Diagnostic Microbiology Lab.

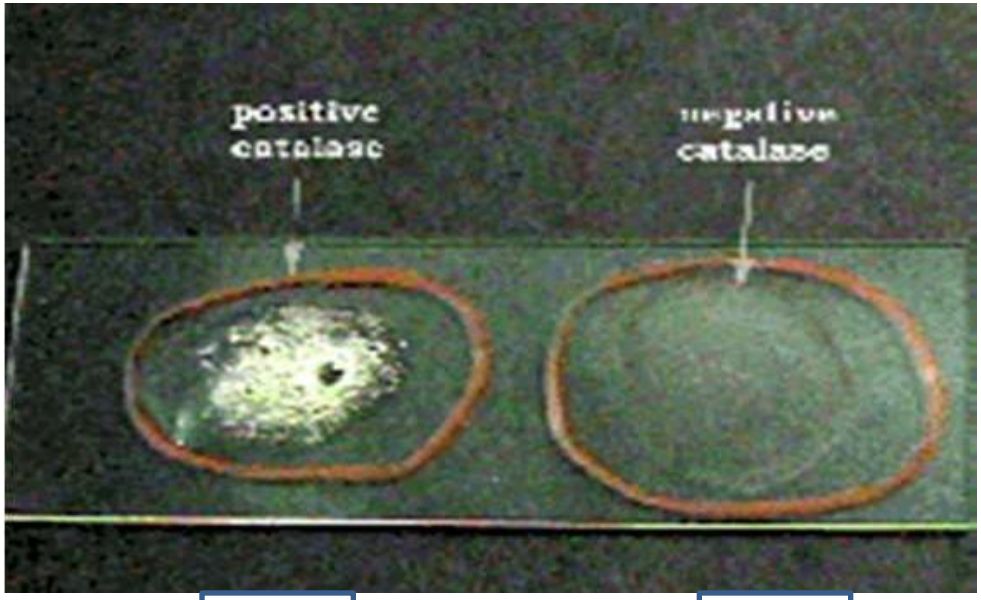
By

Dr. Nabil El Aila

Assistant Professor of Molecular Microbiology

Medical Technology Department

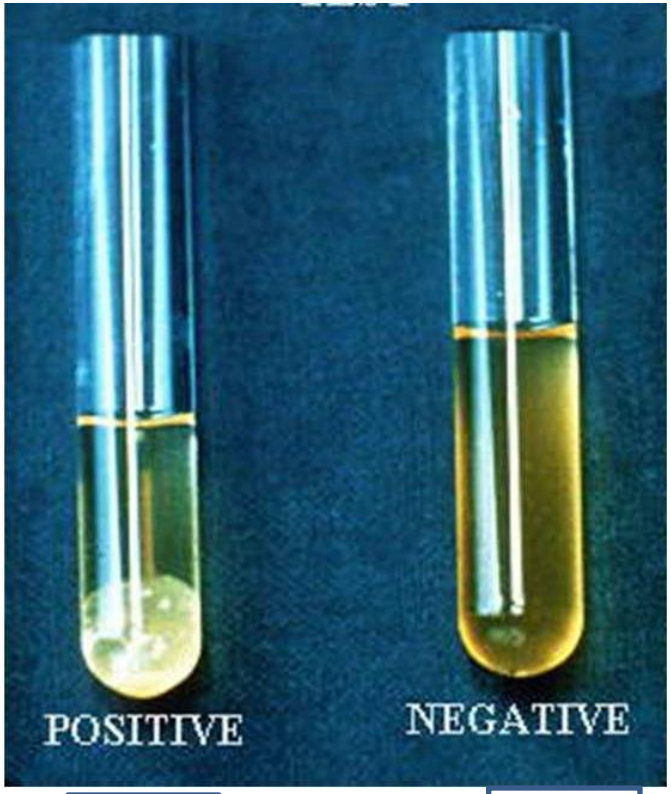
Al -Aqsa University



A B

Name of the test: Catalase test
Example A: positive - Staphylococci
Example B: Negative - Streptococci

Principle: $2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$

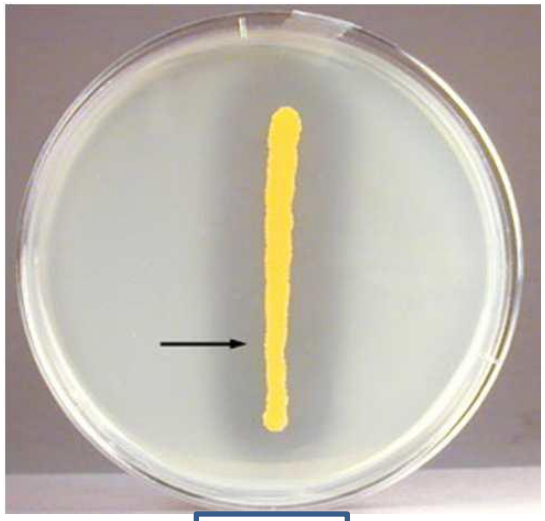


A

B

Name of the test: Coagulase test
Example A: Positive **-** Staph aureus
Example B: Negative - Staph epidermedis

Principle: Fibrinogen in plasma $\xrightarrow{\text{Coagulase}}$ Fibrin clot



A



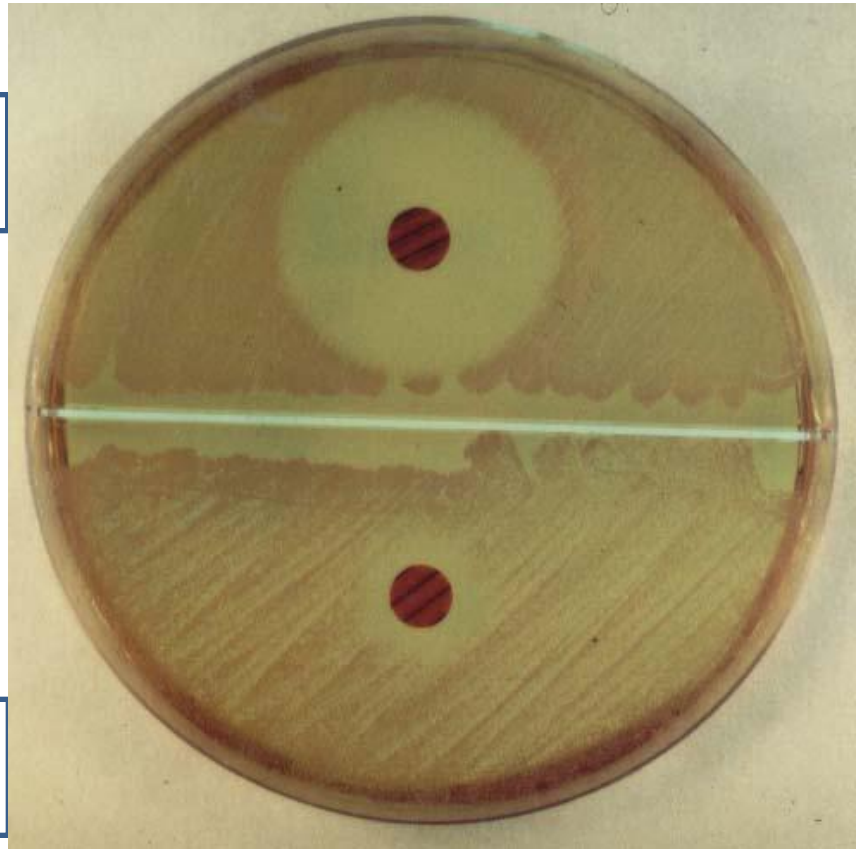
B

Name of the test: DNase test
Example A: Positive - Staph aureus
Example B: Negative - Staph epidermedis
Priciple: DNA is insoluble in acid
DNA is hydrolyzed into oligonucleotides by the action of Dnase
Nucleotides soluble in acid (After addition of 1N HCl)



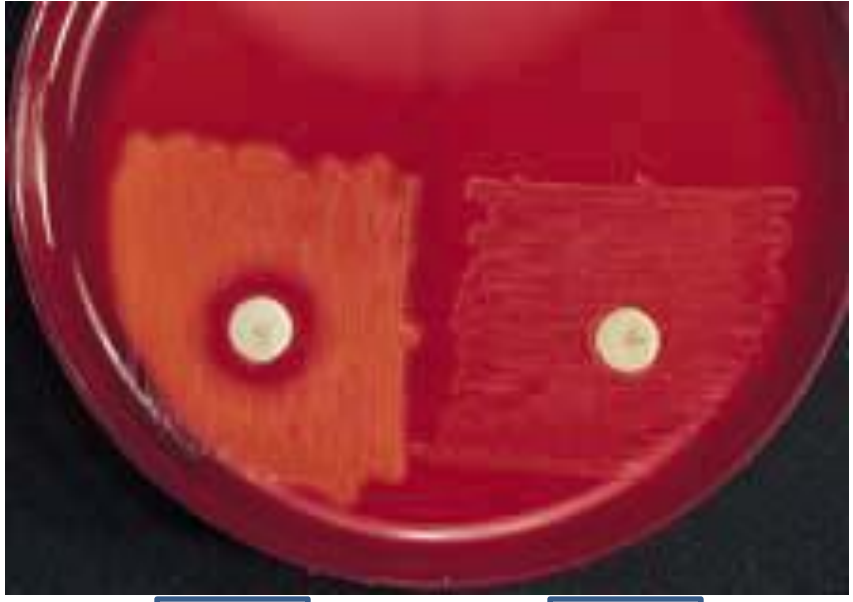
Name of the test: Mannitol salt agar
Example A: Negative - Staph epidermidis
Example B: Positive - Staph aureus
Principle: Staph aureus ferment mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH).

A



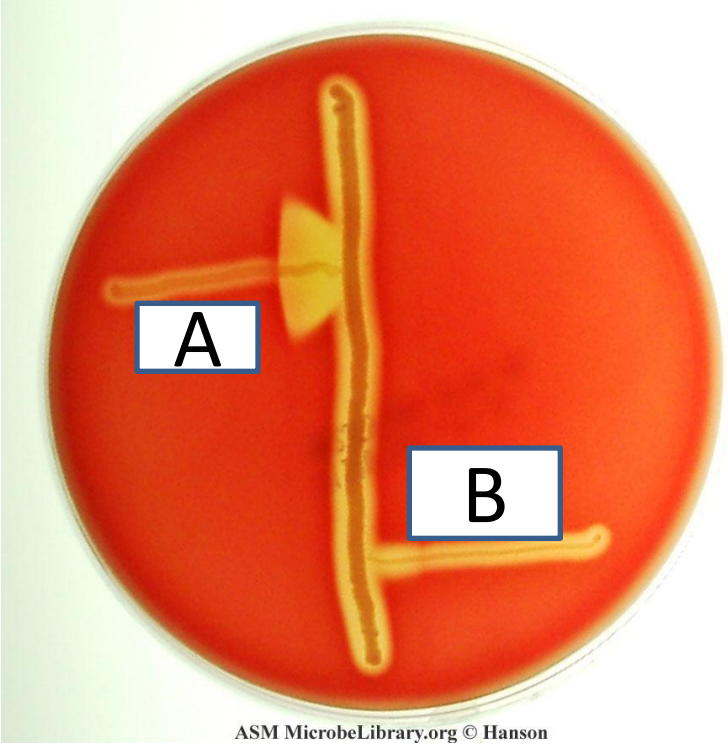
B

Name of the test: Novobiocin sensitivity
Example A: Staph epidermidis (Sensitive)
Example B: Staph saprophyticus (Resistant)

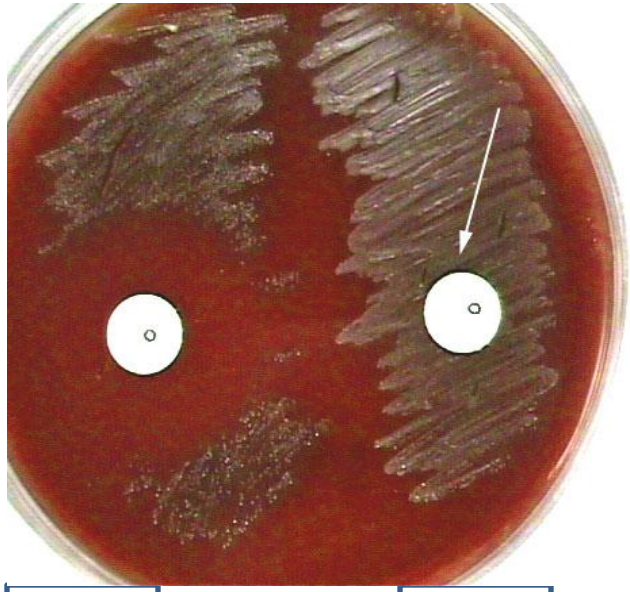


A B

Name of the test: Bacitracin sensitivity
Example A: Strept pyogens (sensitive)
Example B: Strept agalactiae (resistant)



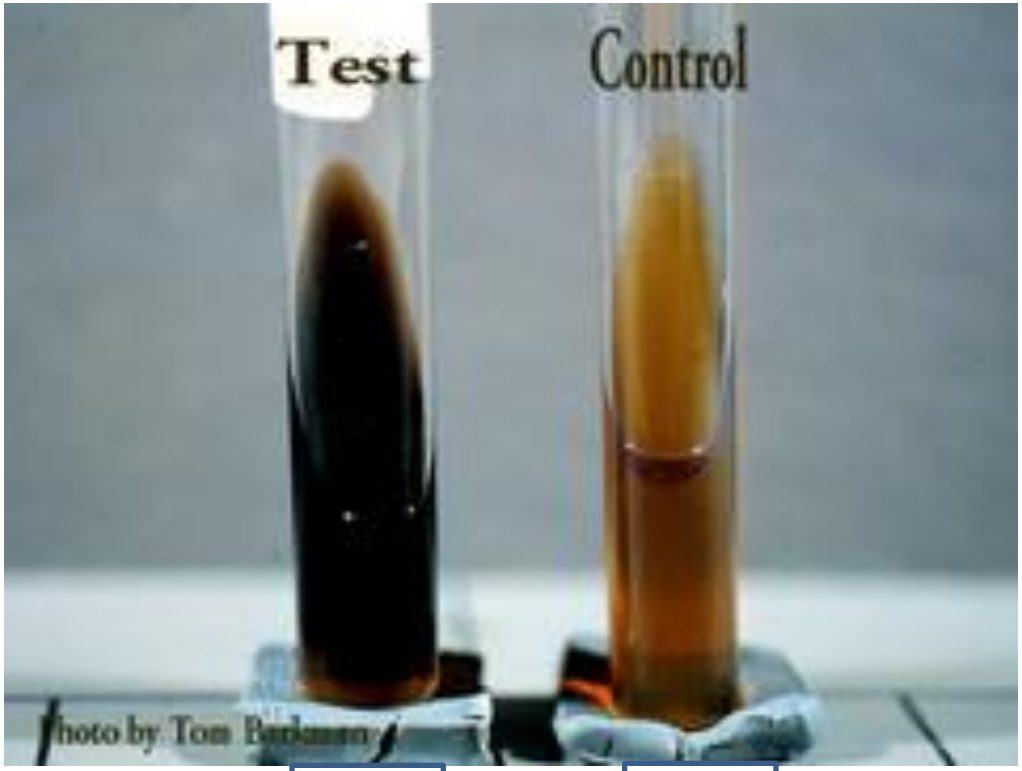
Name of the test: CAMP test
Example A: Positive - Strept agalactiae (Arrow shaped)
Example B: Negative - Strept pyogens
Principle : Strept agalactiae produce CAMP factor (a diffusible extracelluar protein) that synergistically acts with the beta-lysin of *Staphylococcus aureus* and enhances the lysis of red blood cells.



