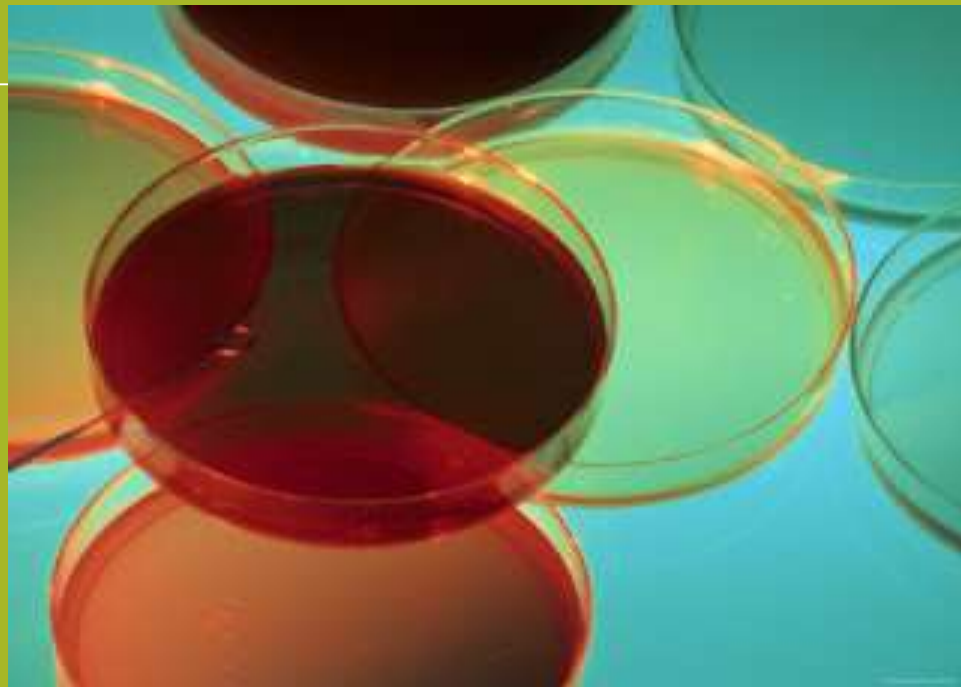


TYPES OF CULTURE MEDIA

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FACULTY OF MEDICINE, MU'TAH UNIVERSITY



Purpose

To become familiar with the selective and differential media used to identify the infections associated bacteria

Principle

- Bacteria and other microbes have particular requirements for growth. Therefore, in order to successfully grow the bacteria in lab so that we can stain and identify them, we must provide an environment that is suitable for growth.
- Growth media are used to cultivate bacteria because it contains essential:
 - ✓ Necessary nutrients
 - ✓ Moisture
 - ✓ pH to support microbial growth

Streaking Microbial Cultures on Agar Plates

Agar plate streaking are an essential tool in microbiology. They allow bacteria and fungi to grow on a semi-solid surface to produce discrete colonies. These colonies can be used to help identify the organism

Quadrant Streak

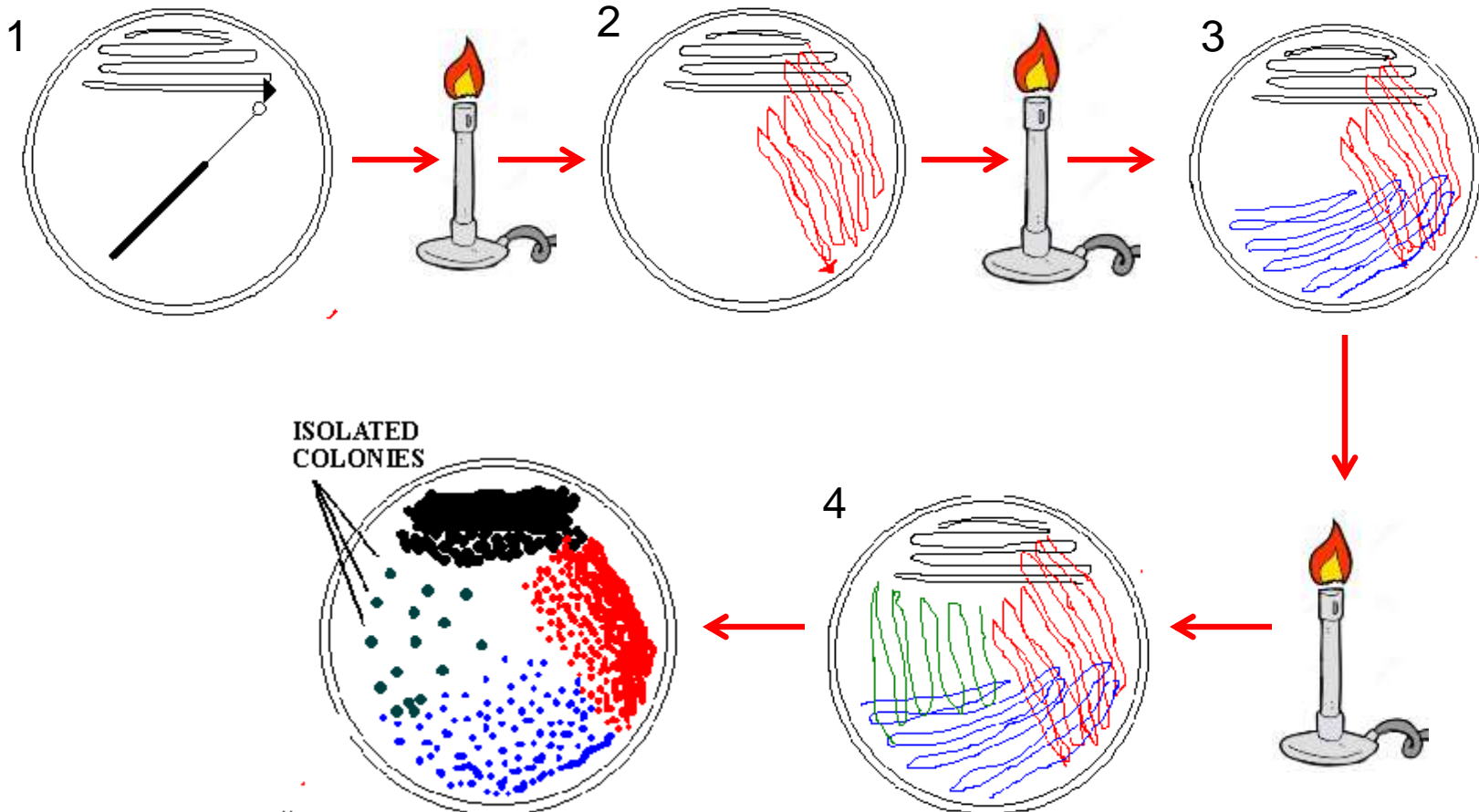
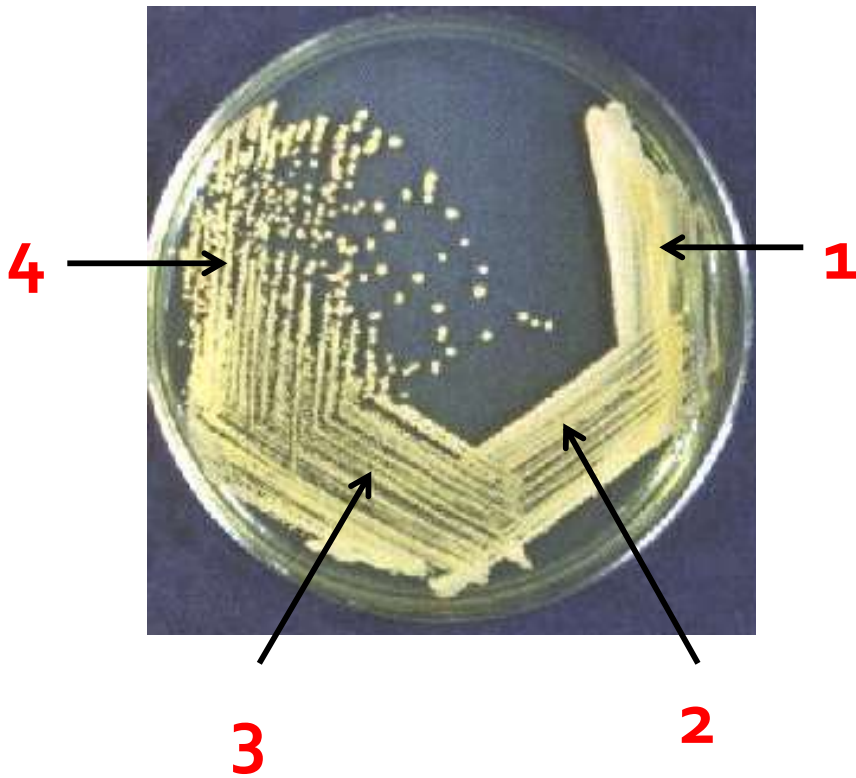
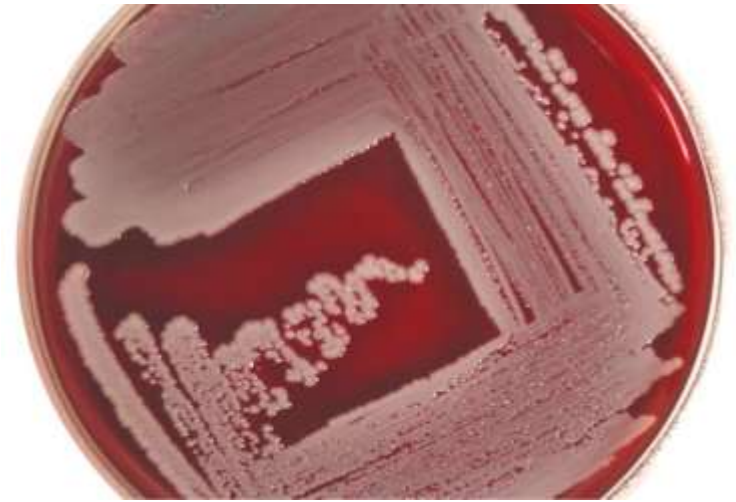


