TYPES OF CULTURE MEDIA

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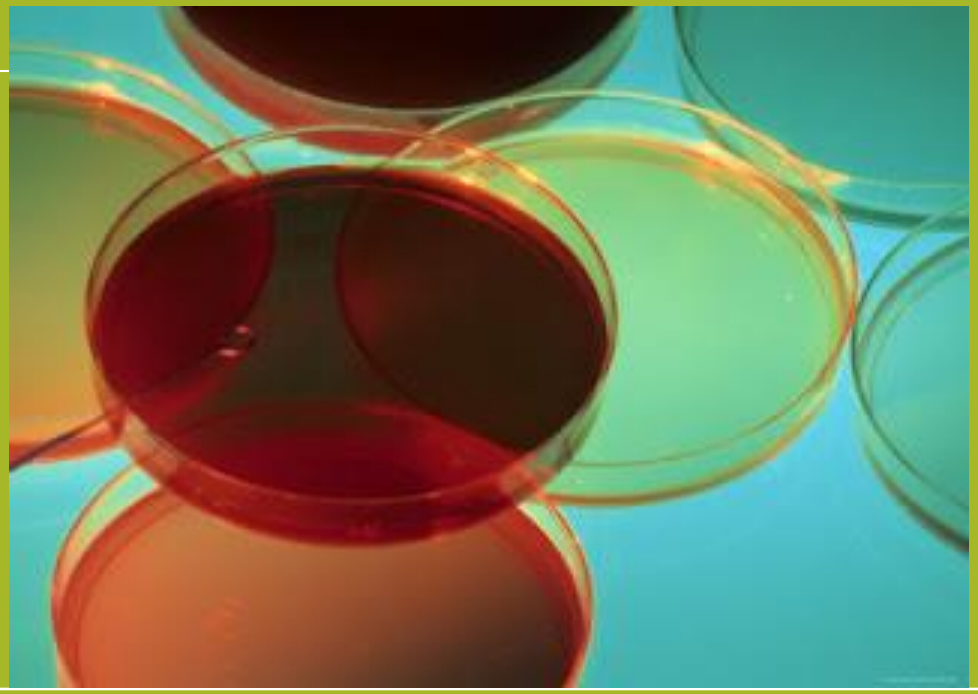
LAB 4