A

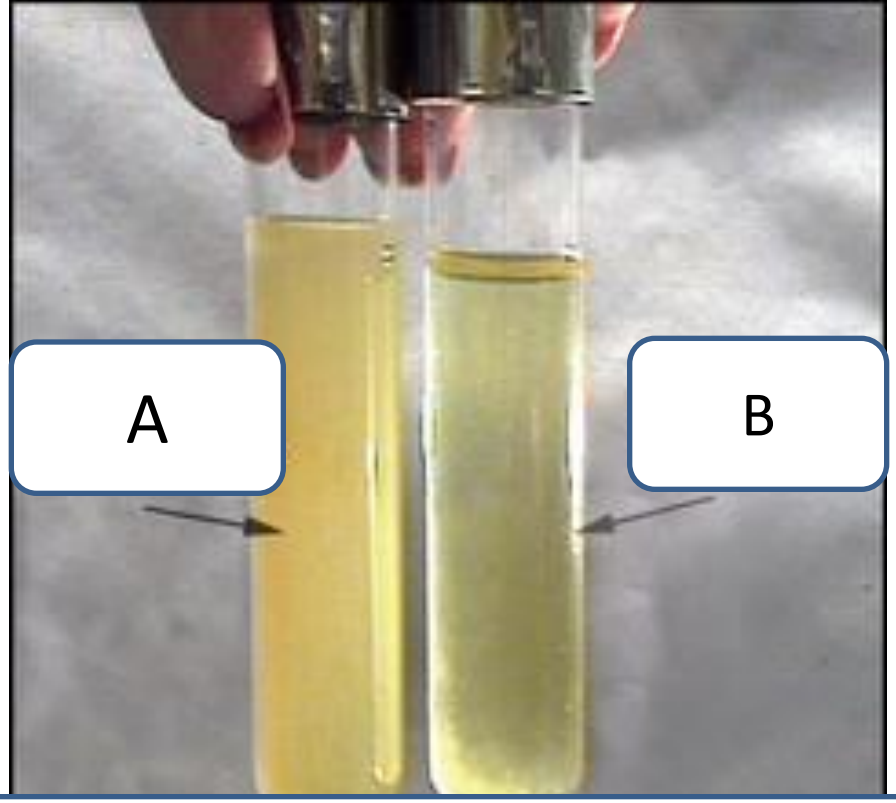
B

Name of the test: Optochin sensitivity
Example A: Strept pneumonia (sensitive)
Example B: Strept viridans (resistant)

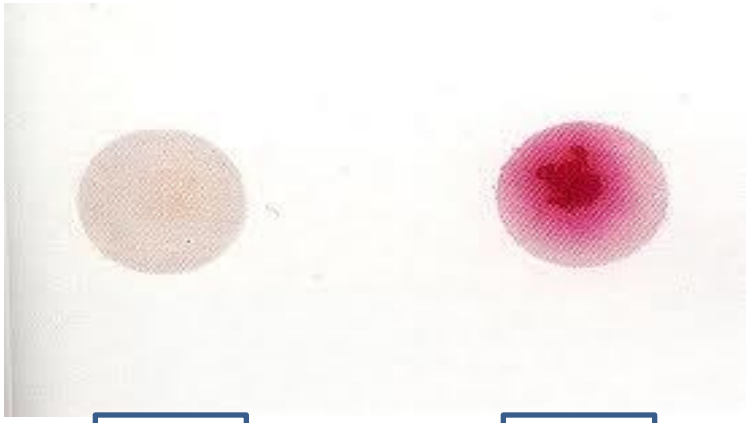


A B

Name of the test: Bile Esculin test
Example A: Positive - Group D streptococcus (Enterococcus species)
Example B: Negative - Group B streptococcus
Principle: The selective agent **bile**, inhibits most gram positive bacteria.
Esculin in the medium is hydrolyzed to esculetin and dextrose.
The esculetin reacts with ferric chloride in the media to form a black-brown color.

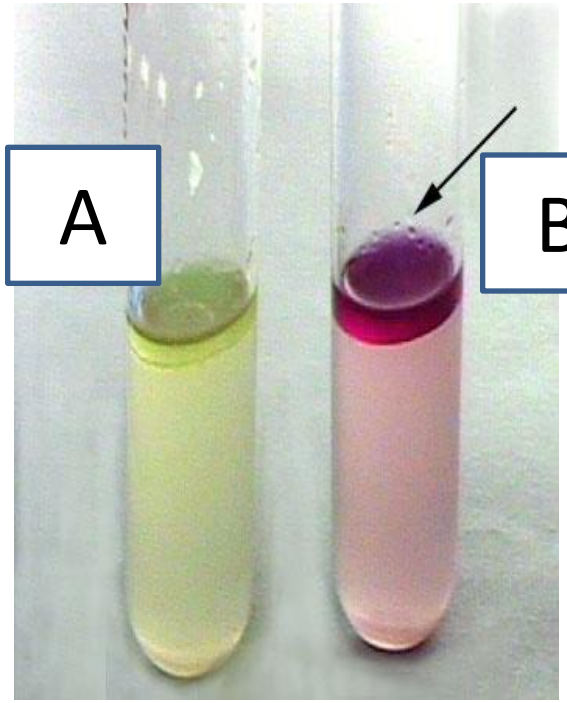


Name of the test: Bile Solubility
Example A: Strept mitis (Negative)
Example B: Strept pneumoniae (positive)
Principle: *S. pneumoniae* is bile soluble whereas all other alpha-hemolytic streptococci are bile resistant. Sodium deoxycholate (2% in water) will lyse the pneumococcal cell wall



A B

Name of the test: **Pyrrolidonyl arylamidase Test (PYR)**
Example A: Strept agalactiae (Negative)
Example B: group A streptococci and Enterococcu s species (positive)
Principle: Group A streptococci and Enterococcu s species produce pyrrolidonyl arylamidase which hydrolyzes the substrate L- pyrrolidonyl -β-naphthylamide to form β-naphthylamine.
A pink to red color forms when p-dimethylaminocinnam-aldehyde (PYR reagent) is added to β-naphthylamine.

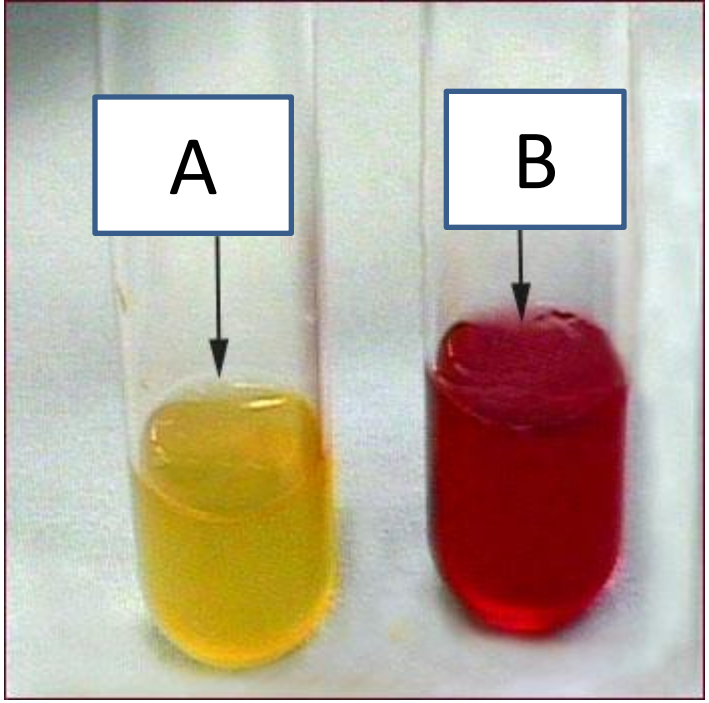


Name of the test: Indole test
Example A: Klebsiella pneumonia (Negative)
Example B: Escherichia coli (Positive)
Indicator: **Kovac's Reagent (4 (p) – dimethylamino benzaldehyde)**
Principle:

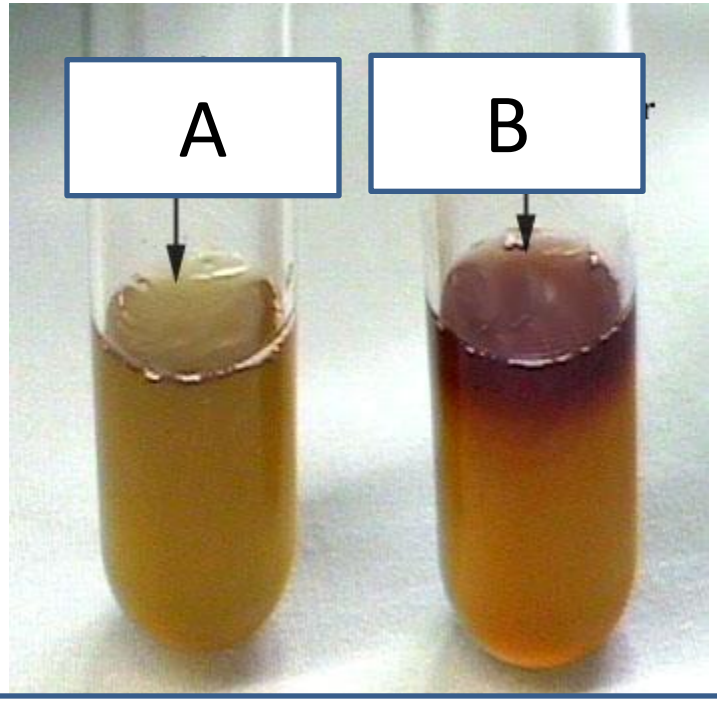
Tryptophane amino acids —**Tryptophanase**→ **Indole + Pyurvic acid + NH3**

Kovac's Reagent ↓

Red color in upper organic layer`



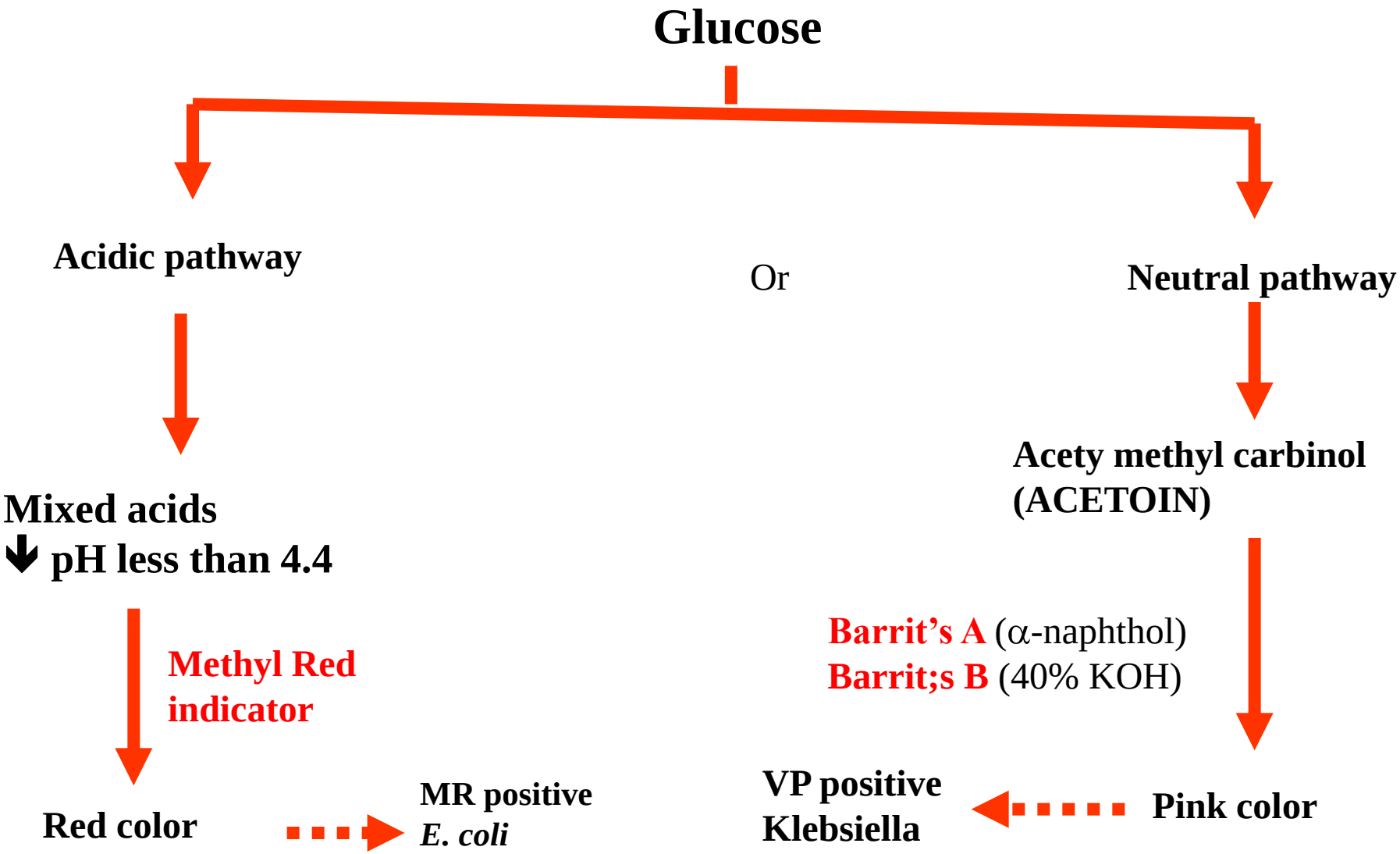
Name of the test: Methyl red
Example A: Klebsiella pneumonia (Negative)
Example B: Escherichia coli (Positive)
Indicator: Methy red indicator
Principle: follow next slides

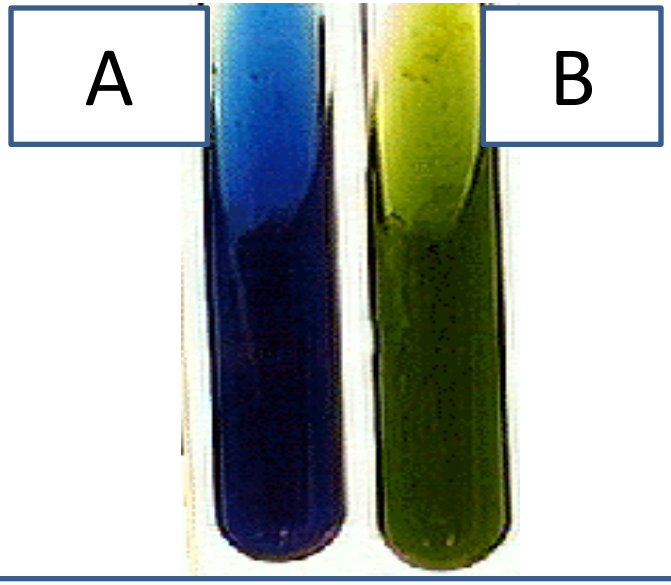


Name of the test: Voges Proskauer test
Example A: Escherichia coli (Negative)
Example B: Klebsiella pneumonia (Positive)
Indicator: α-naphthol and 40% KOH
Principle: follow next slide

IMViC test
Methyl Red-Voges Proskauer (MR-VP) Tests

Principle



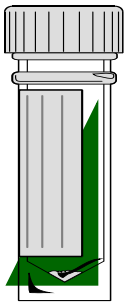


Name of the test: Citrate test
Example A: Klebsiella pneumonia (Positive)
Example B: Escherichia coli (Negative)
Indicator: Bromothymol blue
Principle: follow next slide

IMViC: CITRATE TEST

Principle:

Citrate → Pyruvate → CO2 + Na + H2O → Na_2CO_3

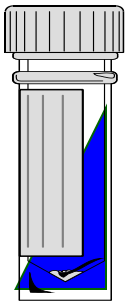


Simmone's Citrate media

Contains Citrate as a sole of C source

Alkaline,↑pH

Bromothymol blue



Positive test

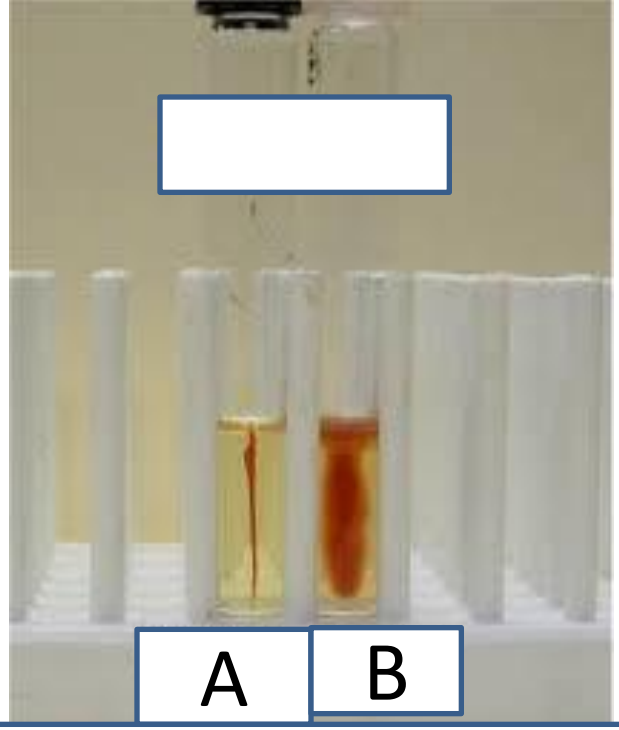
Dr. Nabil El Aila
Diagnostic Microbiology



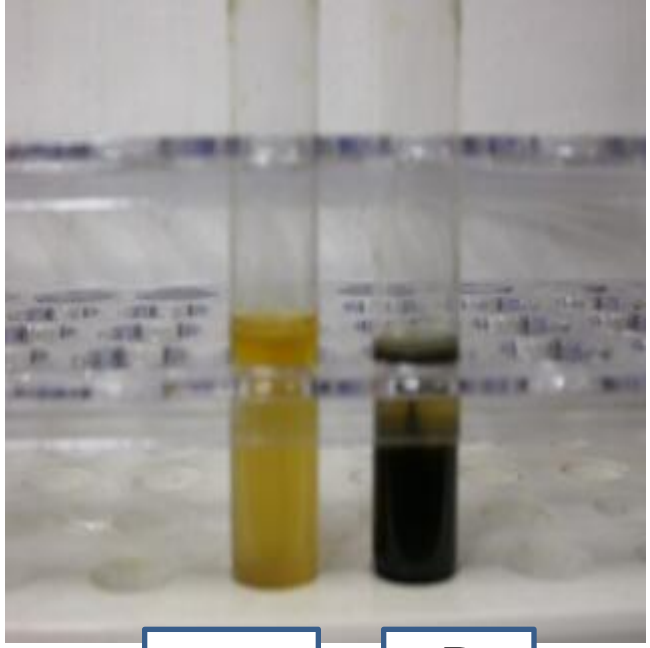
Name of the test: Urea test
Example A: Proteus miribalis (Positive)
Example B: Escherichia coli (Negative)
Indicator: Phenol red
Principle:

Urea —(Urease)→ **$CO_2 + NH_3$** —(H_2O)→ **NH_4OH** → **↑ in pH** → **Phenol Red** → **Pink**

Positive test



Name of the test: Motility test
Example A: Escherichia coli (Negative)
Example B: Proteus miribalis (Positive)



A B

Name of the test: H2S production
Example A: Escherichia coli (Negative)
Example B : Proteus miribalis (Positive)
Principle:
Bacteria use enzyme cysteine desulfurase to hydrolyze the amino acid cysteine, forming hydrogen sulfide as end-product.
The hydrogen sulfide will combine with the iron salt to form a visible **black** ferric sulfide (FeS) in the tube



A B

Name of the test: Triple sugar Iron
Example A: K/A - No H2S – no gas production
Glucose fermenter - Shigella species
Example B: A/A - No H2S – gas production
All three sugars fermented – Escherichia coli

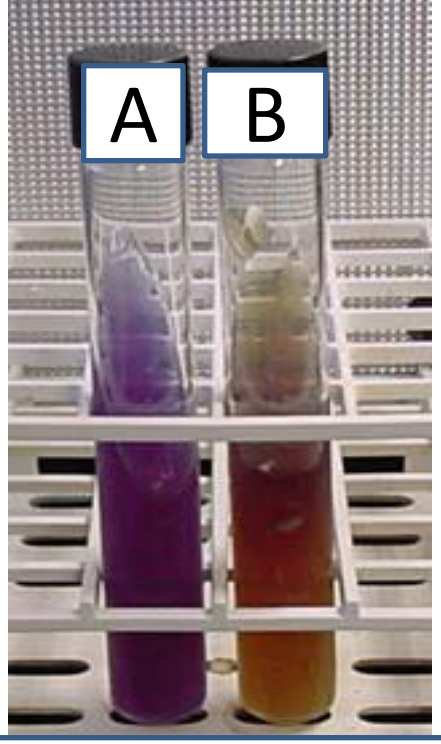


A B

Name of the test: Triple sugar Iron
Example A: A/A - H2S positive –gas production
Glucose fermenter - Proteus species
Example B: K/A - H2S positive – gas production
All three sugars fermented – Salmonella species



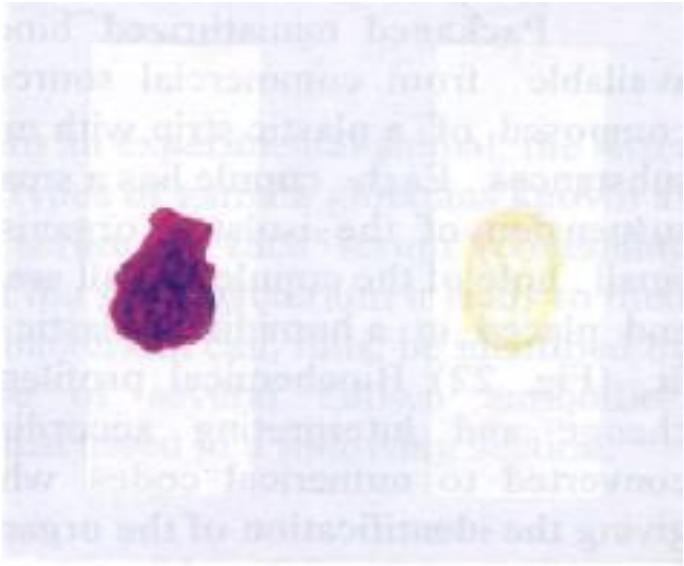
Name of the test: Triple sugar Iron
Example A: K/K – No H2S – No gas production
No action on sugars - Pseudomonas species
Example B: K/A – No H2S – No gas production
Glucose fermenter - Shigella species



Name of the test: Lysine Decarboxylase Agar
Example A: Positive decarboxylation (butt), negative deamination (slant)
: Salmonella species
Example B: Negative decarboxylation (butt), Positive deamination (slant)
: Proteus species



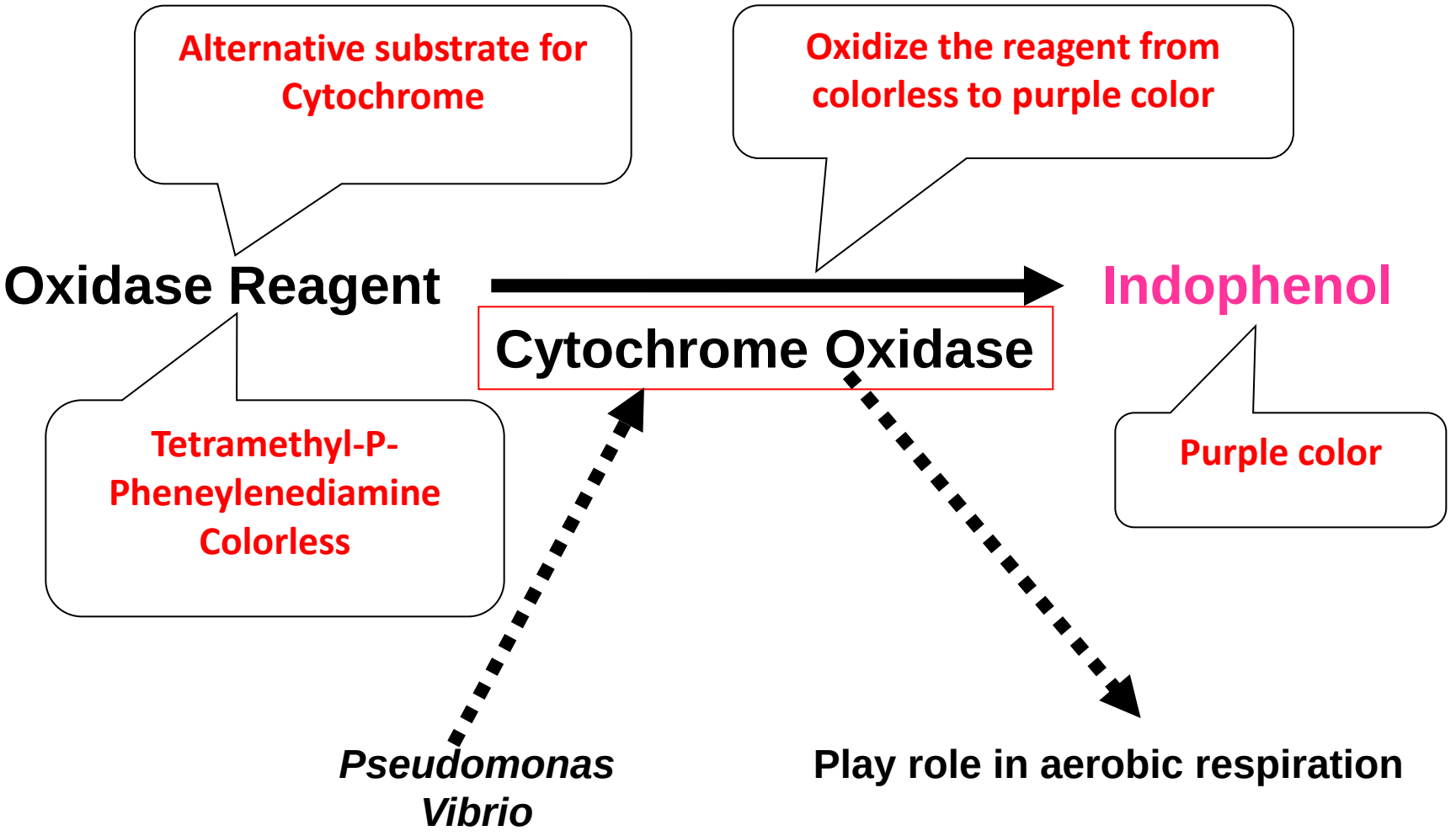
Name of the test: Lysine Decarboxylase Agar
Example A: Positive decarboxylation (butt), negative deamination (slant)
: Salmonella species
Example B: Negative decarboxylation (butt), Negative deamination (slant)
: Citrobacter species

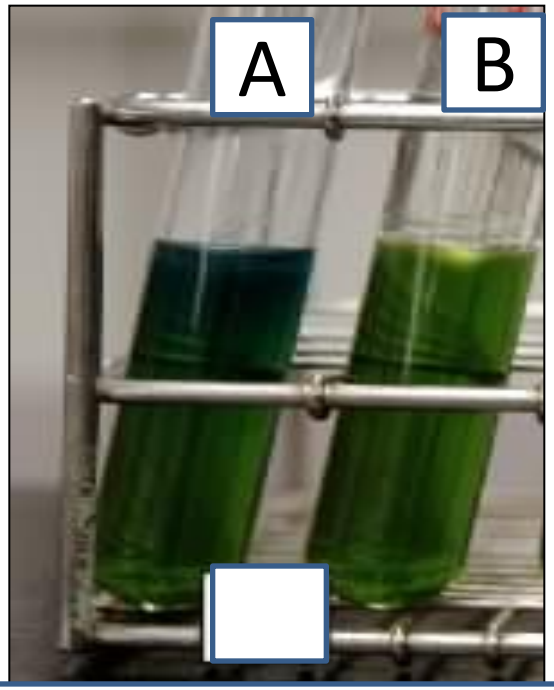


A B

Name of the test: Oxidase test
Example A: Positive - Pseudomonas species
Example B: Negative - Proteus species
Princile: follow next slide

Oxidase Test: Principal

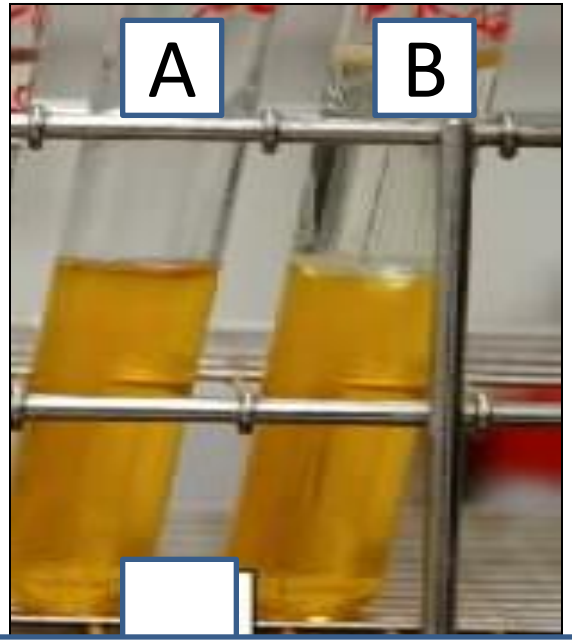




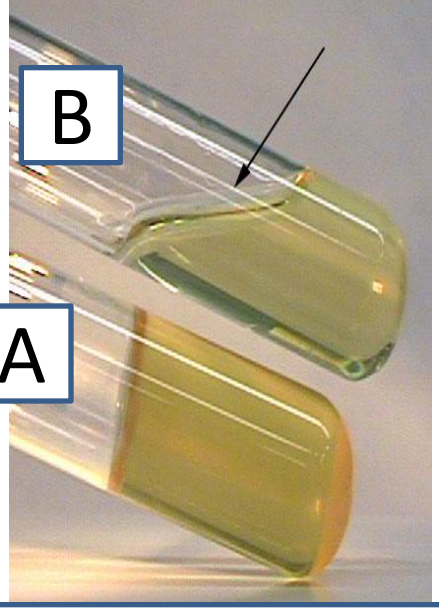
Name of the test: Oxidation Fermentation test
Example A: Oxidation negative (Open tube remain green)
Example B: Fermantation negative (Covered tube remain green)
: Oxidation negative/ Fermentation negative (*Alcaligenes faecalis)*



