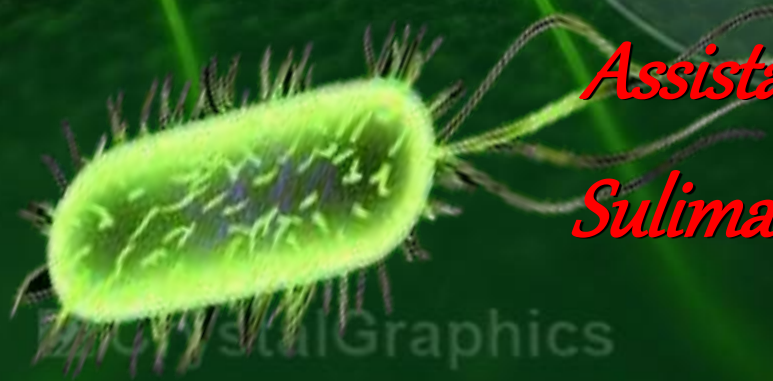
Enterobacteriaceae

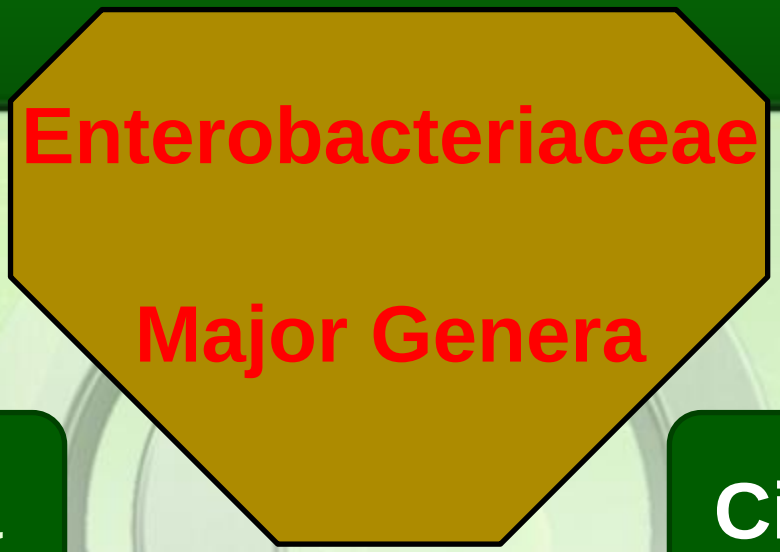
Assistant lecturer

Suliman A. Alqaesi



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

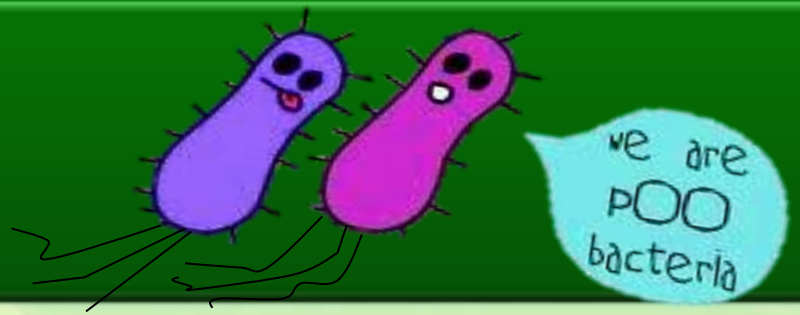


- Escherichia
- Klebsiella
- Salmonella
- Shigella
- Proteus
- Citrobacter
- Enterobacter
- Eduardisella

Major Features

- Gram-negative rods
- Non-spore forming
- All except Klebsiella, and Shigella 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.
- Sometime this bacterium may cause sepsis, secondary pneumoniae and nasal 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.