Plate streaking technique

Streaking Microbial Cultures on Agar Plates

Plate streaking technique



Quadrant Streak



Overview of bacterial infections

Bacterial meningitis

- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Streptococcus agalactiae*
- *Listeria monocytogenes*

Otitis media

- *Streptococcus pneumoniae*

Pneumonia

Community-acquired:

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Staphylococcus aureus*

Atypical:

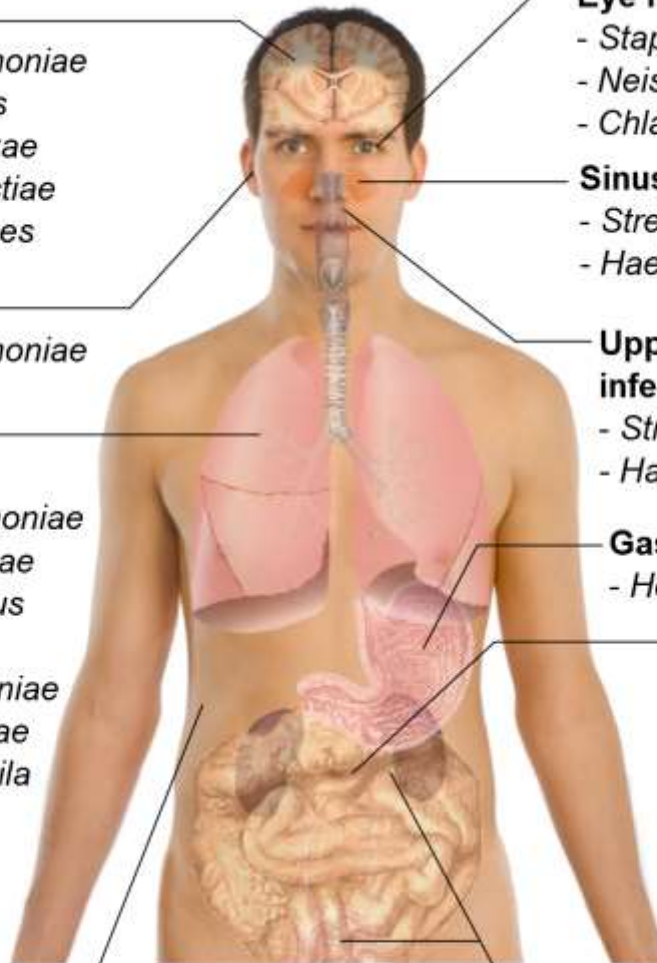
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*
- *Legionella pneumophila*

Tuberculosis

- *Mycobacterium tuberculosis*

Skin infections

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Pseudomonas aeruginosa*



Eye infections

- *Staphylococcus aureus*
- *Neisseria gonorrhoeae*
- *Chlamydia trachomatis*

Sinusitis

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*

Upper respiratory tract infection

- *Streptococcus pyogenes*
- *Haemophilus influenzae*

Gastritis

- *Helicobacter pylori*

Food poisoning

- *Campylobacter jejuni*
- *Salmonella*
- *Shigella*
- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

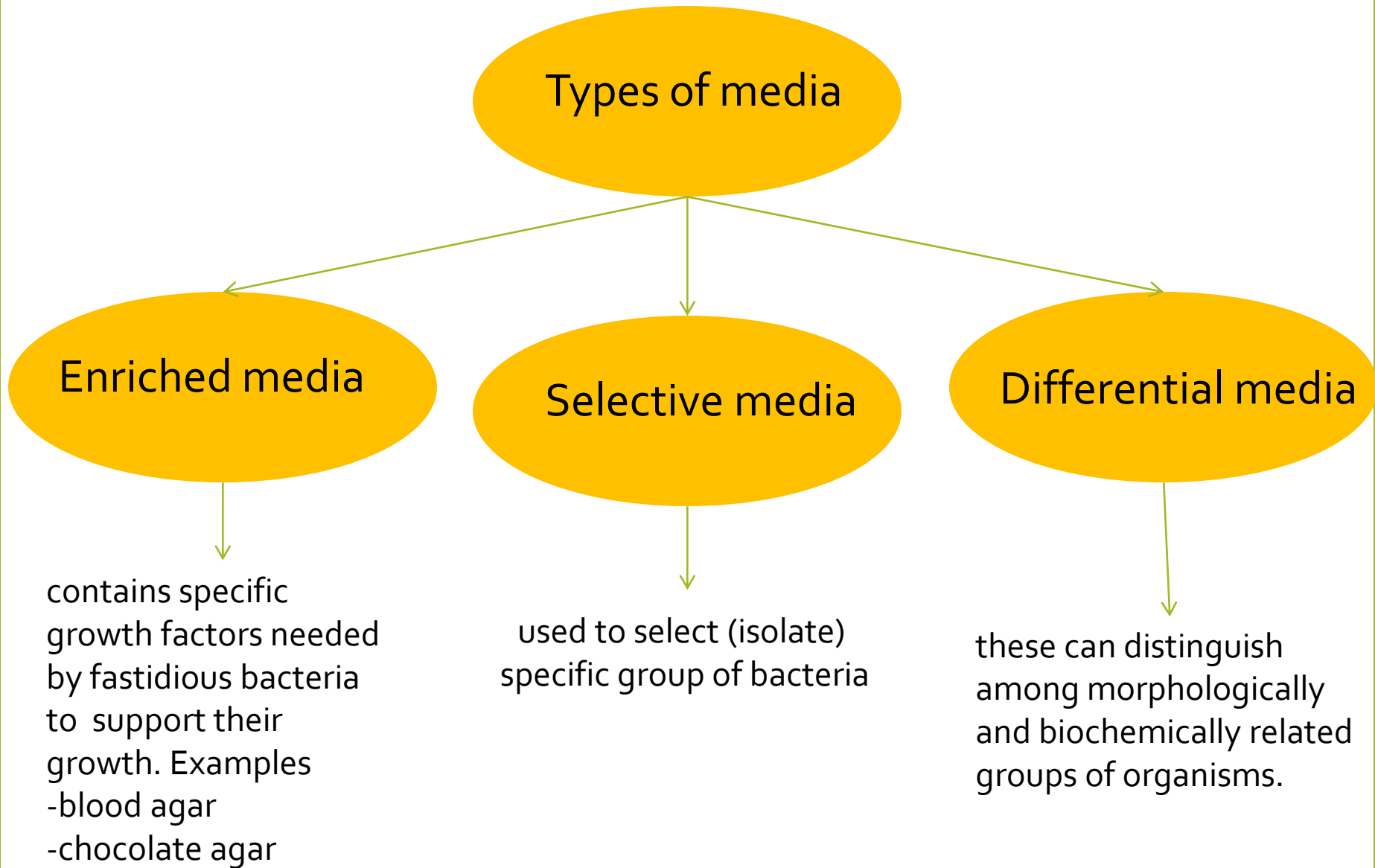
Sexually transmitted diseases

- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Ureaplasma urealyticum*
- *Haemophilus ducreyi*

Urinary tract infections

- *Escherichia coli*
- Other *Enterobacteriaceae*
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*