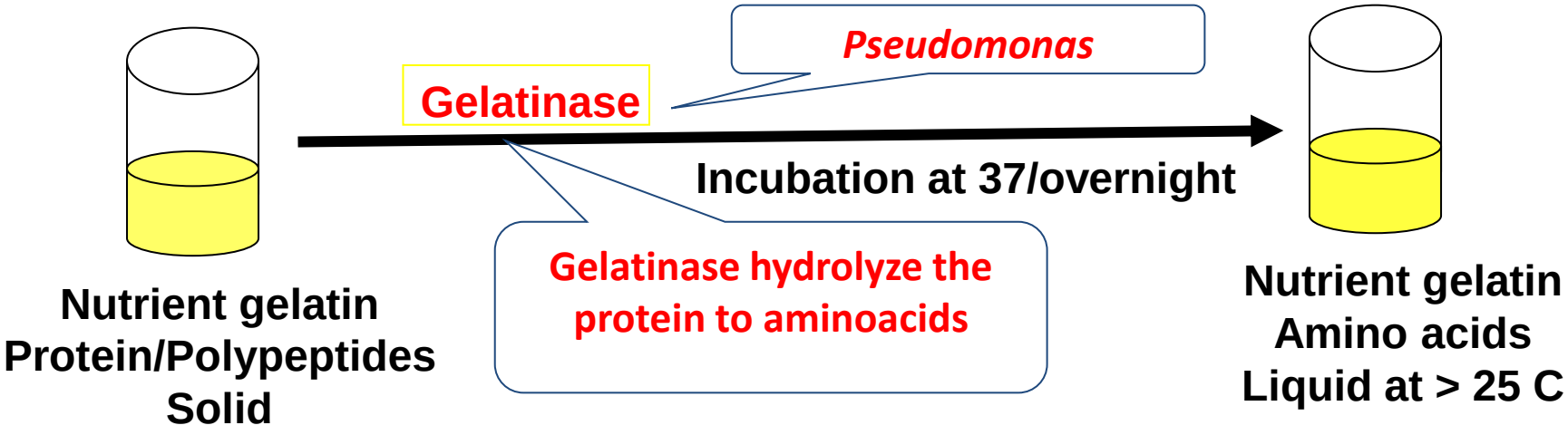
Name of the test: Oxidation Fermentation test
Example A: Oxidation positive (Open tube turns yellow)
Example B: Fermantation negative (Covered tube remain green)
: Oxidation positive/ Fermentation negative (*Pseudomonas species)*

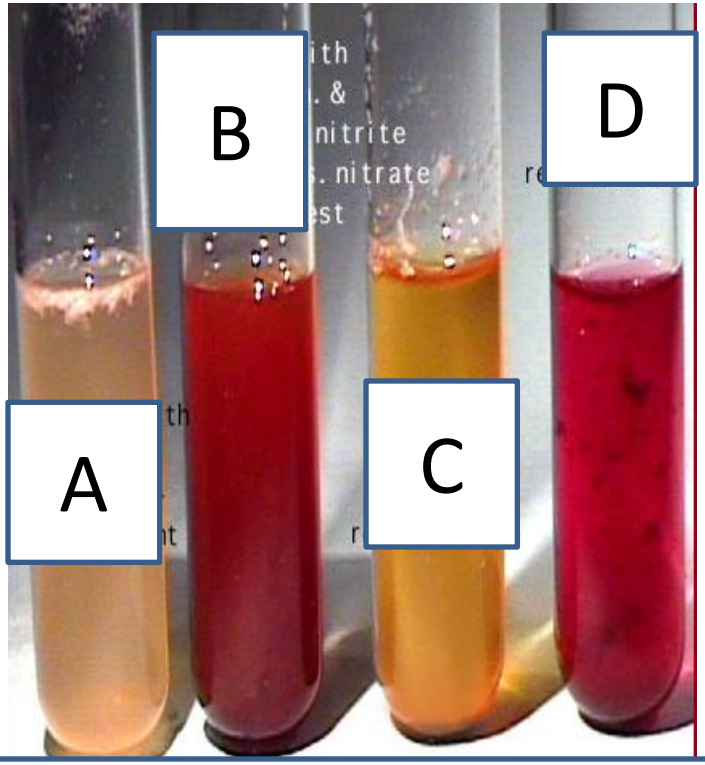


Name of the test: Oxidation Fermentation test
Example A: Oxidation positive (Open tube turns yellow)
Example B: Fermantation positive(Covered tube turns yellow)
: Oxidation positive/ Fermentation positive (*Enterobacteriacea)*



Name of the test: Gelatin Liquification test
Example A: Negative (Proteus species)
Example B: Positive (Pseudomonas species)
Principle :

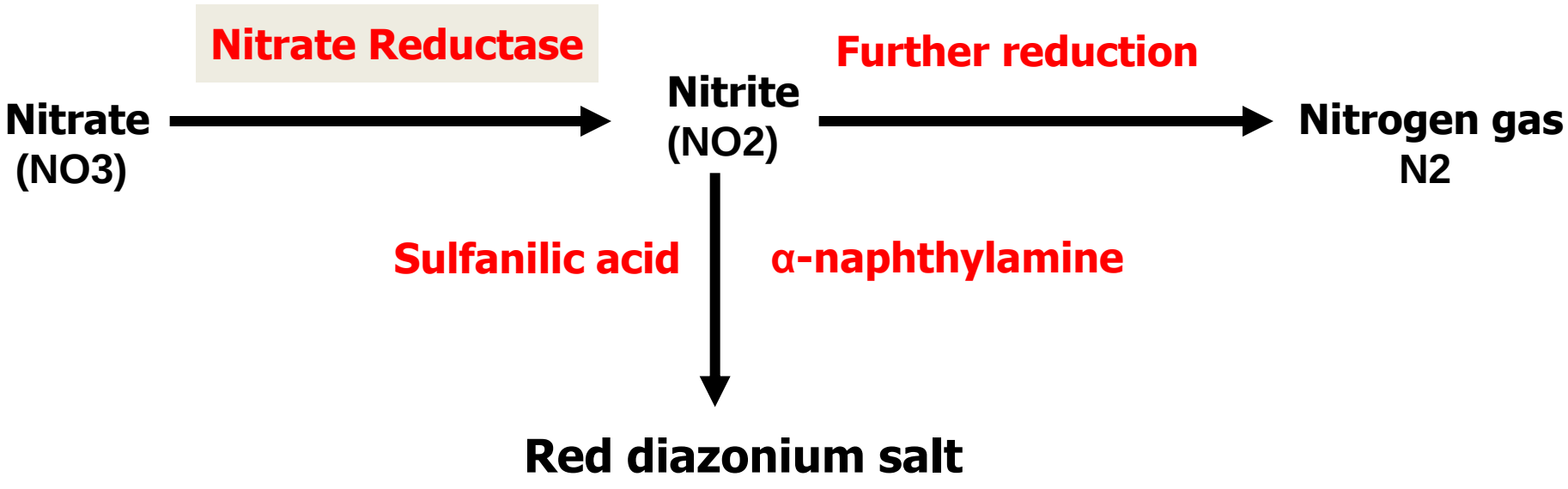




Name of the test: Nitrate reduction test
Example A: Negative nitrate reduction - no change with alpha naphthol and sulf.acid
Example B: Positive nitrate reduction – turns red with alpha naphthol and sulf.acid sulf.acid
Example C: Positive nitrate reduction . No red color after addition of zinc dust (Nitrate reduced into nitrite and further reduction to Nitrogen.
Example D: Negative nitrate reduction red color after addition of zinc dust Nitrate unreduced

Principle : follow next slide

Nitrate Reductase Test: Principal



If no red color! → **Add zinc dust (reducing agent)**

No red color after addition of zinc dust (Nitrate reduced into nitrite and further reduction to Nitrogen (**Positive nitrate reduction)**	**or**	red color after addition of zinc dust Nitrate unreduced (**Negative nitrate reduction)**



Name of the test: ***Satellitism***

Principle:

- A lawn of test bacteria is plated onto a fresh blood agar plate. This provides a supply of haem.
- An inoculum of *Staphylococcus aureus*, which can provide NAD, is placed on the plate and the culture is incubated.
- Haemophili can be seen growing larger near to the staphylococcal colony, where the supply of NAD is greatest.

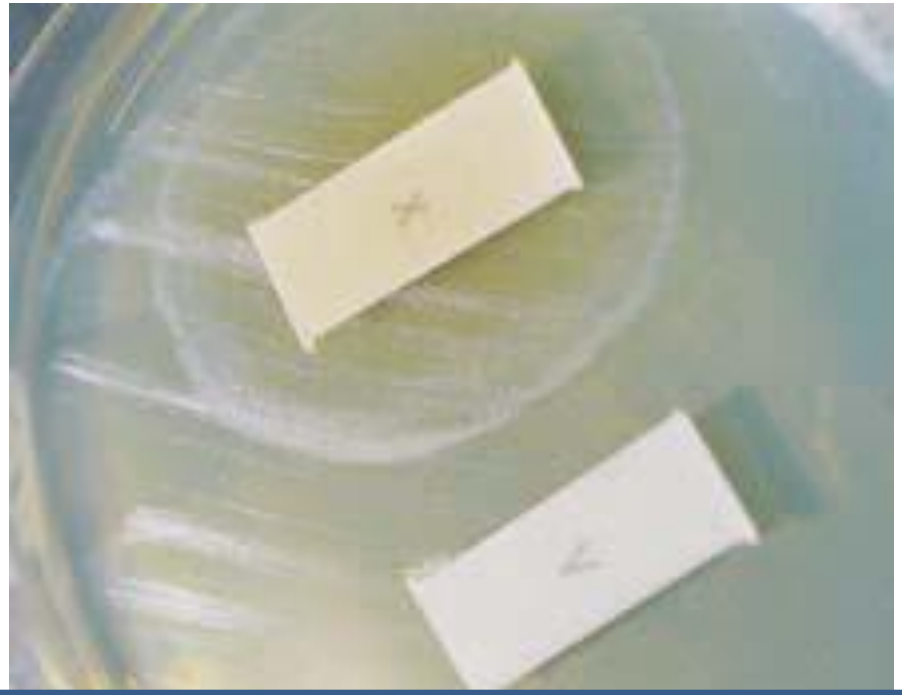


Name of the test: ***Swarming***

Example: *Proteus* species move very actively by means of peritrichous flagella, resulting in "swarming" on solid medium



Name of the test: ***Exopigment production on Muller Hinton agar***
Example: **pseudomonas species** produces exopigments in culture eg pyocyanin, fluorescein, pyorubrin, pyoverdin, and pyomelanin



Bacteria grow in the presence of X factor

Example: Haemophillus aphrophilus

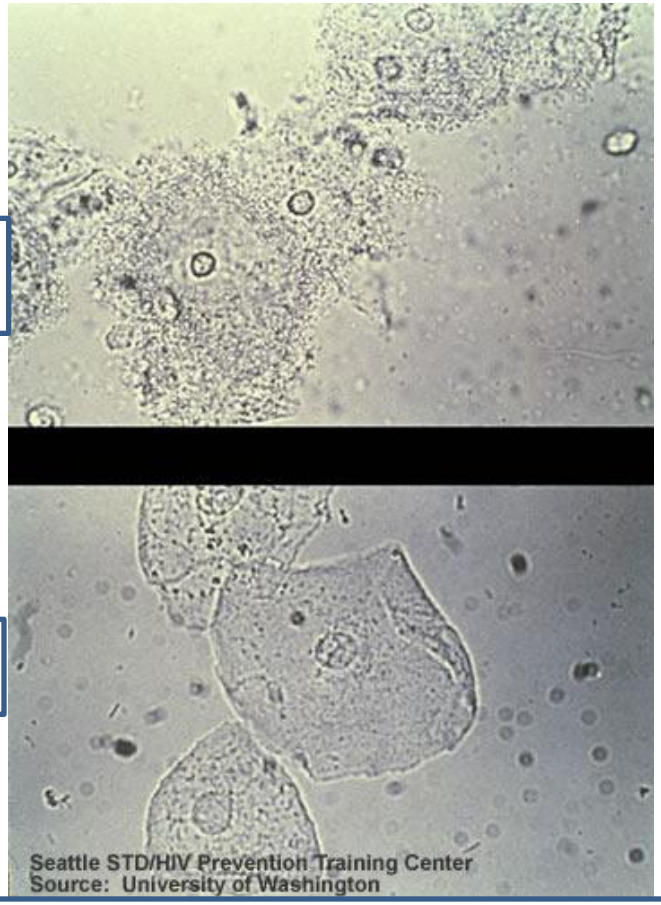


Bacteria grow in the presence of V factor

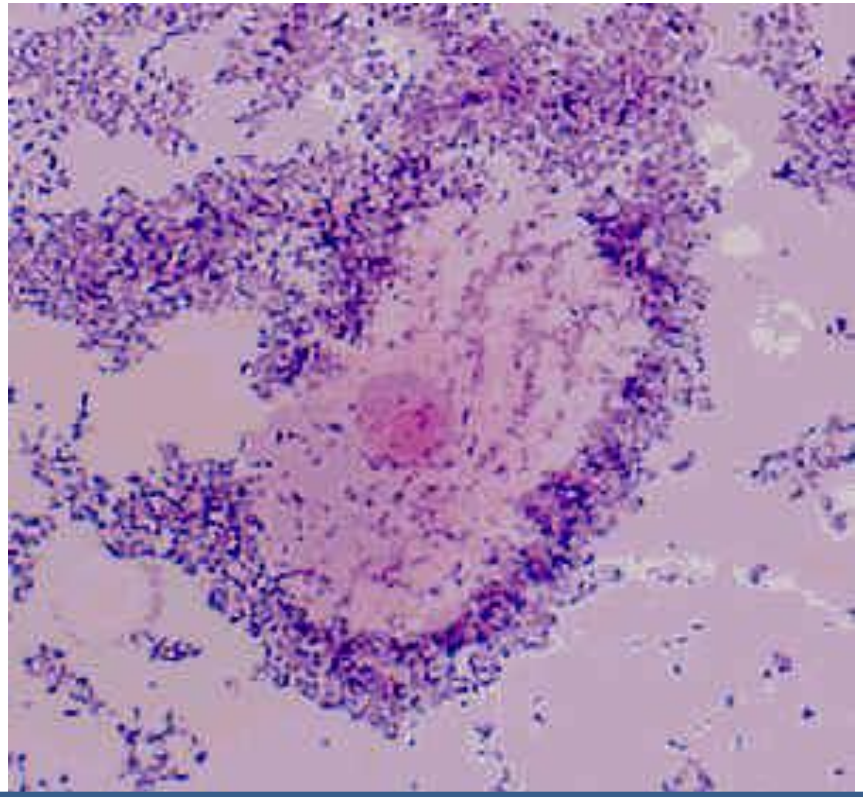
Example: Haemophillus parainfluenza

B

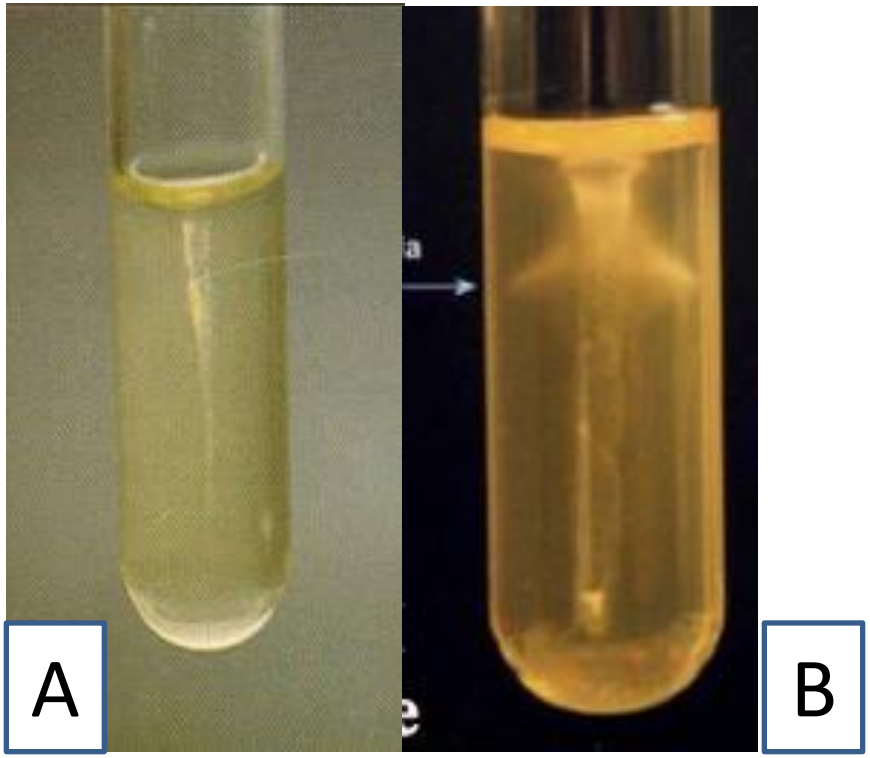
A



A: Vaginal saline preperation: normal epithelial cells
B: Vaginal saline preperation: Clue cells



