ID of Most Common Bacterial Pathogens

CLS 417- Clinical Practice in Microbiology

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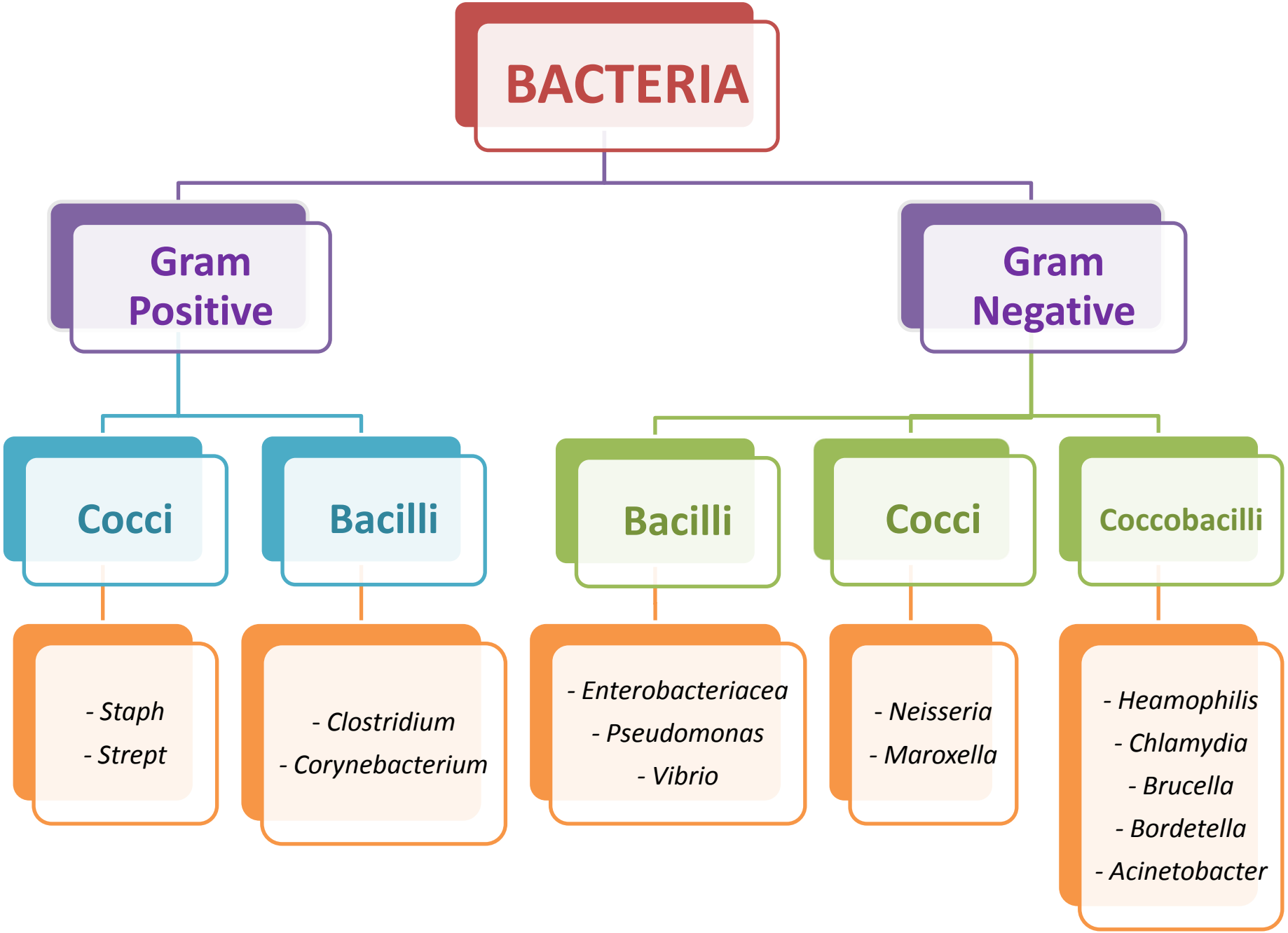
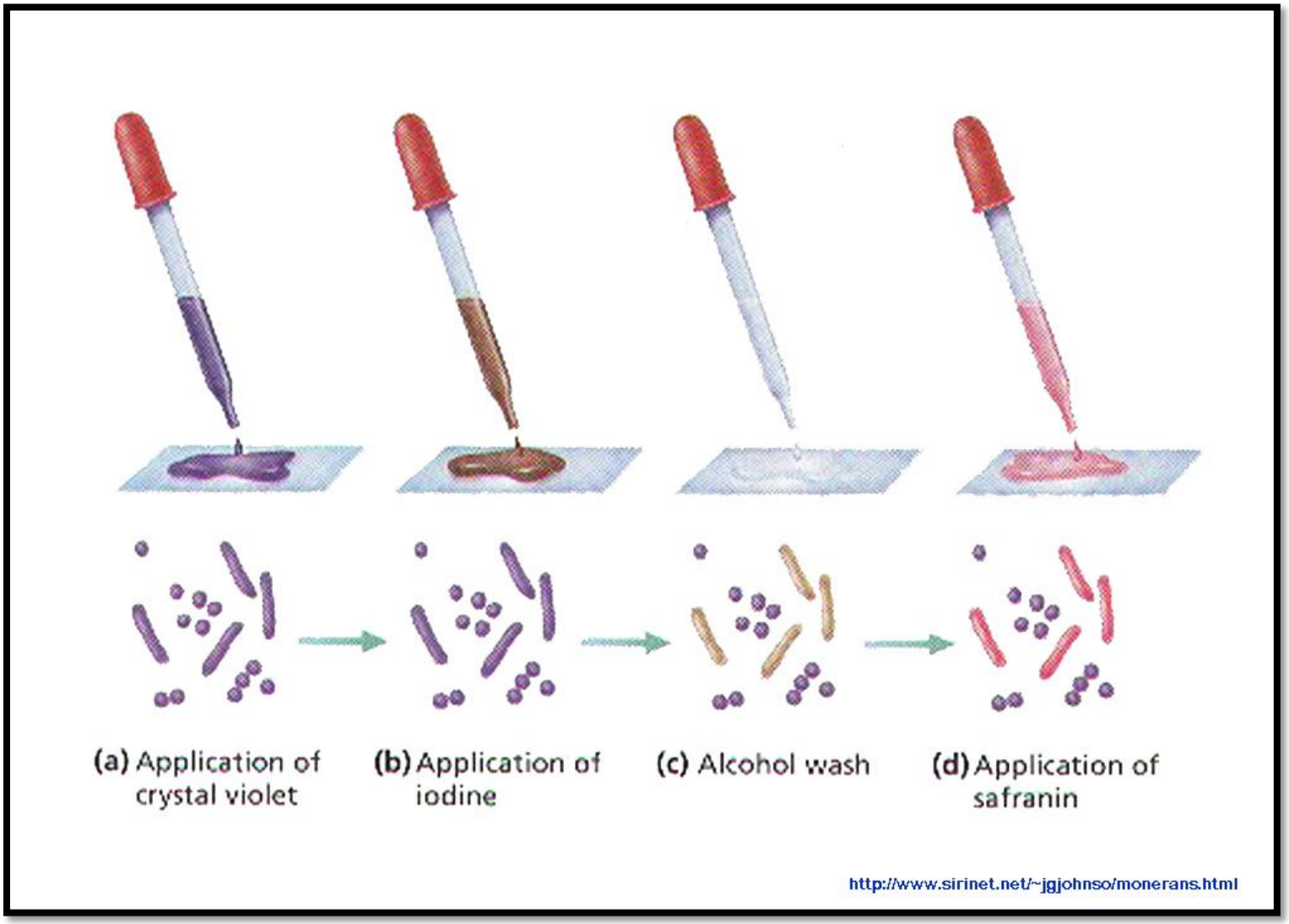
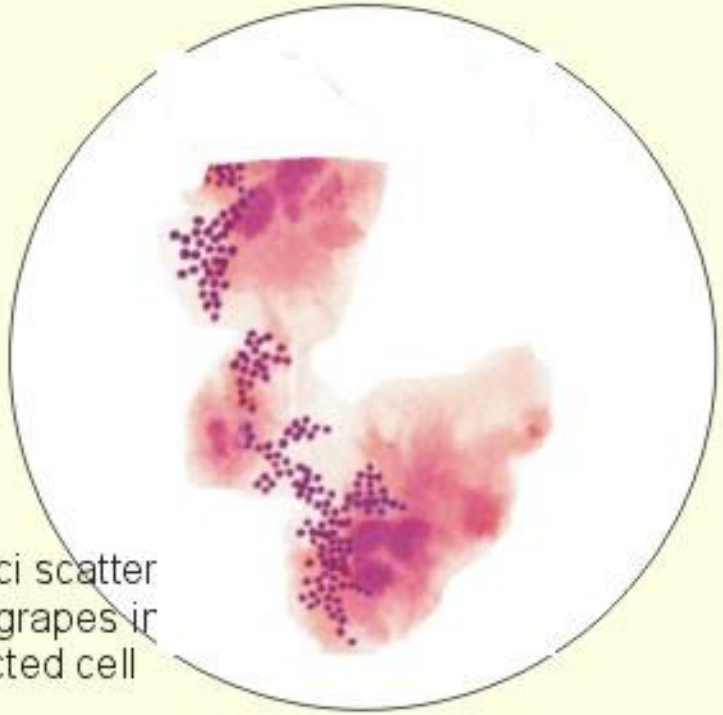

BACTERIA
Gram Positive
Gram Negative
Cocci
Bacilli
Bacilli
Cocci
Coccobacilli
- Staph
- Strept
- Clostridium
- Corynebacterium
- Enterobacteriacea
- Pseudomonas
- Vibrio
- Neisseria
- Maroxella
- Heamophilis
- Chlamydia
- Brucella
- Bordetella
- Acinetobacter

Gram Stain Procedure



Identification of Gram Positive Cocci

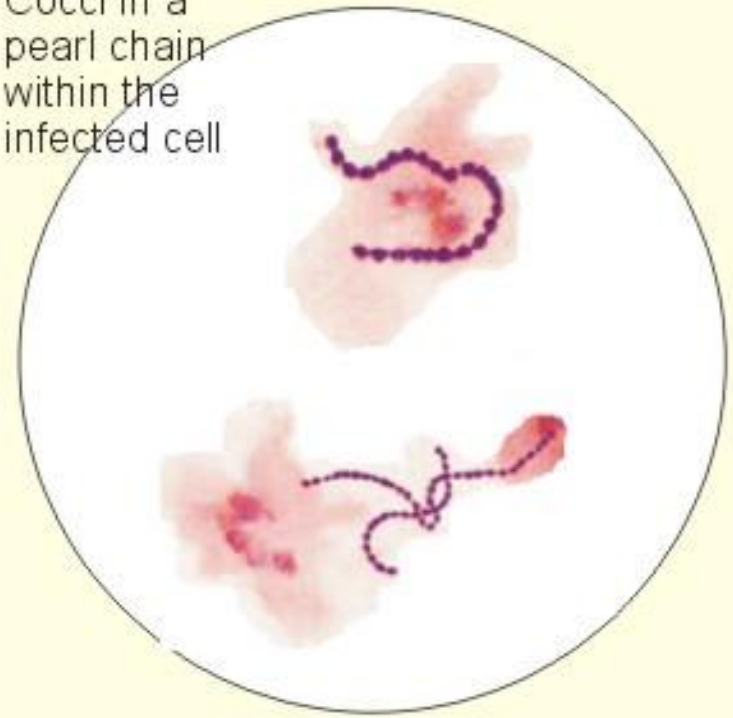
Microscopic Pictures Of Cocci



Cocci scatter like grapes in infected cell

Stafylococci in pus
Therapy: Penicillin

Cocci in a pearl chain within the infected cell



Streptococci in pus
Therapy: Phenoxy-methylpenicillin

Fig. 33-2

KMc

Culture of Staphylococci

- Staphylococci grow well aerobically and in CO_2 enriched atmosphere. Most strains also grow anaerobically.
- Temperature range for growth is 10–42°C, with an optimum of 35–37°C.