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

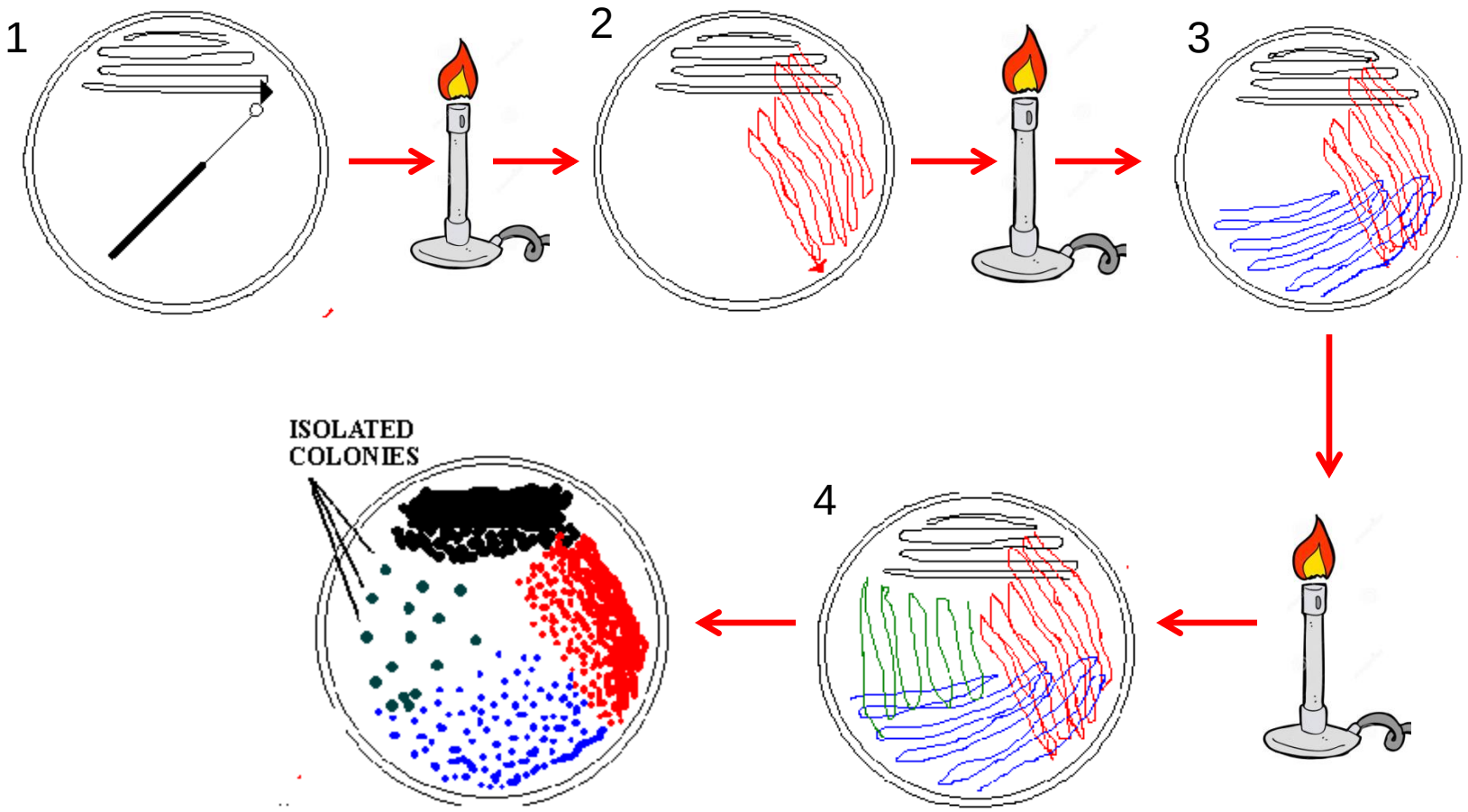
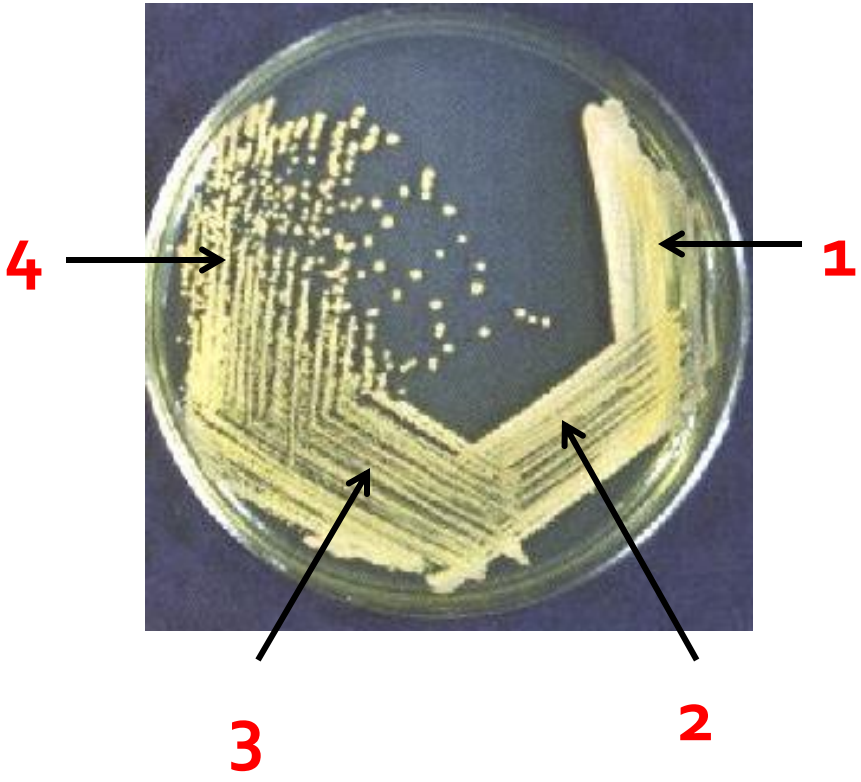


