

# IDENTIFICATION OF PATHOGENIC BACTERIA IN CLINICAL MICROBIOLOGY LABORATORY

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# Identification of Gram Positive Cocci(I)

## Staphylococcus species

**Gram stain** – Gram positive cocci in clusters

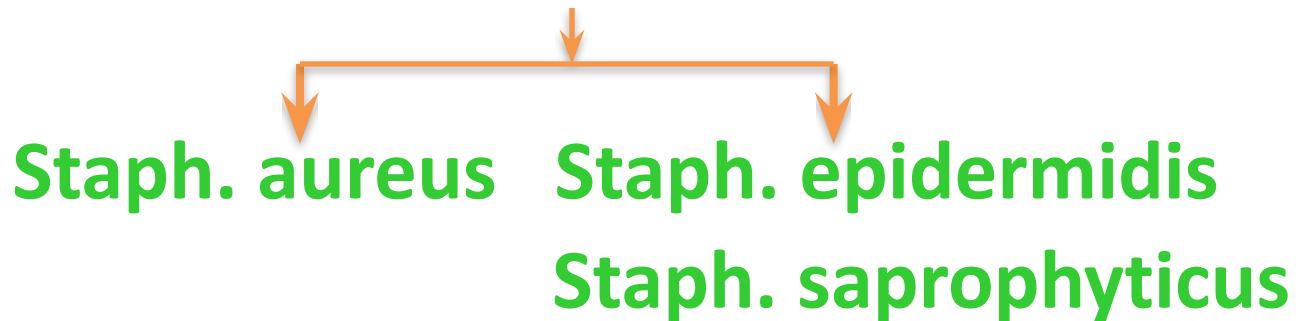
**Motility** – Non-motile

**Catalase** – Positive

**Oxidase** – Negative

So it is Staphylococcus

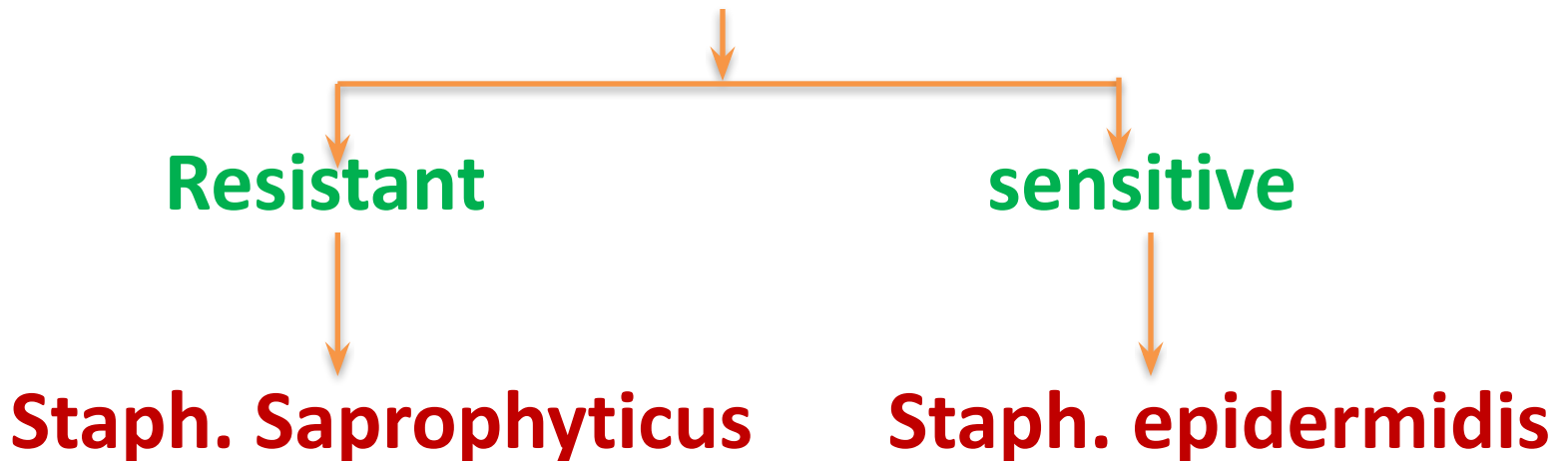
### Coagulase Test



# Identification of Gram Positive Cocci(I)

## Staphylococcus species – cont.,

Novobiocin sensitivity



# Identification of Gram Positive Cocci(I)

## Staphylococcus species- cont.,

- Differentiation between Staphylococci and Micrococci also may be important because both may be similar in microscopic morphology.

TEST	MICROCOCCI	STAPHYLOCOCCI
OF TEST	OXIDATIVE	FERMENTATIVE
MODIFIED OXIDASE TEST	POSITIVE	NEGATIVE
BACITRACIN SUSCEPTIBILITY	SENSITIVE	RESISTANT
FURAZOLIDONE SUSCEPTIBILITY	RESISTANT	SENSITIVE

(contd..)

Note:

**Other characteristic features to identify**

***Staphylococcus aureus***

□ **Mannitol fermentation – Positive**

□ **DNAase test – Positive**

□ **Beta-hemolytic on blood agar**

□ **Golden yellow pigment**

**Mannitol Salt Agar (Selective medium) –**

**Staph. aureus produce yellow color colonies due to mannitol fermentation.**

# IDENTIFICATION OF GRAM POSITIVE COCCI (II)

## STREPTOCOCCUS

- **Gram stain** – Gram positive cocci in chains
- **Motility** – Non-motile
- **Catalase** – Negative
- **Oxidase** – Negative

So it is **Streptococcus**

**Pin-point colonies with wide zone of Beta hemolysis on blood agar (constant property)**

May be Beta-hemolytic streptococci like

- **Streptococcus pyogenes (Group A streptococci)**
- **Streptococcus agalactiae (Group B streptococci)**