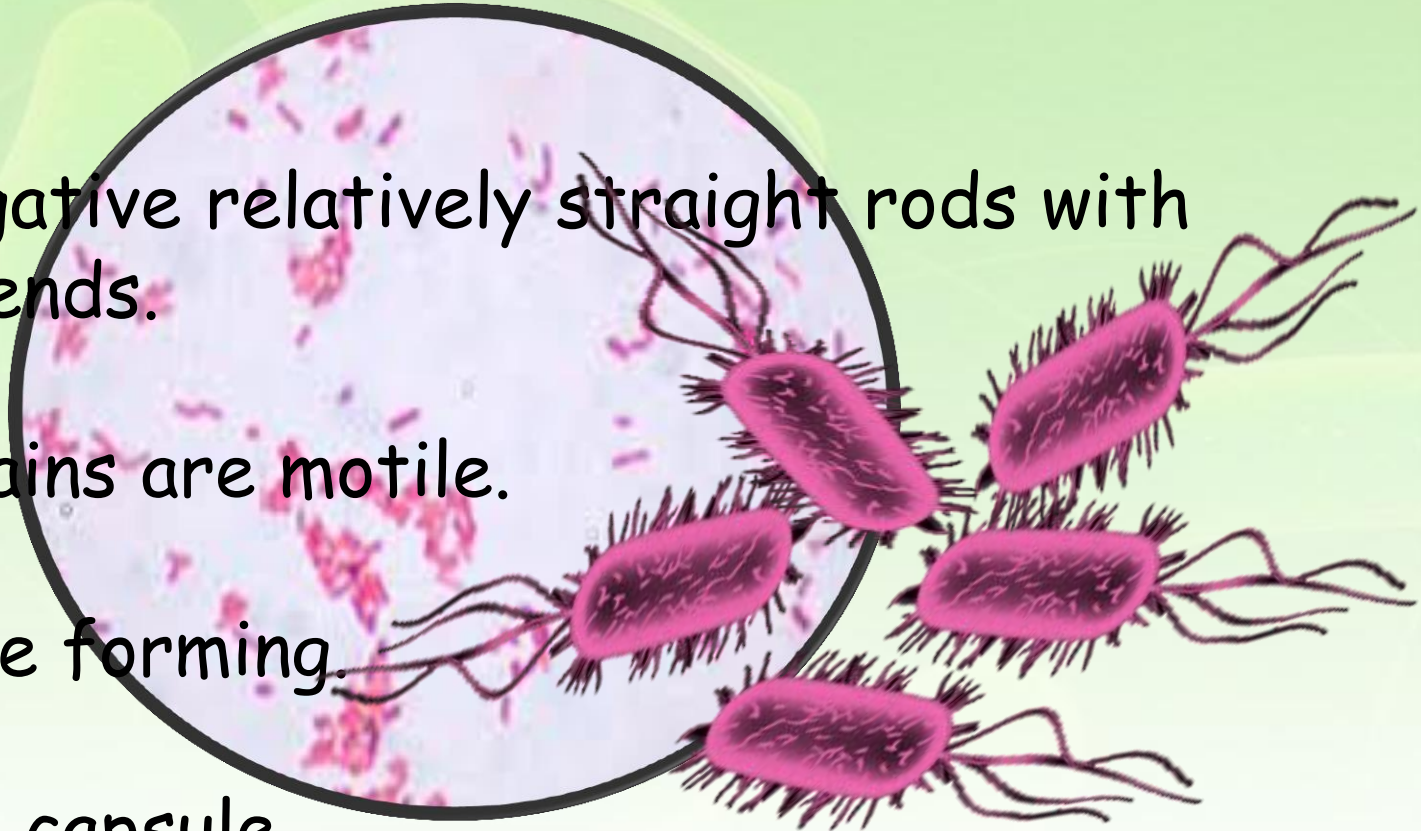
-Faces: when the patient is ill with gastroenteritis.

-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 characters

- ✓ Optimum temperature 37 °C.
- ✓ 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 sheen.
- ✓ Most grow on MacConkey agar, which contain lactose and pH indicator. If lactose is fermented, acid will be generated and the colonies will turn pink.

Klebsiella spp.

It cause a variety of opportunistic infections in debilitated patients. Common klebsiellae infections in humans include

