Microbiology lab 10

Gram-negative Rods

Enterobacteriaceae and *Pseudomonas*

By:

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Learning objectives:

After this lab. You must be able to:

- Describe microscopic morphology and cultural biochemical characteristics of each member in this family.
- List infections caused by each of these members.
- Differentiate each member of this family from each other.
- Discuss principles of biochemical tests of each member in this family.
- Predict enterics causative agents causing clinical cases.

Gram-Negative bacilli

Large group of diverse organisms, they are divided to:

- Gram-Negative Rods related to gastrointestinal tract.
- Gram-Negative Rods related to respiratory tract.
- Gram-Negative Rods related to animal source.

Gram-Negative Rods related to gastrointestinal tract
Enterobacteriaceae and Pseudomonas

- Enterobacteriaceae is a large family of bacteria commonly referred to as the fermentative, gram negative, enteric bacilli, indicating that they are gram-negative rods which can ferment sugars.
- To differentiate them from non-fermentative , gram-negative rods such as *Pseudomonas*.

Clinical Significance of Enterics

- Enterics are ubiquitous in nature, some live in water, soil and sewage and most are present in the intestinal tract of animals and humans as commensal flora; therefore, they are sometimes call "fecal coliforms"
- Based on clinical infections produced, enterics are divided into two categories:
  - True pathogen - *Salmonella*, *Shigella*, *Yersinia* sp and some strains of *E. coli*
  - Opportunistic pathogens – normally part of the intestinal flora that may produce infection outside the intestine

Family Enterobacteriaceae

Certain *E .coli* strains can be considered true pathogens →

True pathogen →

True pathogen →

True pathogen →

Genus	No. of species
Citrobacter	4
Edwardsiella	4
Enterobacter	13
Escherichia	5
Shigella (nonmotile)	4
Ewingella	1
Hafnia	2
Klebsiella (nonmotile)	7
Kluyvera	2
Morganella	2
Proteus	4
Providencia	5
Salmonella	7 subgroups
Serratia	10
Yersinia (nonmotile)	11

Important features

- Gram-negative non spore forming rods.
- Most Enterobacteriaceae are motile, with the exception of the common isolates *Klebsiella*, *Shigella*, and *Yersinia*, the motile strains possess peritrichous flagella.
- Many Enterobacteriaceae also possess virulence factors: fimbriae, sex Pilli, capsule, and endotoxin.
- Facultative anaerobes.
- All members ferment glucose
- All members reduce nitrate to nitrite.
- All members are catalase positive and oxidase negative.

Colonial Morphology

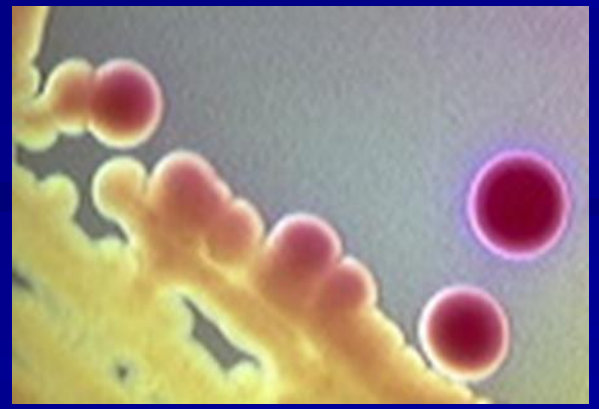
- **Ability to ferment lactose:**
  - lactose-fermenting strains *pink-purple colonies* (e.g., *Escherichia, Klebsiella, Enterobacter, Citrobacter*)
  - Non lactose-fermenting strains * colorless colonies* (e.g., *Proteus, Salmonella, Shigella, and Yersinia* spp).
  - Delayed lactose fermenter (DLF) (e.g., *Morganella, Providencia, Serratia, Edwardsiella, Erwinia, Hafnia*).
- **Ability to grow on a large number of selective and differential media:**
  - Eosin-Methylene blue (EMB) agar: contains bile salt and dyes eosin and methylene blue, inhibit gram-positive bacteria.
  - Hektoen Enteric agar(HE): contains high concentration of bile salt and dyes bromothymol blue and acid fuchsin.
  - Xylose Lysine Deoxycholate (XLD) agar contains: sodium desoxycholate inhibits the growth of gram positive bacteria.
- **Most have similar colonial morphology in blood agar plate.**
  - moist, smooth, gray colonies and some strains are beta hemolytic.