# Bacitracin sensitivity

Sensitive

Resistant

Strep. pyogenes  
(PYR+ve)

Other Beta hemolytic  
Streptococci  
(Strep. agalactiae)

(Camp test +ve)  
(Hippurate hydrolysis +ve)

# IDENTIFICATION OF GRAM POSITIVE COCCI (III)

## PNEUMOCOCCUS

- **Gram stain** – Gram positive cocci in pairs  
(lanceolate shape)
- **Motility** – Non-motile
- **Catalase** – Negative
- **Oxidase** – Negative

**On blood agar – ALPHA HEMOLYTIC**

**It may be *Streptococcus pneumoniae***

**(or) Viridans streptococci (though it occur in chains predominantly sometimes may occur in pairs)**



# Optochin sensitivity test

(Sensitive)

(Resistant)

**Pneumococcus**

**(Capsulated)**

**(Bile solubility test - Pos)**

**(Capsule swelling test – pos)**

**(Bile Esculin test – Neg)**

**Viridans streptococci**

**(Non-capsulated)**

**(Bile solubility test - Neg)**

**(Capsule swelling test – Neg)**

**(Bile Esculin Negative)**

**Note: Because the isolate is Gram positive cocci in pairs, we may also suspect Enterococcus sp., which may be alpha, beta or gamma hemolytic pattern on blood agar, hence Bile Esculin test, to which Enterococcus sp., is positive, can be used.**

## Note:

**Pneumococcus – cause of Lobar pneumonia so it is most likely to be present in sputum of infected person.**

**Remember pneumococcus is also cause of meningitis ( so also found in CSF)**

**Along with sputum, viridans streptococcus, which is a normal flora in oral cavity, may be present when sputum contaminated with saliva.**

**Viridans streptococcus, usually arranged in chains, may break into pairs looking like Streptococcus pneumoniae.**

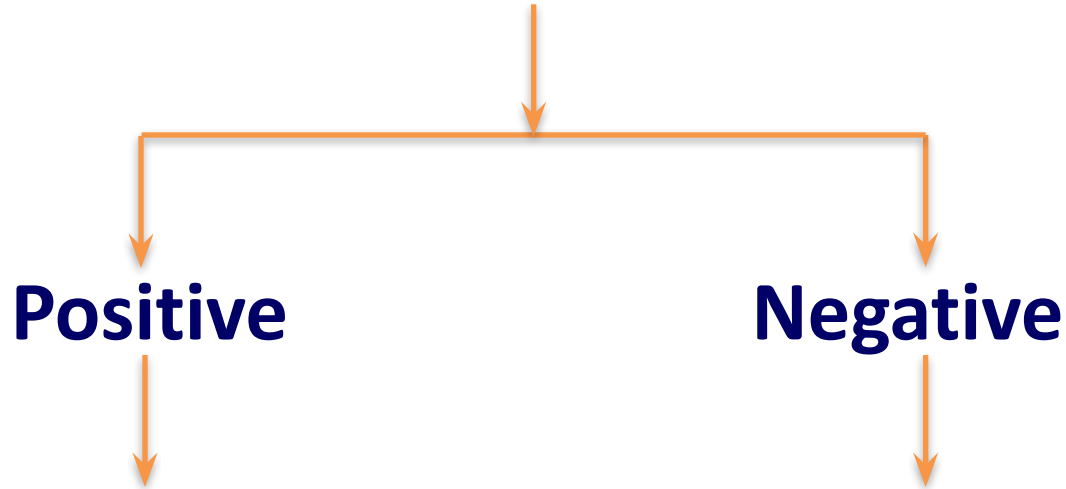
**Viridans streptococcus and Streptococcus pneumoniae are always alpha-haemolytic.**

# IDENTIFICATION OF GRAM POSITIVE COCCI- IV ENTEROCOCCUS

- **Gram stain** – Gram positive cocci in pairs
- **Motility** – Non-motile
- **Catalase** – Negative
- **Oxidase** – Negative

So it may be Enterococcus (or) Pneumococcus  
(or) viridans streptococci

# BILE ESCULIN HYDROLYSIS TEST



**Group D streptococcus**

**Enterococcus**

**(Grow in MacConkey's agar)**

**(Tiny deep pink (LF) colonies)**

**Negative**

**Pneumococcus**

**Viridans streptococci**

**(Not Grow in MacConkey's agar)**

# Growth in 6.5% salt (Salt tolerance test)

Positive (Growth)

Negative (No growth)

Enterococcus

Group D streptococcus

PYR

(+ve)

(-ve)

Sensitivity

(R)

(S)

to SXT

Ability to

grow at 45°C

Yes

No

## **Note:**

**Enterococcus can be alpha or beta or gamma hemolytic on blood agar**

**Enterococcus faecalis and Enterococcus faecium are important pathogenic members in Genus Enterococcus**

# IDENTIFICATION OF GRAM NEGATIVE COCCI (I) NEISSERIA SPECIES

- **Gram Stain – Gram negative diplococci**
- **Motility – Non- motile**
- **Catalase – Positive**
- **Oxidase – Positive**

So it may be

**Pathogenic Neisseria** (or) **Non-pathogenic Neisseria**

  
(Neisseria gonorrhoeae)