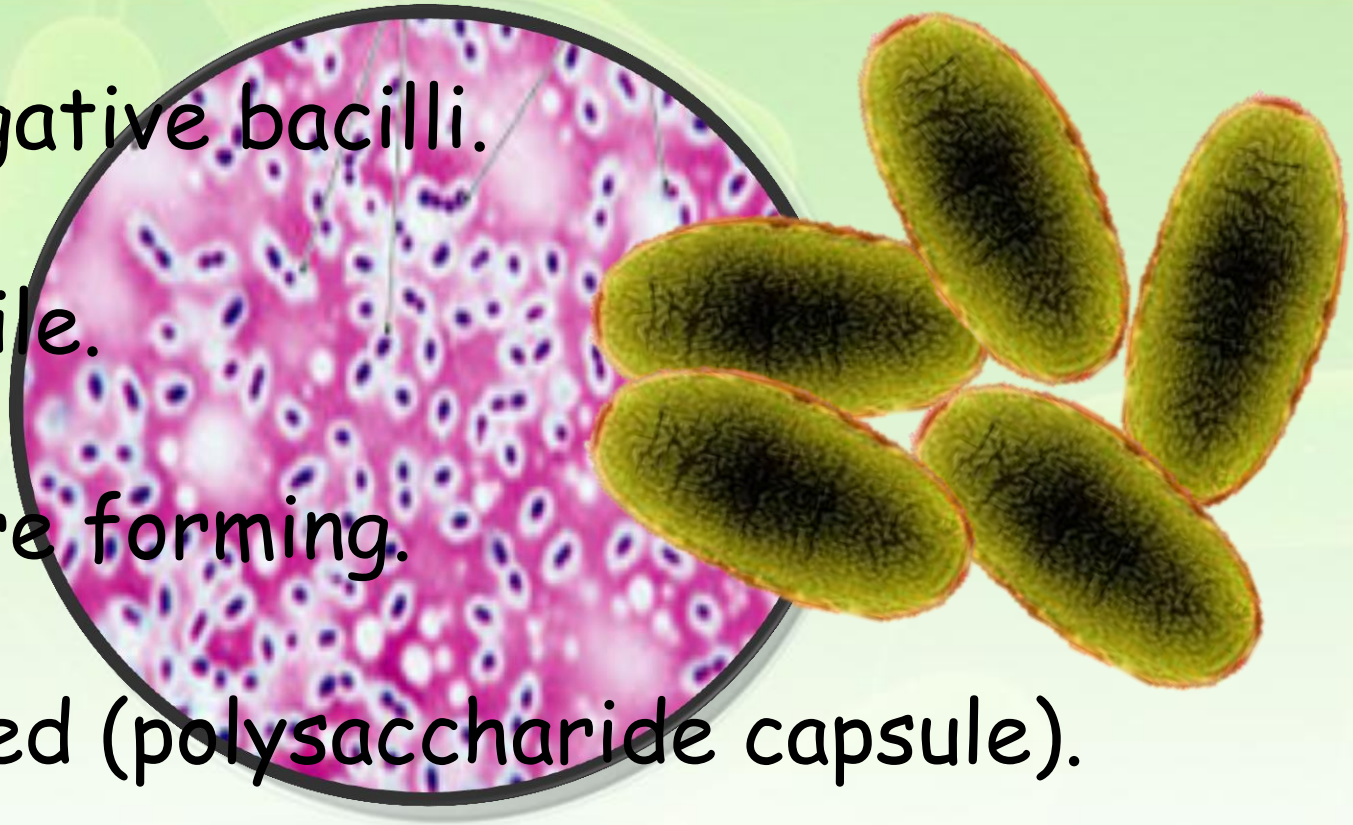
(1)pneumonia, (2) UTI,

(3) nasal infection, (4) septicemia,

(5) soft tissue infection

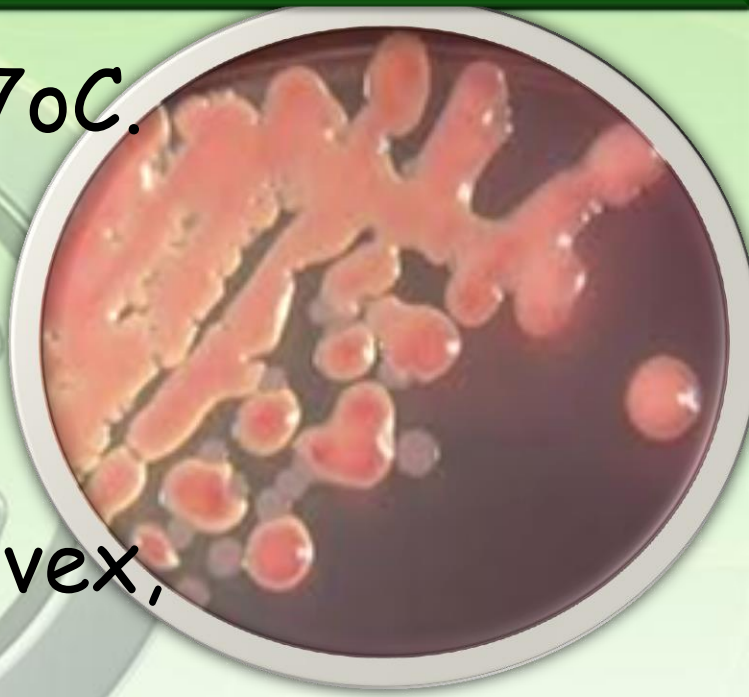
Microscopical appearance

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



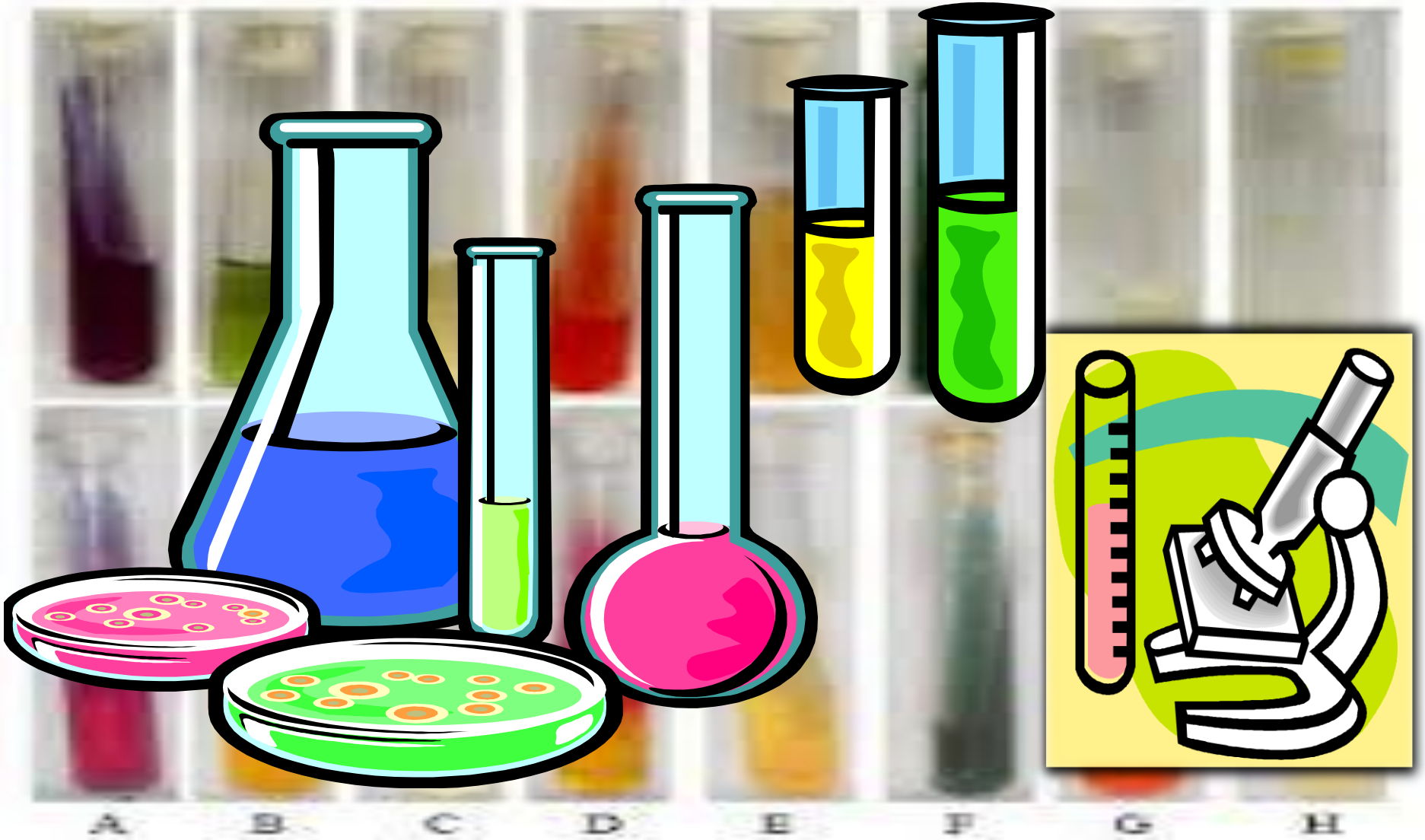