Types of media



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- *Haemophilus influenzae*

Upper respiratory tract infection

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- *Haemophilus influenzae*

Gastritis

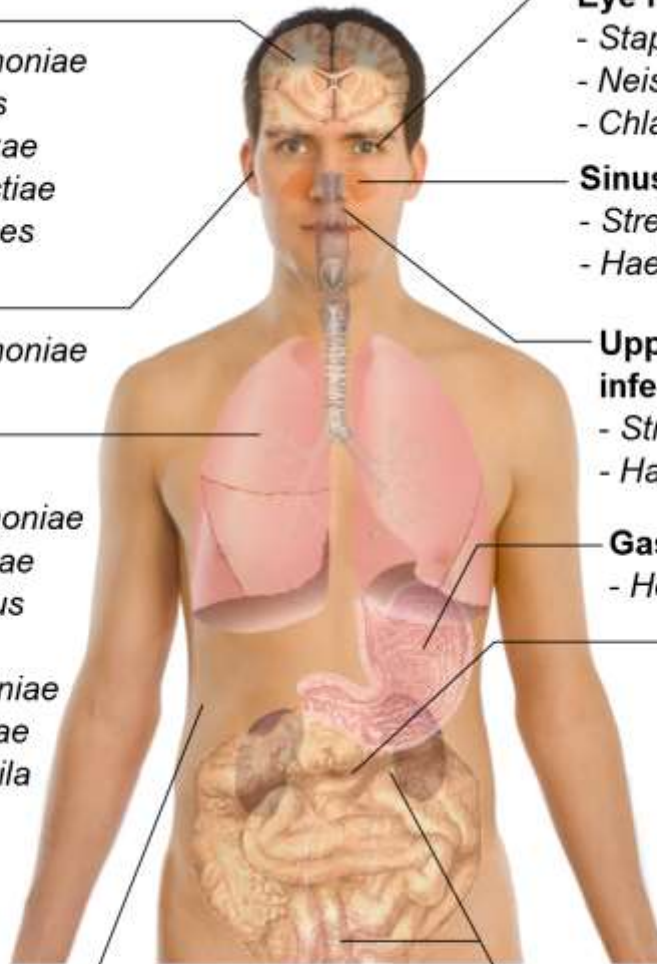
- *Helicobacter pylori*

Food poisoning

- *Campylobacter jejuni*
- *Salmonella*
- *Shigella*
- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

Urinary tract infections

- *Escherichia coli*
- Other Enterobacteriaceae
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*



Microbiological Analysis of Urine Specimens

<i>Stapylococcus aureus</i>		
<i>Streptococcus pyogenes</i>		
Bacteria	Gram positive	Enterococci
	Gram negative	
		<i>Streptococcus facalis</i> <i>Streptococcus faecium</i>
		<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Proteus vulgaris</i> <i>Klebsiella pneumoniae</i>
Viruses _____	Venereal Disease	<i>Treponema pallidum</i> <i>Neisseria gonorrhoeae</i> <i>Hemophilus ducreyi</i>
	—	<i>Calymnatobacterium granulomatis</i> <i>Herpes hominus (type 11)</i>
Fungi	<i>Candida albicans</i> <i>Blastomyces dermatitidis</i> <i>Coccidioides bancrofti</i>	
Protozoa	<i>Trichomonas vaginalis</i> <i>Entameoba histolytica</i>	

Urine analysis

Midstream urine sample
Sample inoculation

Blood agar

Significant growth

Gram stain

Gram positive cocci

catalase

positive

Staphylococci

Mannitol salt agar

MacConkey agar

Abundant growth

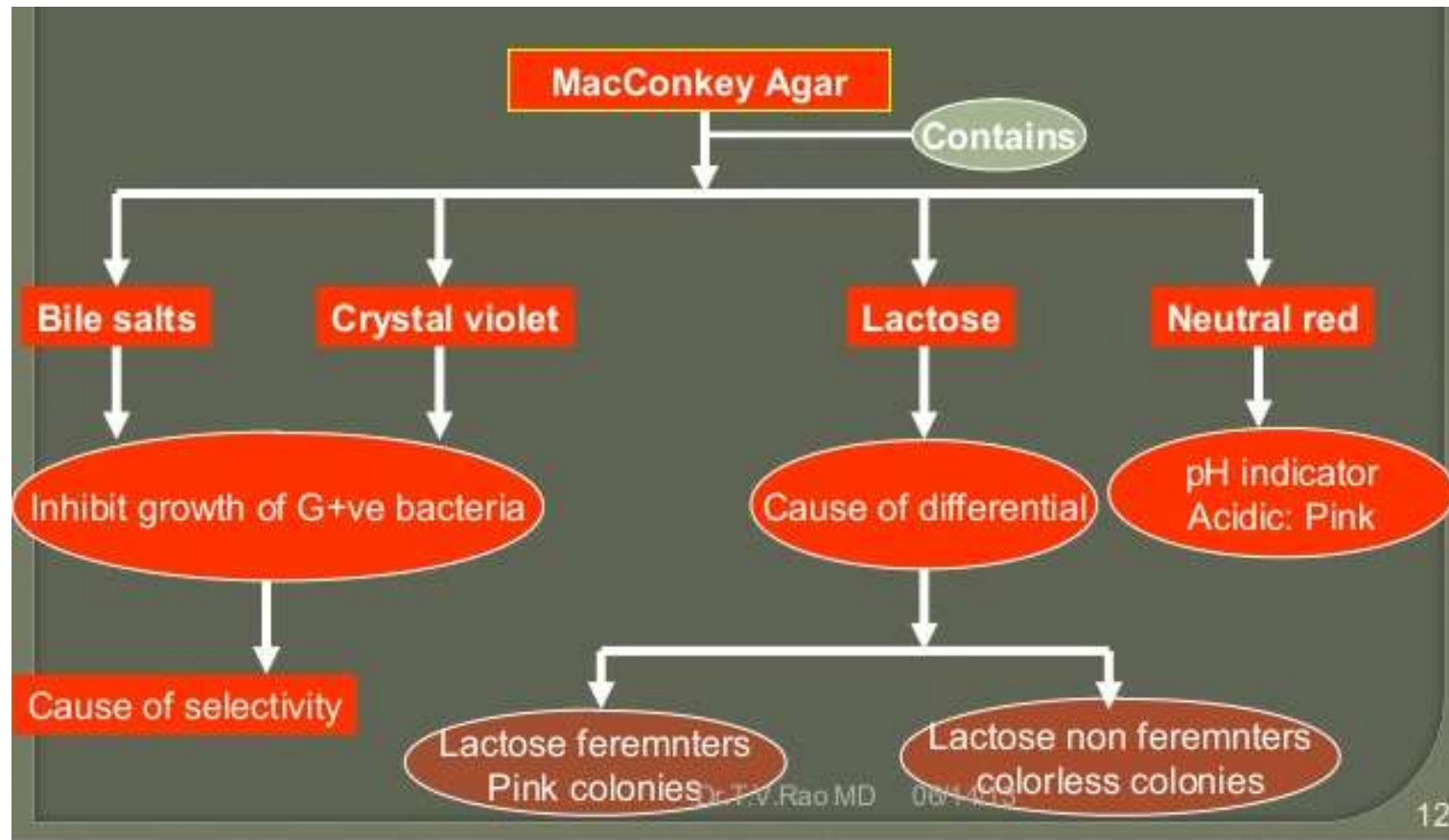
Gram stain

Gram negative bacilli

Escherichia coli, *Pseudomonas aeruginosa*
Proteus vulgaris, *Klebsiella pneumoniae*

Biochemical reactions

MacConkey agar is a selective and differential media for Enterobacteriaceae



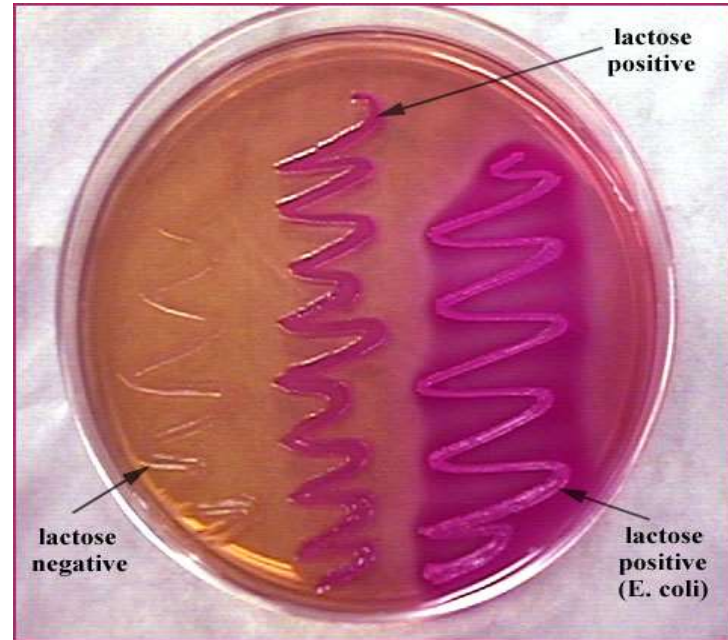
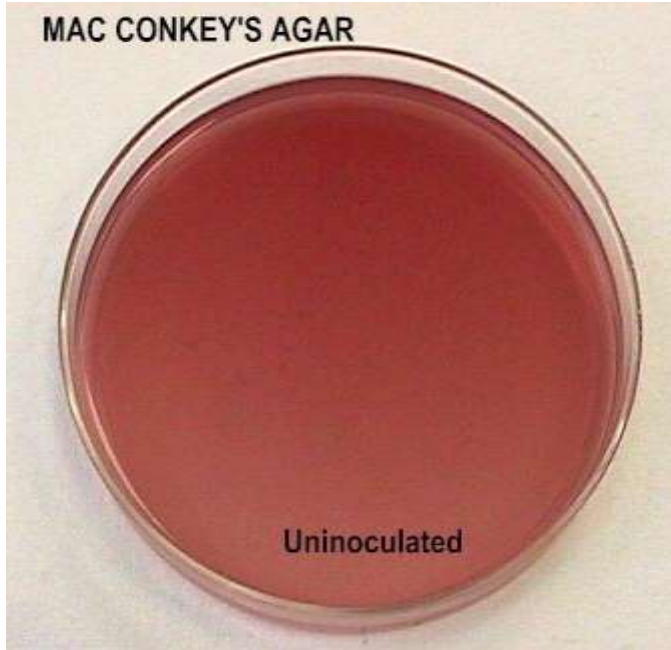
↓

Escherichia coli
Klebsiella spp
Enterobacter spp
Citrobacter spp

↓

Salmonella spp
Schigella spp
Proteus spp
Yersinia spp

MacConkey agar



Blood agar (BA)

Enriched medium: containing peptones, yeast extracts, liver or heart extracts (depending on the medium), and blood.



