence of color means that indole was not produced indole test is negative



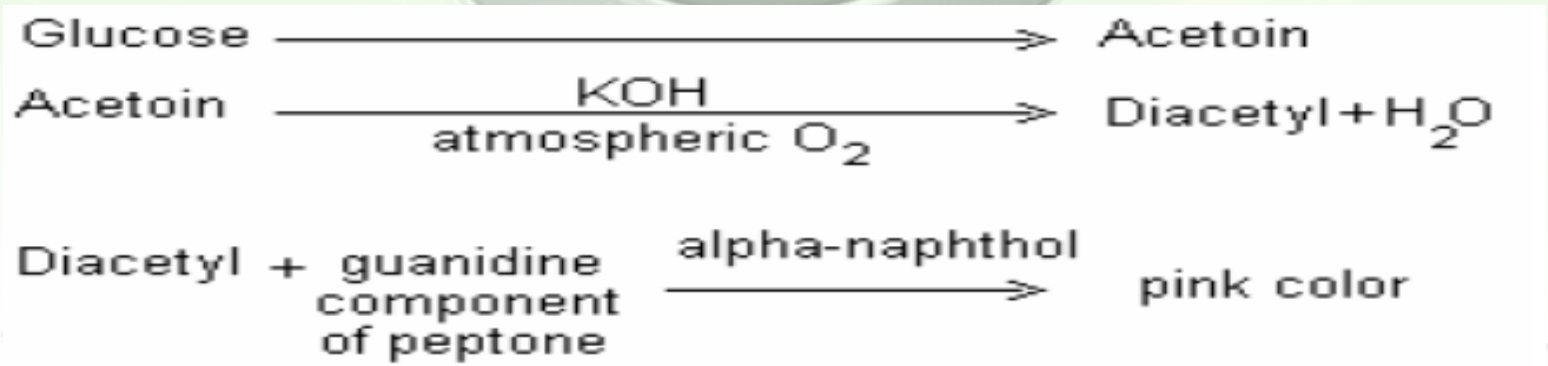
# Methyl Red-Voges Proskauer (MR-VP) Tests