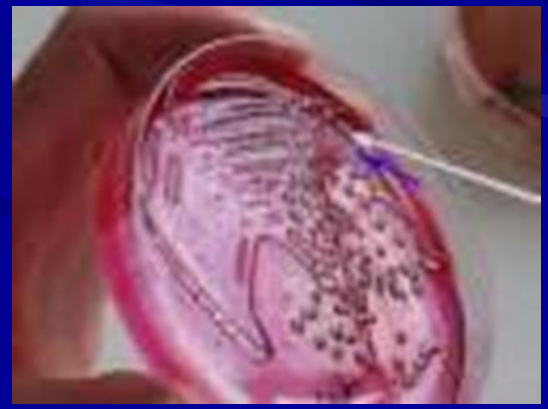
Escherichia coli

- *One of the most predominant intestinal flora, causes wide range of infections including: UTI, gastrointestinal infection, meningitis, wound infection and bacteremia.
- **Ferments lactose.
- Have characteristic metallic sheen on EMB.
- Positive in indole and methyl red tests
- Negative in Voges-Proskauer and Citrate tests
- Does NOT produce H_2S
- Usually motile.
- IMViC test: + + - -
- TSI test: A/A/ -

Klebsiella pneumoniae

- Usually found in intestinal tract and frequent cause of nosocomial pneumonia.
- Ferments lactose.
- Possess a polysaccharide capsule makes the colonies mucoid and moist.
- Negative in indole and methyl red tests
- Positive in Voges-Proskauer and Citrate tests
- Does NOT produce H_2S
- Non motile.
- IMViC test: - - + +
- TSI test: A/A/ -





Enterobacter

- The most clinically important species are: *E. cloacae* and *E. aerogenes*
- Isolated from wounds, urine, blood and CSF
- Ferments lactose. (Colonies resemble *Klebsiella*)
- Motile (differ from *Klebsiella*)
- Negative in indole and methyl red tests
- Positive in Voges-Proskauer and Citrate tests
- Urease test positive
- IMViC test: - - + +
- TSI test: A/A/ -