CrystalGraphics

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

- Indole, Methyl Red, Voges-Prosakaur, Citrate (IMVC) Tests:

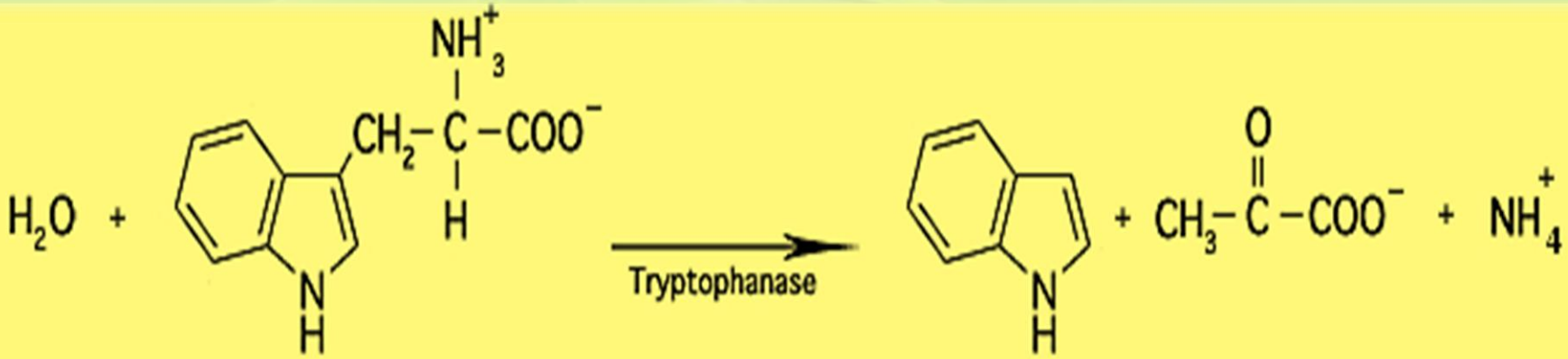
- The IMViC series of reactions allows for the differentiation of the various members of Enterobacteriaceae.