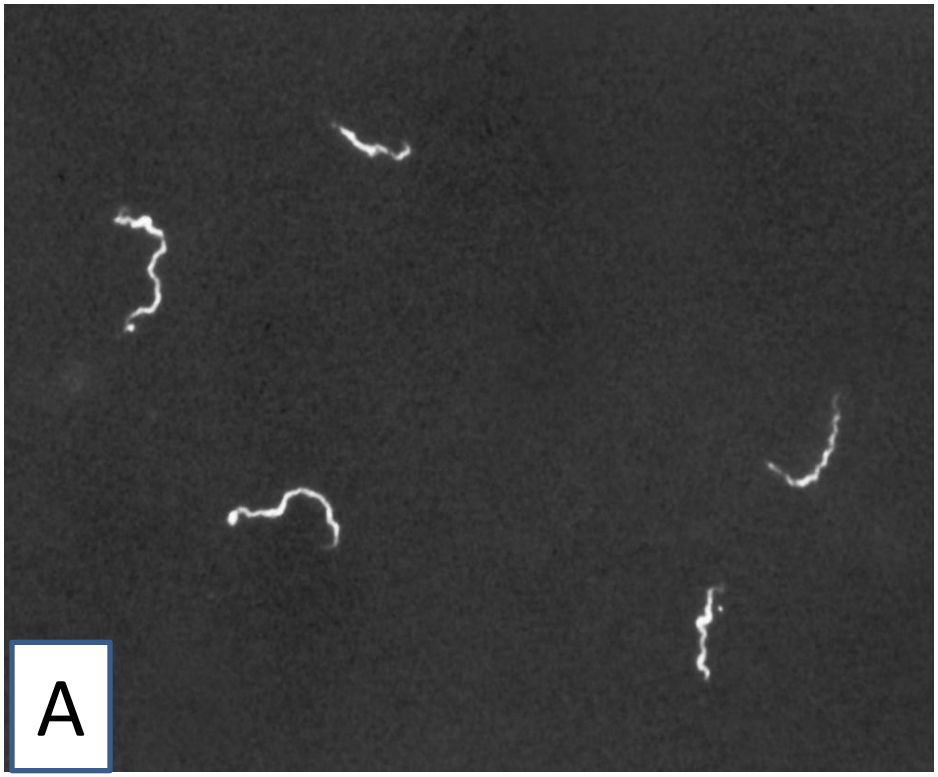
Gram variable bacteria
(Gram negative and positive coccobacilli)
Gardnerella vaginallis

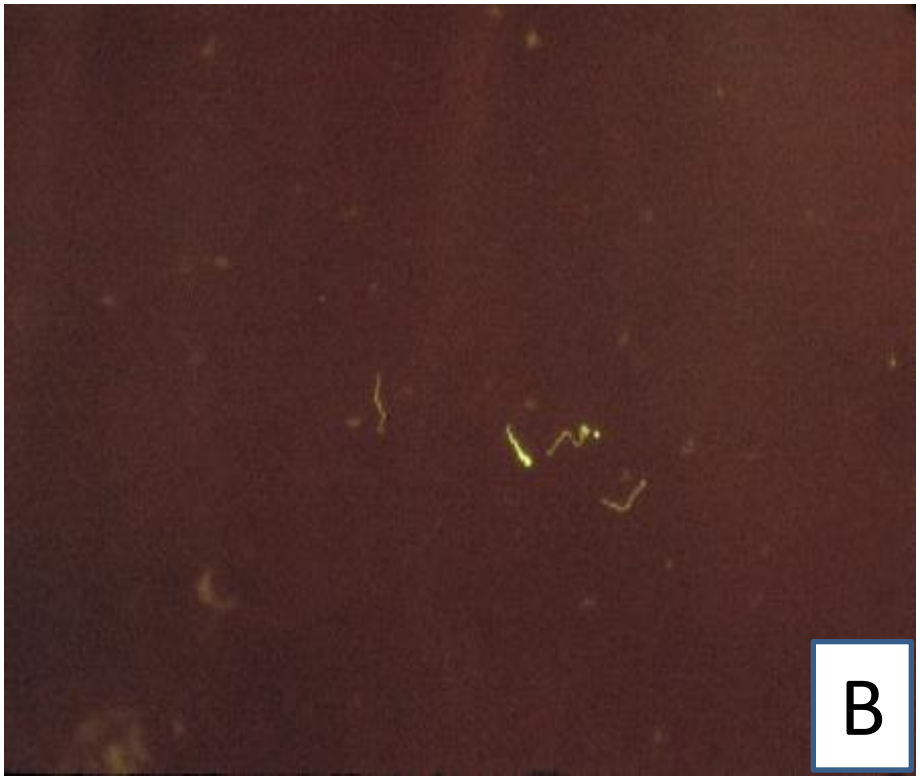


Name of test: Motility test listeria

A: Non motile

B: umbrella shape indicative of motility



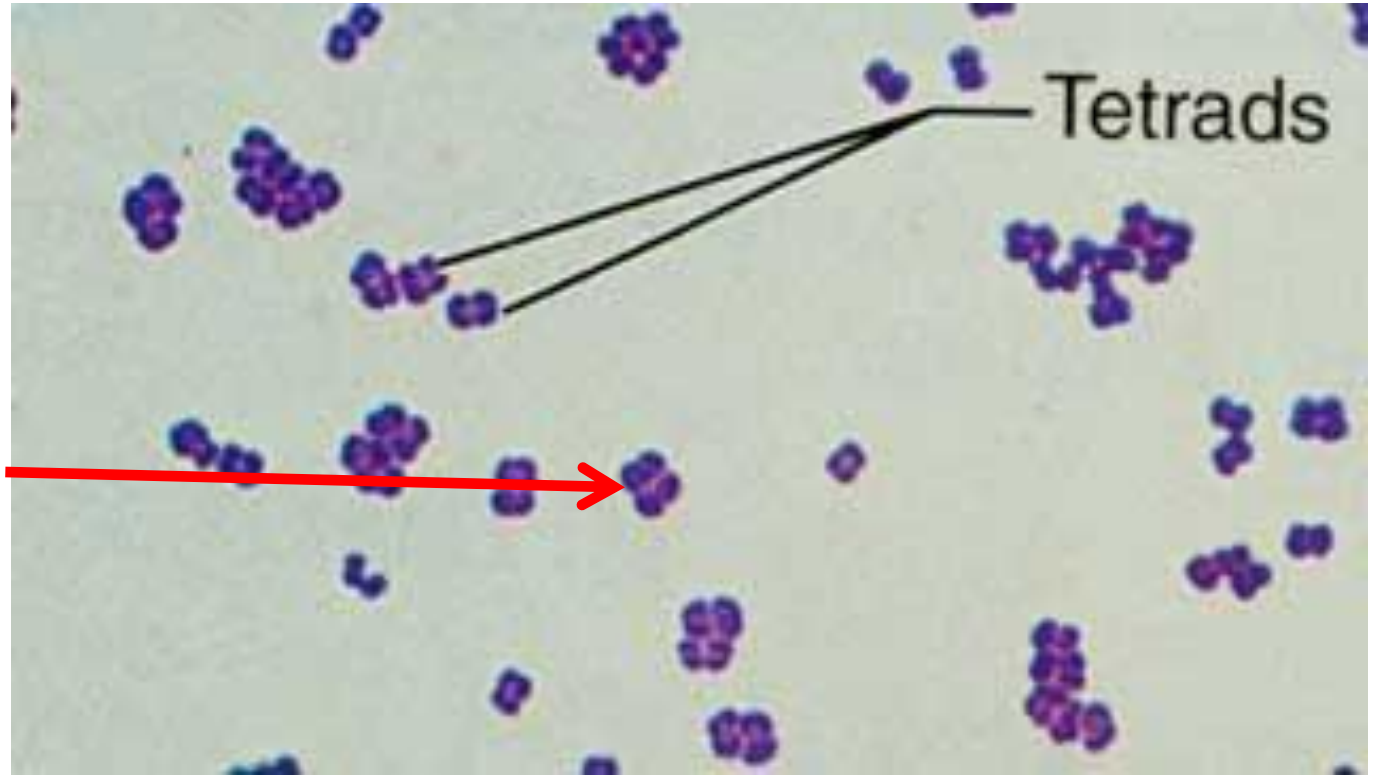


A: Dark field microscopy

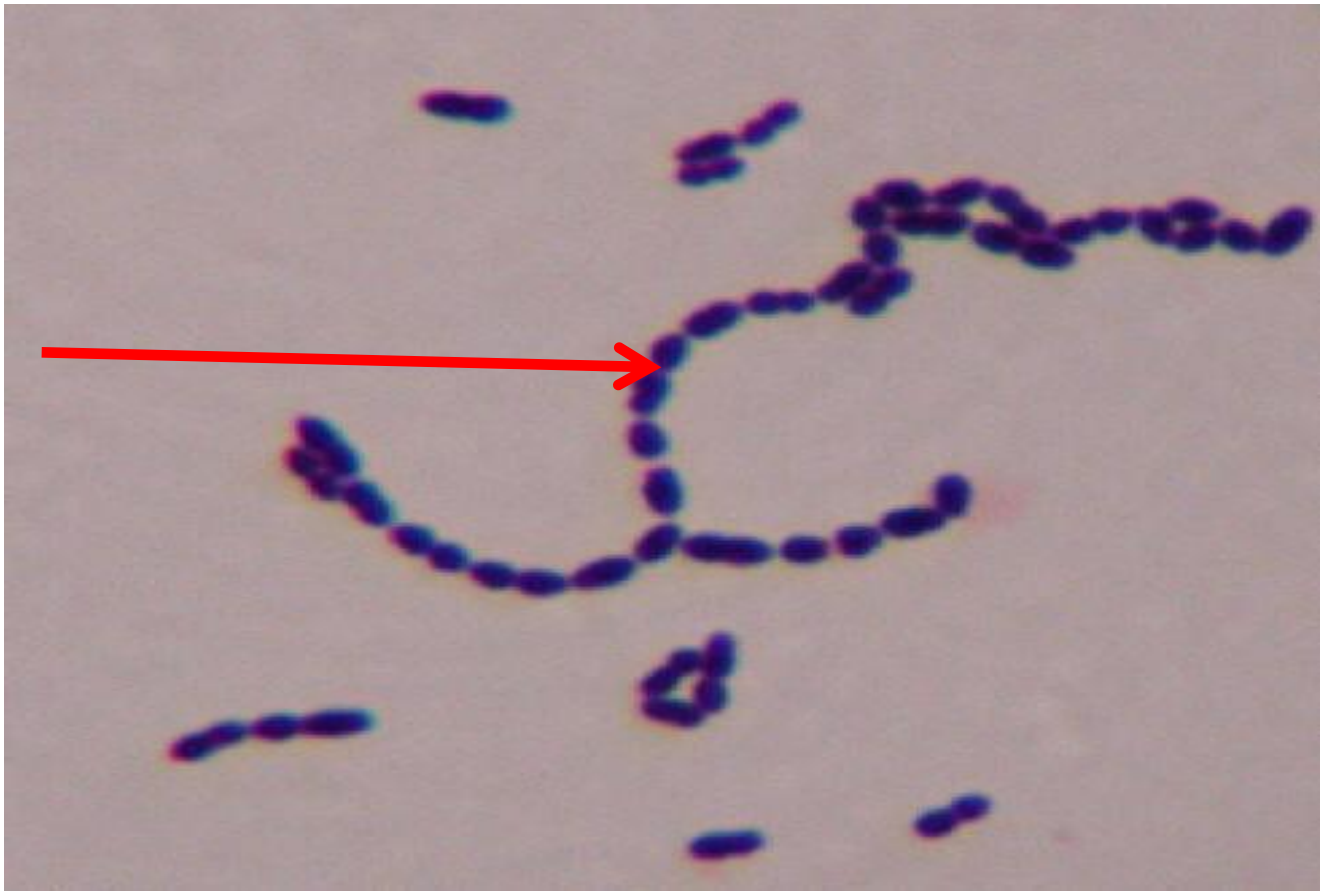
B: Fluorescence microscopy antibody staining



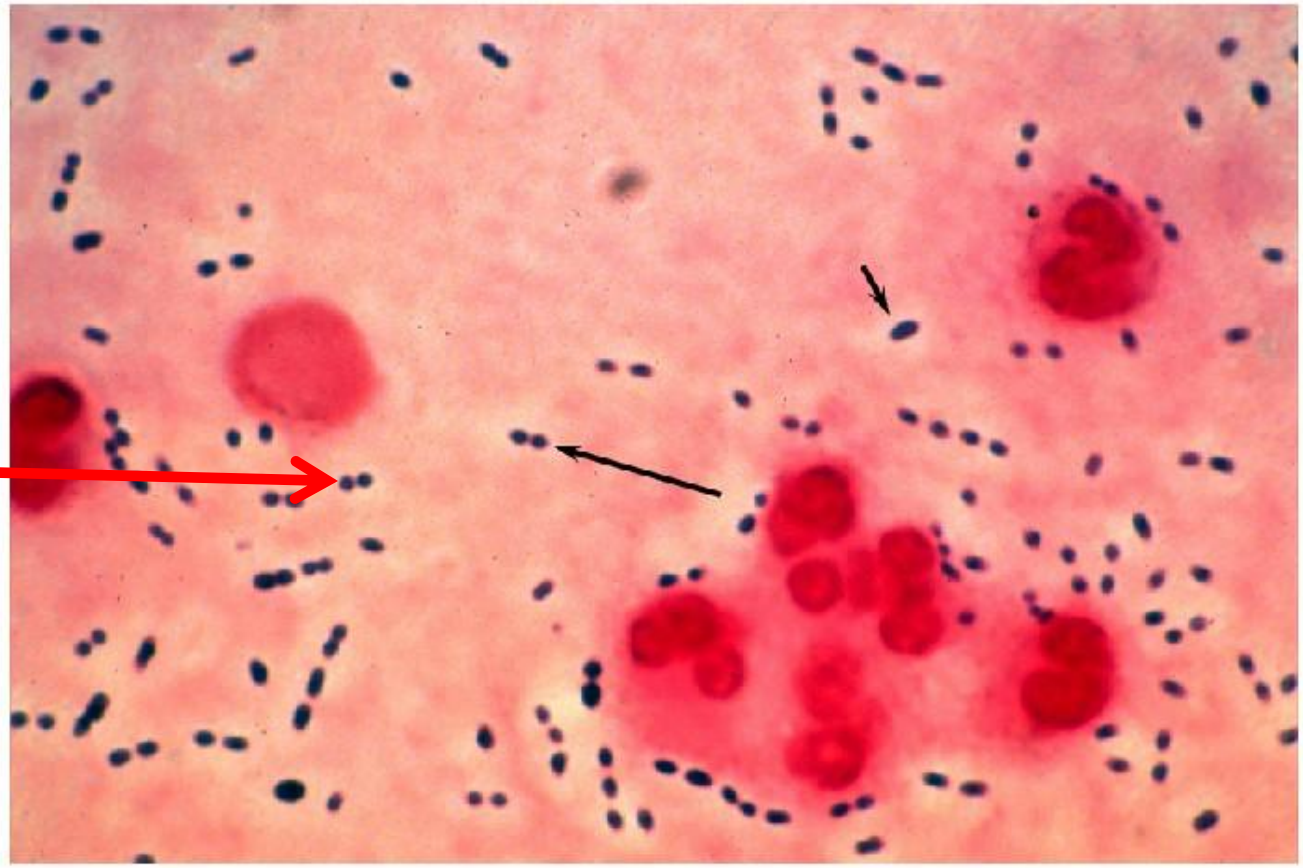
Grram positiv cocci in Clusters
Example: *Staph aureus*