## Principle

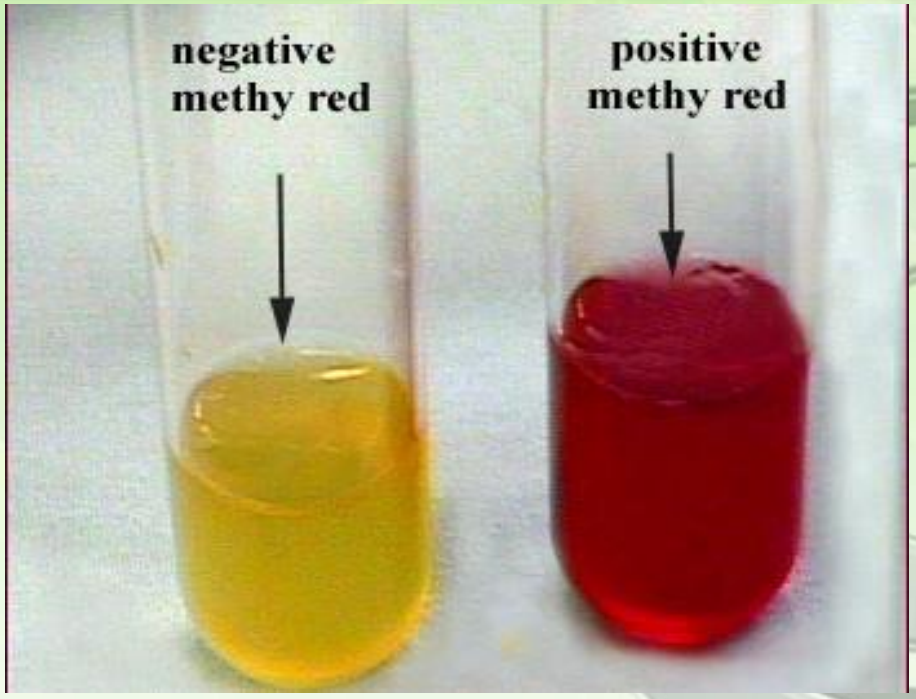


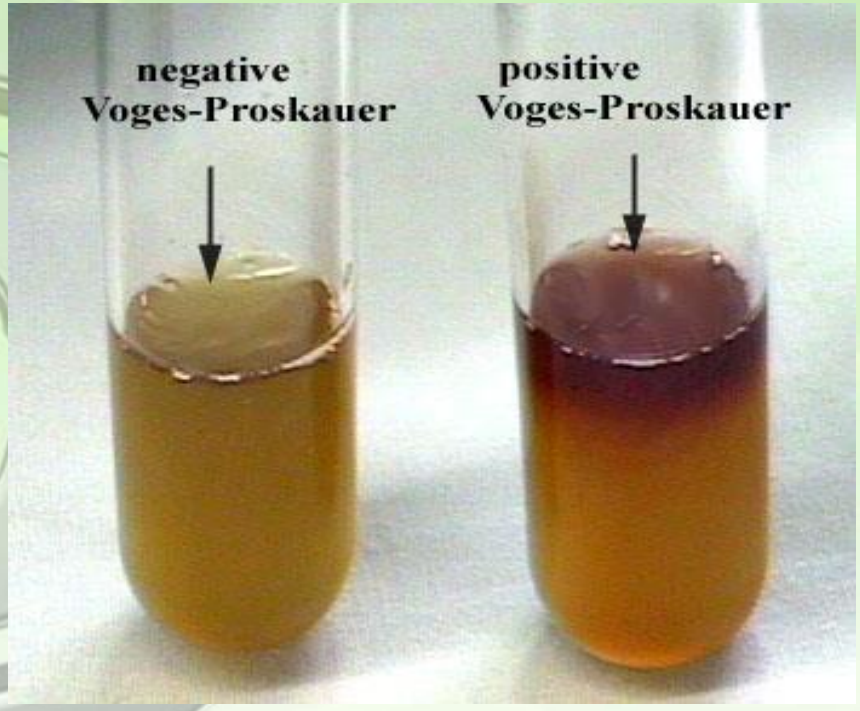
# Method

- Inoculate the tested organism into **two tubes** of MRVP broth
- Incubate the tubes at 37°C for 48 hours .
  - For methyl red: Add 6-8 drops of methyl red reagent.
  - For Voges-Proskauer: Add 12 drops of Barritt's A (α-naphthol), mix, 4 drops of Barritt's B (40% KOH), mix



# Results




## Methyl Red test

- ✓Red: Positive MR (*E. coli*)
- ✓Yellow or orange: Negative MR (Klebsiella)

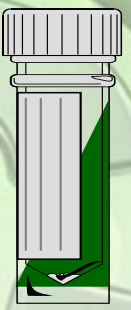
## Voges-Proskauer test

- ✓Pink: Positive VP (*Klebsiella*)
- ✓No pink: Negative VP (*E. coli*)