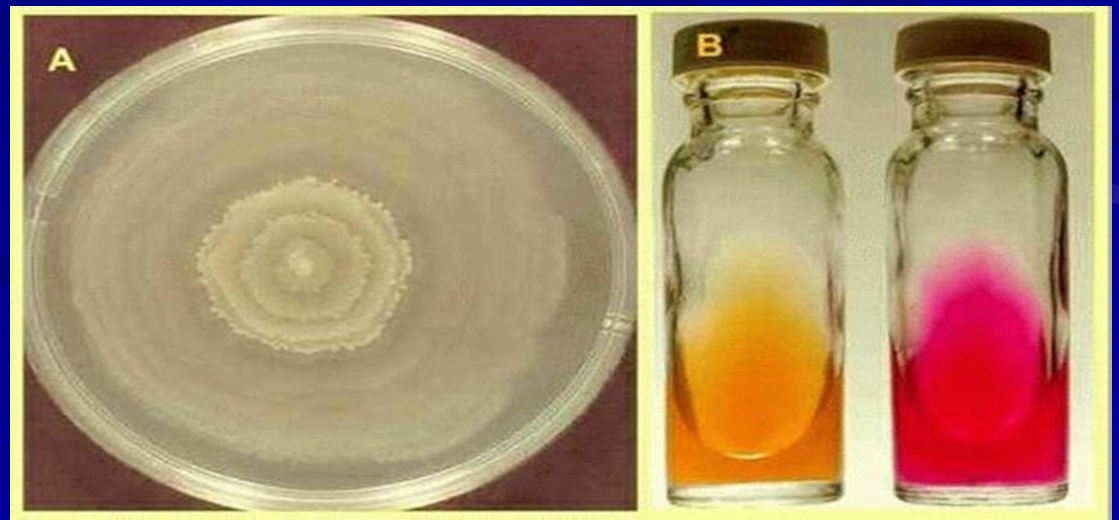
Serratia marcescens

- Causes nosocomial UTI, and respiratory tract infections.
- Ferments lactose slowly.
- Motile
- Produce characteristic reddish-pink color on nutrient agar when cultured on room temp.

S. marscens on nutrient agar →

Proteus

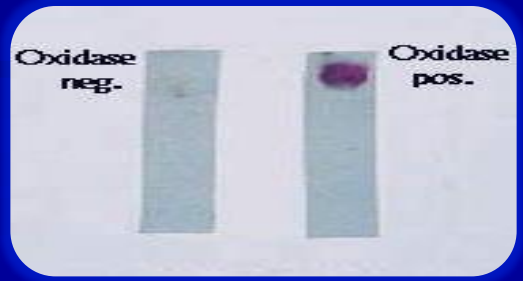
- The most clinically important species are: *P. mirabilis* and *P. vulgaris*
- Isolated from urine, wound, ear and bacteremic infections.
- Do not ferment lactose.
- Motile (produce swarming on non selective media).
- Negative in indole and Voges-Proskauer tests
- Positive in methyl red and Citrate tests
- Produce H_2S
- Urease test positive
- IMViC test: - + - +
- TSI test: Alk/A/ +



Pseudomonas aeroginosa

- Non -fermenter gram-negative bacilli.
- Strict aerobes (acquire energy by oxidation not by fermentation)
- Oxidase-positive.
- Some *Pseudomonads* are motile by means of polar flagella.
- produces a characteristic fruity or sweety grape juice-like aroma.

Oxidase test



-Commonly habitat soil and water and found in small numbers in human feces.

-Have the ability to grow in lower nutrient environment, and have the ability to grow in disinfectant, so they persist in hospital environment.

- It is especially dangerous to the debilitated or compromised patient (burn and cystic fibrosis), it cause a nosocomial UTIs, wound infections, pneumonia, and septicemia.

- Produce two characteristic pigments, diffused in agar:

✓ **Pyocyanin:** color the pus in **wound (blue)**

✓ **Pyoviridin** (fluorescein): fluoresces under UV light (yellow green), help in early diagnosis of skin infection.

pyocyanin

pyoviridin

Laboratory diagnosis:

- Specimen: site of origin must be considered
- Gram-stain: not of value
- Culture: blood agar and a selective-differential medium such as MacConkey's agar

- Lactose-fermenter: **pink colonies**
- Non lactose fermenter: colorless colonies.
- For stool: highly selective media such as Hekton- enteric, SS agar is used along with Mac.

Laboratory diagnosis:

- Biochemical tests:
  - IMViC test
  - TSI tests
  - API test
  - Urease test
  - Oxidase test
- Serological test