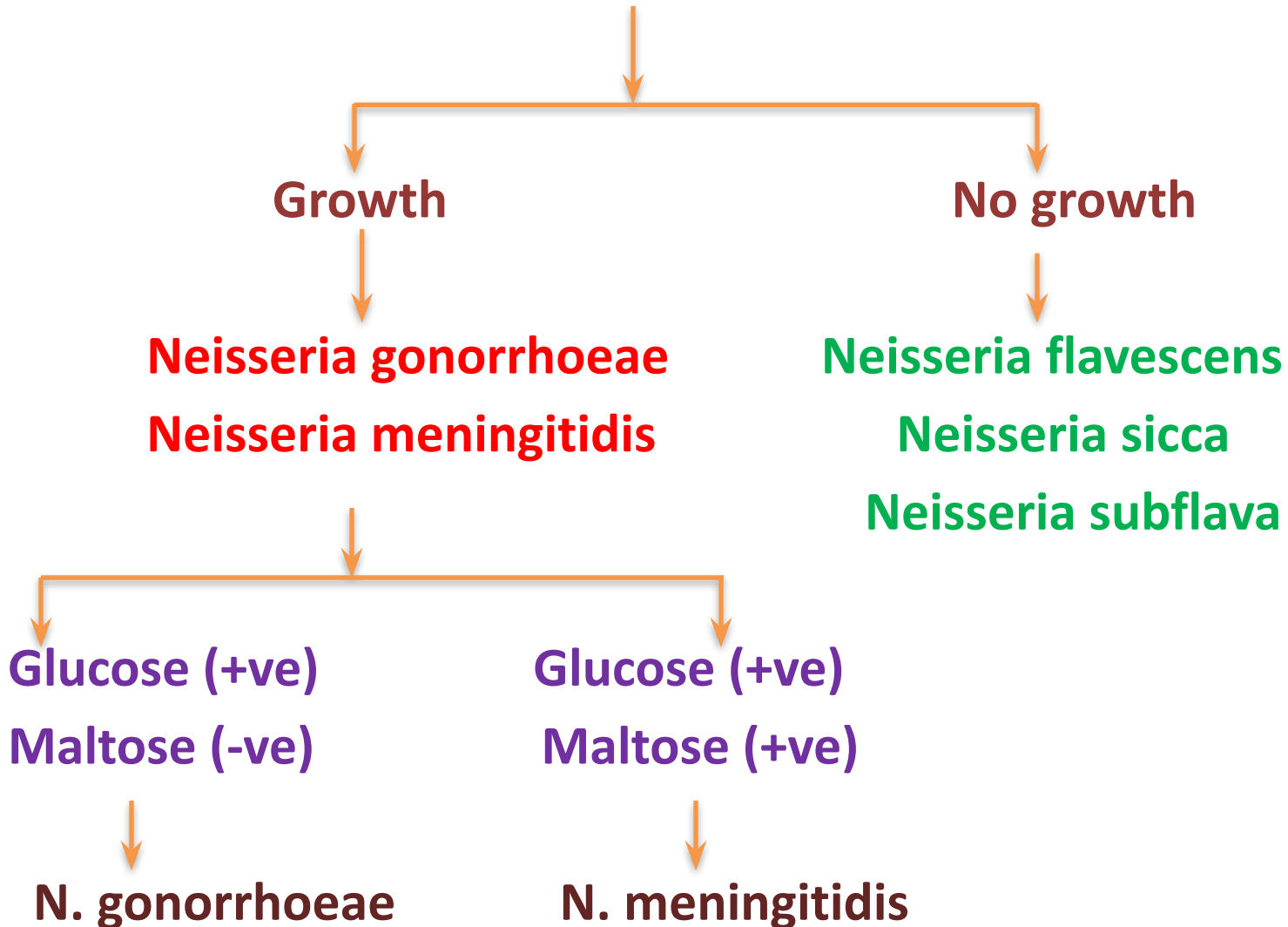
(Neisseria meningitidis)

  
(Neisseria flavescens)

(Neisseria sicca)

(Neisseria subflava)

# Growth on THAYER-MARTIN MEDIUM (selective medium)





## Note:

- Non-pathogenic Neisseria like *N. flavescens*, *N. sicca* & *N. subflava* produce yellow pigment
- Non-pathogenic Neisseria can grow on Nutrient agar but pathogenic Neisseria don't.
- Catalase test for Neisseria can be done by superoxol test using 30% Hydrogen peroxide.
- *N. gonorrhoeae* – most likely to be present in urethral pus.
- *N. meningitidis* – most likely to be present in CSF.