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.

Chemical equitation



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

↓ Kovac's Reagent

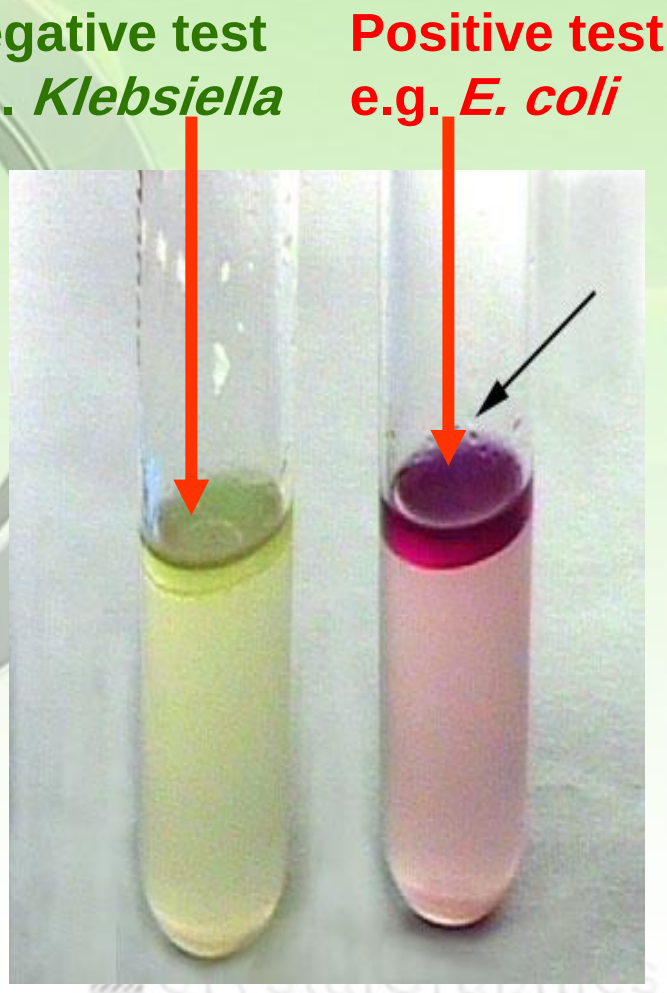
Red color ring in upper layer

Method:

- Inoculate tryptone 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

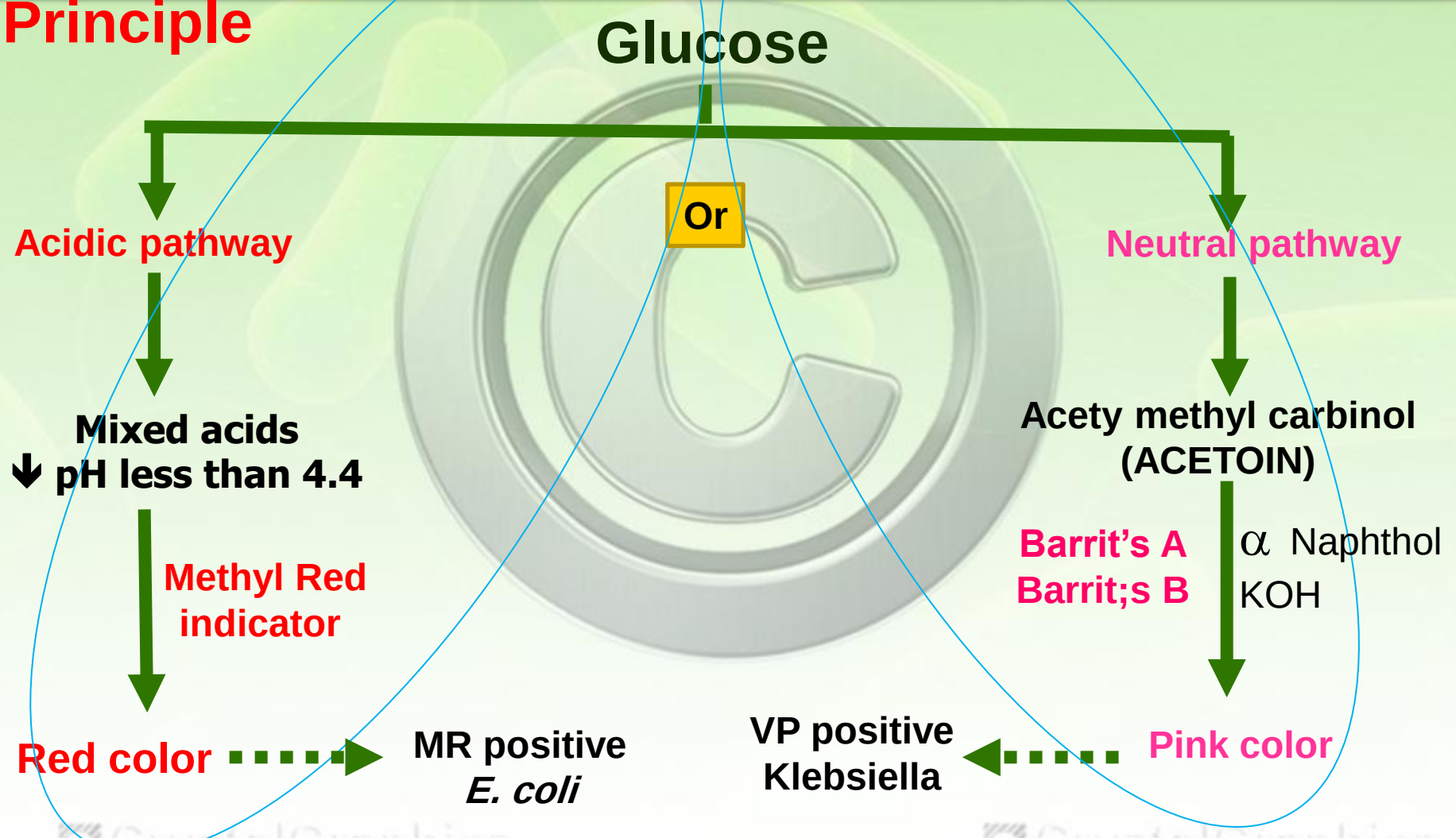
Result

- 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

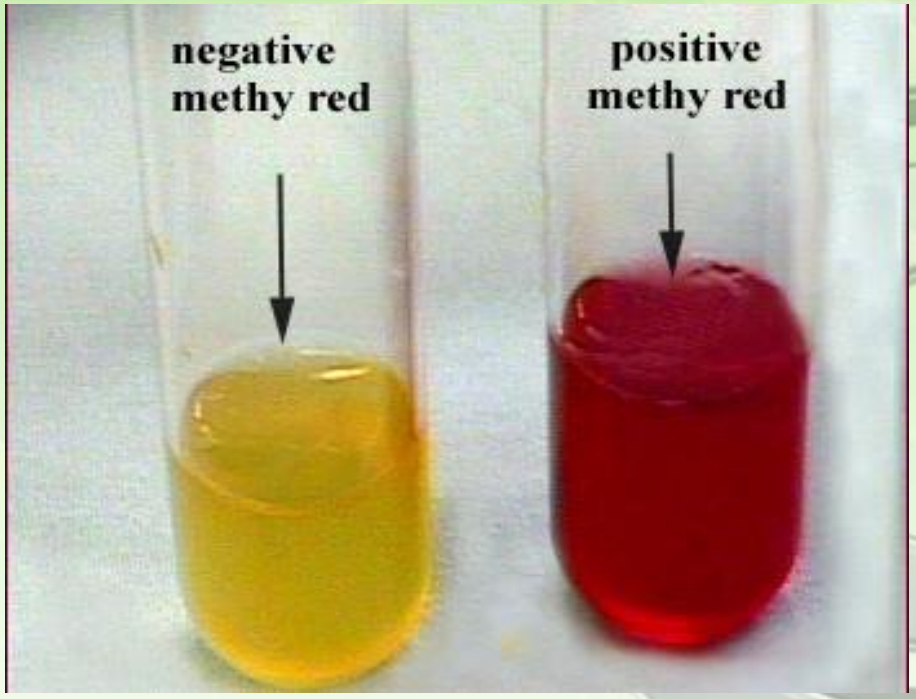
Principle

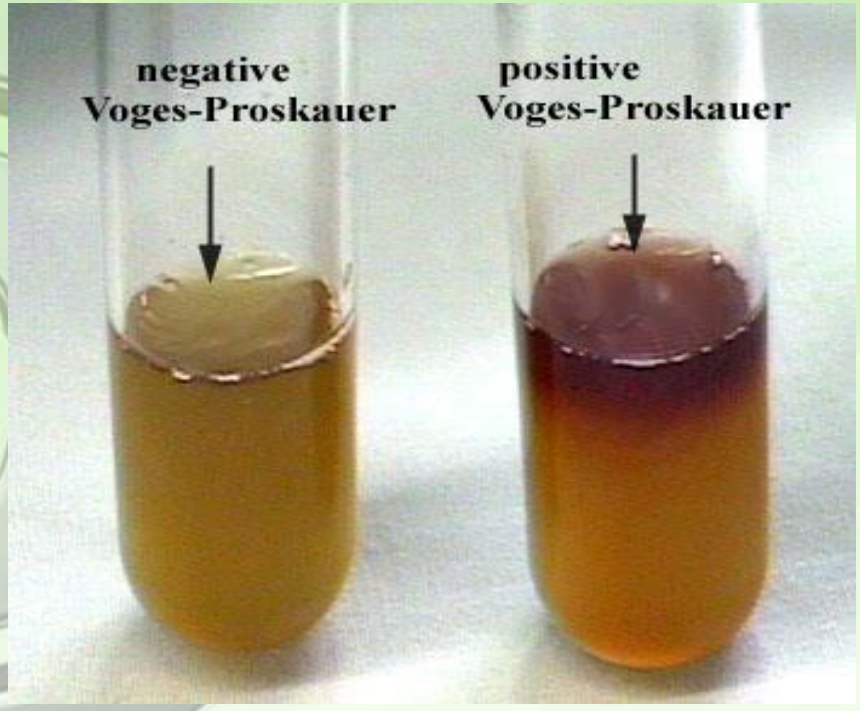


Method

- Inoculate the tested organism into **two tubes** of MRVP broth
- Incubate the tubes at 37°C for 24 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

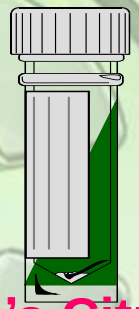
- ✓Pink: Positive VP (*Klebsiella*)