Plate streaking technique

Streaking Microbial Cultures on Agar Plates

Plate streaking technique



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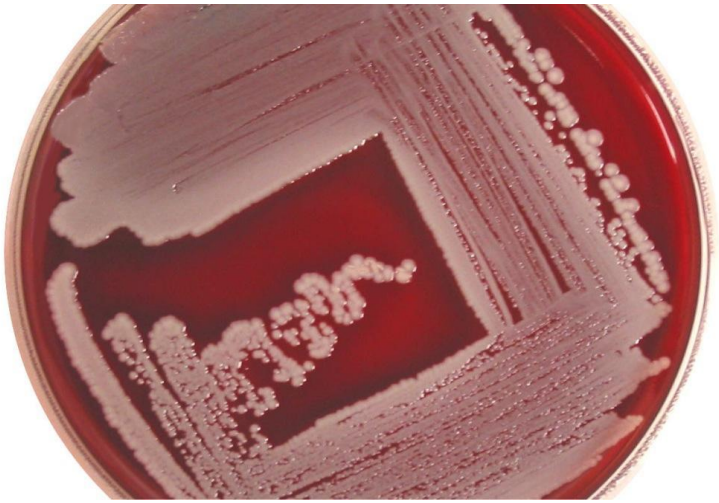
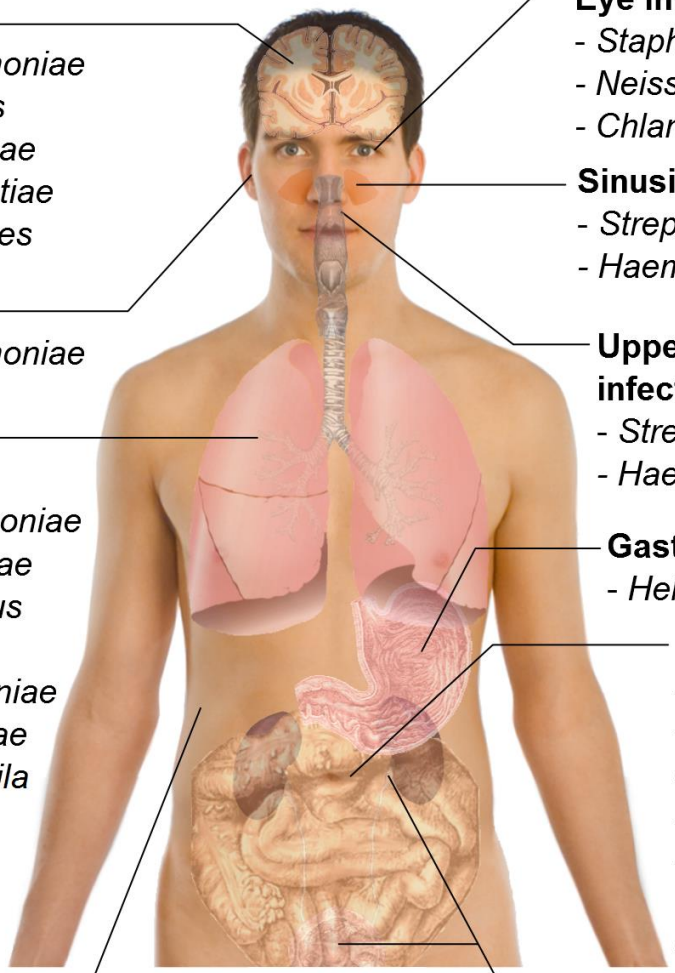