# IDENTIFICATION OF GRAM POSITIVE BACILLI

## List of Gram positive Bacilli

**Corynebacterium sp.**

**Listeria sp.**

**Erysipelothrix rhusiopathiae**

**Lactobacillus sp.**

**Kurthia sp.**

**Actinomyces sp.**

**Bacillus sp.**

**Clostridium sp.**

**Spore**

**(Present)**

**(Absent)**

**Bacillus sp.**

**Clostridium sp.**

**Corynebacterium sp.**

**Listeria sp.**

**Ersipelothrix sp.**

**Lactobacillus sp.**

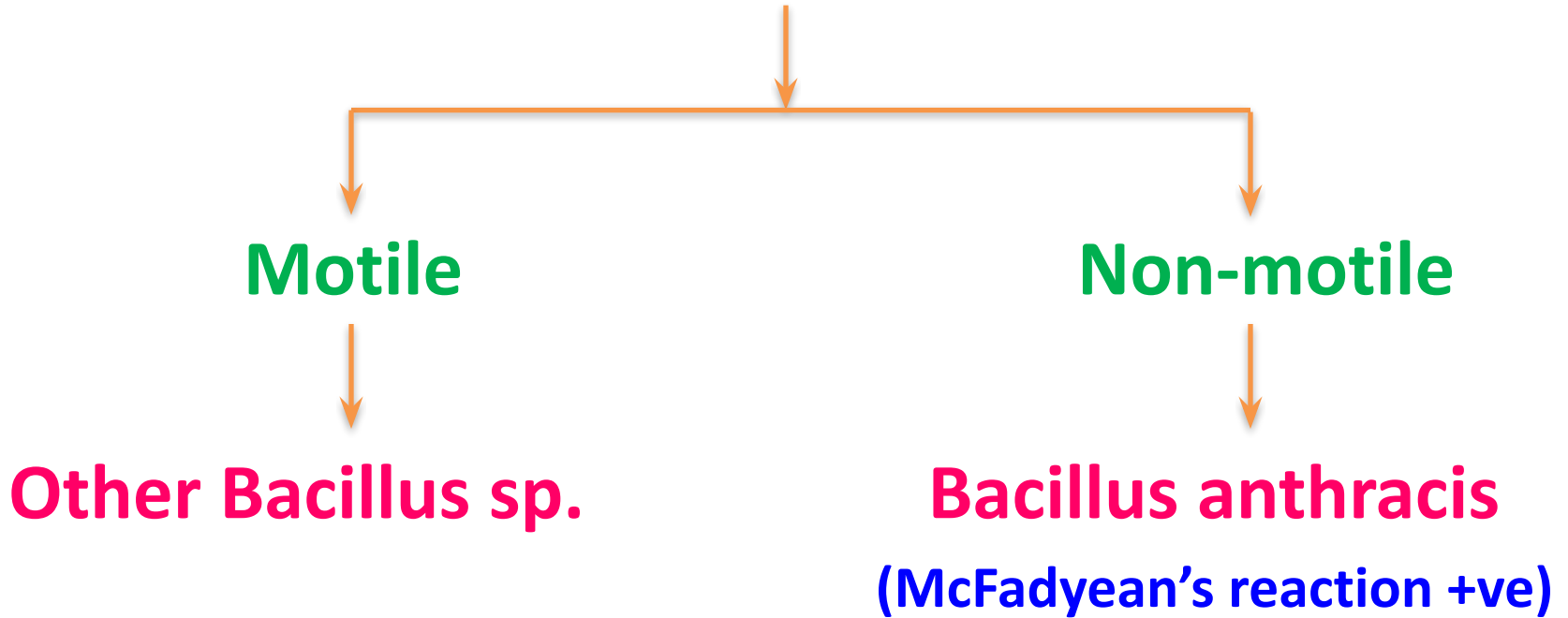
**Bacillus**

**Clostridium**

**(Aerobic)**

**(Anaerobic)**

# Bacillus species



# Catalase

(+)

(-)

**Corynebacterium**

**Listeria**

**kurthia**

**Erysipelothrix**

**Lactobacillus**

**Actinomyces**

## Beta hemolytic on BA

**(Yes)**

**(Listeria)**

**(Corynebacterium)**

**(No)**

**(Kurthia)**

## H<sub>2</sub>S Production

**(+)**

**Erysipelothrix**

**(-)**

**Lactobacillus**

**Actinomyces**

**Listeria**

**Corynebacterium**



**(Esculin Hydrolysis)**



**(+)**

**(-)**



**Listeria**

**Corynebacterium**

**(Motile at 25<sup>0</sup>C)**

**(Non-motile)**

**(Non-Motile at 37<sup>0</sup>C)**

**Lactobacillus**  
**Actinomyces**

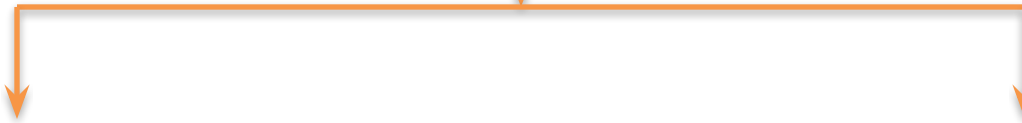


**(Branching filaments)**



**(+)**

**(-)**



**Actinomyces**

**Lactobacillus**

## Note:

Other examples of anaerobic Gram positive bacilli – Eubacterium, Propionibacterium, Bifidobacterium, Mobilincus.

Remember, Actinomyces and Lactobacillus also contains anaerobic species and microaerophilic species.

Clostridium species (anaerobes) – another example of Gram positive bacilli

Diphtheroids, morphologically similar to C. diphtheriae can grow on ordinary media like Nutrient agar. But Corynebacterium diphtheriae can only grow on enriched media like Blood agar and Loeffler serum slope.



# IDENTIFICATION OF GRAM NEGATIVE BACILLI (I) ESCHERICHIA COLI /E.COLI

- Gram stain – **Gram negative bacilli**
- Motility – **Motile**
- Catalase – **Positive**
- Oxidase – **Negative**

**So it is one of the members of  
Enterobacteriaceae**

**Enterobacteriaceae includes E.coli, Klebsiella,  
Citrobacter, Enterobacter, Serratia, Salmonella,  
Shigella, Proteus**