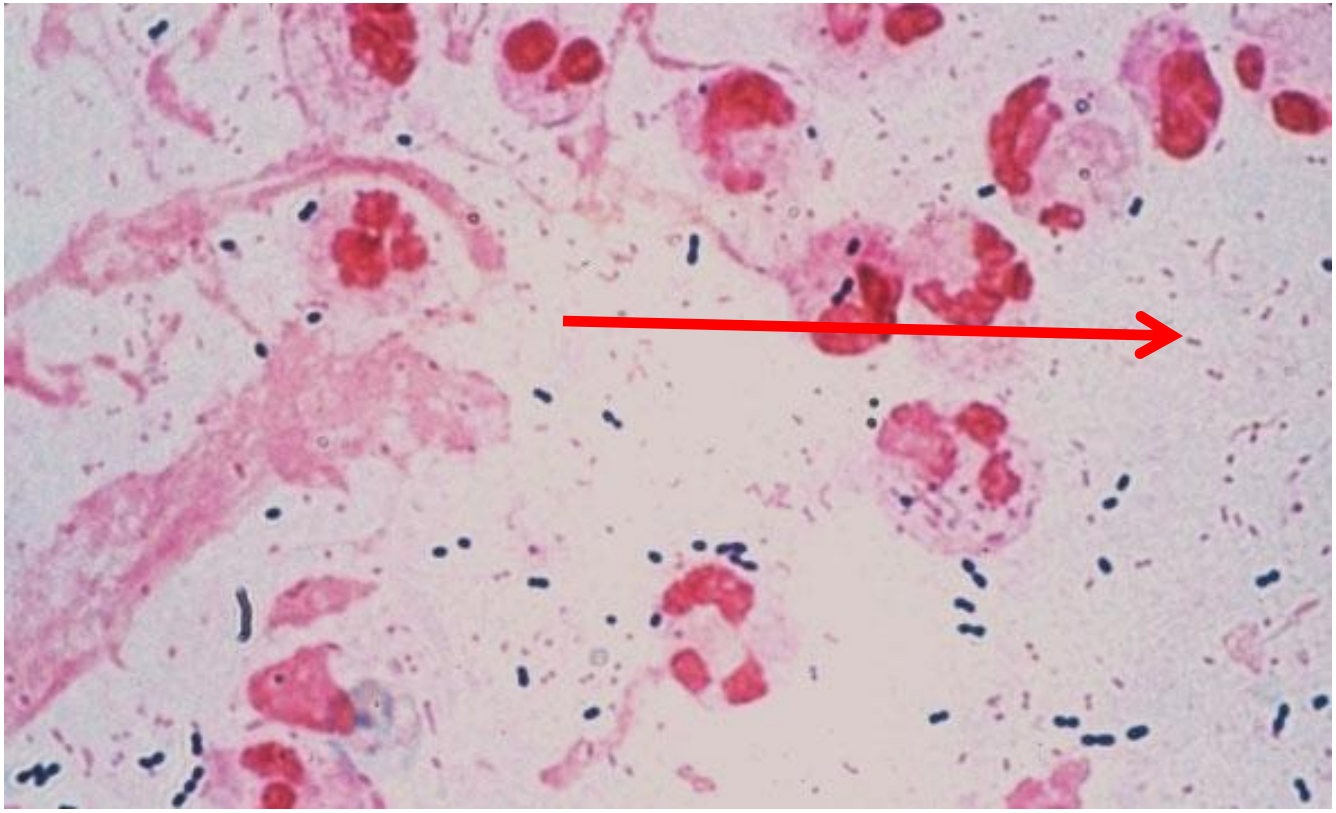
Grram positiv cocci in Tetrads
Example: *Micrococcus species*



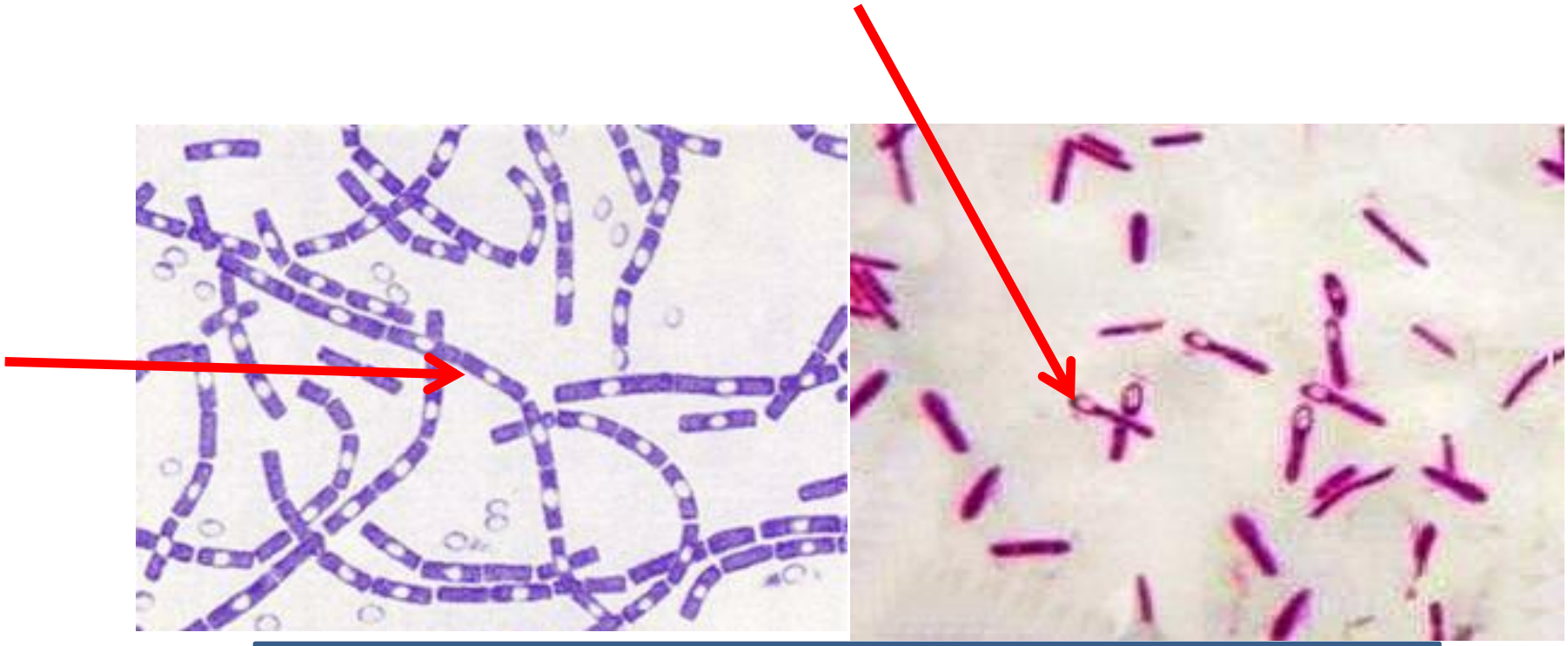
Grram positiv cocci in Chains
Example: *Streptococcus pyogens*



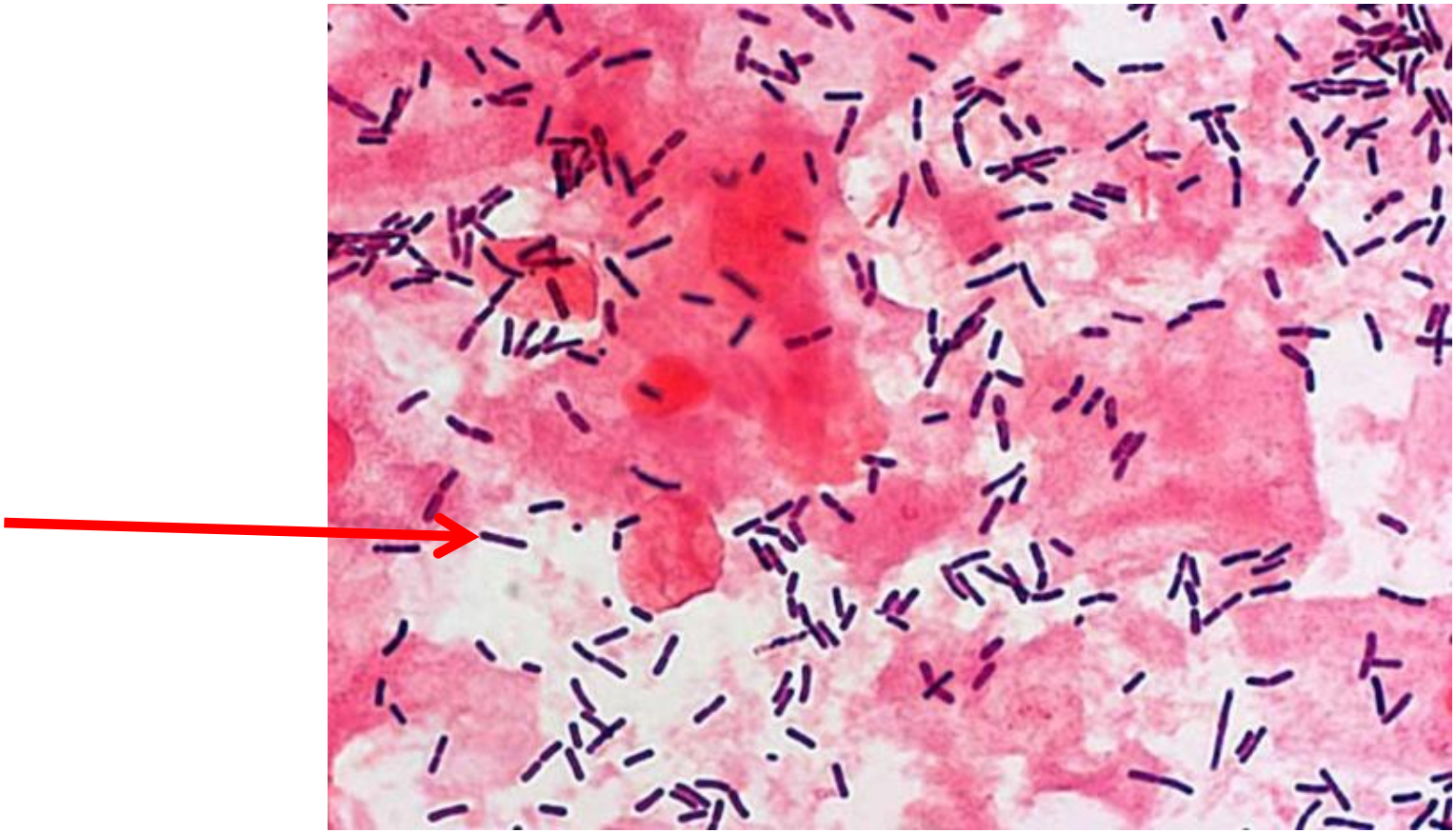
Grram positiv cocci in pairs
Example: *Streptococcus pneumoniae*



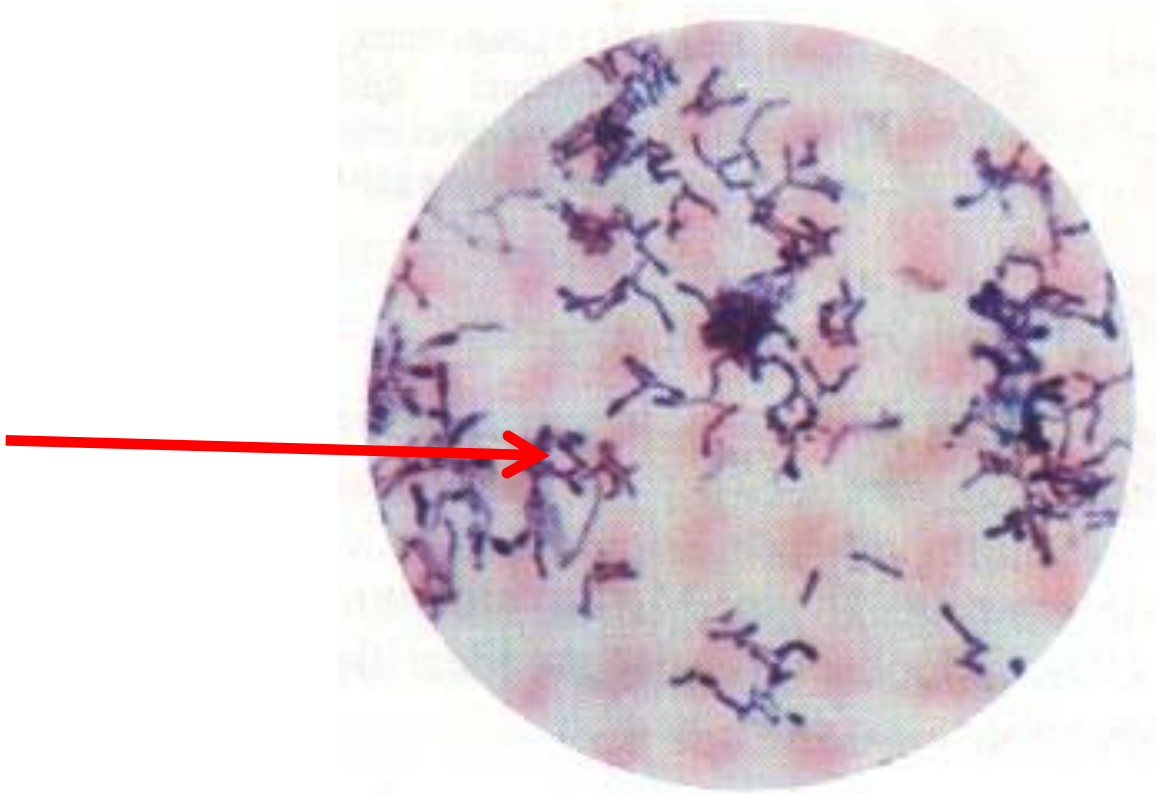
Gram negative coccobacilli
Example: *Haemophilus influenza*



Grram positiv bacilli (spore forming)
Example: *Bacillus anthracis*



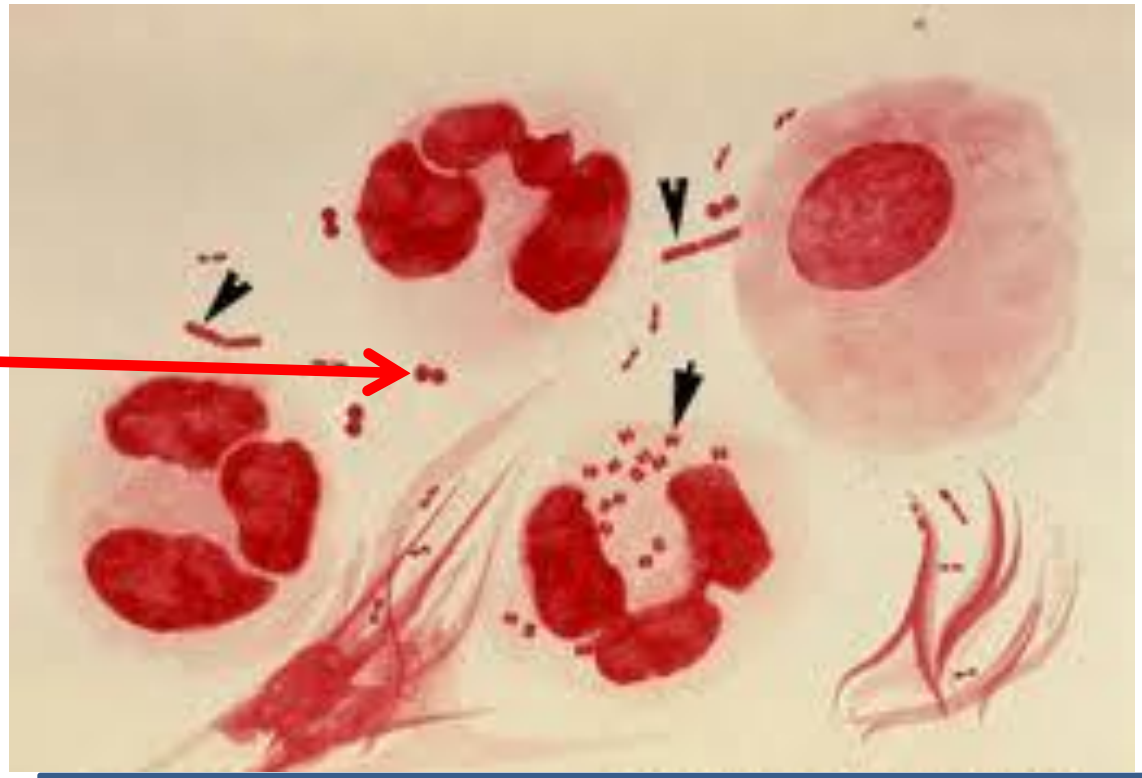
Gram positiv bacilli (non spore forming)
Example: *Lactobacillus species*