Blood agar, chocolate agar:

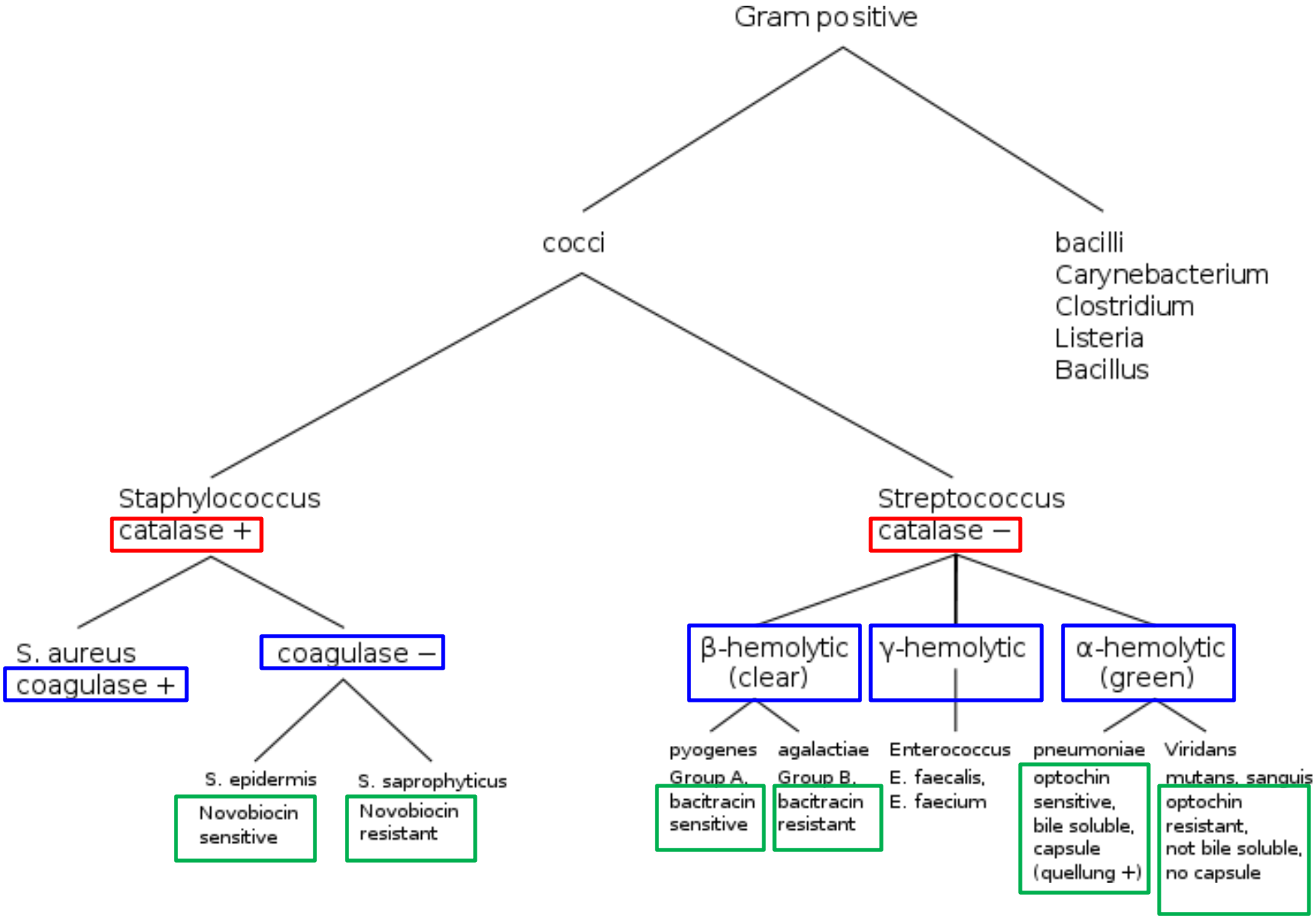
S. aureus produces yellow to cream or occasionally white (1–2 mm in diameter) colonies after overnight incubation. Some strains are beta haemolytic when grown aerobically. Colonies of *S. epidermidis* are white and usually non-haemolytic. The colonies of *S. saprophyticus* may be white or yellow.

MacConkey agar:

Smaller (0.1–0.5 mm) colonies and most strains are lactose fermenting. Growth may not occur on MacConkey agar for *S. epidermidis* and *S. saprophyticus*.

Mannitol salt agar:

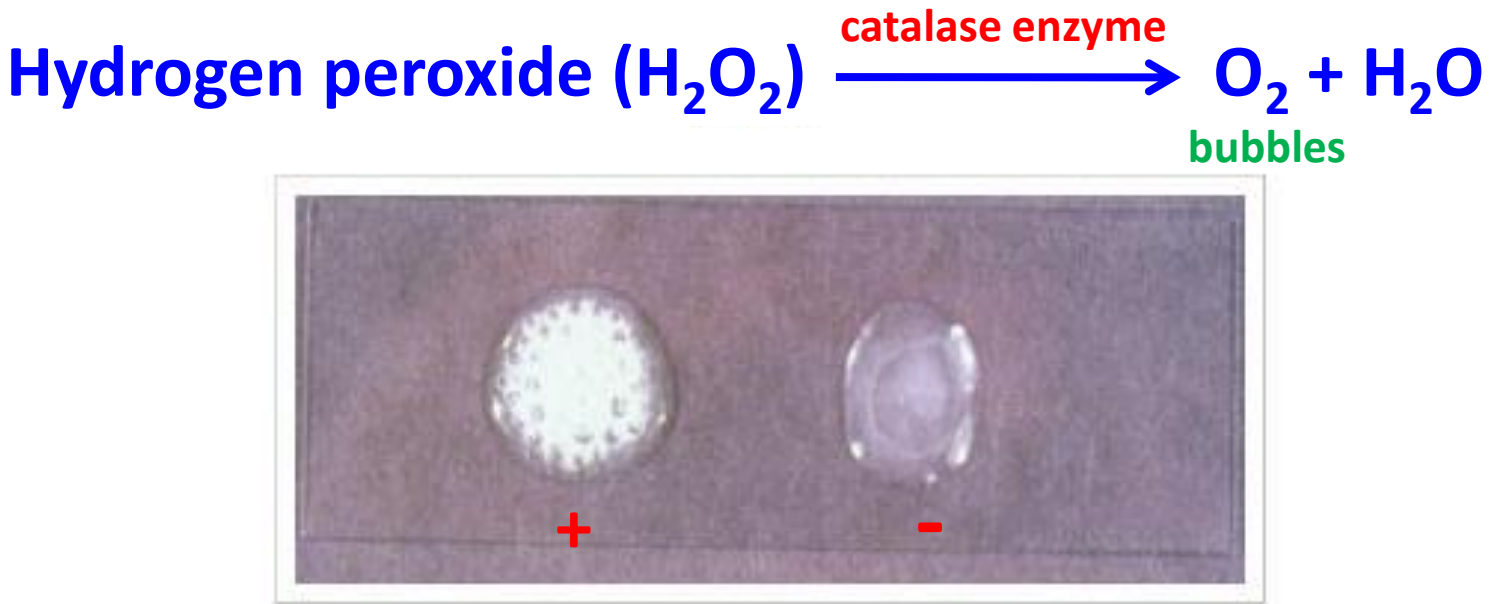
A useful selective medium for recovering *S. aureus* from faecal specimens when investigating staphylococcal food-poisoning.


Gram positive
cocci
bacilli
Carynebacterium
Clostridium
Listeria
Bacillus
Staphylococcus
catalase +
Streptococcus
catalase −
S. aureus
coagulase +
coagulase −
β-hemolytic
(clear)
γ-hemolytic
α-hemolytic
(green)
S. epidermis
Novobiocin
sensitive
S. saprophyticus
Novobiocin
resistant
pyogenes
Group A,
bacitracin
sensitive
agalactiae
Group B,
bacitracin
resistant
Enterococcus
E. faecalis,
E. faecium
pneumoniae
optochin
sensitive,
bile soluble,
capsule
(quellung +)
Viridans
mutans, sanguis
optochin
resistant,
not bile soluble,
no capsule

Catalase

With an inoculating loop, pick a small colony and swab it onto a glass slide. Then, add 2-3 drops of hydrogen peroxide to the colony. A catalase positive test will show bubbles form immediately.

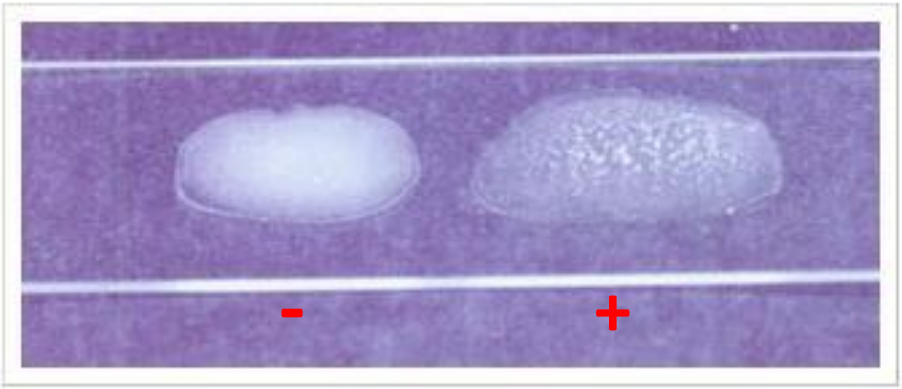
The catalase test differentiates the *Staphylococci*, which are positive, from the *Streptococci*, which are negative. When a catalase test is positive, it will produce bubbles. This is because *Staphylococci* produce catalase that will convert hydrogen peroxide to water and oxygen, producing bubbles.