**MacConkey's agar – Dry, flat LF colonies**

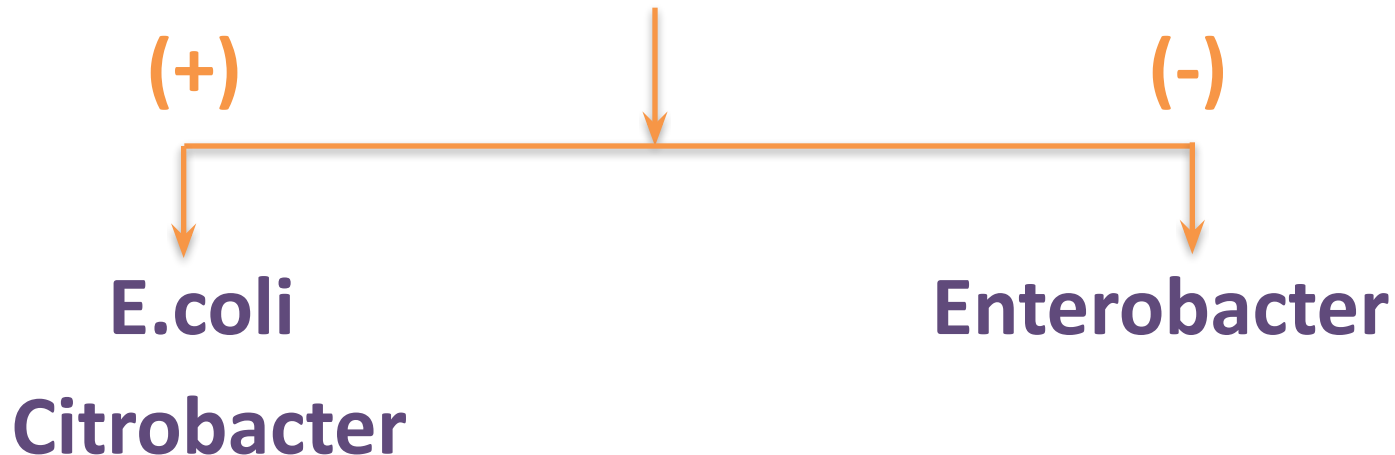
**Motility – Motile**

**So it may be E.coli, Citrobacter, Enterobacter, Serratia**

**Klebsiella being non-motile, it is omitted**

**Indole test**

**Methyl-Red test**



# Citrate test

(+)

(-)

Citrobacter

E.coli

Serratia

Citrobacter

Serratia

(No red pigment)

(Red pigment)

## IMVIC REACTIONS FOR E.COLI

- Indole – (+)
- Methyl red – (+)
- Voges-Proskauer – (-)
- Citrate - (-)

### Other biochemical reactions:

TSI – A/A, Gas (+), H<sub>2</sub>S (-)

Urease – (-)

### Remember:

E.Coli is the most common cause of lower urinary tract infection. In this case, E.coli is most likely to be present in urine sample of infected persons.

## IDENTIFICATION OF GNB (II) KLEBSIELLA SPECIES

- Gram stain – **Gram Negative bacilli**
- Motility – **Non-motile**
- Catalase – **Positive**
- Oxidase – **Negative**

**So it is one of the members of  
Enterobacteriaceae**

**On MacConkey's agar – Mucoid LF Colonies**

**Motility – Non-motile**

**So it may be Klebsiella species**

**But it may not be E.coli, Citrobacter,  
Enterobacter or Serratia ( because all are  
motile)**

**Klebsiella species**

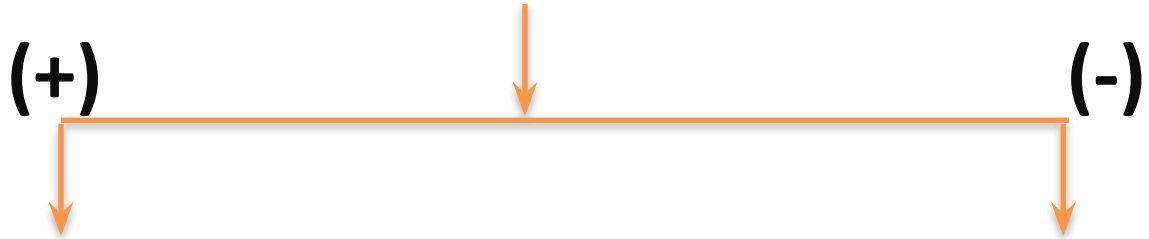
**Klebsiella pneumoniae**

**Klebsiella oxytoca**

**Klebsiella rhinoscleromatis**

**Klebsiella ozanae**

# Indole test



**Klebsiella oxytoca**

**Klebsiella rhinoscleromatis**

**Klebsiella pneumoniae**

**Klebsiella ozanae**

**(Urease +ve)**

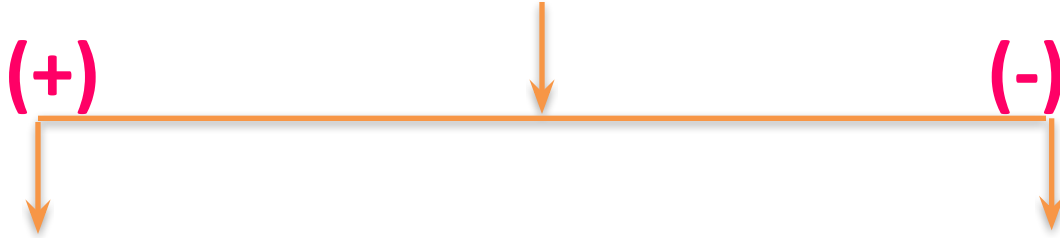
**(Urease -ve)**

**Klebsiella pneumoniae**

**Klebsiella rhinoscleromatis**

**Klebsiella ozanae**

# Malonate



**Klebsiella rhinoscleromatis**

**(VP POSITIVE)**

**Klebsiella ozanae**

**(VP POSITIVE)**



## IMVIC REACTIONS FOR KLEBSIELLA PNEUMONIAE

- Indole – (-)
- Methyl red – (-)
- Voges-Proskauer – (+)
- Citrate - (+)

### Other biochemical reactions:

TSI – A/A, Gas (+), H<sub>2</sub>S (-)

Urease – (+)

### Remember:

**Klebsiella pneumoniae is also the most commonest cause of lower urinary tract infection. In this case, it is most likely to be present in urine sample of infected persons.**

# IDENTIFICATION OF GNB (III)

## CITROBACTER

- Gram stain – Gram negative bacilli
- Motility – Motile
- Catalase – Positive
- Oxidase – Negative