Some bacteria produce an enzyme called hemolysin that is able to lyse RBCs (hemolysis)

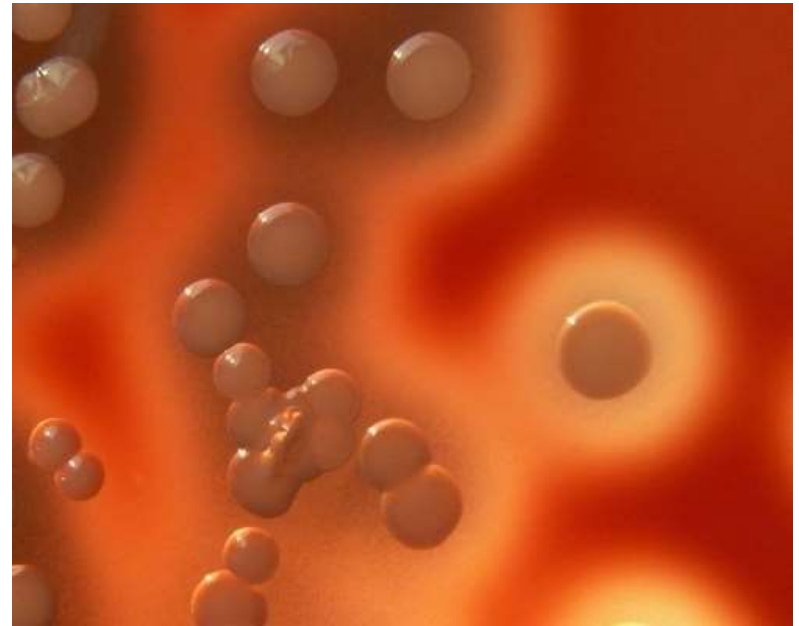
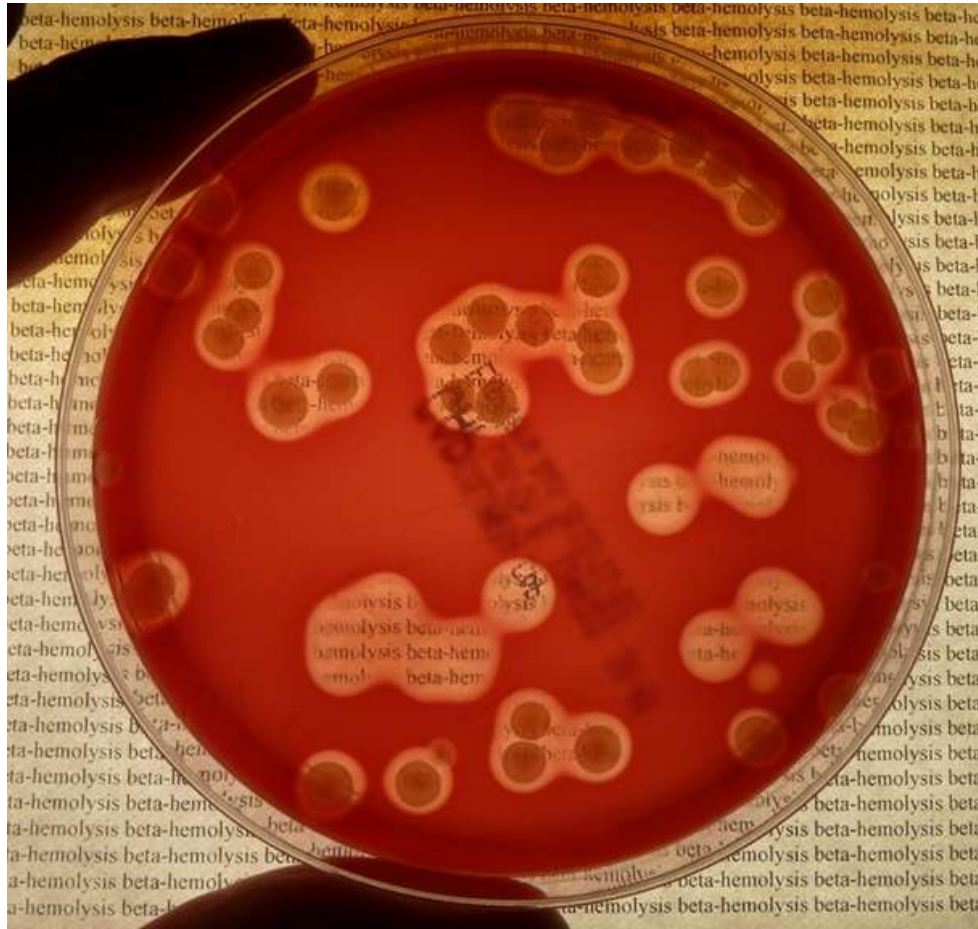
Differential medium: containing blood

If hemolysin is produced by the bacteria it will be secreted into the medium and the RBCs will be lysed

Growth on BA differentiates between the three groups of Bacteria:

- 1- Alpha hemolytic bacteria
- 2- Gamma hemolytic bacteria
- 3- Beta hemolytic bacteria

Beta hemolysis = Complete hemolysis



Alpha hemolysis

Hemoglobin containing
 Fe^{2+} (ferrous)



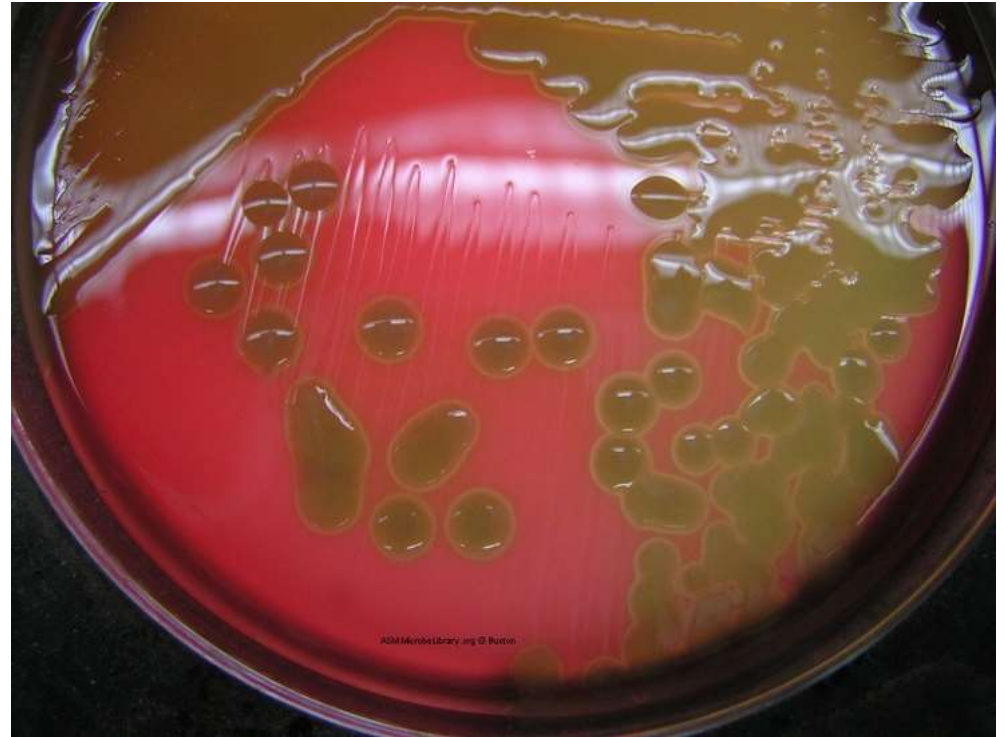
hydrogen peroxide
produced by the bacterium



**Oxidation of Fe^{2+}
into Fe^{3+} (ferric) state**



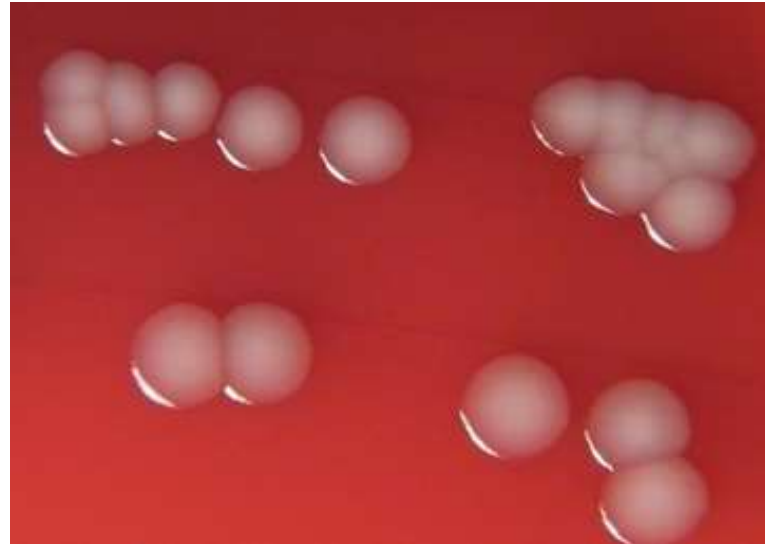
Hemoglobin converted into
methemoglobin (greenish color)