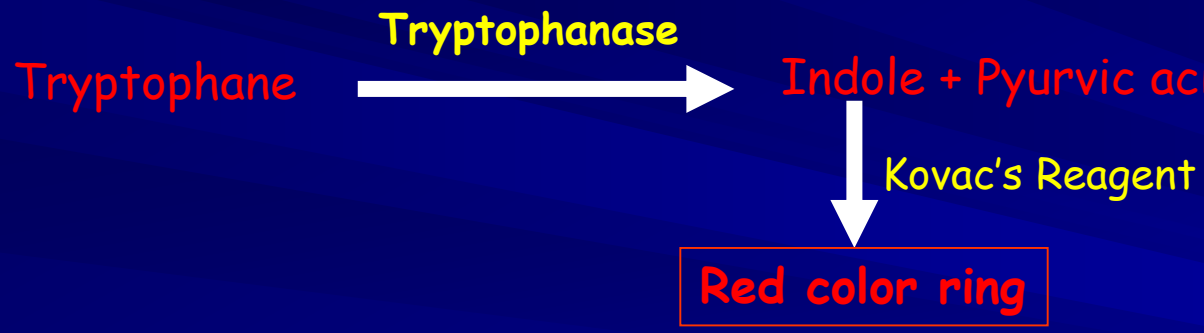
IMViC Test

- **Indole, Methyl red, Voges-ProsKauer, Citrate tests**
  - **Indole test:**

Principle

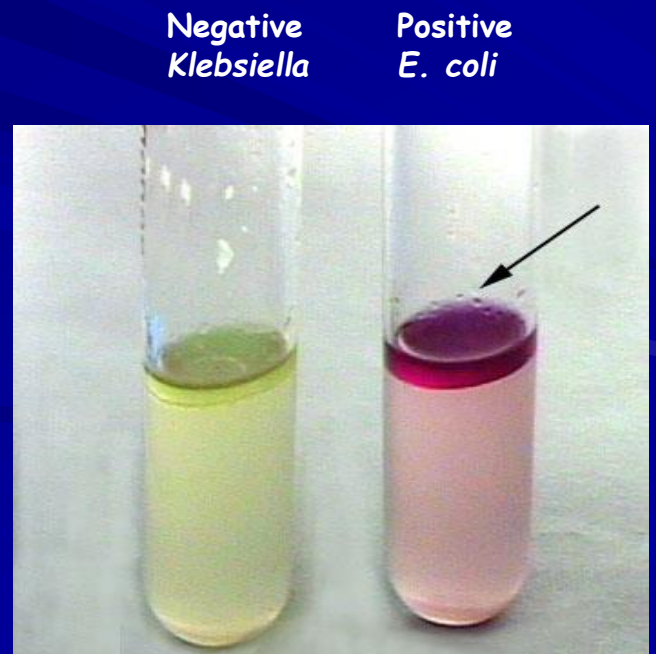
- Certain microorganisms can metabolize tryptophan by tryptophanase to pyruvic acid, indole and ammonia.

The presence of indole is detected by addition of **Kovac's reagent**.