So it is one of the members of Enterobacteriaceae

On Mac – LF colonies

Being motile

It may be E.coli or Enterobacter or Citrobacter

**E.coli**

**Enterobacter**

**Citrobacter**



**Citrate test**



**(+)**

**(-)**

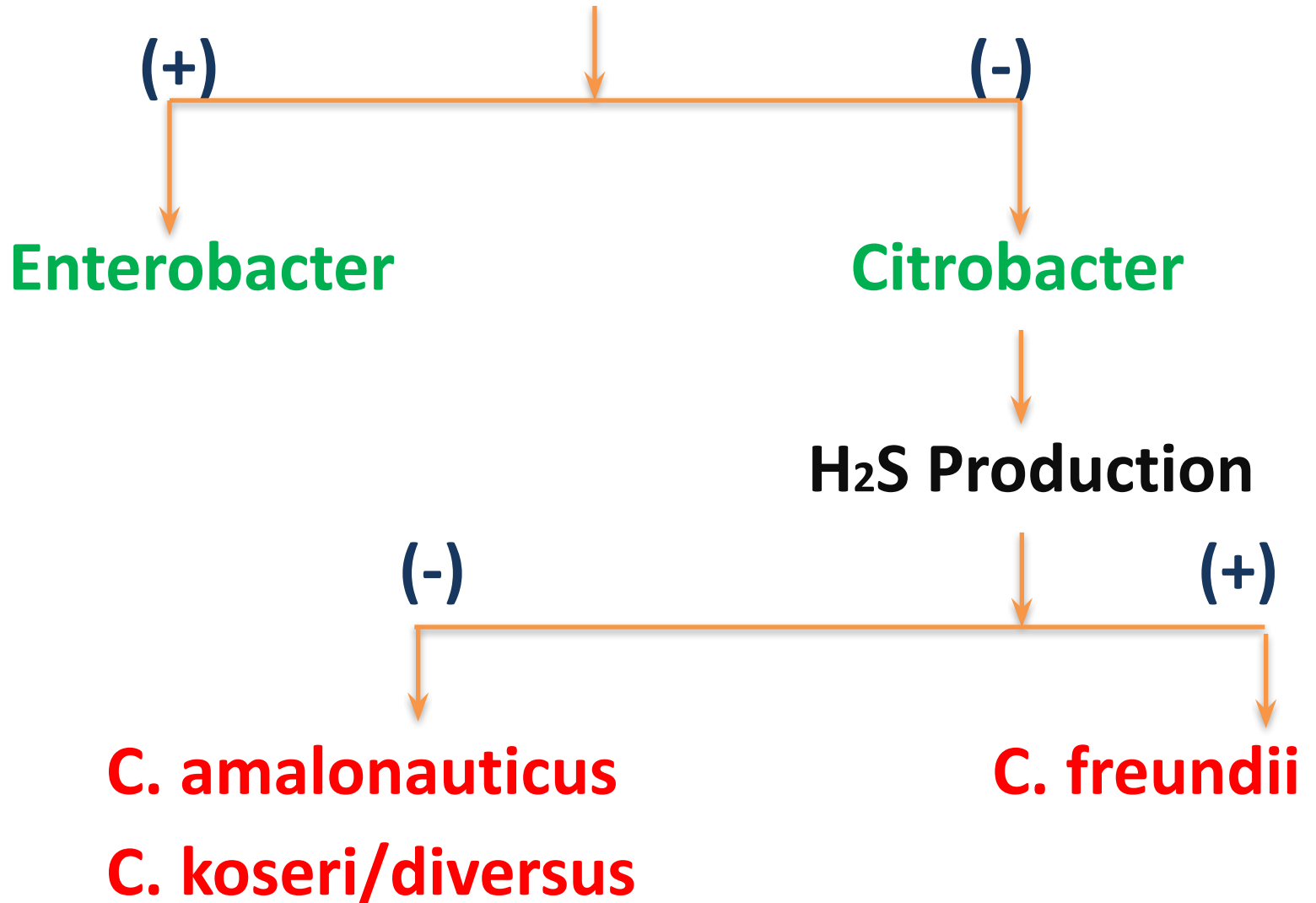


**Citrobacter**

**E. coli**

**Enterobacter**

# VP TEST



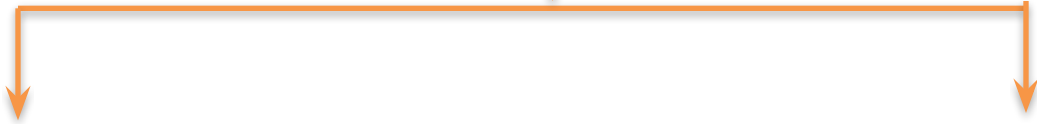
**C. amalonauticus**  
**C. koseri/diversus**



**MALONATE**  
**ADONITOL**

**(+)**

**(-)**



**C. koseri/diversus**

**C. amalonauticus**

## Other reactions of Citrobacter

- Indole – (+/-)
- MR – (+)
- VP – (-)
- Urease – (weakly positive)
- TSI – A/A, Gas (+ve), H<sub>2</sub>S (+/-)

Remember citrobacter sometimes also may be  
Late lactose fermenter ( ONPG – +VE)

Remember Citrobacter always Citrobacter (+)

# IDENTIFICATION OF GNB (IV)

## ENTEROBACTER

- Gram stain – **Gram Negative Bacilli**
- Motility – **Motile**
- Catalase – **Positive**
- Oxidase – **Negative**

**So it is one of the members of Enterobacteriaceae**

**On Mac- LF colonies (Less mucoid)**

**Being motile**

**It may be E.coli (or) Citrobacter (or) Enterobacter**

# CITRATE TEST

(+)

(-)

Citrobacter  
Enterobacter

E.coli

# VP TEST

(+)

(-)