Incomplete (partial) lysis of RBCs

Gamma hemolysis

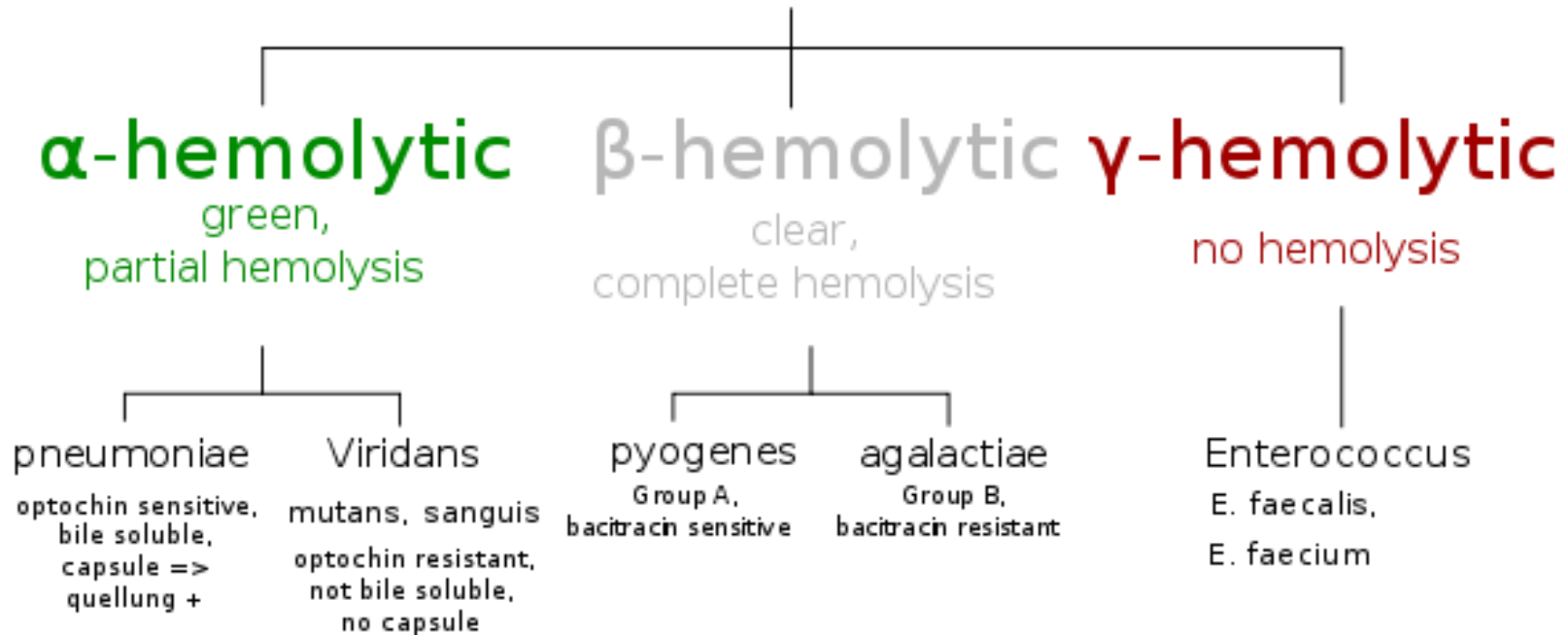
No hemolysis, and no change in the medium



The three types of hemolysis

The hemolytic pattern of different Streptococci

Streptococcus



Mannitol salt agar

Selective and Differential for Staphylococci



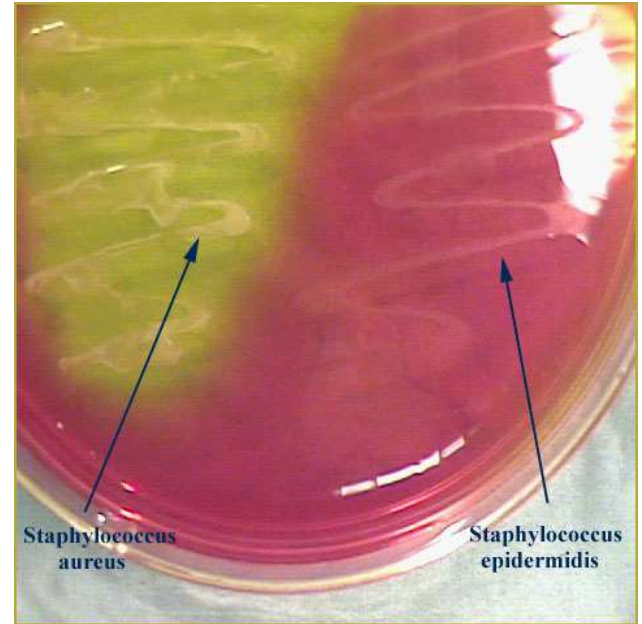
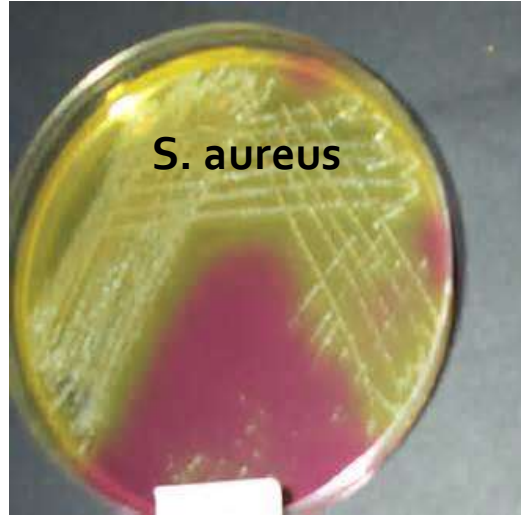
- **Selective agent:** 7.5% NaCl
- **Differential agent:** mannitol to differentiate between mannitol Fermenters and nonfermenters
- pH indicator: Phenol red

Mannitol salt agar

MSA



Non-cultured



Blood agar



Beta hemolytic Staphylococci

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- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Streptococcus agalactiae*
- *Listeria monocytogenes*

Otitis media

- *Streptococcus pneumoniae*

Pneumonia

Community-acquired:

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Staphylococcus aureus*

Atypical:

- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*
- *Legionella pneumophila*

Tuberculosis

- *Mycobacterium tuberculosis*

Skin infections

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Pseudomonas aeruginosa*

Sexually transmitted diseases

- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Ureaplasma urealyticum*
- *Haemophilus ducreyi*

Eye infections

- *Staphylococcus aureus*
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- *Chlamydia trachomatis*

Sinusitis

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*

Upper respiratory tract infection

- *Streptococcus pyogenes*
- *Haemophilus influenzae*

Gastritis

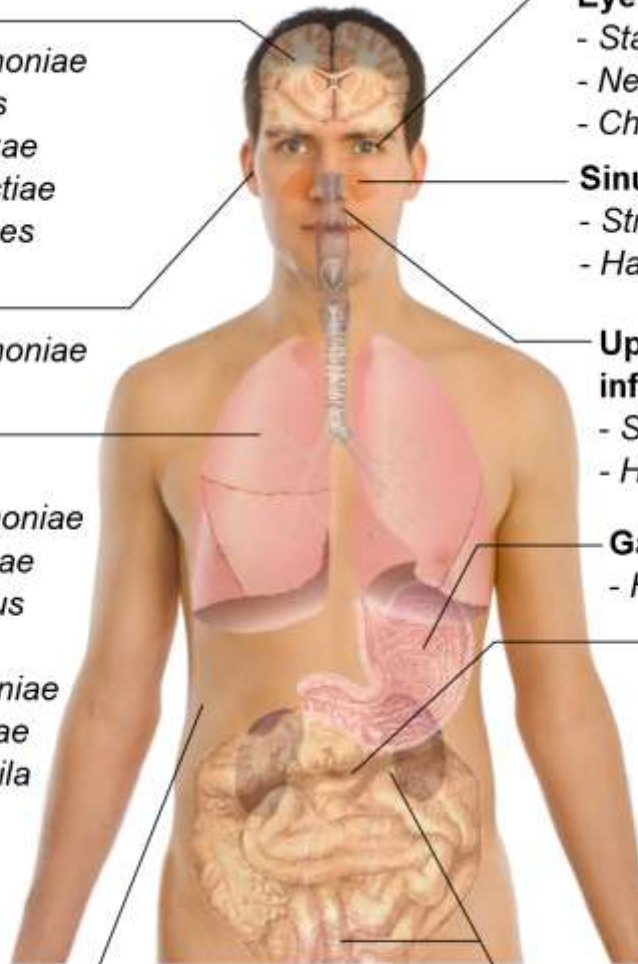
- *Helicobacter pylori*

Food poisoning

- *Campylobacter jejuni*
- *Salmonella*
- *Shigella*
- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

Urinary tract infections

- *Escherichia coli*
- Other *Enterobacteriaceae*
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*

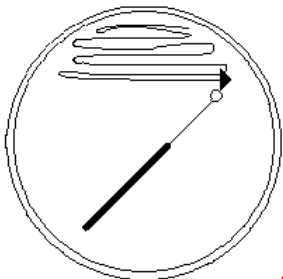


Processing of stool samples

Patient with diarrhea



Stool sample received in the lab



Culture for bacteria



Microscopy for parasites

Salmonella -*Shigella* agar (SS agar)

Purpose

For isolation and differentiation of *Salmonella* & *Shigella*

Components

- ✓ Bile salt: inhibit the growth of gram-positive bacteria (**selective agent**)
- ✓ Lactose: carbon source
- ✓ Neutral red: pH indicator, red in acidic conditions

Salmonella Shigella agar (SS agar)

Why black colonies?