Overview of bacterial infections



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*

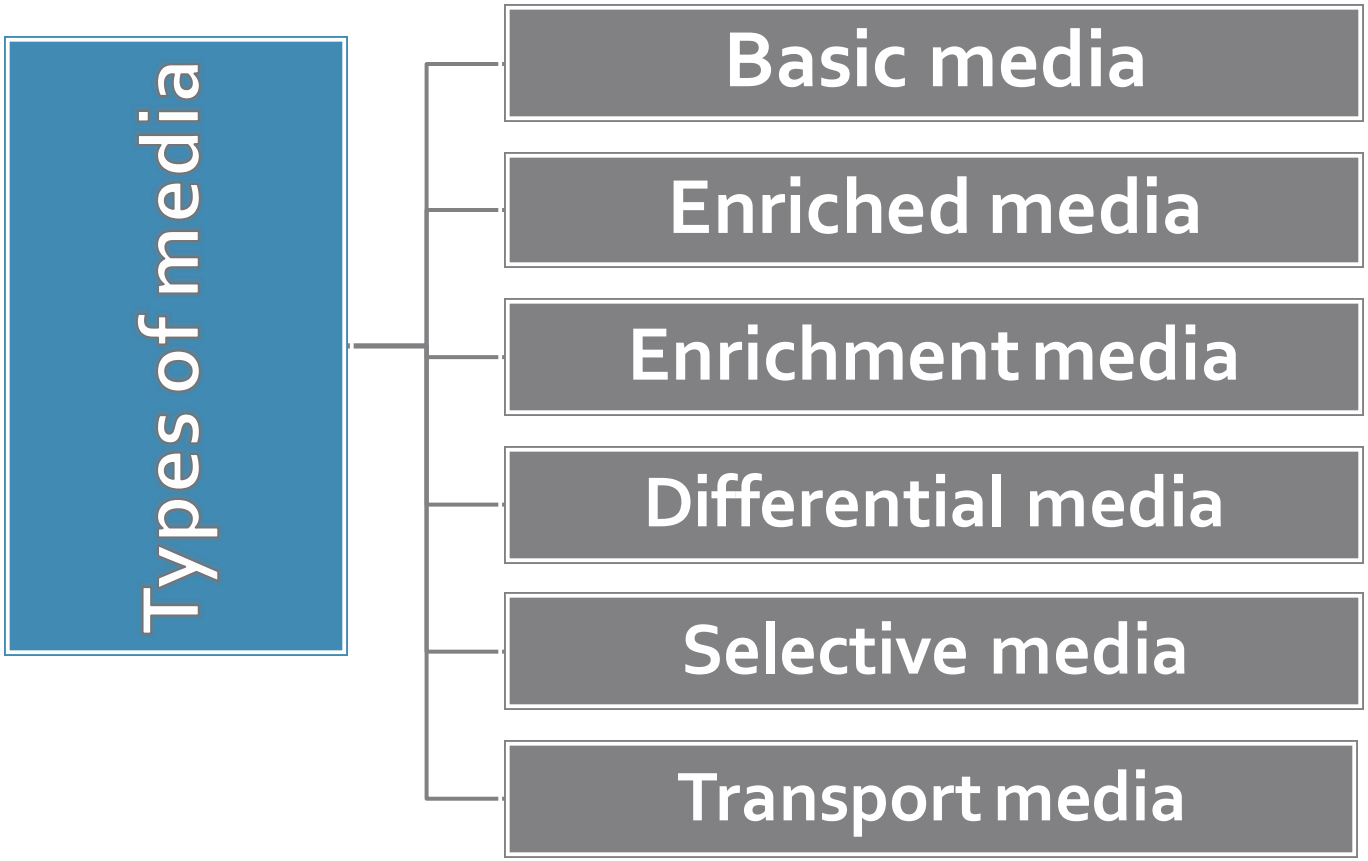
Upper respiratory tract infection
- *Streptococcus pyogenes*
- *Haemophilus influenzae*

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*

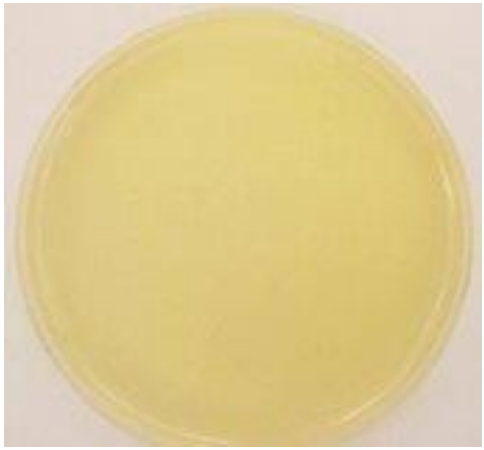

Types of media
Basic media
Enriched media
Enrichment media
Differential media
Selective media
Transport media

BASIC MEDIA

- Simple media
- Support growth of microorganisms
- No special nutritional requirements
- **Examples:**

  Nutrient agar

  Nutrient broth



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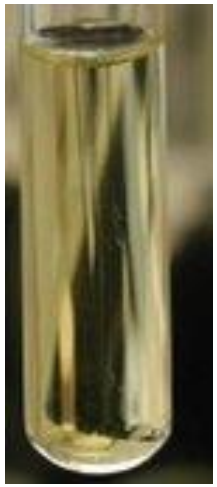