Enterobacter

Citrobacter



**Enterobacter cloacae**  
**Enterobacter aerogenes**

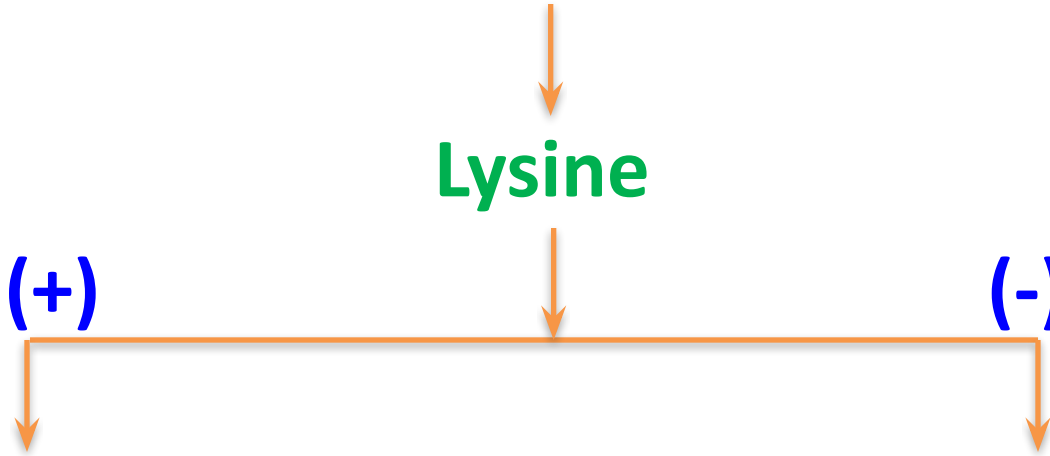
**Lysine**

**(+)**

**(-)**

**Ent. aerogenes**  
**(Arginine- Pos)**

**Ent. Cloacae**  
**(Arginine- Neg)**



# IDENTIFICATION OF GNB-V PROTEUS, MORGANELLA, PROVIDENCIA

- **Gram stain – Gram negative bacilli**
- **Motility – Motile**
- **Catalase – Positive**
- **Oxidase – Negative**

**So it is one of the members of  
Enterobacteriaceae**

**Being motile – Shigella is omitted**

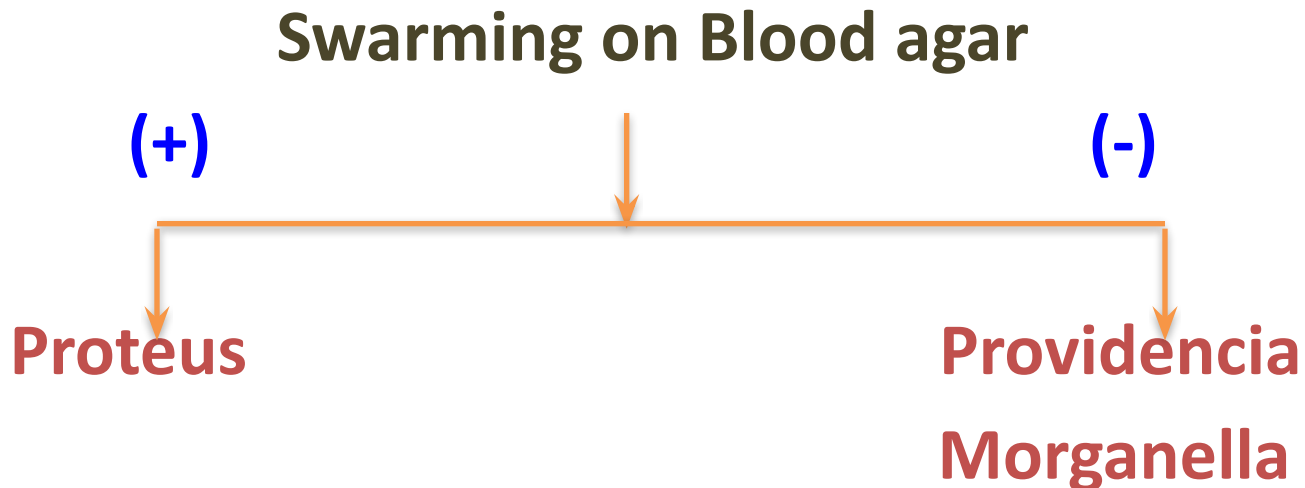
**On Mac – NLF – so E.coli, Citrobacter, Enterobacter are  
omitted**

- It may be Salmonella or Proteus or Morganella or Providencia

Being PPA/PAD test positive

It may not be Salmonella

It may be Proteus (or) Morganella (or) Providencia



**Proteus species**



**Indole test**

**(+)**



**(-)**



**Proteus vulgaris**

**(Ornithine decarboxylase (+))**

**Proteus mirabilis**

**(Ornithine decarboxylase (-))**

**Morganella  
Providencia**



**Ornithine decarboxylase**



**(+)**

**(-)**



**Morganella morgani**



**Prov. alcalifaciens**

**Prov. rettgeri**

**Prov. stuartii**

**Providencia alcalifaciens**

**Providencia rettgeri**

**Providencia stuartii**



**Gas from glucose**



**(-)**

**(+)**



**Prov. rettgeri**

**Prov. stuartii**



**Prov. alcalifaciens**

**Prov. rettgeri**

**Prov. Stuartii**



**Fermentation of trehalose**



**(+)**

**(-)**



**Prov. stuartii**

**Prov. rettgeri**

## Other biochemicals for Proteus species

**MR – (+)**

**VP – (-)**

**Urease - (+)**

**Citrate – (-)**

**TSI – K/A, Gas(+), H<sub>2</sub>S (+)**



# IDENTIFICATION OF GNB – VI

## SHIGELLA SPECIES

- **Gram stain – Gram negative bacilli**
- **Motility – Non-motile**
- **Catalase – Positive**
- **Oxidase – Negative**

so it is one of the members of enterobacteriaceae

On mac – NLF colonies

Being non-motile

It is not Salmonella (or) Proteus (being motile)