Due to the production of FeS (ferrous sulfide) forming black precipitate presented by black-centered colonies)

SS agar

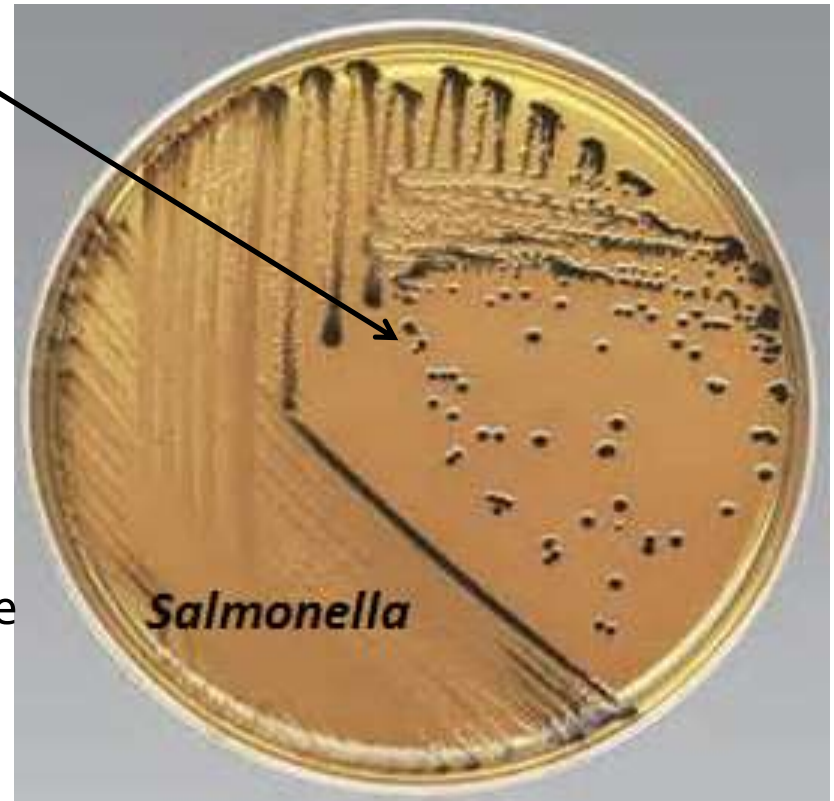


- Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$): sulfur source
- Fe^{3+} (ferric) H_2S indicator



$\text{Na}_2\text{S}_2\text{O}_3$ + thiosulfate reductase \longrightarrow sulfite + H_2S

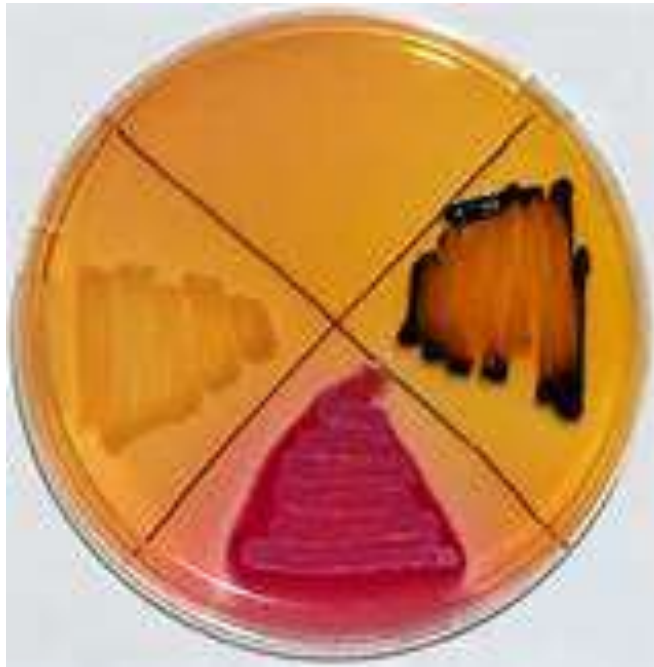
H_2S + Fe^{3+} \longrightarrow FeS (black precipitate presented by black-centered colonies)



Salmonella Shigella agar (SS agar)

Results

- ✓ Lactose fermenters: pink to red colonies (few can grow)
- ✓ Non lactose fermenters: translucent, colorless colonies with or without black centers



Lactose fermenter flora:
pink to red colonies



Salmonella:
colorless colonies
with black centers



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Skin infections

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- *Streptococcus pyogenes*
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Sexually transmitted diseases

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Eye infections

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- *Chlamydia trachomatis*

Sinusitis

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- *Haemophilus influenzae*

Upper respiratory tract infection

- *Streptococcus pyogenes*
- *Haemophilus influenzae*

Gastritis

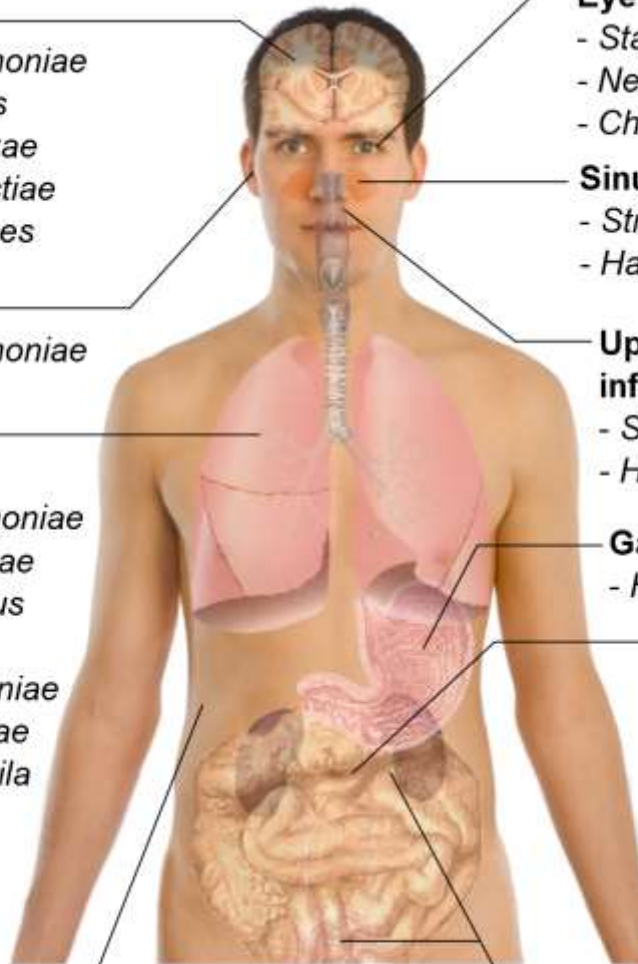
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Food poisoning

- *Campylobacter jejuni*
- *Salmonella*
- *Shigella*
- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

Urinary tract infections

- *Escherichia coli*
- Other *Enterobacteriaceae*
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*



Cholera identification

Identification

- ✓ Thiosulfate citrate bile salt sucrose agar or TCBS agar
- ✓ The medium is alkaline (pH 8.6) which enhances the growth of *Vibrio* species

Important components

- ✓ Sucrose: sugar source
- ✓ Bromothymol blue: pH indicator
 - pH < 6.0 - yellow
 - pH > 7.6 - blue

Cholera identification

Results

- ✓ *Vibrio cholera*: Ferment sucrose smooth yellow colonies
- ✓ *Vibrio parahemolyticus*: non-sucrose fermenter, green colonies