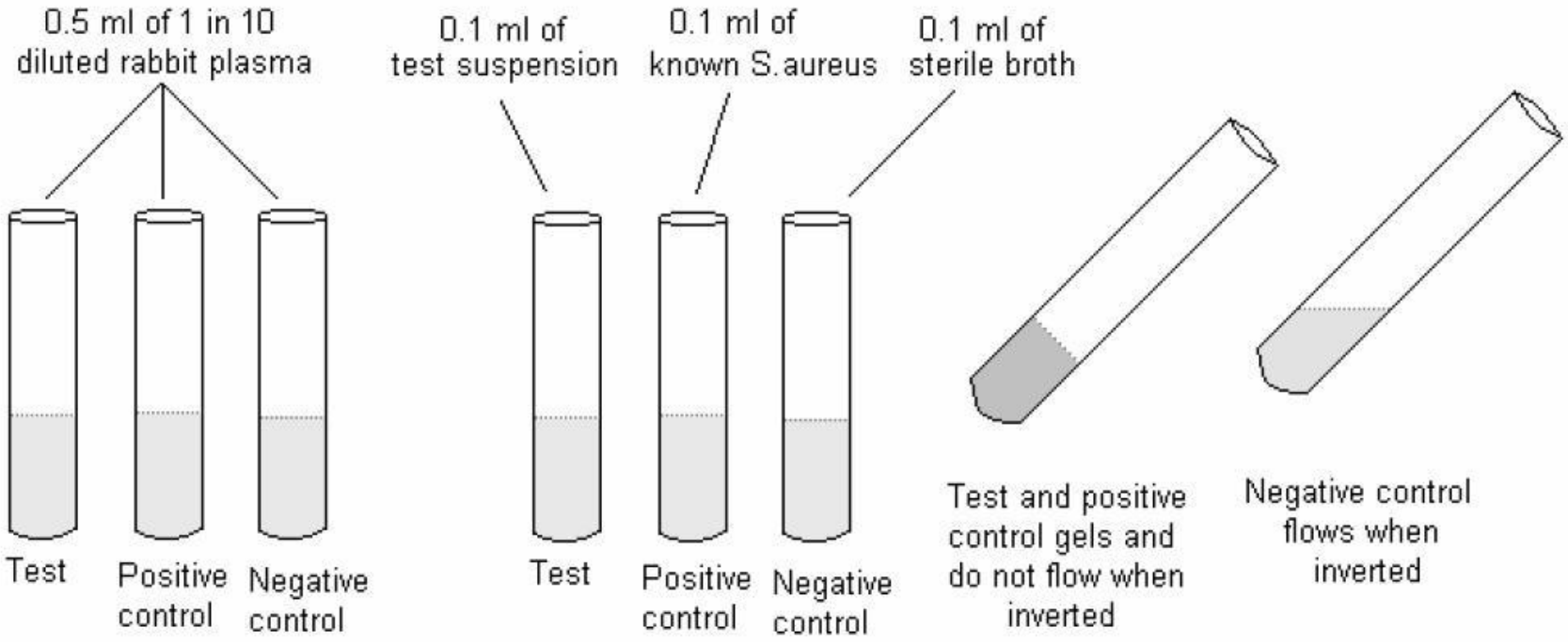
Coagulase

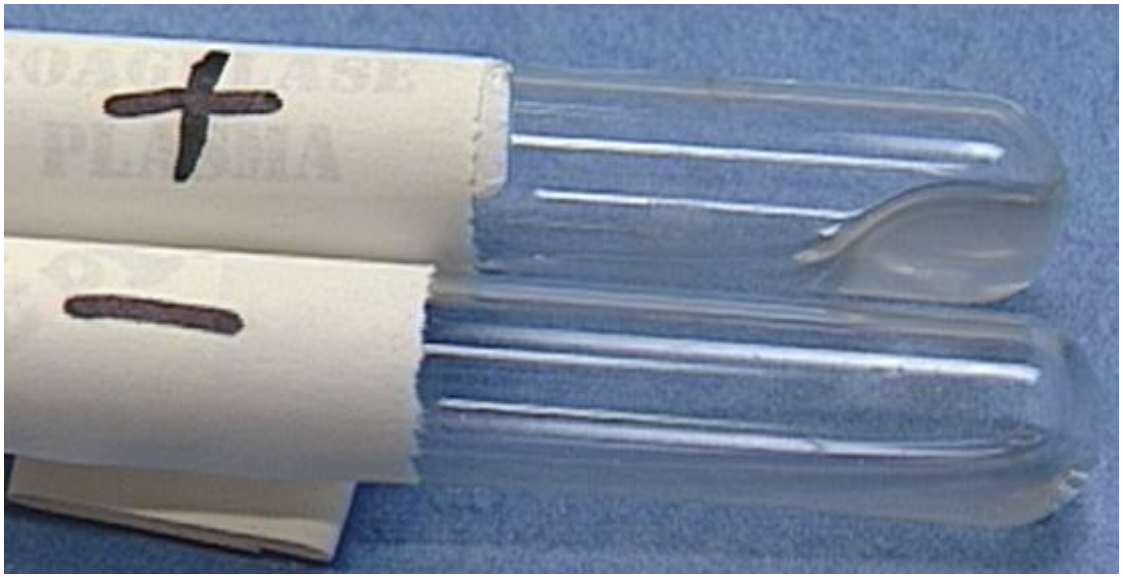
With an inoculating loop, pick a small colony and swab it onto a glass slide. Then, add 2-3 drops of coagulase to the colony. Mix the coagulase with the colony well and observe for agglutination. When a catalase is positive, proceed to do a coagulase test to differentiate *S.aureus* from other *Staphylococci*. *S.aureus* produce coagulase, an enzyme-like protein that clots citrated plasma. Coagulase will bind to prothrombin and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of *Staphylococci*. When this happen, *S. aureus* will produce a clumping factor on its surface for the organism's adherence to fibrinogen and fibrin. Agglutination indicates coagulase positive, indicating that the organism is most likely a *S. aureus*. Coagulase negative suggest it might be other species of staphylococci. In the case of UTI, it might be a *S. saprophyticus*.

Plasma Fibrinogen —(coagulase enzyme)→ **Fibrin Clot**



TUBE COAGULASE TEST





DNase Test

Test Principle

1. DNA —(DNA-ase enzyme)→ hydrolysed DNA (appear as clear zone) = +ve
2. HCL ——→ Precipitate unhydrolysed DNA = -ve

1. Inoculate plates by spotting or streaking a heavy inoculum of test organism. Use a spot approximately 5 mm in diameter or a 1 – 2 cm streak approximately 5 mm wide.
2. Incubate plates at 35 ± 2°C for 18 – 24 hours and up to 48 hours.
3. Flood plates with 1 N HCl. Tip off excess acid.

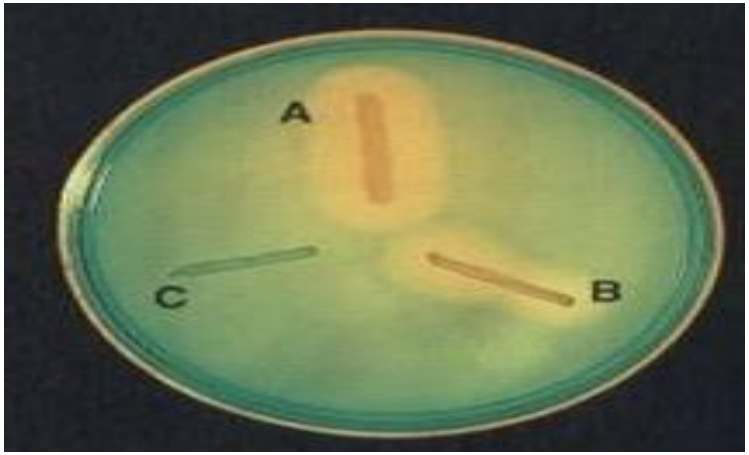
Results

A zone of clearing around the spot or streak indicates DNase activity.

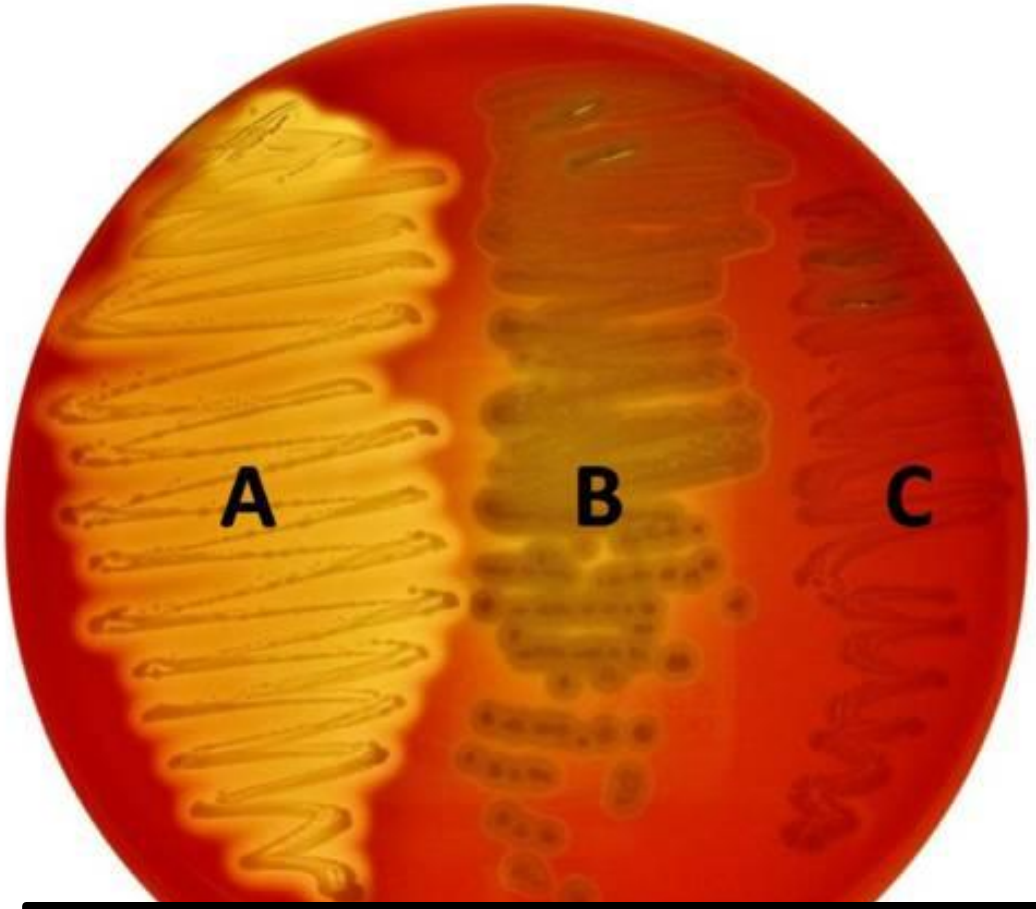
+ve result: *S. aureus*

week +ve *S. epidermidis*

-ve result: *S. saprophyticus*



Types of Haemolysis on BA

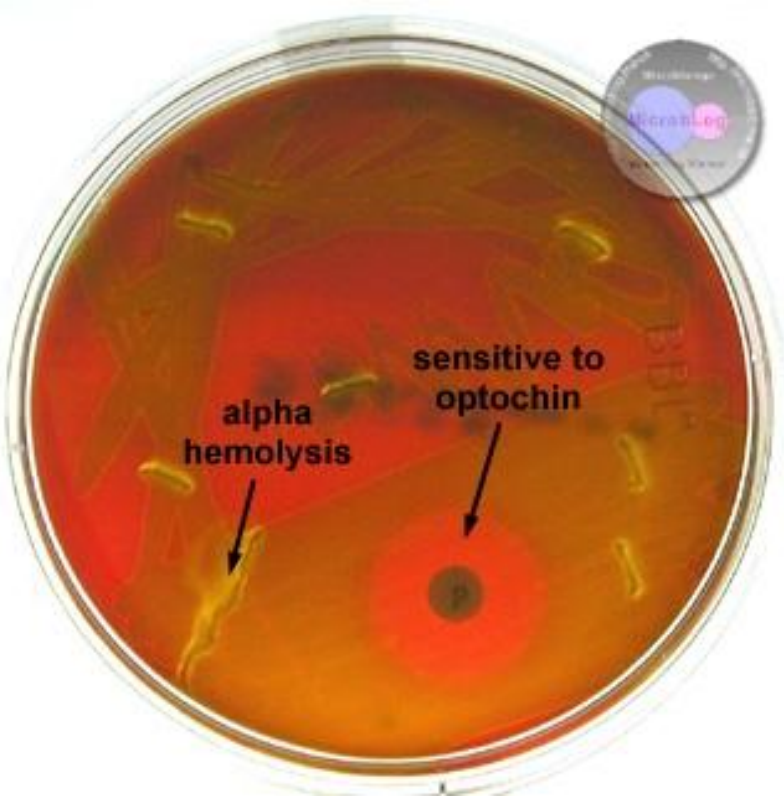


Beta = Clear Alpha = Partial greenish Gamma = No

To Differentiate α-Haemolytic *Streptococci*

Add an Optochin disc to the blood agar plate within the area of 2nd spread before incubation. Incubate and describe inhibition zone.