- ✓No pink: Negative VP (*E. coli*)

Citrate Utilization Test

Principle:

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

Alkaline, ↑pH

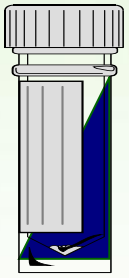


Simmone's Citrate media

Contains Citrate as a sole of C source

Bromothymol blue

Positive test



Blue color

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

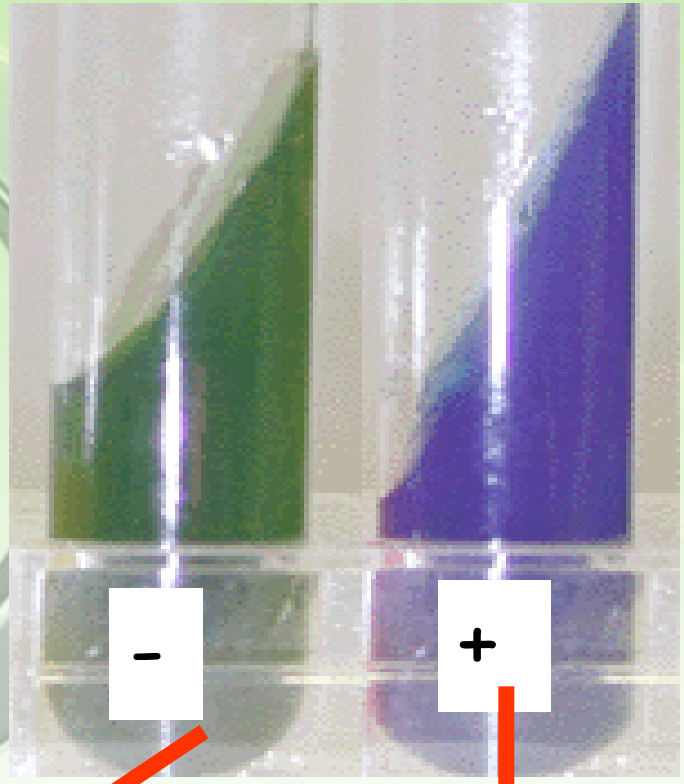
Method

- Streak a **Simmon's Citrate agar** with the organism

- Incubate at 37°C for 24 hours.