Nutrient broth

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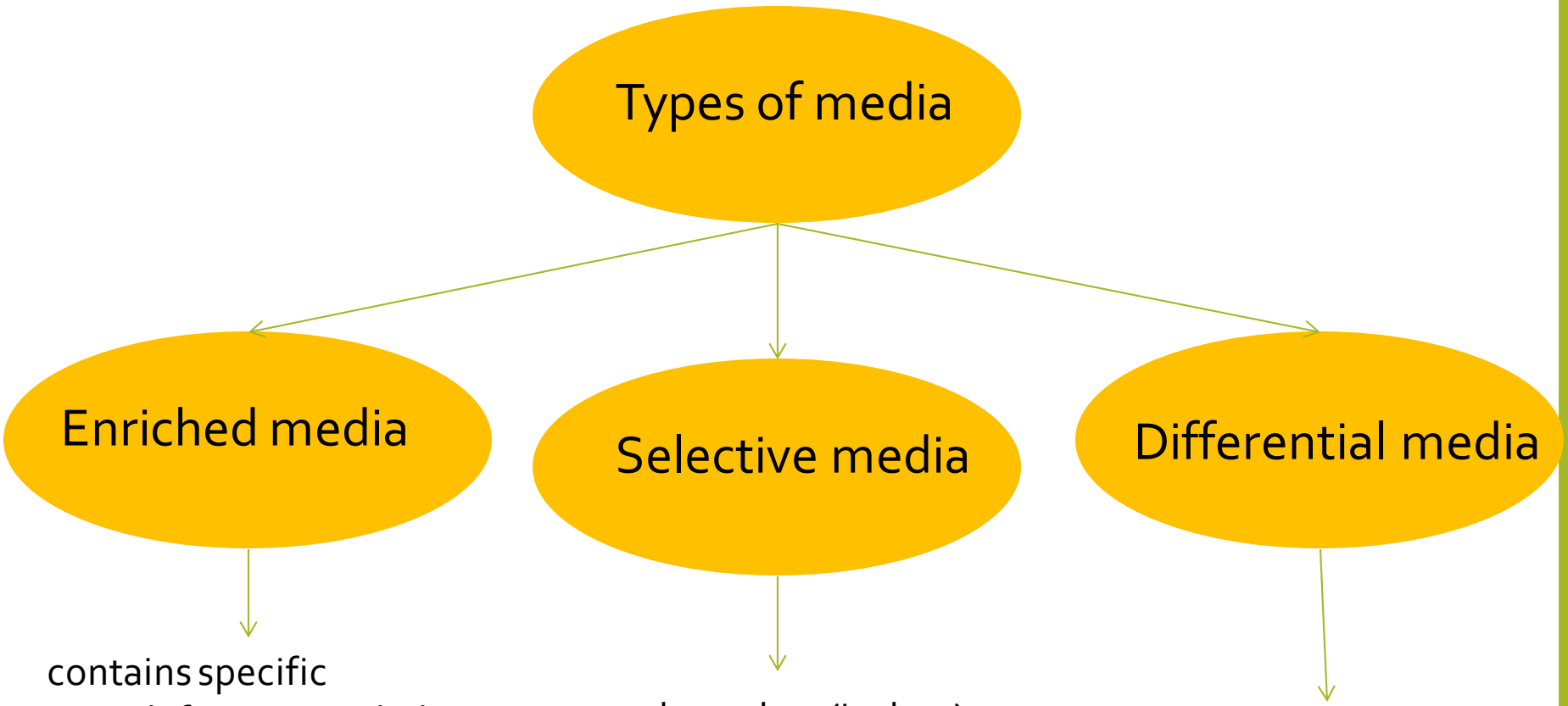

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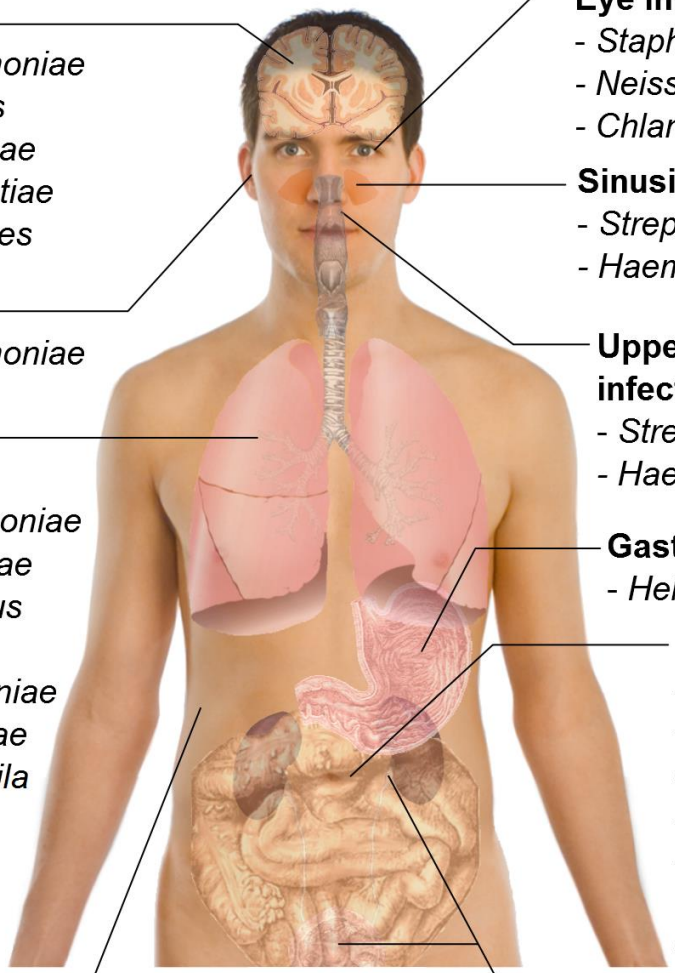