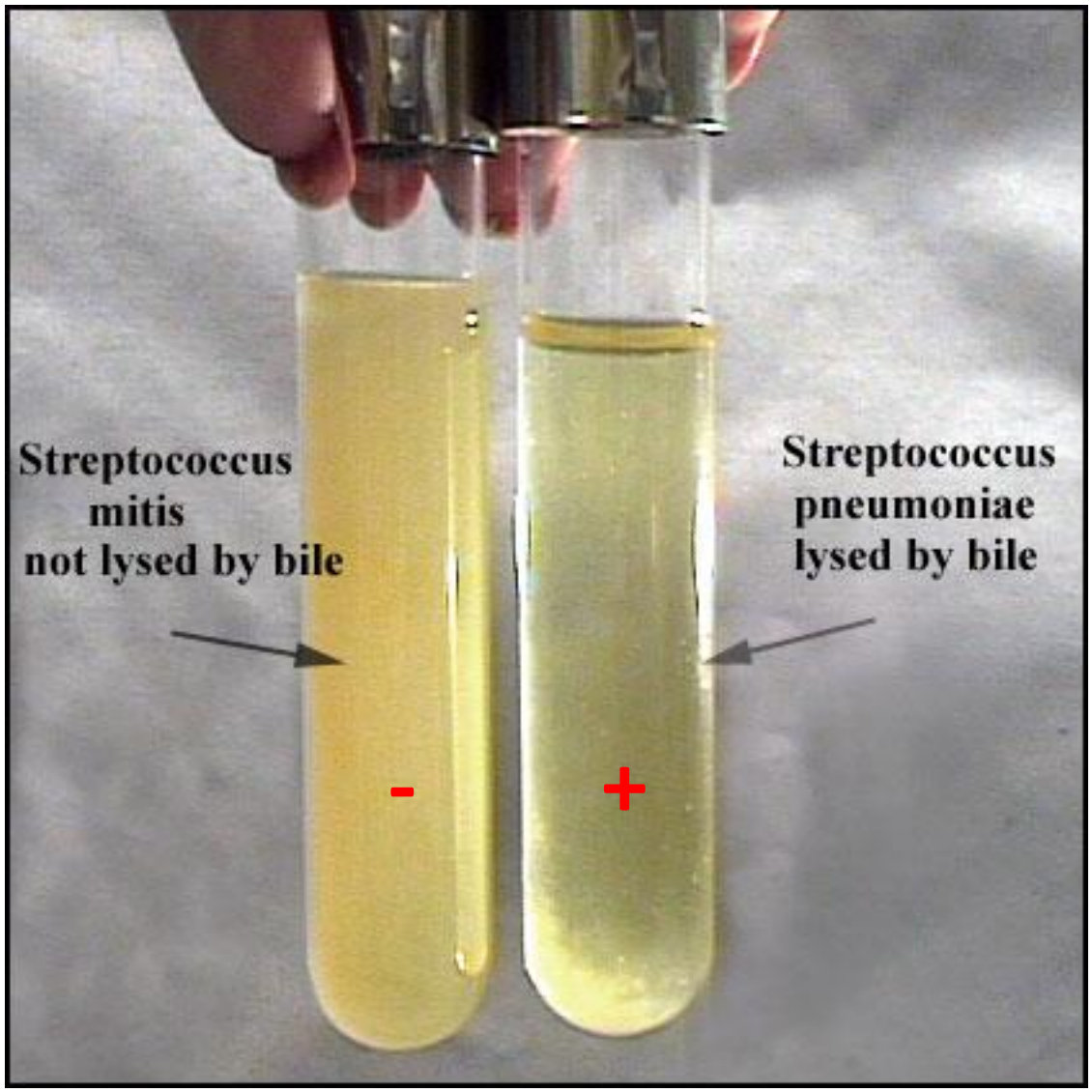
- *S. pneumoniae* is Sensitive (14mm zone of inhibition)
- *S. viridans* is Resistant



Bile Solubility Test

When a bile salt (such as Na- deoxycholate) is added directly to *S. pneumoniae* growing on an agar plate or in a broth culture the bacteria will lyse and the area become clear.

Other alpha-hemolytic strept are resistant to (not lysed by) bile and will stay visible or turbid (cloudy).



To Differentiate β-Haemolytic *Streptococci*

Swab the bacterial suspension on blood agar plate , add a Bacitracin disc then incubate.

- *Strept group A* is Sensitive
- *Strept group B* is Resistant

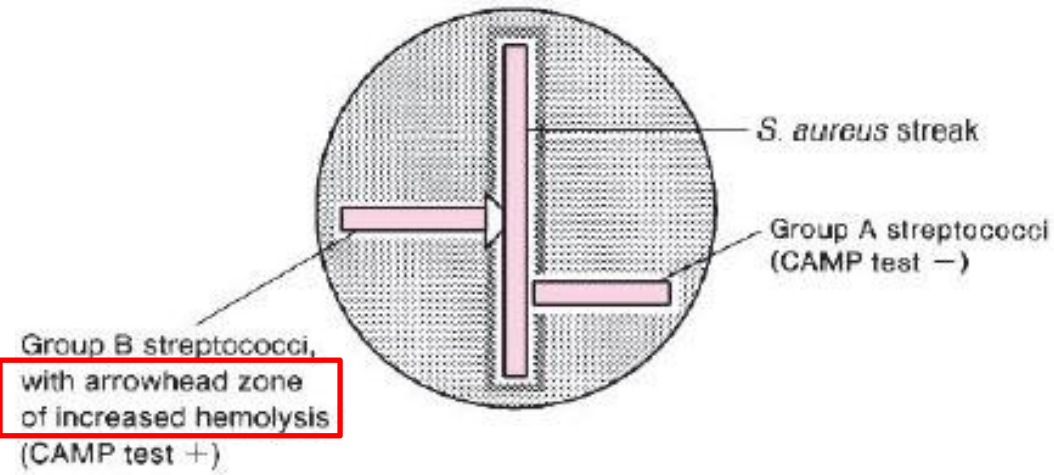
CAMP Test

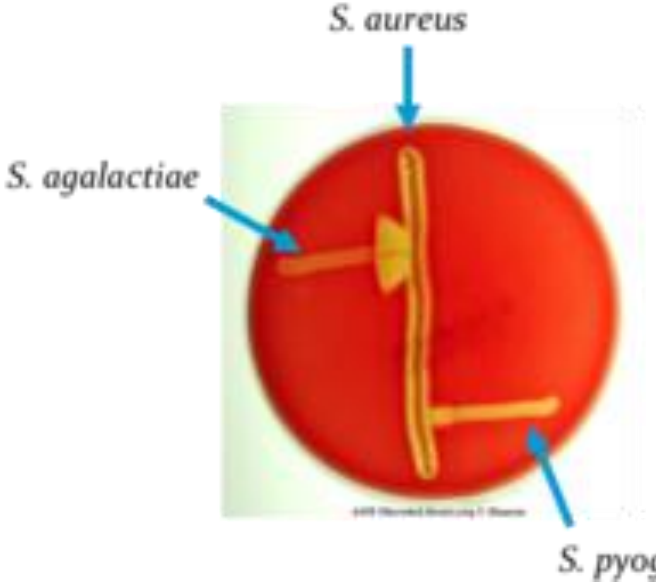
CAMP test to identify *Strept agalactiae*

Principle

This test requires the use of a beta-lysin producing strain of *S. aureus* to detect the CAMP factor produced by *S. agalactiae.* This protein factor interacts with the staphylococcal beta-lysin on sheep blood agar producing enhanced haemolysis.

+ve test: Arrowhead zone of haemolysis.





Hippurate Hydrolysis Test

S. agalactiae hydrolyzes hippurate

Principle

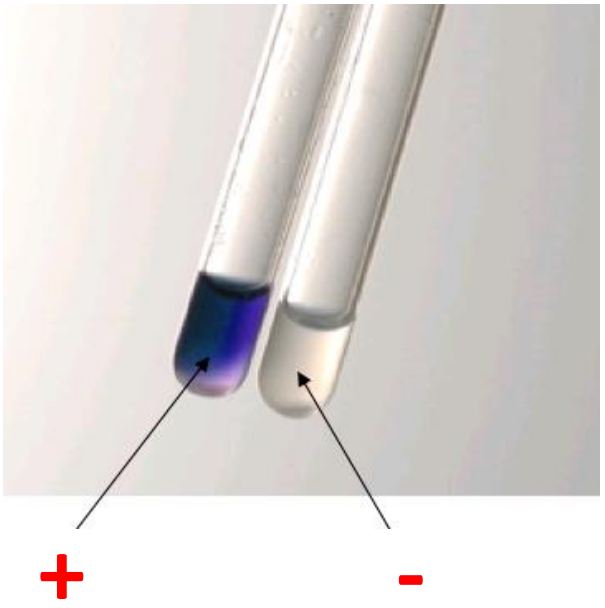
Hippurate acid $\xrightarrow{\text{hippuricase}}$ Benzoic acid + Glycine

The glycine end product is detected by the addition of ninhydrin reagent.

Positive result:

deep purple color within

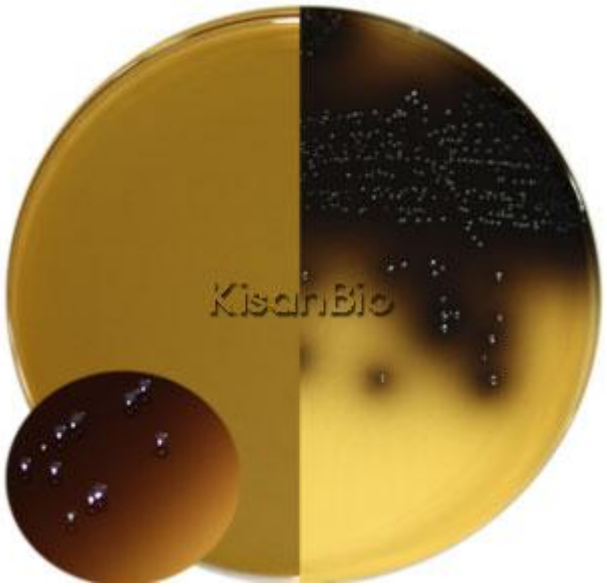
5-10 minutes.