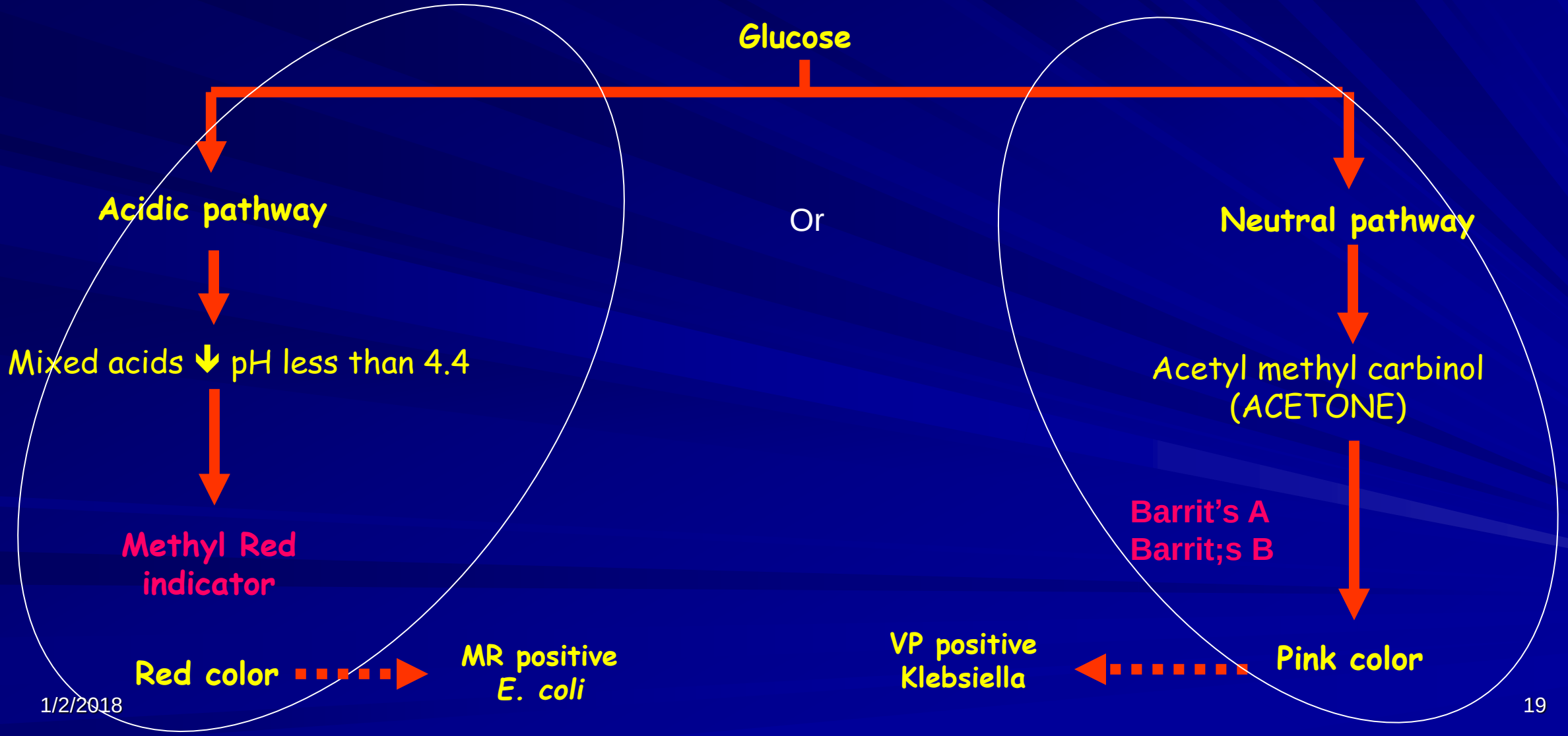
Method:

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



Methyl Red-Voges Proskauer (MR-VP) Tests

Principle: depend on the patterns of glucose metabolism



- Methods:

- Inoculate the tested organism into tubes of MRVP broth, incubate the tubes at 37°C for 24-48 hours

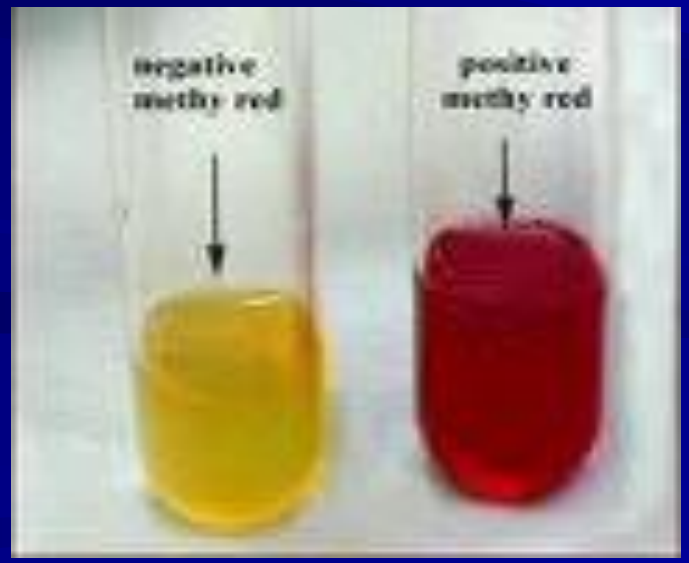
- AFTER INCUBATION:

  For methyl red: Add 6-8 drops of methyl red reagent.