Gram positiv bacilli (non spore forming)

(Chines letter)

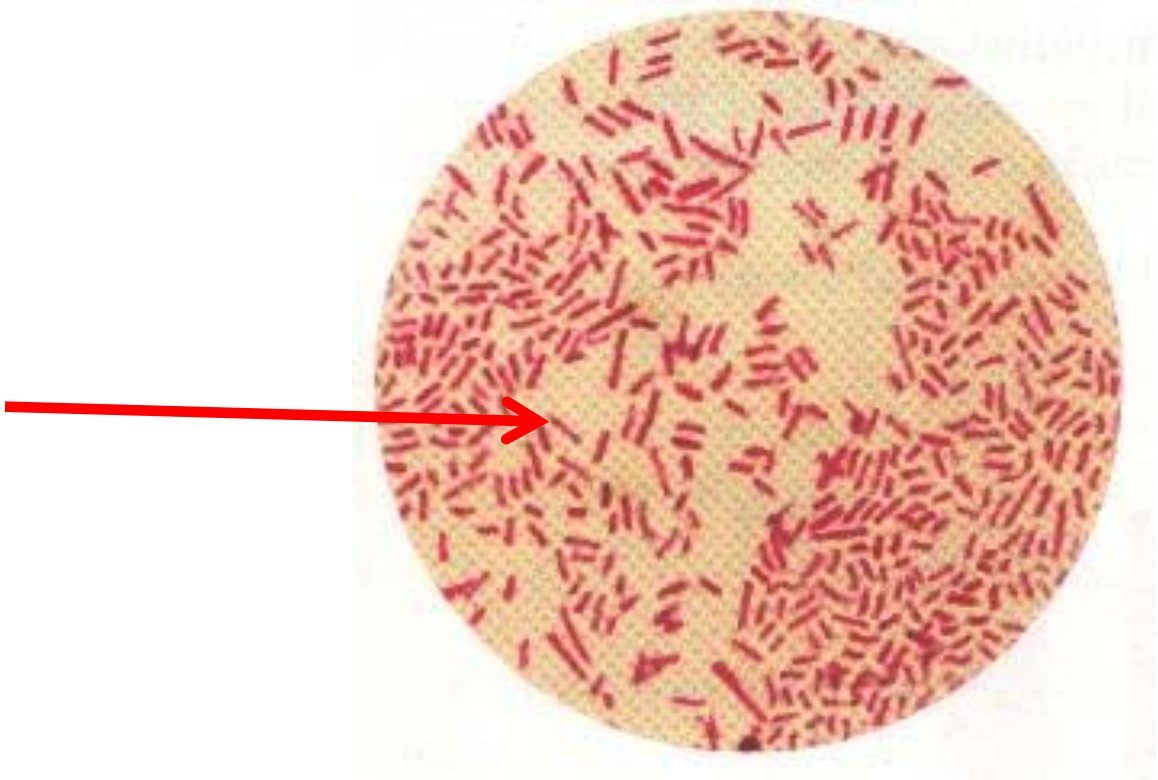
Example: *Corynebacterium diphtheria*



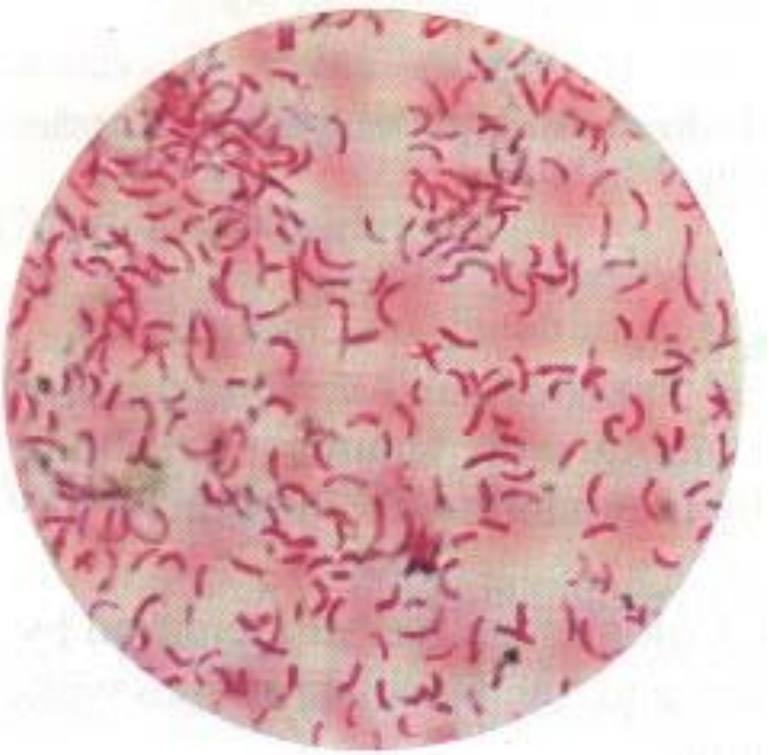
Gram Negative diplococci

Example: *Neisseria gonorrhoeae*

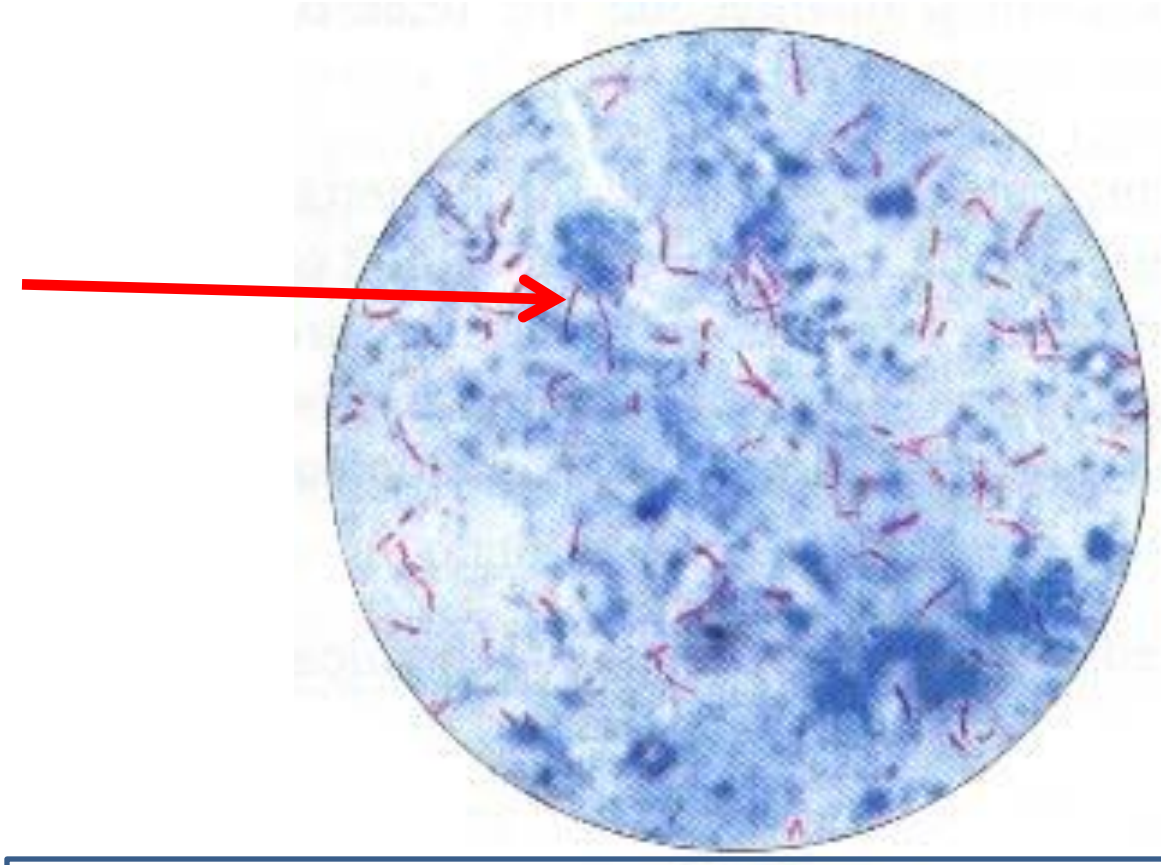
Neisseria meningitidis



Gram Negative bacilli
Example: *Escherichia coli*

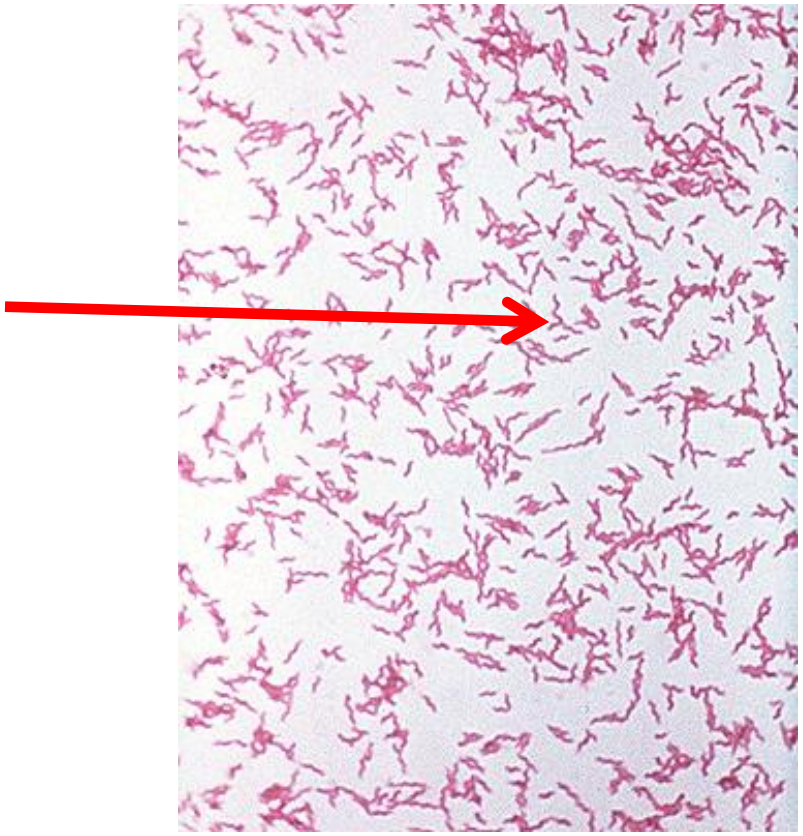


Gram Negative curved rods (Comma shaped)
Example: *Vibrio cholera*

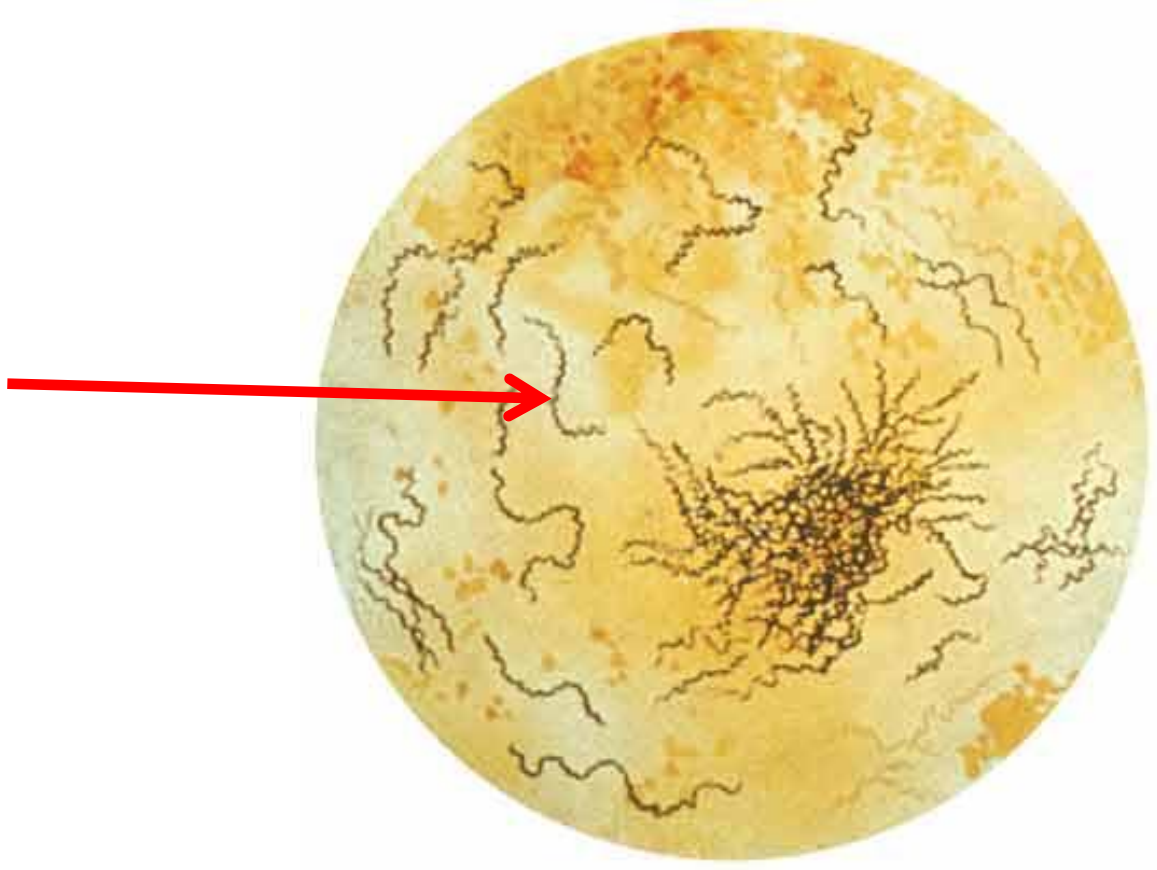


Acid fast bacilli

Example: *Mycobacterium tuberculosis*



Gram Negative spiral rods
Example: *Campylobacter species*



Spirochates
Example: *Treponema pallidum*



Type of culture media: Basic Media

Example: Nutrient agar

Microbe: Staph epidermidis



Type of culture media: Enriched Media
Example: Blood agar (golden yellow)
Microbe: *Staph aureus*



Type of culture media: Enriched Media
Example: Blood agar (white colonies)
Microbe: *Staph epidermidis*
Staph saprophyticus



Type of culture media: Enriched Media
Example: Blood agar (Alpha haemolysis)
Microbe: *Strept pneumoniae*



Type of culture media: Enriched Media
Example: Blood agar (Beta haemolysis)
Microbe: *Strept pyogens*



Type of culture media: Enriched Media
Example: Blood agar (Gamma haemolysis)
Microbe: *Enterococcus faecalis*