Bile Esculin Test

To identify non-haemolytic Group D *Strept* (*Enterococcus*)

Bile esculin agar slants contain bile salts, esculin, ferric ammonium citrate, and beef and gelatin extracts. Organisms that are able to hydrolyze the carbohydrate esculin in the presence of bile will produce glucose and esculetin. The esculetin reacts with ferric ammonium citrate to form a blackish precipitate. The black precipitate must be in >50% of the tube to be considered positive.



Tube 1: **Positive** for esculin hydrolysis in the presence of bile. Note black precipitate in > 50% of tube.

Tube 2: **Negative** for esculin hydrolysis in the presence of bile.

Serology Testing for *Streptococci* Agglutination Test

1. Lancefield grouping: to differentiate beta-haemolytic Strept into groups A,B,C, D & G.
2. Differentiate group A beta-haemolytic Strept into types.
3. Differentiate alpha-haemolytic Strept pneumoniae into 80 capsular serotypes.

Identification of Gram Negative Cocci

Neisseria meningitidis

- Gram negative diplococci inside and outside pus cells.
- Grey colonies on Chocolate & Blood Agar in CO_2.
- Catalase test +ve / Oxidase test +ve.
- DNAse test -ve.
- Carbohydrates Utilization test (Hiss Media):

  glucose & maltose +ve

  sucrose & lactose –ve
- **3 Serology testing:** capsular serogroups A, B, C, W135 + protein subtypes + protein serotypes.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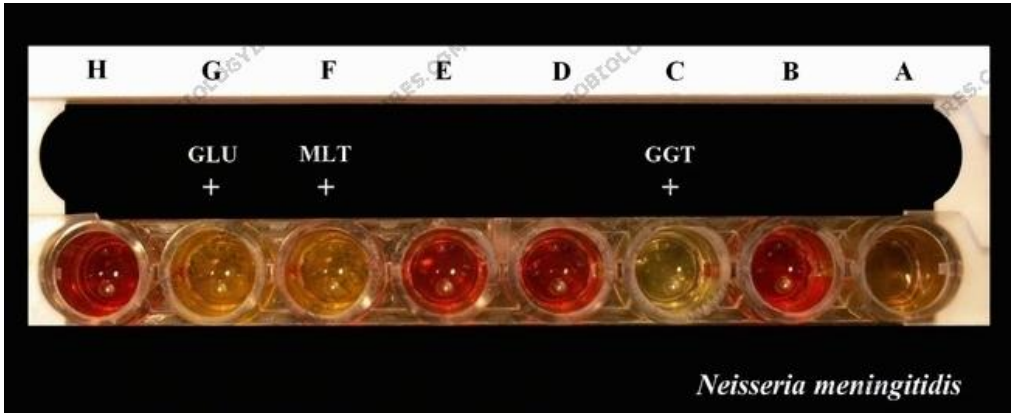
GRAM-NEGATIVE
DIPLOCOCCI
OXIDASE TEST
POSITIVE
Neisseria meningitidis
VANCOMYCIN RESISTANT
HansN.

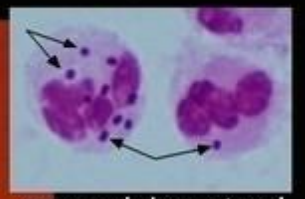

H
G
F
E
D
C
B
A
GLU
+
MLT
+
GGT
+
Neisseria meningitidis

Neisseria gonorrhoeae

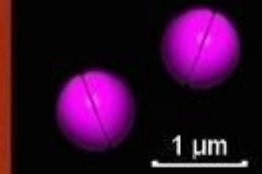
- Gram negative diplococci mostly inside pus cells.
- Grey colonies on Chocolate, modified New York City media (MNYC) in Co_2 or Thayer Martin Media. No growth on blood agar.
- Catalase test +ve / Oxidase test +ve.
- DNAse test -ve.
- Carbohydrates Utilization test (Hiss Media):

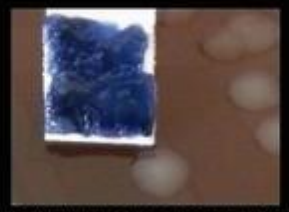
  glucose +ve

  maltose, sucrose, & lactose –ve
- **Serology testing:** W-antigen serogroups (I, II, III) and protein serotypes.

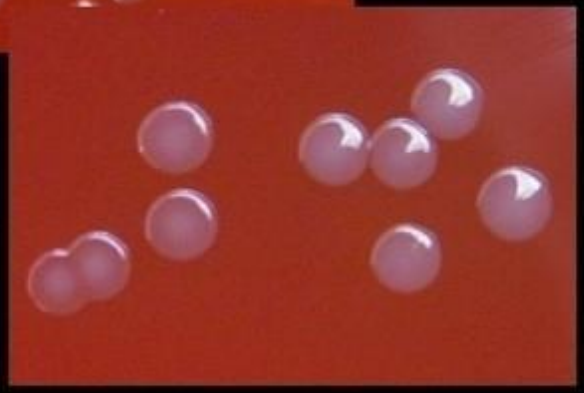


gonococci phagocytosed by polymorphonuclear leukocytes (PMN's)

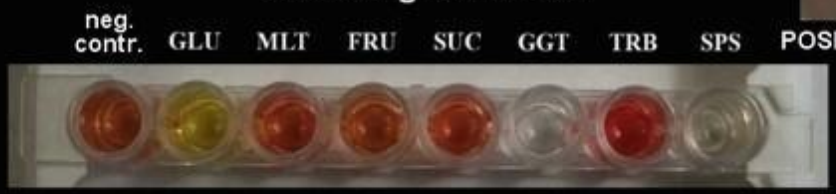




POSITIVE OXIDASE TEST



BIOCHEMICAL TESTS FOR
Neisseria gonorrhoeae

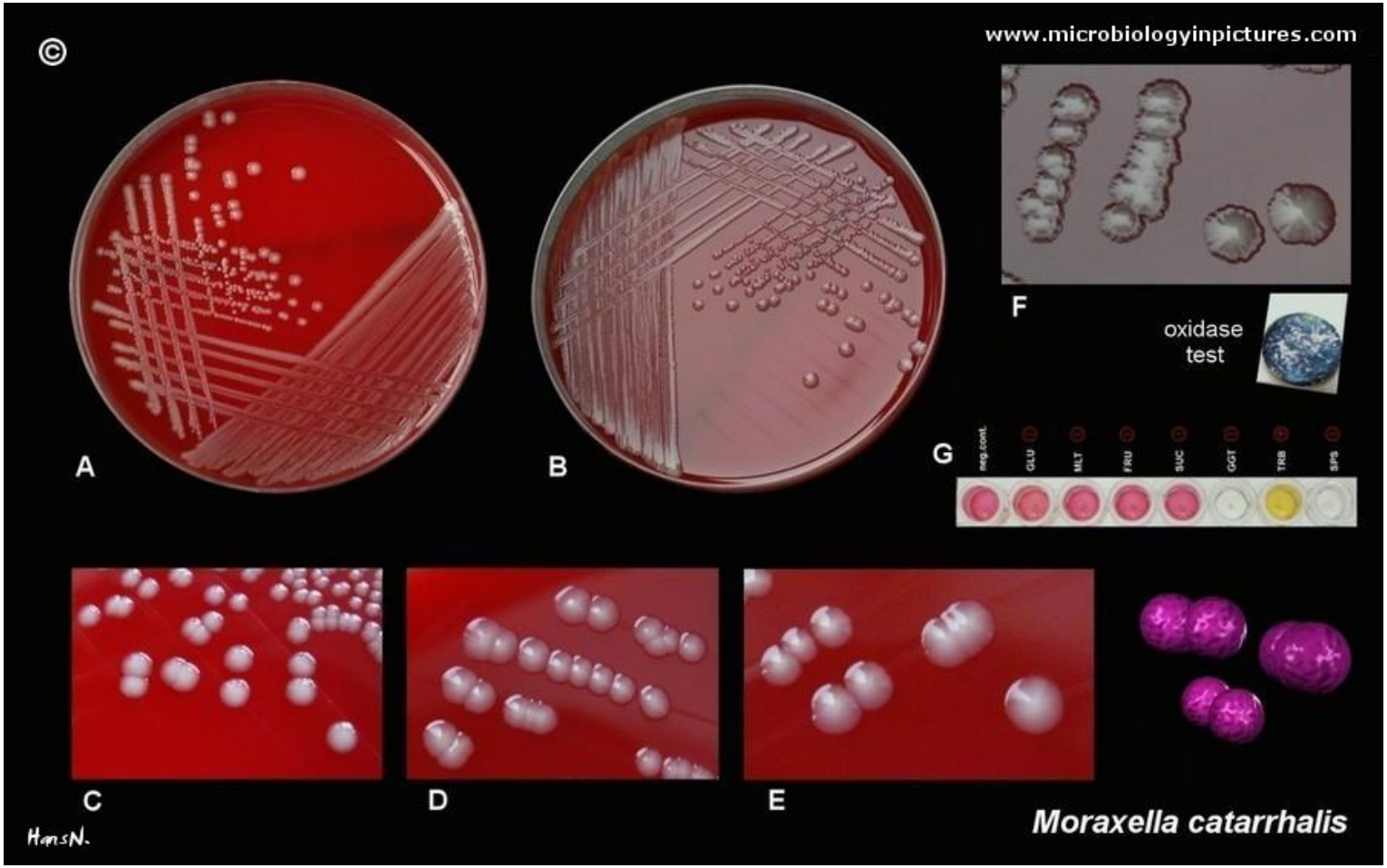


Neisseria gonorrhoeae
cultivation 48 hours, 37°C, 5% CO_2

Moraxella catarrhalis

- Gram –ve diplococci.
- Grey colonies on Chocolate & Blood Agar in CO_2.
- Catalase test +ve / Oxidase test +ve.
- **DNAse test +ve.**
- Carbohydrates Utilization test (Hiss Media):

  glucose, maltose, sucrose & lactose –ve


©
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A
B
F
oxidase
test
G
neg cont.
GLU
MLT
FRU
SUC
GGT
TRE
SPS
C
D
E
HansN.
Moraxella catarrhalis


B. catarrhalis
C
G
M
L
S
DNase
ß-Lac

Oxidase

Oxidase test is done to determine the presence of cytochrome oxidase activity in bacteria. Cytochrome oxidase is an enzyme found in certain bacteria that is able to transfer electrons to oxygen. The enzyme oxidizes reduced cytochrome c to make this transfer of energy. Presence of cytochrome oxidase can be detected through the use of oxidase disk which acts as an electron donator to cytochrome oxidase. If the bacteria oxidize the disk, the disk will turn purple, indicating a positive test. No colour change indicates a negative test. (8)