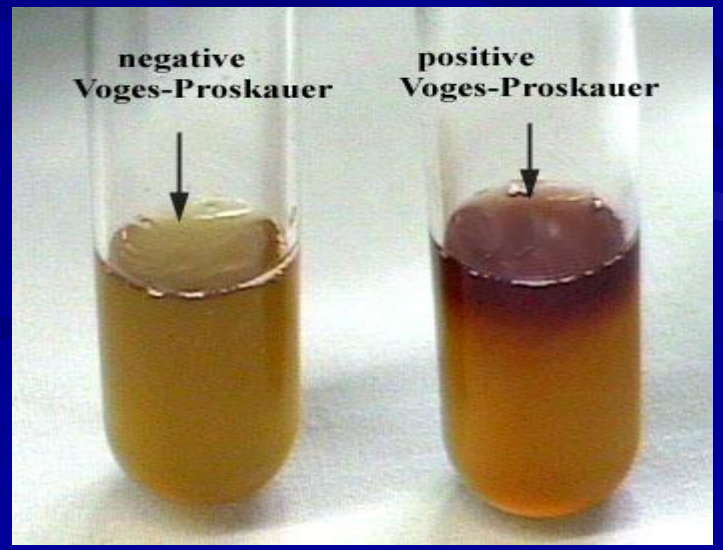
  For Voges-Proskauer: Add 6 drops of Barritt's A (α-naphthol), and 2 drops of Barritt's B (40% KOH), mix

  Let, undisturbed, for at least 1 hour, then note color change.

Methyl Red test



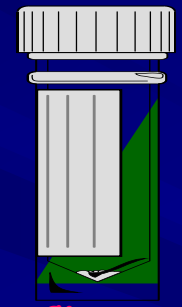
Voges-Proskauer test



❖ Citrate Utilization Test

- Principle: citrate utilized by bacteria produces citrase enzyme.