❖Result

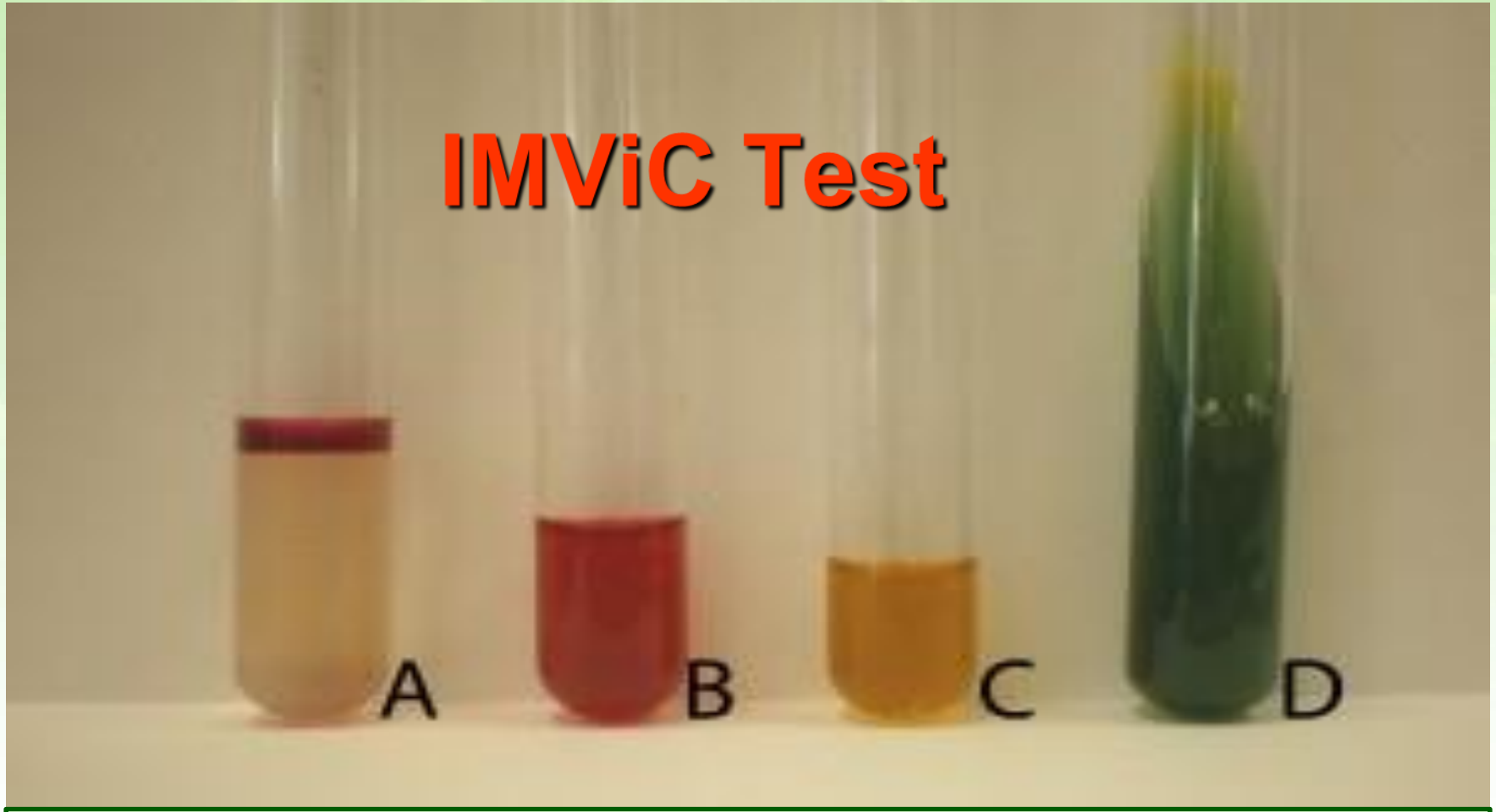
Growth on the medium is accompanied by a rise in pH to change the medium from its initial green color to deep blue



Negative
E. coli

Positive
Klebsiella

IMViC Tests for *E. coli*



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

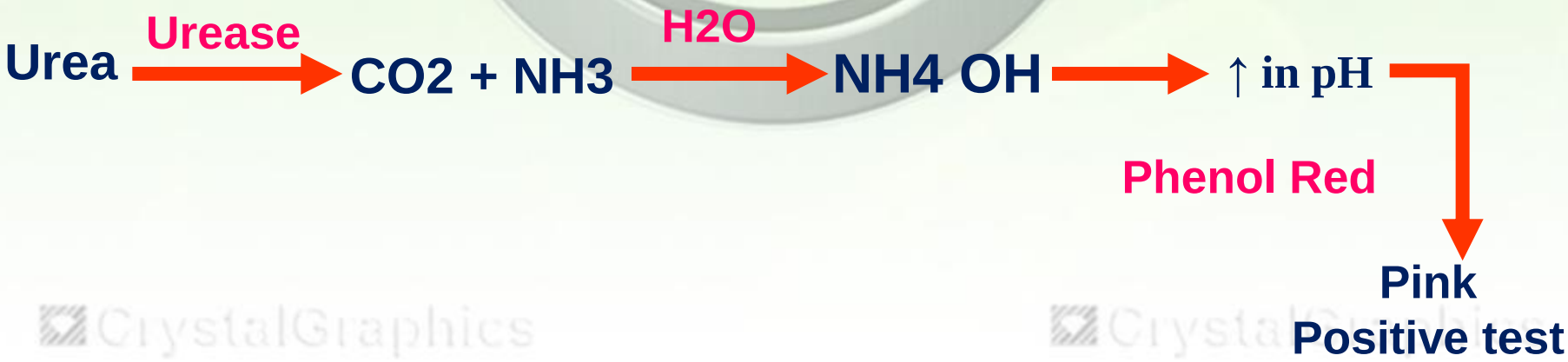
IMViC Tests for *Klebsiella*

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.
- *Kelebsiella* gave positive results after 24 hrs

Positive test

Negative test

CrystalGraphics

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