Identification of Gram Positive Bacilli

Corynebacterium diphtheriae

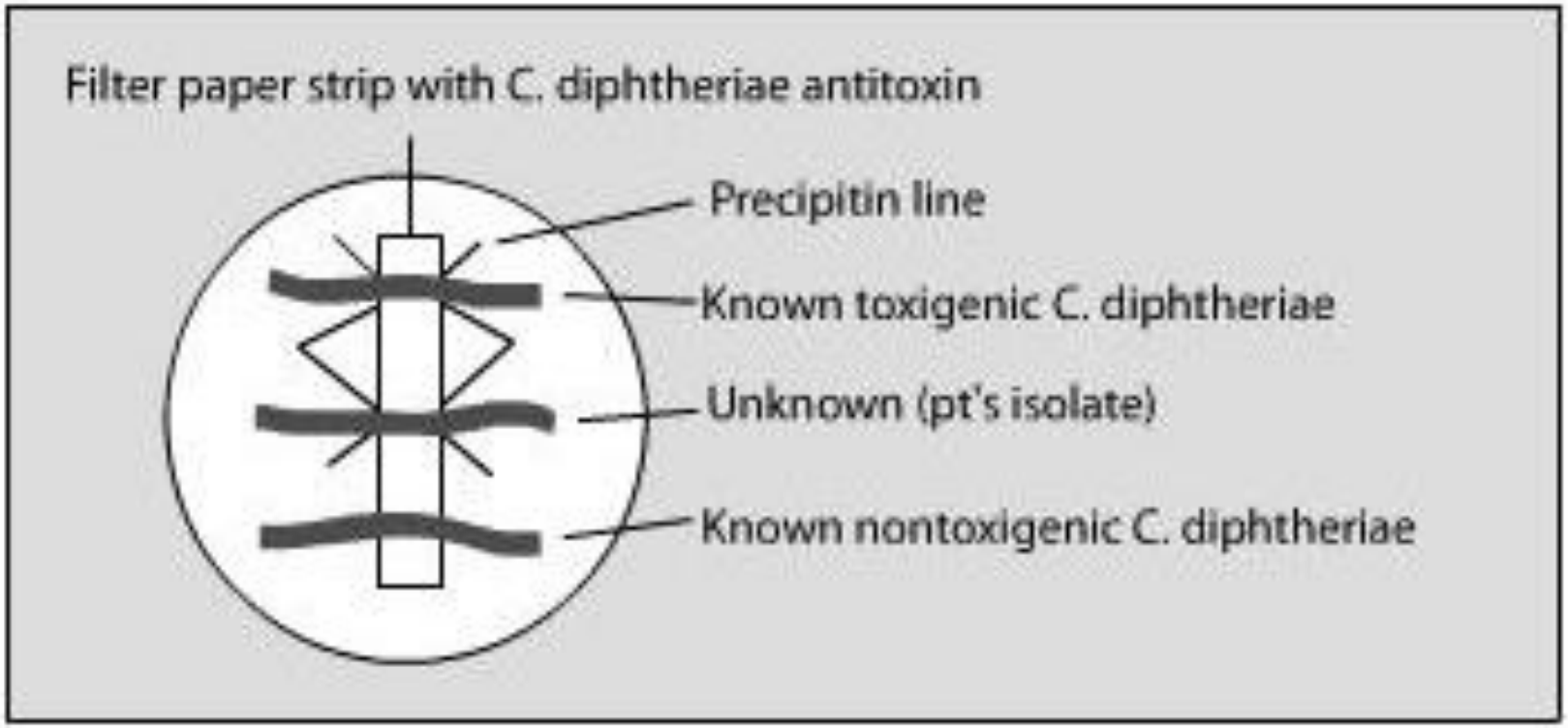
- Gram +ve bacilli arranged as “Chinese letters”.
- Black colonies on Tellurite blood medium and Tinsdale medium.
- Catalase and nitrate +ve.
- Oxidase and Urease -ve.
- Carbohydrates Utilization test (Hiss Media):

  glucose & maltose +ve

  sucrose & lactose –ve
- **Serology testing:** 3 biotypes- gravis, intermedius, and mitis.

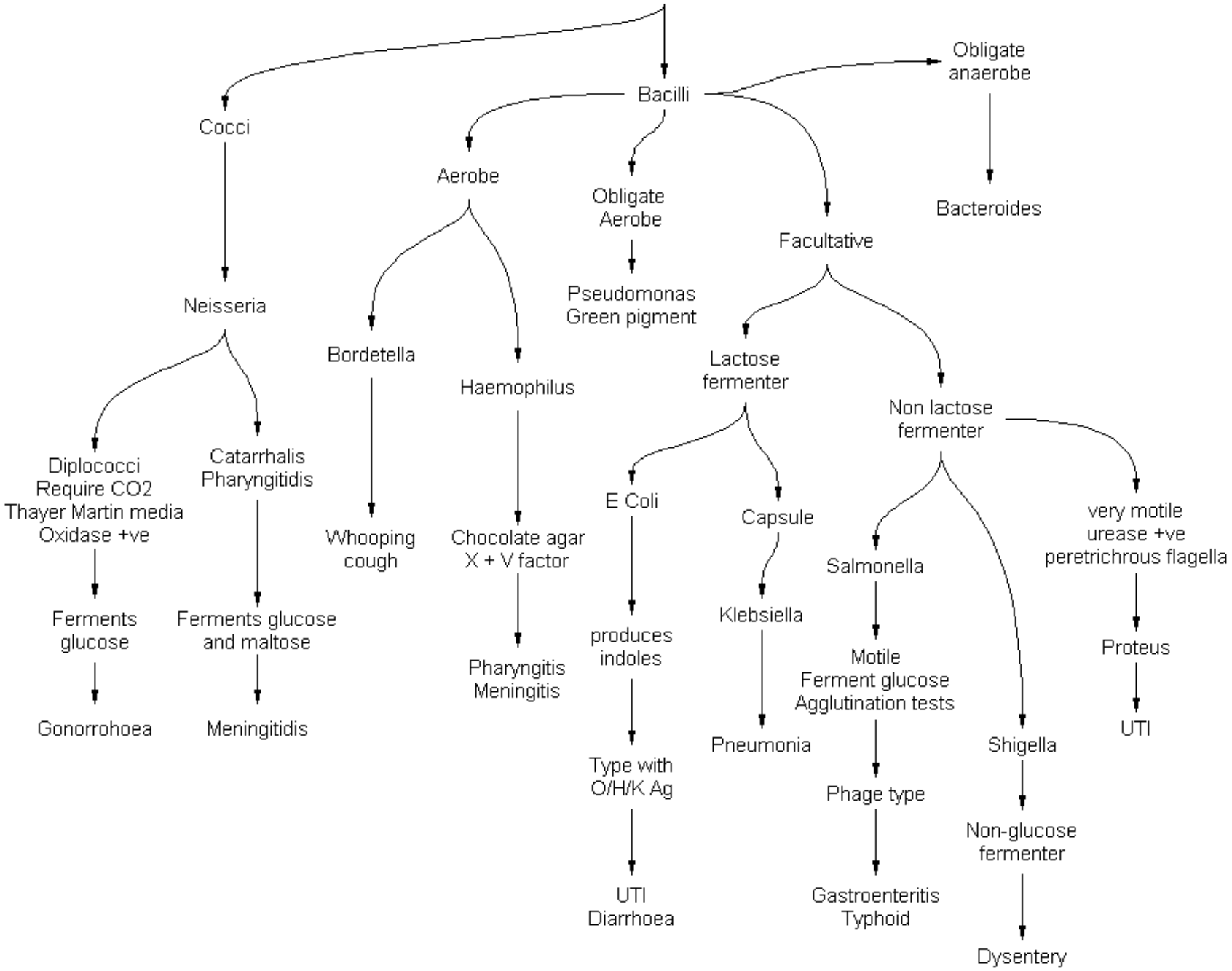
ELEK Test

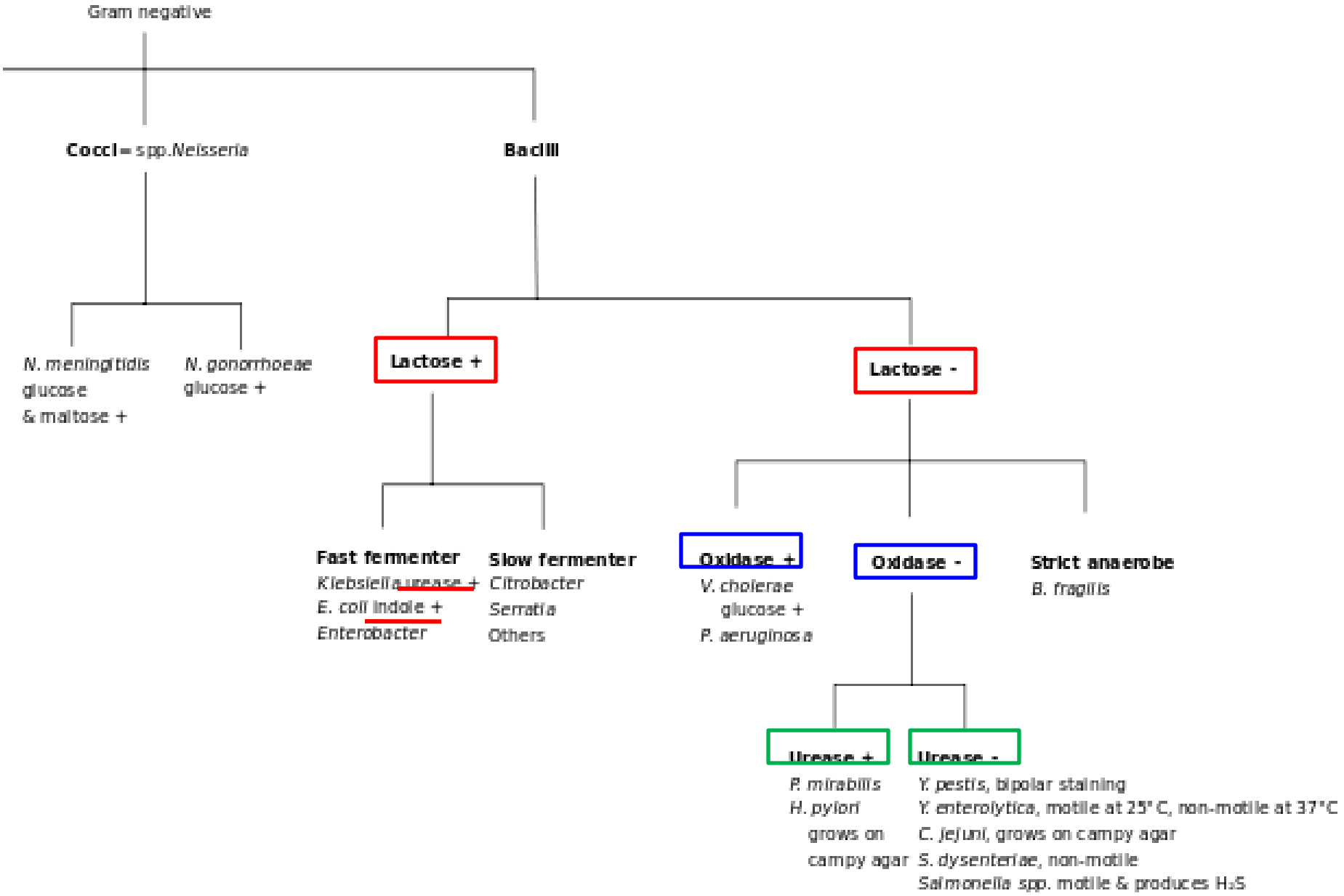
to identify toxin producing *C. diphtheriae* causing the disease diphtheria