TCBS media

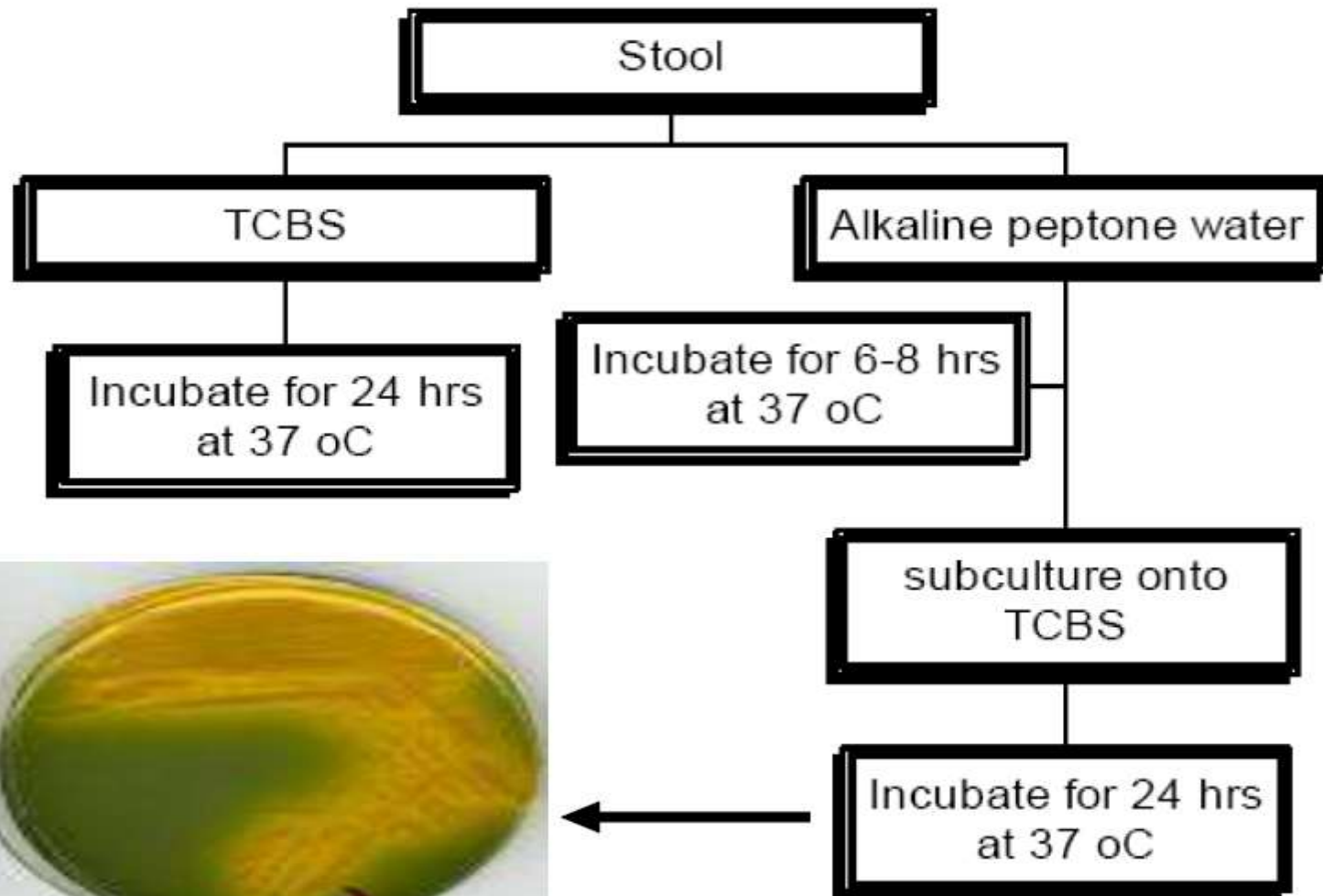


V. cholera



V. parahemolyticus

Cholera identification



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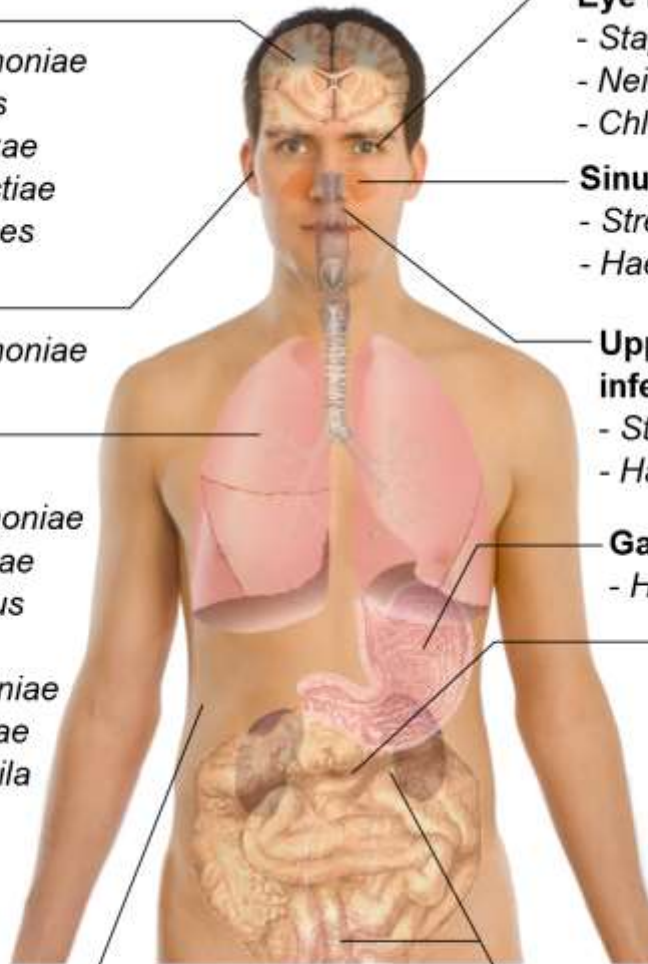
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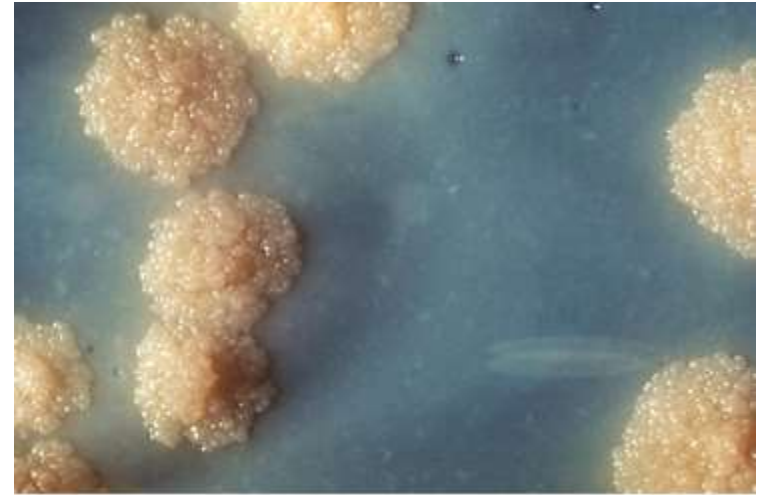
Urinary tract infections

- *Escherichia coli*
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- *Pseudomonas aeruginosa*



Löwenstein–Jensen (LJ) medium

- Is a growth medium specially used for culture of *Mycobacterium*, notably *Mycobacterium tuberculosis*.



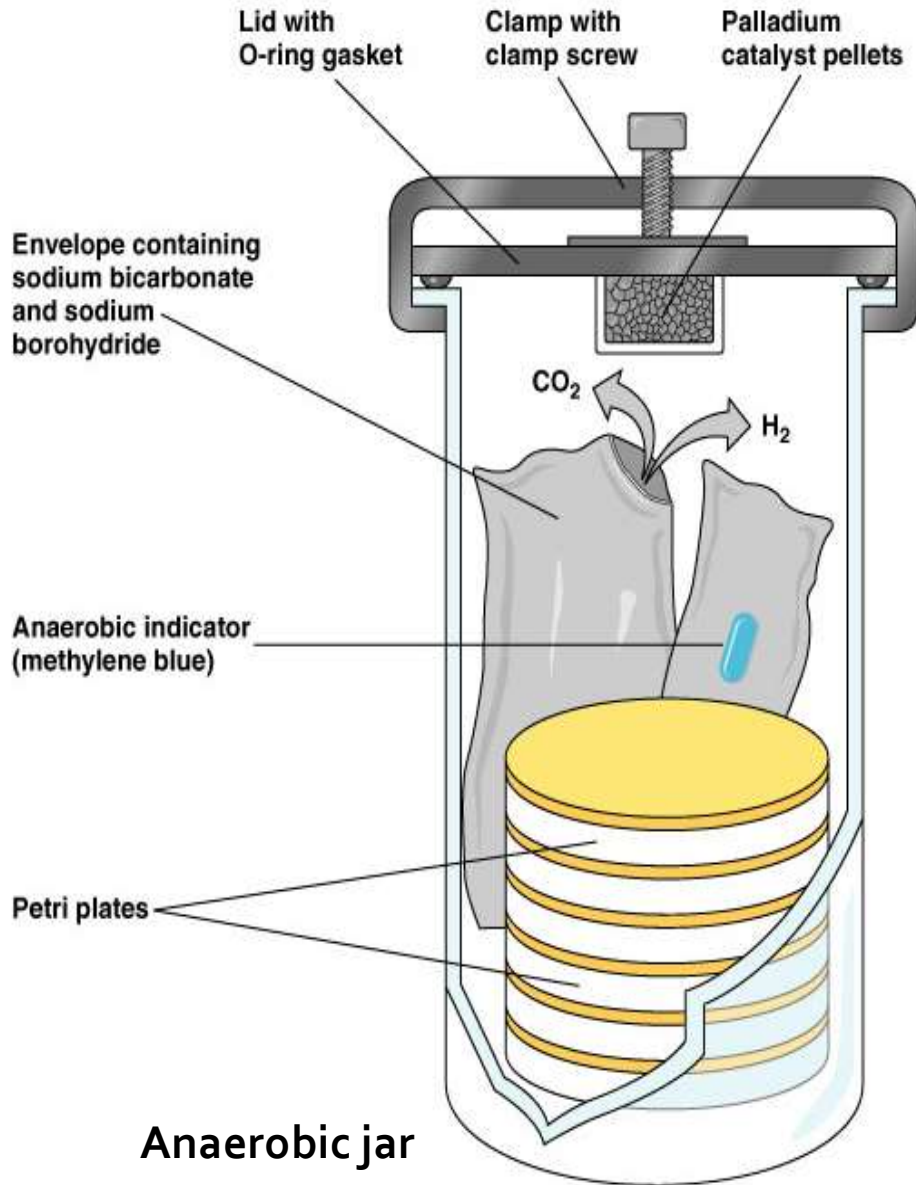
M.tuberculosis produces rough and tough colonies