# Citrate Utilization Test

## Principle:

Citrate → oxaloacetate and acetate → Pyruvat+CO2 → CO2 + Na + H2O → $Na_2CO_3$



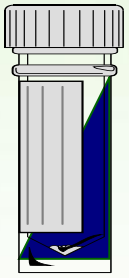
Alkaline, ↑pH

Simmone's Citrate media

Contains Citrate as a sole of C source

Bromothymol blue

Positive test



**Blue color**

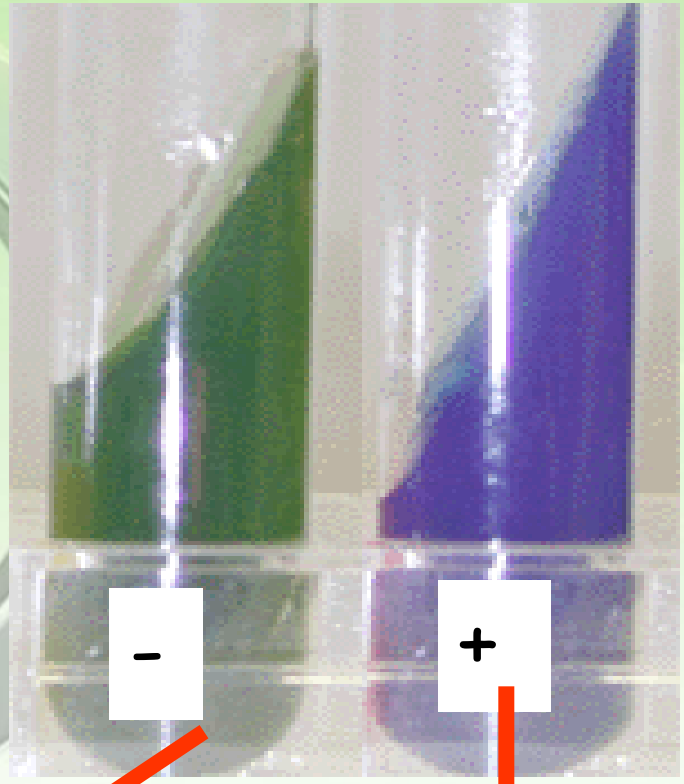
- Positive test: *Klebsiella*
- Negative test: *E. coli*

# Method

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

# ❖Result

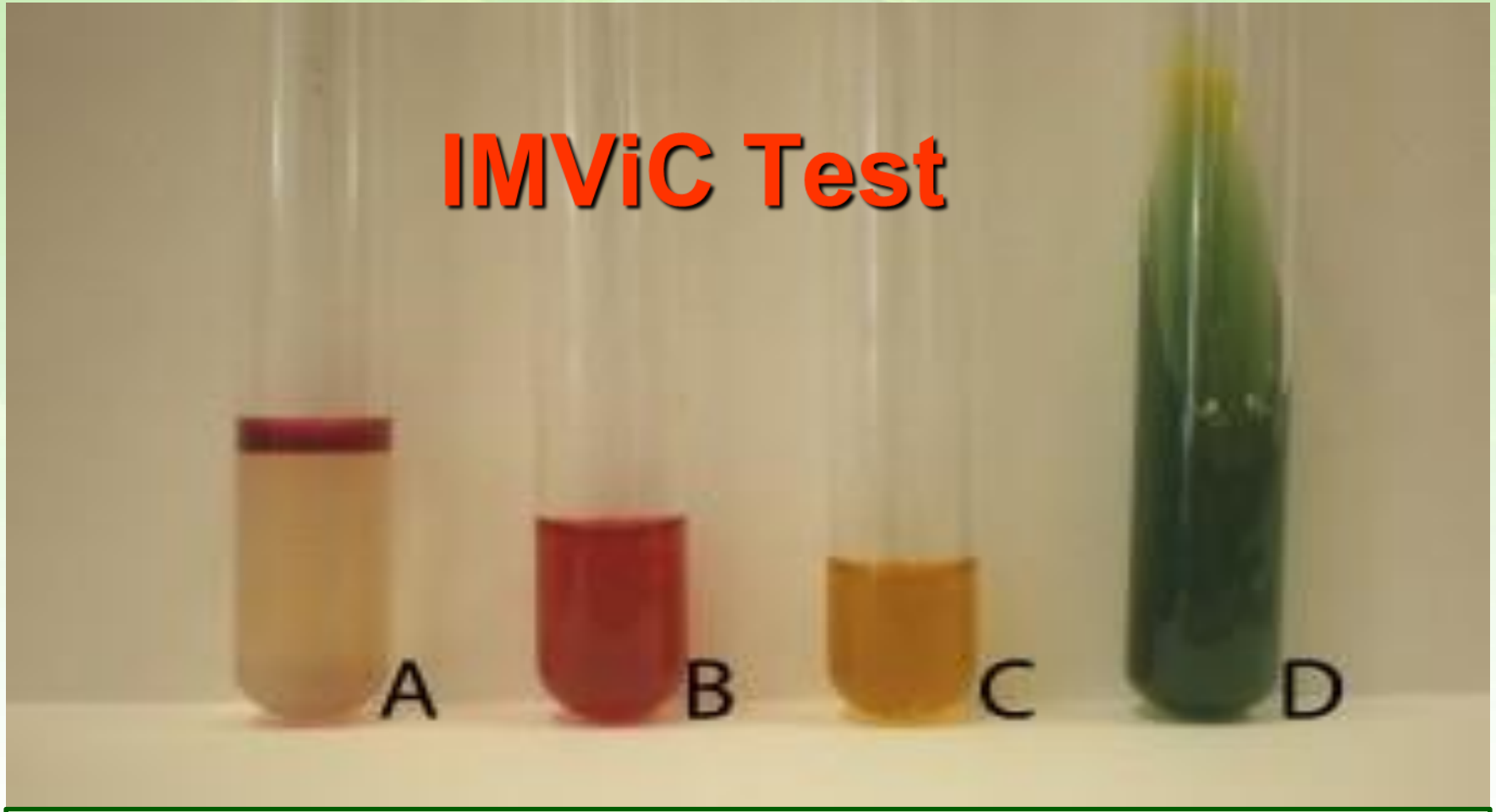
Growth on the medium is accompanied by a rise in pH to change the medium from its initial green color to deep blue