Citrate → Pyruvate → CO_2 + Na + H_2O → Na_2CO_3



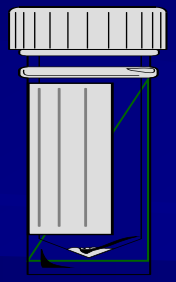
Simmon's Citrate media

Contains Citrate as a sole of C source

Alkaline, ↑pH

Bromothymol blue

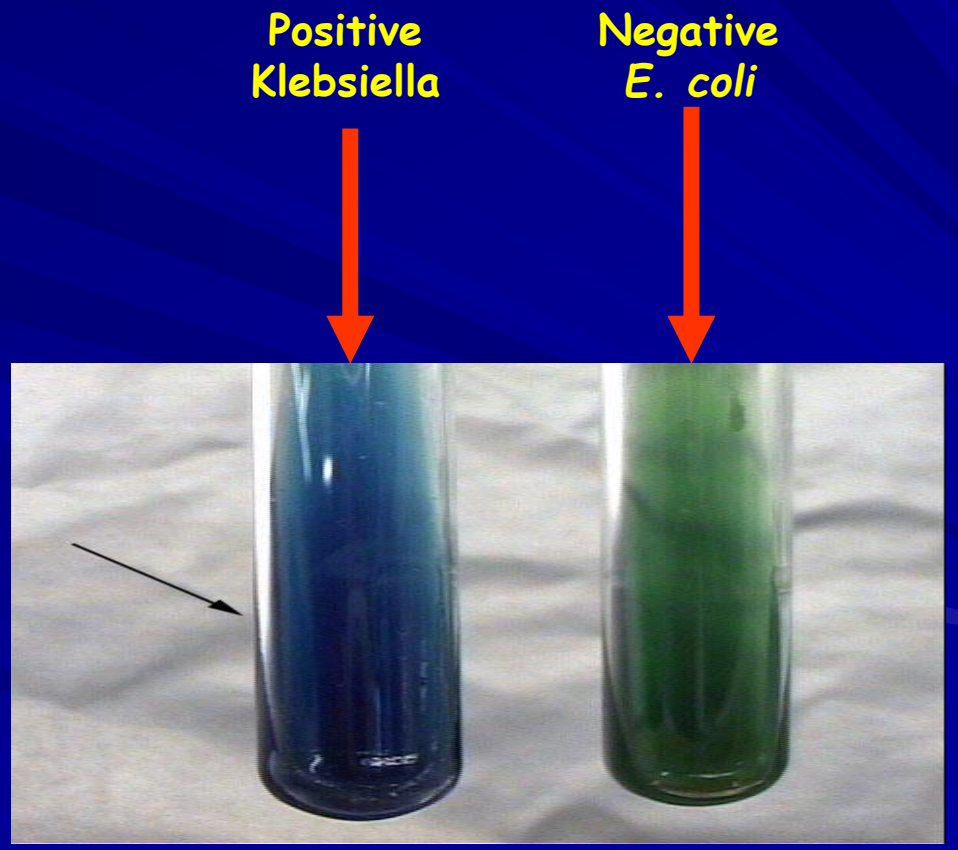
Blue color



Positive test

- **Method:**

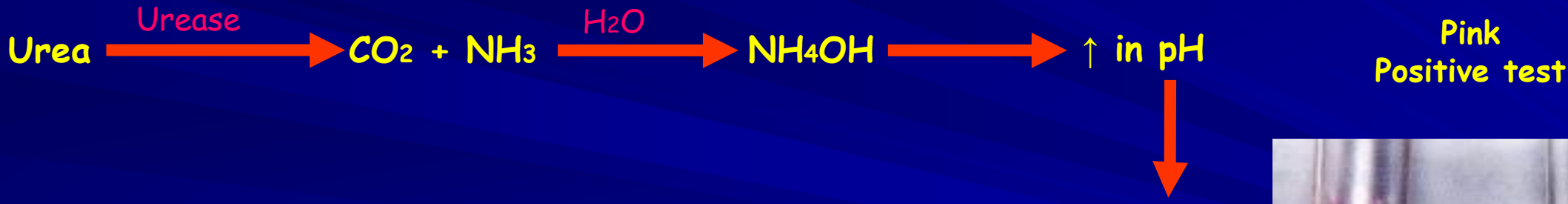
Streak a **Simmon's citrate agar** slant with the organism and incubate at 37°C for 24 hours. Growth on the medium is accompanied by a rise in pH to change the medium **(containing bromothymol blue indicator)** from its initial green color to deep blue.



Citrate utilization test

Urease Test

- **Principle:**
  - Urea agar contains urea and phenol red, Urease is an enzyme that catalyzes the conversion of urea to CO_2 and NH_3
  - In solution reacts with **CO_2** to produce ammonium hydroxide, a strong base which ↑ pH of the medium.
  - ↑ in the pH causes phenol red to turn a deep pink.



- **Method:**
  - Streak a urea agar tube with the organism incubate at 37°C for 24 h
  - If color of medium turns from yellow to pink indicates positive test.

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

H_2S Production

- Principle:
  - Bacteria use the enzyme cysteine disulfurase to hydrolyze the amino acid cysteine, forming hydrogen sulfide as end product.

- $H_2S + FeSO_4 \longrightarrow FeS + H_2SO_4$

Result

Reaction on TSI			Result	Example
Slant color	Butt color	H_2S		
Red	Red	Negative	Alk/Alk/- (No action on sugars)	Non fermenter e.g. *Pseudomonas*
Red	yellow	Negative	Alk/A/- (Glucose fermented without H_2S)	LNF e.g. *Shigella*
Red	yellow	Positive black in butt	Alk/A/+ (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*