Identification of Gram Negative Bacilli

GRAM NEGATIVE BACTERIA

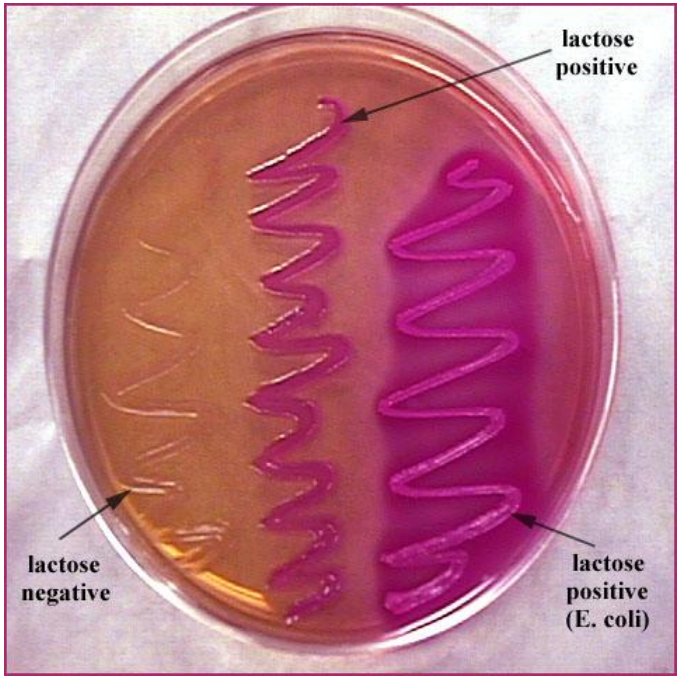



Gram negative
Cocci = spp. Neisseria
Bacilli
N. meningitidis
glucose
& maltose +
N. gonorrhoeae
glucose +
Lactose +
Lactose -
Fast fermenter
Klebsiella urease +
E. coli indole +
Enterobacter
Slow fermenter
Citrobacter
Serratia
Others
Oxidase +
V. cholerae
glucose +
P. aeruginosa
Oxidase -
Strict anaerobe
B. fragilis
Urease +
Urease -
P. mirabilis
H. pylori
grows on
campy agar
Y. pestis, bipolar staining
Y. enterolytica, motile at 25°C, non-motile at 37°C
C. jejuni, grows on campy agar
S. dysenteriae, non-motile
Salmonella spp. motile & produces H2S

Lactose Fermentation

Grow Gram negative bacteria on MacConkey Agar @35±2°C overnight or for 24hours.

- Lactose fermenters = Pink colonies
- Non-Lactose fermenters = Colorless colonies



Identification of *Enterobacteriaceae*

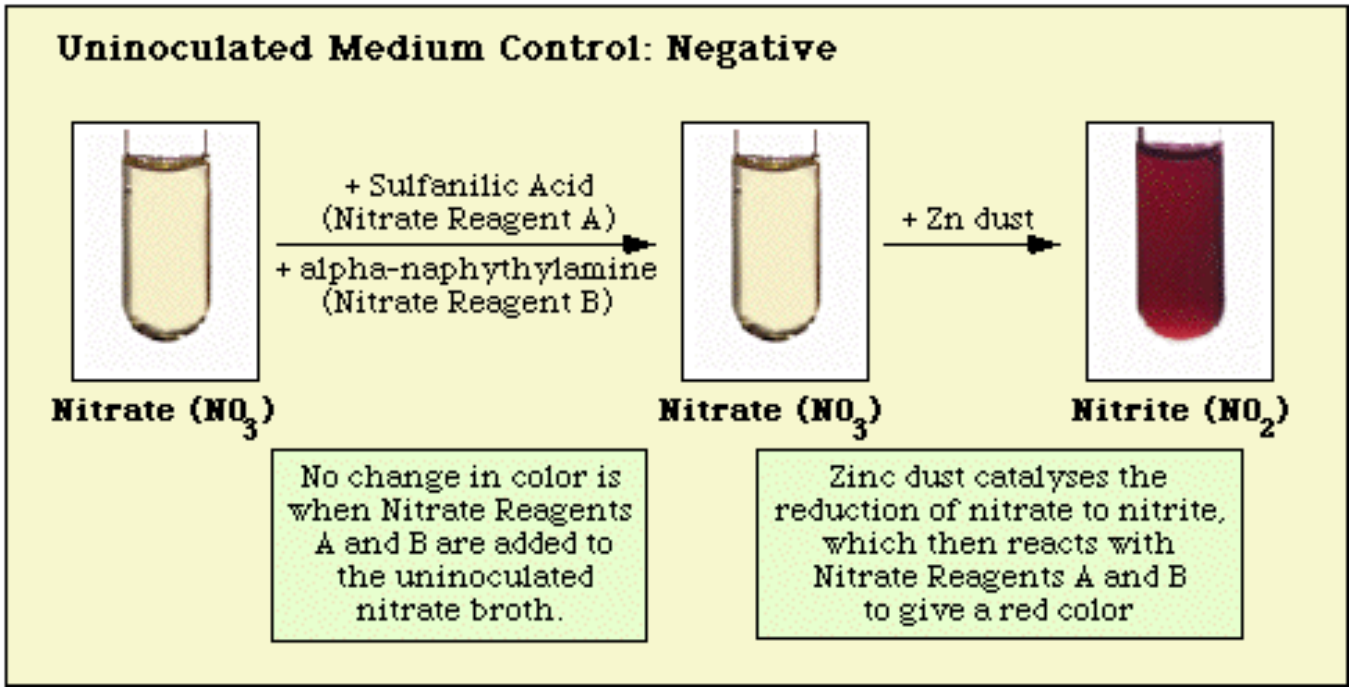
All are: facultative anaerobes, ferment glucose, Oxidase –ve, Nitrate +ve

	Urea	MR	VP	Motility	Indole	TSI			
						Slope	Butt	H_2S	Gas
E. coli	-	+	-	+	+	A	A	-	+
Klebsiella pneumonia	+	-	+	-	-	A	A	-	+
Shigella sp.	-	+	-	-	-/+	K	A	-	-
Salmonella typhi	-	+	-	+	-	K	A	+	-
Salmonella paratyphi	-	+	-	+	-	K	A	-	+
Salmonella sp.	-	+	-	+	-	K	A	+	-/+
Proteus vulgaris	+	+	-	+	+	K	A	+	-/+
Proteus mirabilis	+	-/+	-/+	+	-	K	A	+	+

Nitrate Test

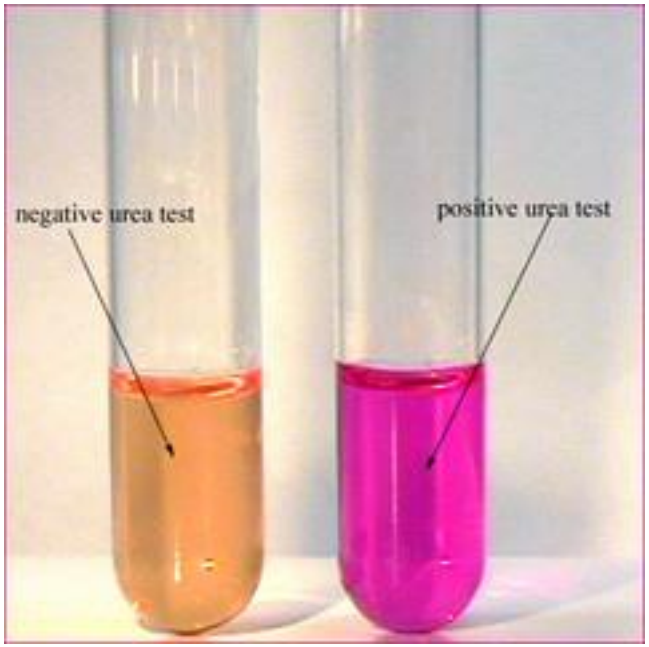
Nitrate (NO3) $\xrightarrow{\text{nitrate reductase}}$ Nitrite (NO2)

Nitrous acid reacts with reagent 1 then reagent 2 and finally form a red color.



Urease Test

- The test organism is inoculated into a urea broth that contains phenol red, a pH indicator, and has a pH of 6.8.
- At this pH phenol red is salmon color. However, when the pH rises above 8.1 phenol red turns into pink in color.
- Positive result: organisms that produce urease will change color of tube to pink.
- Negative result: organisms that are unable to synthesize urease is indicated by the continuance of a salmon color in the urea broth.
- The urease test is useful for differentiating *Salmonella* , which is urea negative, from *Proteus* , which is urea positive.

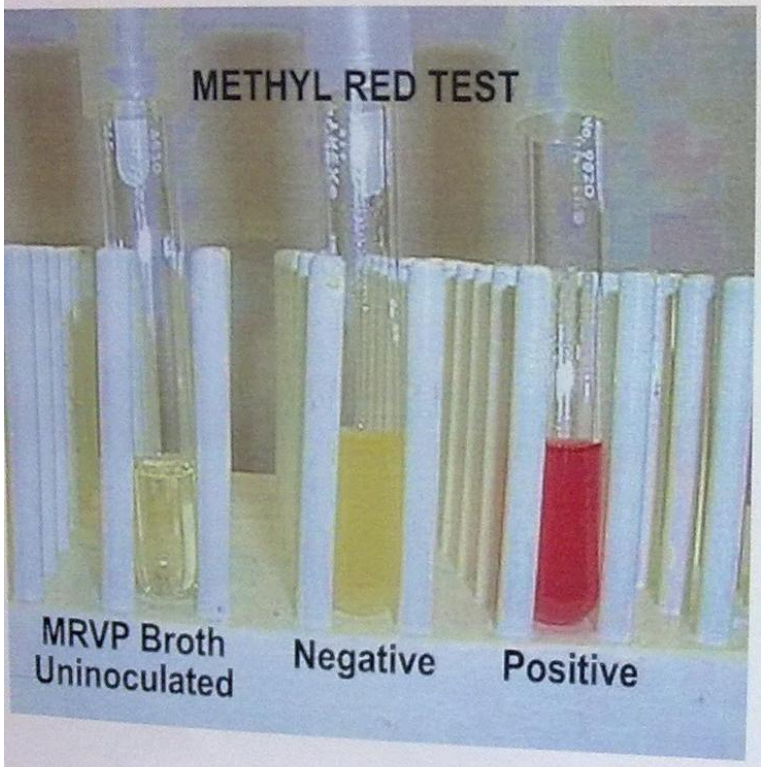


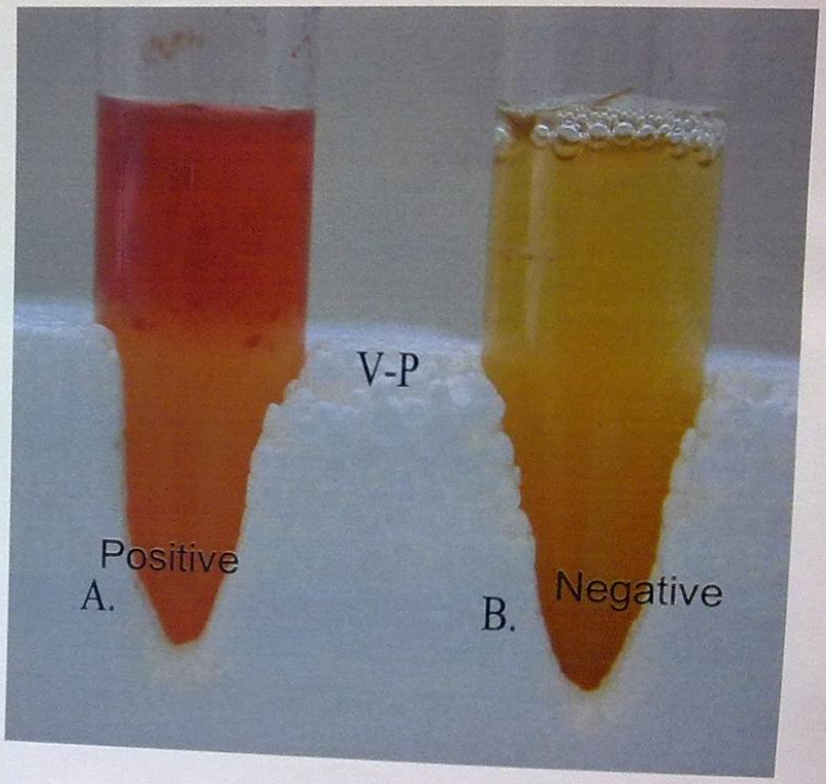
MR-VP Test

- Members of Enterobacteriaceae convert glucose to pyruvate.
- Some bacteria metabolize pyruvate and produce acidic end products (pH < 4.4).
- Other bacteria metabolize pyruvate and produce neutral end products (pH > 6.0) = acetoin.
- In the MR test the pH indicator, methyl red, detects acidic end products.
- In the VP test, acetoin is oxidized into red color.
- **Methyl Red (MR) Test:**

  Positive – bright red color; Negative – yellow-orange color.
- **Voges-Proskauer (VP) Test:**

  Positive – red color, Negative – no red color.