It is Shigella species

# Mannitol fermentation

(+)

(-)

**Shigella dysenteriae**

**Shigella flexneri**

**Shigella boydii**

**Shigella sonnei**

**Shigella flexneri**  
**Shigella boydii**  
**Shigella sonnei**

**ONGP**

**(-)**

**(+)**

**Shigella flexneri**  
**Shigella boydii**

**Shigella sonnei**

**Note:**

**Shigella dysenteriae type 1 is always catalase negative.**

**Differentiation between**

**Sh. Flexneri and Sh. Boydii can be done only by serological method using specific antisera because of non-availability of suitable biochemical reactions.**

## Other biochemicals for Shigella species

Indole – (+/-)

MR – (+)

VP – (-)

Citrate – (-)

Urease – (-)

TSI – K/A, Gas (-), H<sub>2</sub>S (-)

# IDENTIFICATION OF GNB – VII

## SALMONELLA SPECIES

- **Gram stain** – Gram negative bacilli
- **Motility** – Motile
- **Catalase** – Positive
- **Oxidase** – Negative

So it is one of the members of Enterobacteriaceae

On Mac – NLF colonies

Being motile

It may not be Shigella (being non-motile)

It may be Salmonella or Proteus

**PPA/PDA**

**(+)**

**(-)**

**Proteus sp.**

**(Swarming on BA (+))**

**Salmonella sp.**

**(Swarming on BA (-))**

**S. typhi**

**S. paratyphi A**

**S. paratyphi B**

# H<sub>2</sub>S Production

(+)

(-)

*S. typhi*

*S. paratyphi A*

*S. paratyphi B*

# Gas production

(-)

(+)

*S. typhi*

*S. paratyphi B*



## Another way of differentiation of Salmonella species

- Remember *S. paratyphi* B alone is citrate positive.
- Remember *S. paratyphi* A and *S. paratyphi* B both are xylose and arabinose positive
- Remember *S. typhi* is both xylose and arabinose negative.

# Differentiation of Salmonella species also may be possible by TSI reaction

## S. typhi

K/A, Gas (-), speck of H<sub>2</sub>S (+)

## S. paratyphi A

K/A, Gas (+), H<sub>2</sub>S (-)

## S. paratyphi B

K/A, Gas (+), abundant H<sub>2</sub>S (+)

## Other biochemicals for Salmonella species

**Indole – (-)**

**MR – (+)**

**VP – (-)**

**Citrate – (only *S. paratyphi* B positive)**

**Urease – (-)**

# IDENTIFICATION OF GNB – VIII

## PSEUDOMONAS SPECIES

- **Gram stain – Gram negative bacilli**
- **Motility – Motile**
- **Catalase – Positive**
- **Oxidase – Positive**

So it is one of the members of  
Non-enterobacteriaceae like  
**Pseudomonas species**  
**Vibrio species**

**On MAC – NLF colonies (irregular)**

**On NA – Bluish green (pyocyanin) pigmentation**

**OF –TEST – Oxidative reaction seen**



**It is more likely to be Pseudomonas aeruginosa**

**(because it is only pseudomonas species produce such type of bluish green pigment)**

**It may not be Vibrio species because it is fermentative in OF-TEST and not produce bluish green pigment**

## Other biochemicals for *Pseudomonas aeruginosa*

Indole – (-)

MR – (-)

VP – (-)

Citrate – (+)

Urease – (-)

TSI - K/K (or) K/no change, Gas (-), H<sub>2</sub>S (-)

Nitrate reduction test – (+)

# IDENTIFICATION OF GNB – IX

## VIBRIO AND ASSOCIATED SPECIES

- Gram stain – Gram negative bacilli
- Motility – Motile (Darting motility)
- Catalase – Positive
- Oxidase – Positive

So it is one of the members of

Non- Enterobacteriaceae

It may be Vibrio (or) Aeromonas (or) Plesiomonas

It may not Pseudomonas because no bluish green pigment seen

**Vibrio**

**Aeromonas (A. hydrophila)**

**Plesiomonas (Pl. shigelloides)**



**Lysine**

**Ornithine**

**(+)**

**(-)**



**Vibrio**

**Aeromonas**

**Plesiomonas**



**Arginine**

**(+)**

**(-)**

**Vibrio species**

**Plesiomonas species**

**TCBS Medium**

**Yellow colonies**

**Green colonies**

**V. Cholerae**

**V. parahaemolyticus**

**V. alginolyticus**

**Vibrio cholerae**  
**Vibrio alginolyticus**

**Swarming on Blood agar**

**(+)**

**(-)**

**V. alginolyticus**

**V. cholerae**

**Two biotypes**

**Classical**

**ElTor**

# Vibrio Cholerae

```
graph TD; A[Vibrio Cholerae] --> B[Classical biotype]; A --> C[ElTor biotype];
```

## Classical biotype

VP – (-)

Non- haemolytic

Chick RBC agglutination (-)

## ElTor biotype

VP – (+)

Haemolytic

Chick RBC  
agglutination (+)

## Specific tests for Vibrio cholerae

- String test – (+)
- Cholera Red Reaction – (+)
- Gelatin liquefaction – (+)

---

## Other reactions of Vibrio cholerae

MR – (-)

VP – (+/-)

Citrate (+)

Urease – (-)

TSI – A/A, Gas (-), H<sub>2</sub>S (-)

## **Serotyping of Vibrio cholerae**

**Vibrio cholerae O1 antiserum helps to identify Vibrio cholerae O1 strain. This is followed by identification of specific subtypes like Inaba (or) Ogawa (or) Hikojima by using specific antisera.**

- **This presentation was created for those who are working as a laboratory technician in clinical microbiology diagnostics.**
- **Also may find useful for UG, PG, DMLT, PGDMLT in Microbiology.**

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