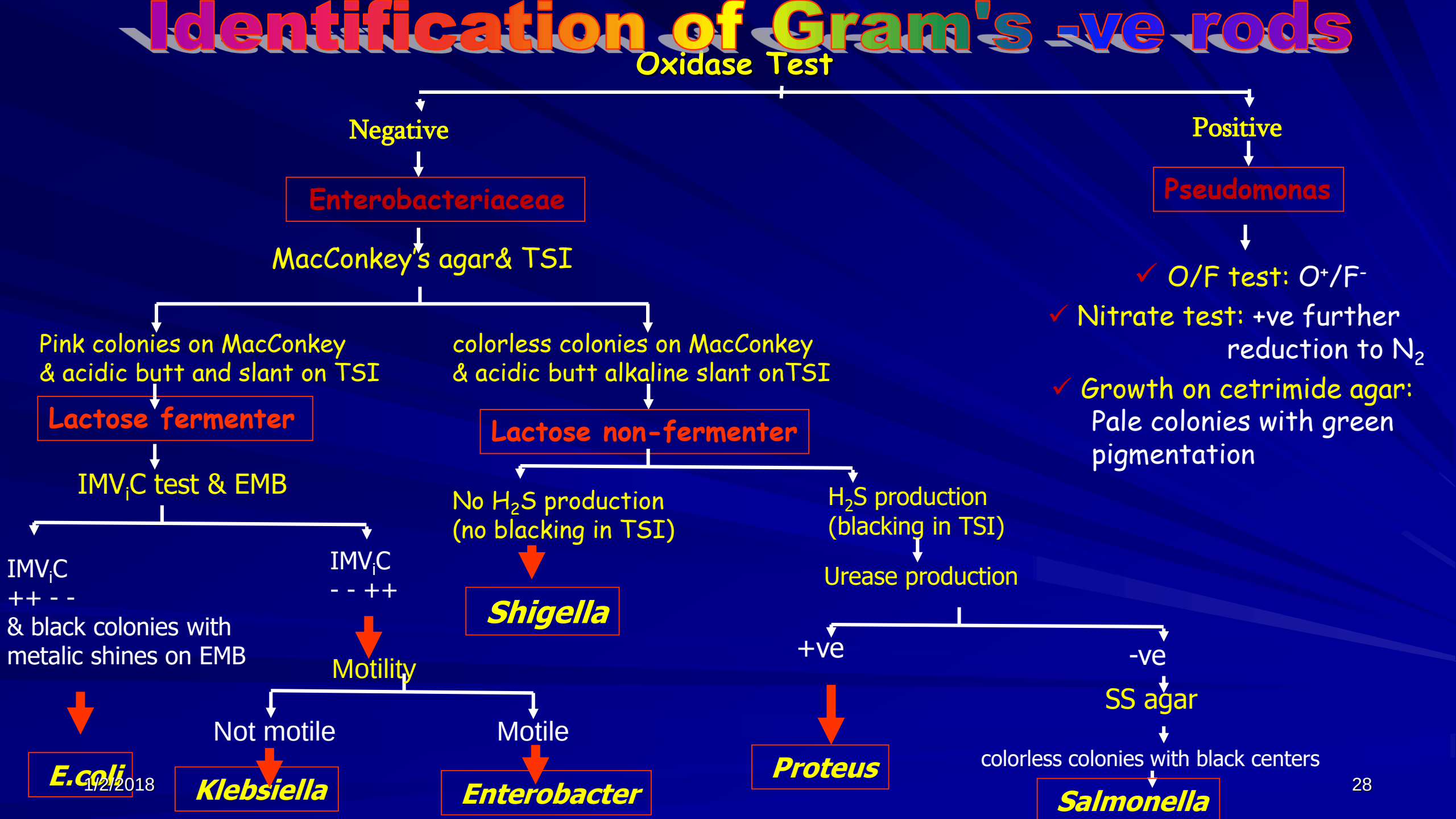
Summary of morphology, cultural characteristics, and biochemical reactions of *Enterobacteriaceae*

	Gram stain	Oxidase	Nitrate reductase	O/F	Mac	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/H_2S	Colorless
Shigella	-ve rods	-ve	+ve	O+/F+	NLF	NLF	Colorless
Proteus	-ve rods	-ve	+ve	O+/F+	NLF	NLF/H_2S	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	Alk/A/+	-ve	+ve	-ve	+ve	-ve	Motile
Shigella boydii	Alk/A/-	-ve	+ve	-ve	-ve	-ve	Non motile
Proteus mirabilis	Alk/A/+	-ve	+ve	-ve	+ve	+ve	Motile Swarwing

Identification of Gram's -ve rods



THANK YOU FOR ATTENTION