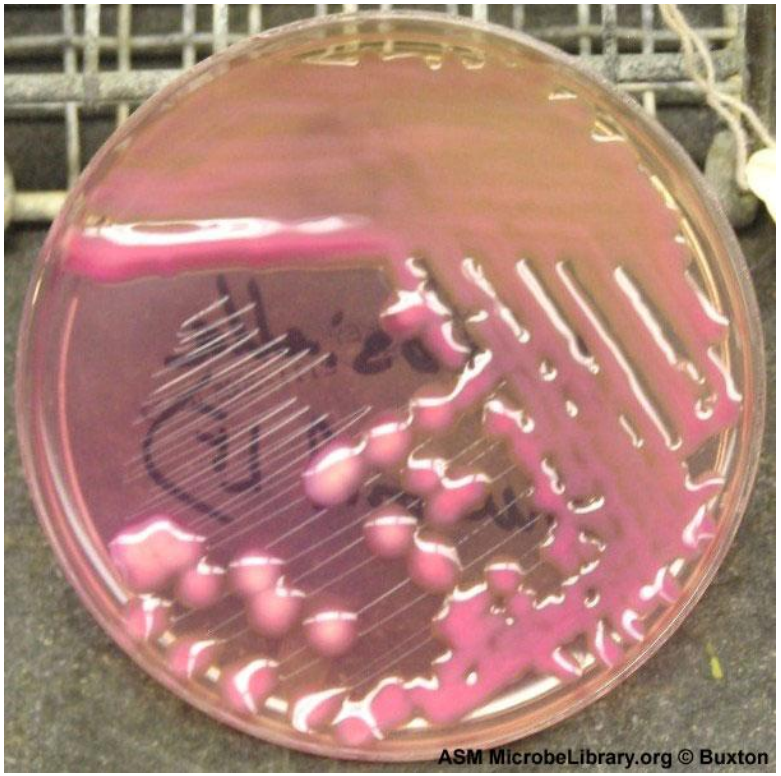


Type of culture media: Enriched Media
Example: Chocalate agar
Microbe: *Haemophilus influenza*





Type of culture media: Differential Media
Example: MacConkey agar
Microbe: *Escherichia coli*



Type of culture media: Differential Media
Example: MacConkey agar
Microbe: *Klebsiella pneumoniae*



Type of culture media: Differential Media
Example: TCBS (Thiosulfate citrate bile salts sucrose agar)
Microbe: *Vibrio cholera*

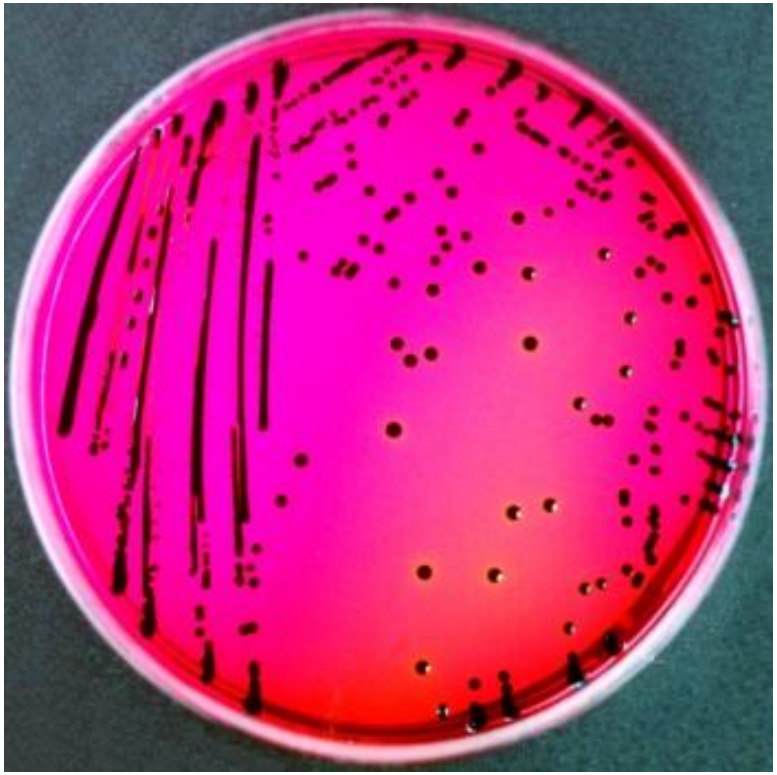


Type of culture media: Differential Media
Example: CLED (Cystine-Lactose-Electrolyte-Deficient Agar)
Microbe: *Escherichia coli*

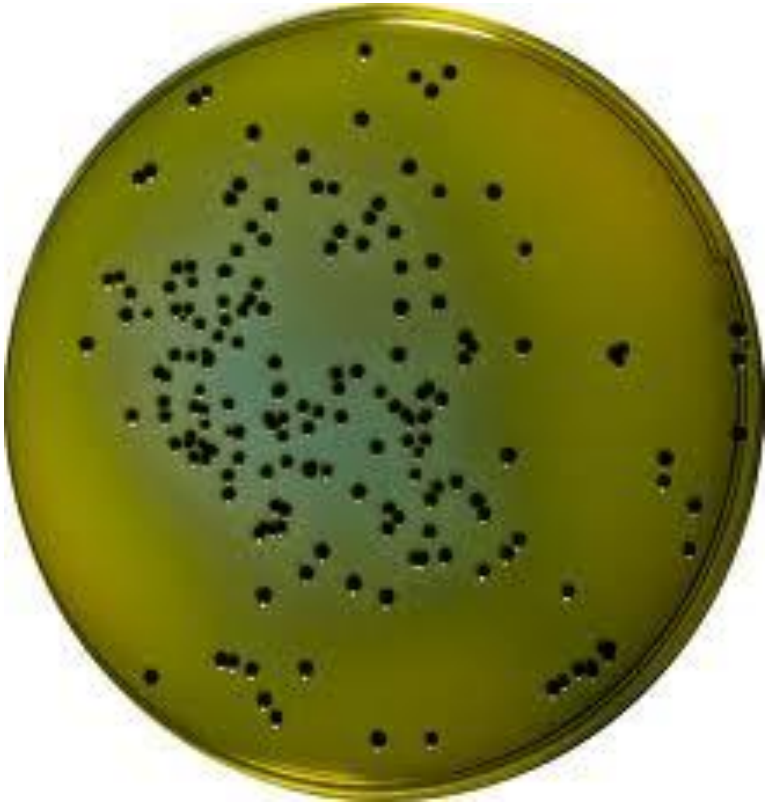
Dr. Nabil El Aila
General Microbiology



Type of culture media: Selective Media
Example: Salmonella shigella agar
Microbe: *Salmonella typhi*



Type of culture media: Selective Media
Example: Xylose lysine Deoxycholate
Microbe: *Salmonella typhi*



Type of culture media: Selective Media
Example: Hecktoen Enteric
Microbe: *Salmonella typhi*



Type of culture media: Selective Media
Example: Mannitol salt agar
Microbe: *Staph aureus*

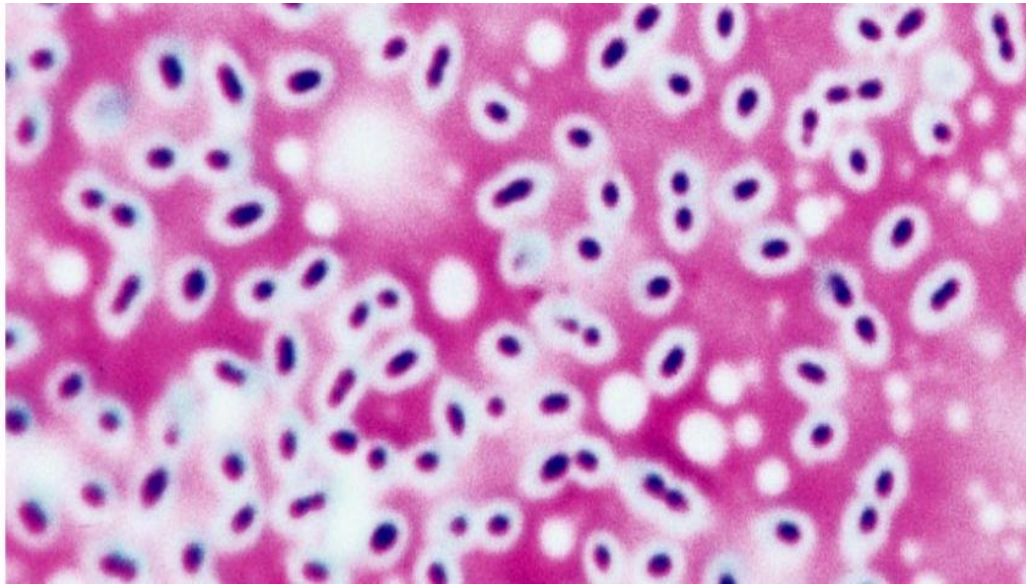


Type of culture media: Selective Media
Example: Lewenstein Jensen Medium
Microbe: *Mycobacterium tuberculosis*

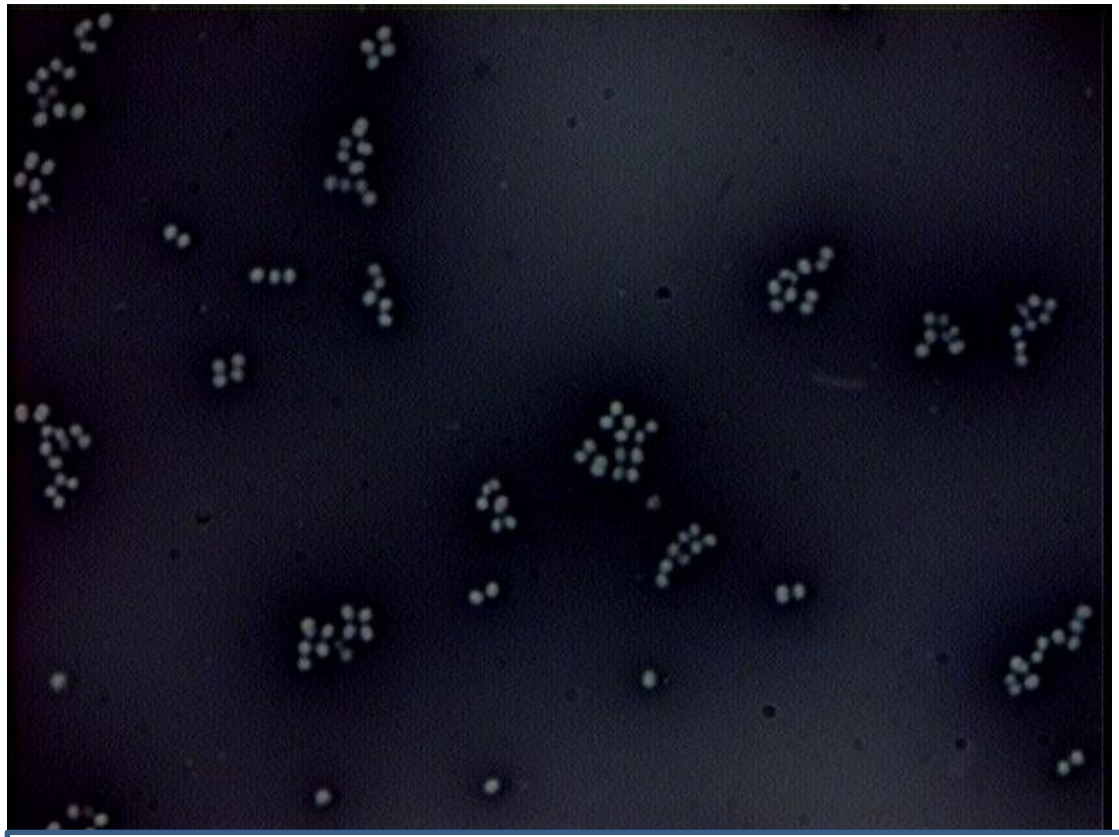


Muller Hinton Agar

Used for antibiotic susceptibility testing



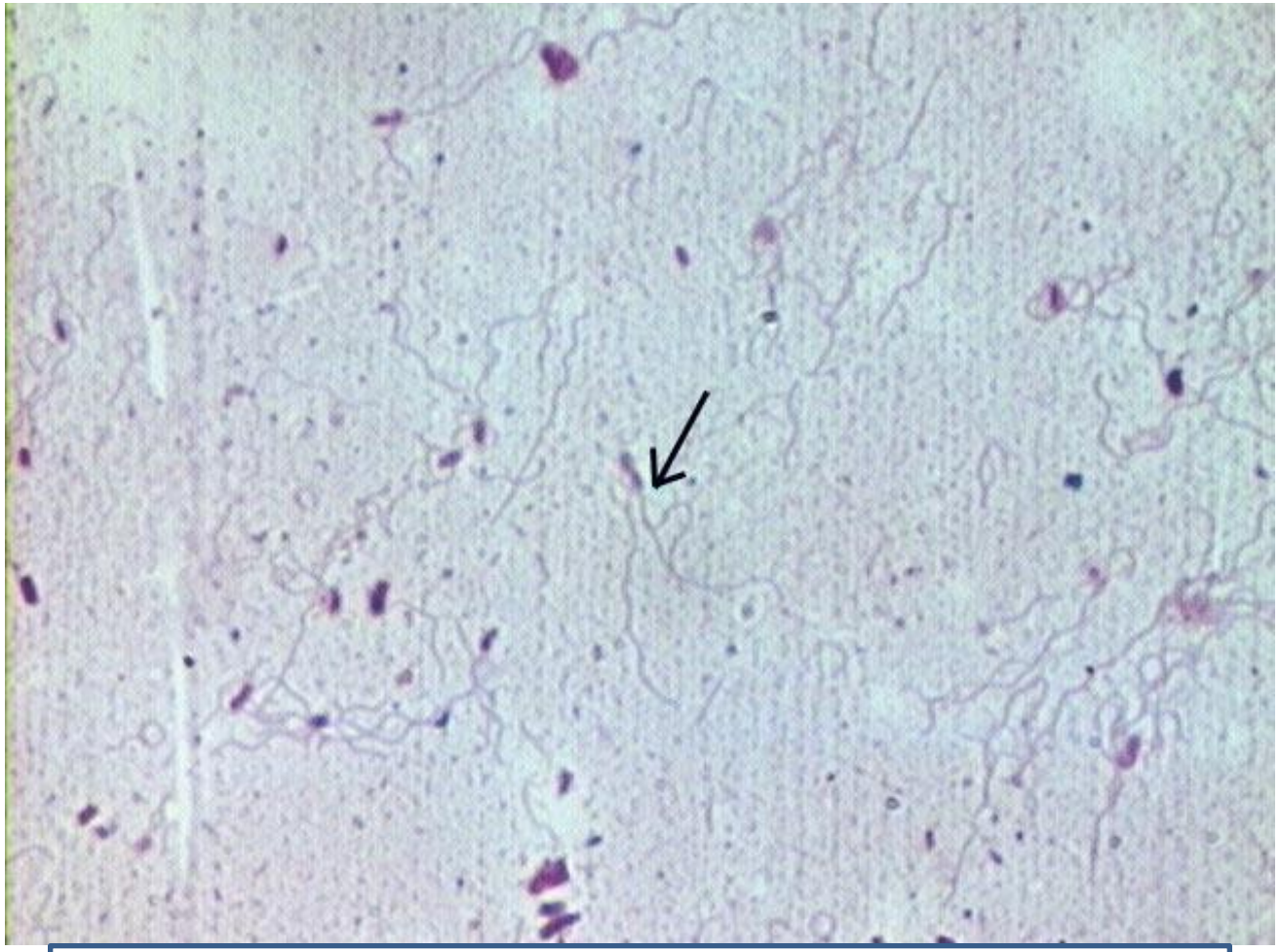
Capsule stain



Negative stain



Spore stain



Flagellar stain