METHYL RED TEST
MRVP Broth
Uninoculated
Negative
Positive


V-P
A.
Positive
B.
Negative

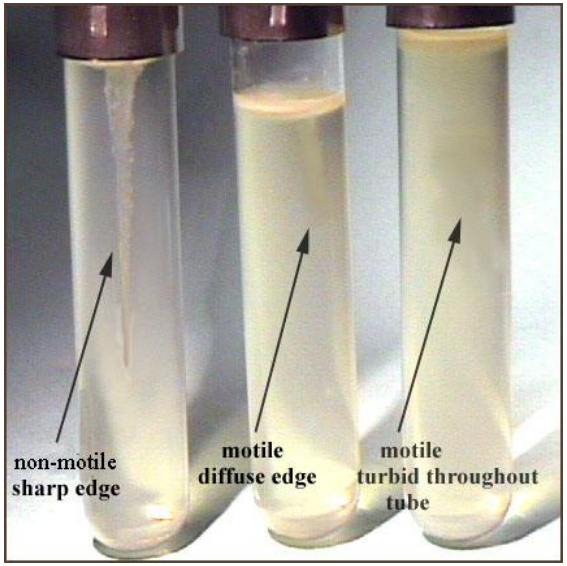
Motility Test

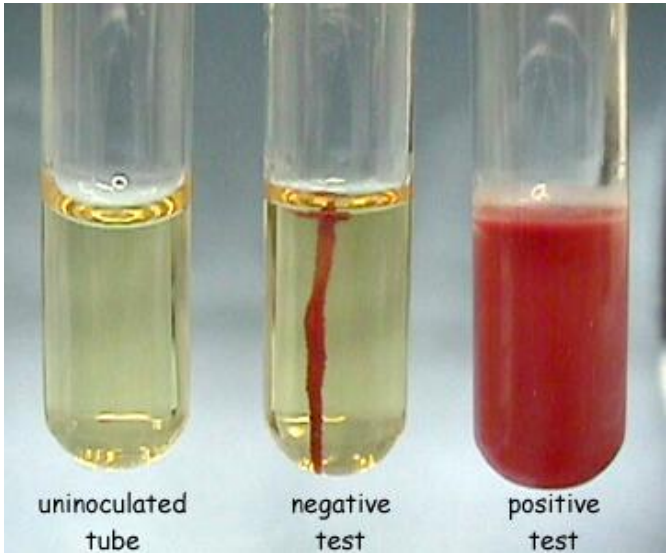
Test Procedure

Inoculate tubes by stabbing through center of the medium with inoculating needle to approximately one-half the depth of the medium. Incubate @37°C for 24hours.

Results

Motility is observed visually by diffuse growth spreading from the line of inoculation.

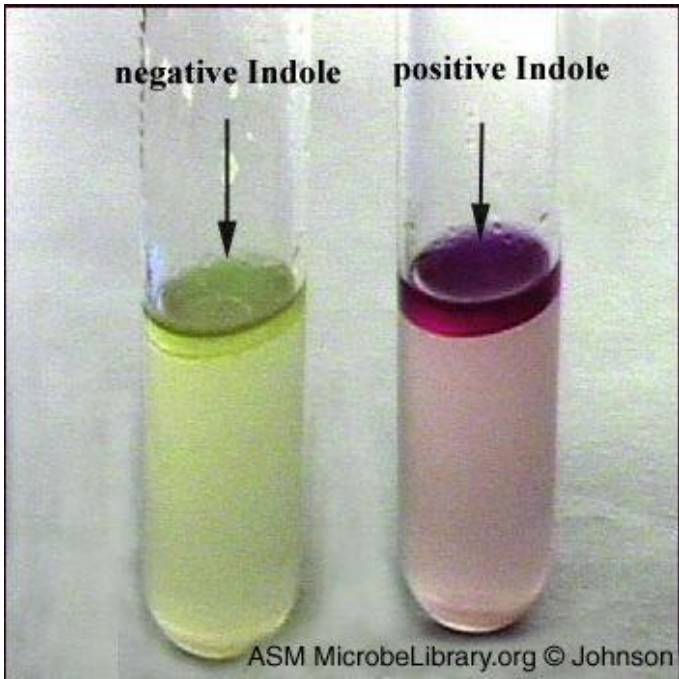




Indole Test

H_2O + Tryptophan $\xrightarrow[\text{enzyme}]{\text{tryptophanase}}$ Indole + Pyruvate + Ammonium

- The test organism is inoculated into tryptone broth, a rich source of the amino acid tryptophan.
- After 48hours of incubation, add Kovac's reagent.
- **Positive result:** dark pink color develops.
- *E. coli* is indole positive.



TSI= Triple Sugar Iron Agar

Principle

Differentiation of bacteria on the basis of dextrose, lactose, and sucrose fermentation and hydrogen sulfide production.

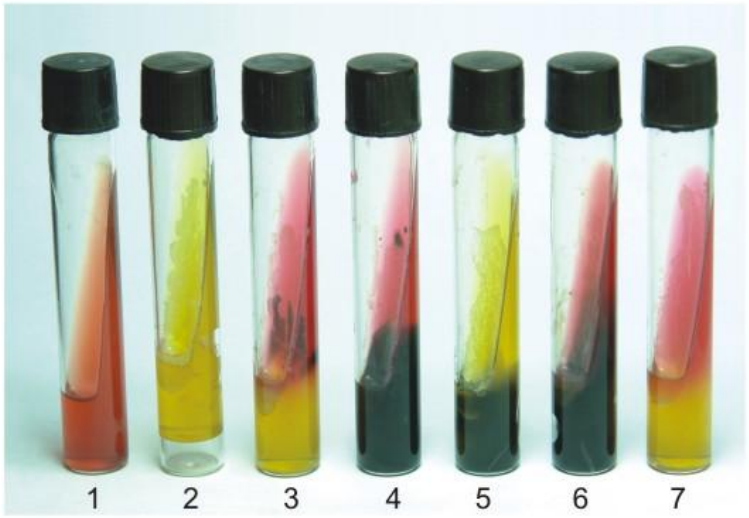
- Sugar fermentation= acid production (Yellow color), No fermentation= alkaline (Red color).
- Sodium Thiosulfate is reduced to hydrogen sulfide, and hydrogen sulfide reacts with an iron salt yielding the typical black iron sulfide.

TSI

Results

- An alkaline slant-acid butt (red/yellow= K/A) indicates fermentation of dextrose only like ***Salmonella, Shigella, & Proteus.***
- An acid slant-acid butt (yellow/yellow= A/A) indicates fermentation of dextrose, lactose and/or sucrose like ***E. coli & Klebsiella.***
- An alkaline slant-alkaline butt (red/red= K/K) indicates dextrose or lactose were not fermented (non-fermenter) like ***Pseudomonas aeruginosa.***
- Cracks, splits, or bubbles in medium indicate gas production.
- A black precipitate in butt indicates hydrogen sulfide production.

TSI Results



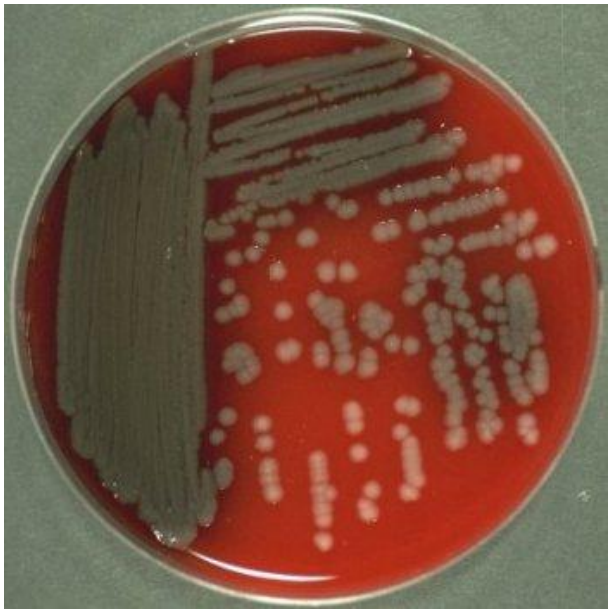
Triple Sugar Iron Agar (M021)

1. Control
2. Escherichia coli ATCC 25922
3. Salmonella Typhi ATCC 6539
4. Proteus vulgaris ATCC 13315
5. Citrobacter freundii ATCC 8090
6. Salmonella Typhimurium ATCC 14028
7. Shigella flexneri ATCC 12022

Pseudomonas aeruginosa

- Gram -ve motile obligate aerobe bacilli.
- Dark greenish-blue heamolytic colonies on blood agar. Green colonies on MacConkey and CLED.
- Cultures have a distinctive smell= grape odour.
- Oxidase +ve.
- TSI test: K/K, no gas, no H_2S.

P. aeruginosa



Identification of Gram Negative Coccobacilli

Haemophilus influenzae

- Gram -ve non-motile coccobacilli.
- Culture media *must contain haemin or* other iron-containing porphyrin (factor X) and the NAD or NADP (factor V). So it grows on Chocolate agar and not blood agar in moist CO_2 medium. It can grow on blood agar around X and V discs.
- *H. parainfluenzae* needs only V factor to grow.
- Satellitism test +ve.
- **Serology:** divided into six serogroups a-f. type b is the most invasive.

Satellitism test

identify H. influenzae

- Make suspention of *Haemophilus* in sterile physiological saline. Inoculate on blood agar and nutrient agar.
- Streak a pure culture of *S. aureus across* the inoculated plates. Incubate in CO_2 at 35–37 ºC overnight.
- Examine the cultures for growth and satellite colonies.

Results:

- *H. influenzae* shows growth on BA but not on NA, and the colonies near the column of *S. aureus* growth are larger than those furthest from it.
- If satellite colonies are present on both plates the organism is probably an *Haemophilus sp.* that requires only factor V (*H. parainfluenzae).*