**Negative**
***E. coli***

**Positive**
**Klebsiella**

# IMViC Tests for *E. coli*




**A. Indole (+) , B. Methyl red (+) , C. Voges-Proskauer (-) D. Citrate (-)**

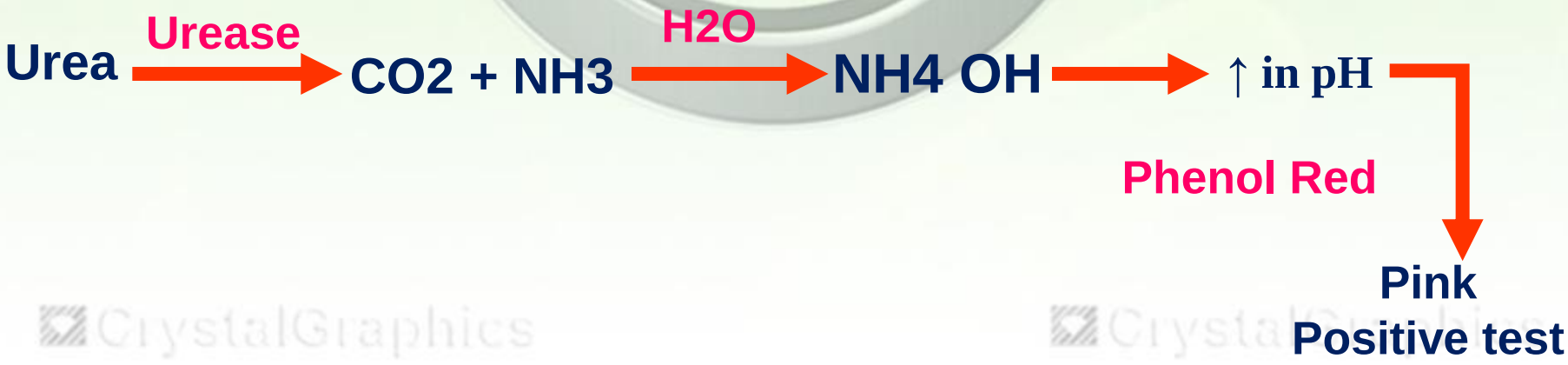
# IMViC Tests for *Klebsiella*


**A. Indole (-) , B. Methyl red (-) , C. Voges-Proskauer (+) D. Citrate (+)**

# Urease Test

## Principle

- Christenson medium contains urea 40% and phenol red
- Urease is an enzyme that catalyzes 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 r 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

# Result

- If color of medium turns from yellow to pink indicates positive test.
- *Klebsiella* gave positive results after 24 hrs

Positive test

Negative test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# Conclusion

| Test | Media | Substrate | Reagent | positive | negative |
|---|---|---|---|---|---|
| Indole | Pepton water | Tryptophan | Kovacs | Red ring | No red ring |
| Methyl red | MR-VP | Glucose | Methyl red | Red color | No red color |
| Voges-Proskauer | MR-VP | Glucose | α-naphthol + KOH 40% | Pink color | No pink color |
| Citrate | Simmon‘s citrate | Citrate | Bromothymol blue | Blue | Green |
| Urease | Christenson medium | urea | phenol red | Pink color | yellow |


Thank You