Löwenstein–Jensen (LJ) medium

Penicillin and Nalidixic acid (FD053) along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of Mycobacteria

Composition	Ingredients Gms / 600 ml
L-Asparagine	3.600
Monopotassium phosphate	2.400
Magnesium sulphate	0.240
Magnesium citrate	0.600
Potato starch, soluble	30.000
Malachite green	0.40

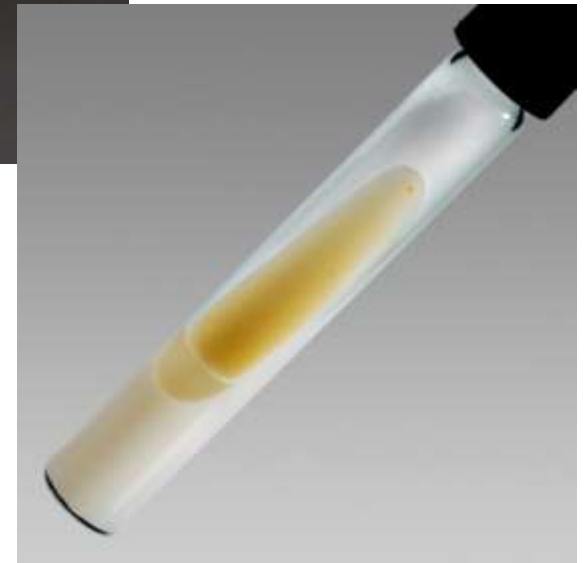
Anaerobic jars



Anaerobic candle jar

Löffler's medium

Is a special substance used to grow *Corynebacterium diphtheriae* bacilli to confirm the diagnosis.



Gram-positive rod-shaped bacteria that are straight or slightly curved. The bacteria group together in a characteristic way (Chinese letters)

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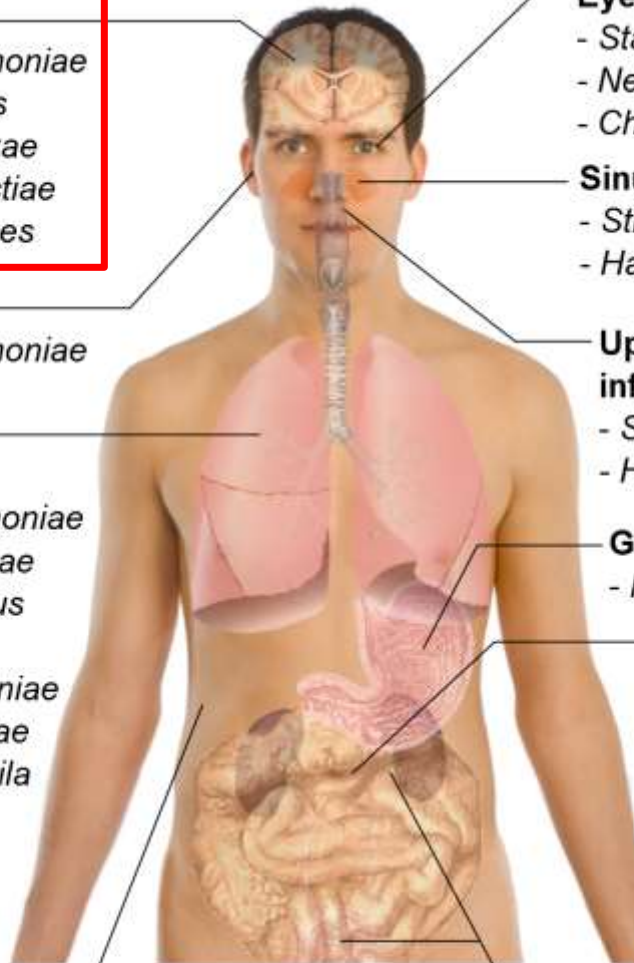
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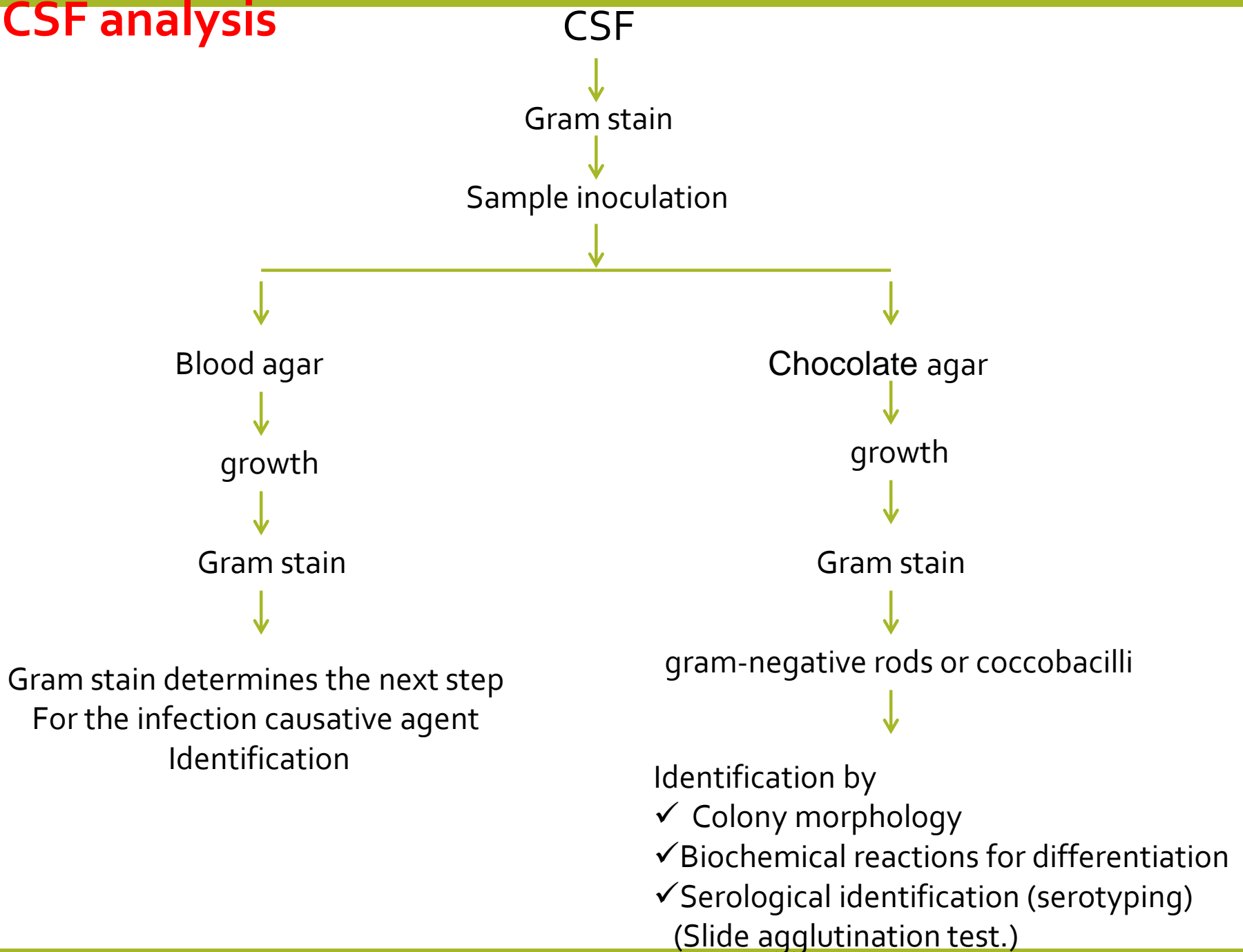
- *Campylobacter jejuni*
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- *Clostridium*
- *Staphylococcus aureus*
- *Escherichia coli*

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CSF analysis



Chocolate agar

- Used to isolate *Haemophilus influenzae*
- Is a hemolysed blood either by heating blood to 80°C or using enzyme treatment
- Treatment result in browning of the medium, therefore, it is called chocolate agar.



Chocolate agar



Haemophilus influenzae growth on Chocolate agar