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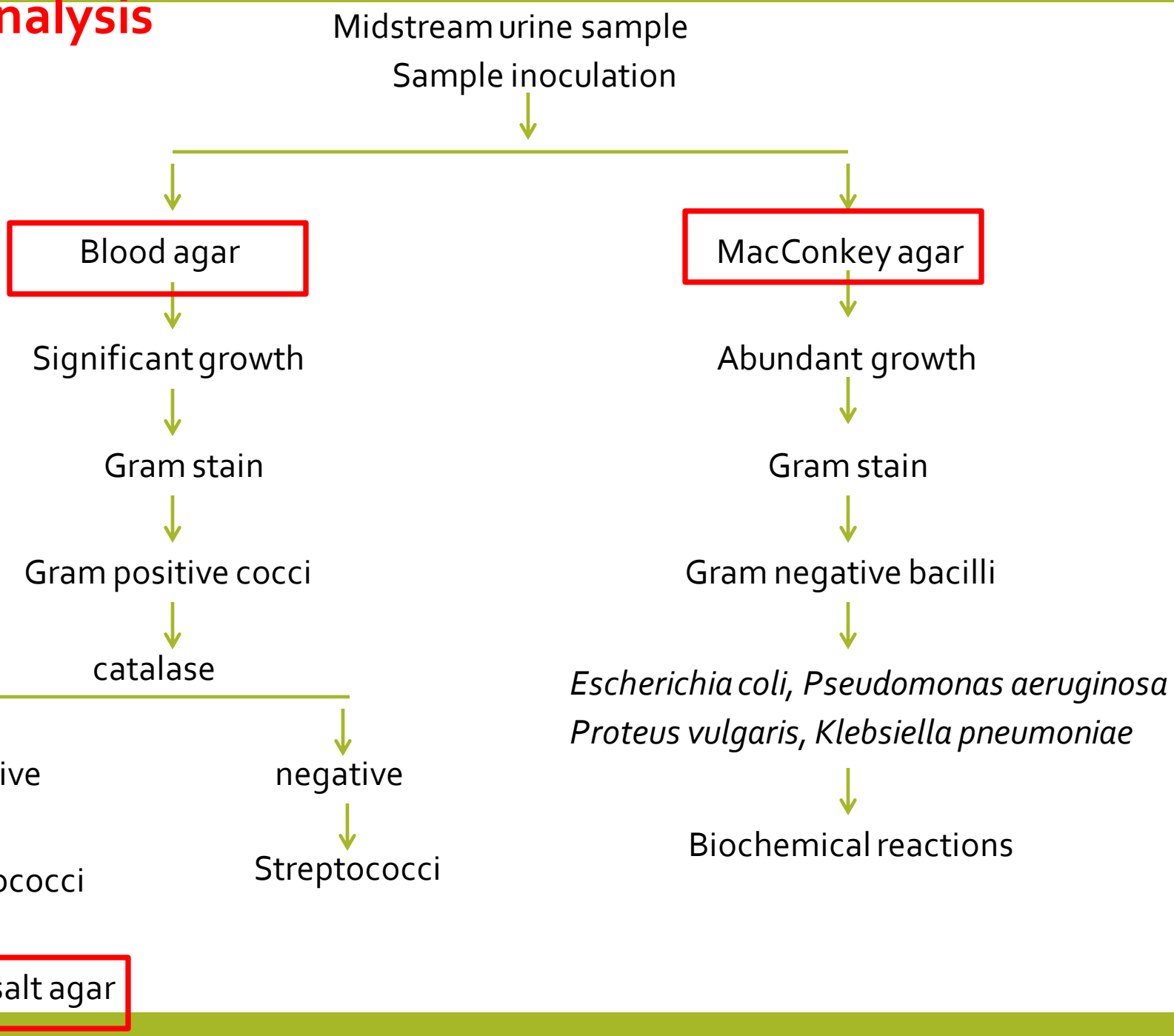
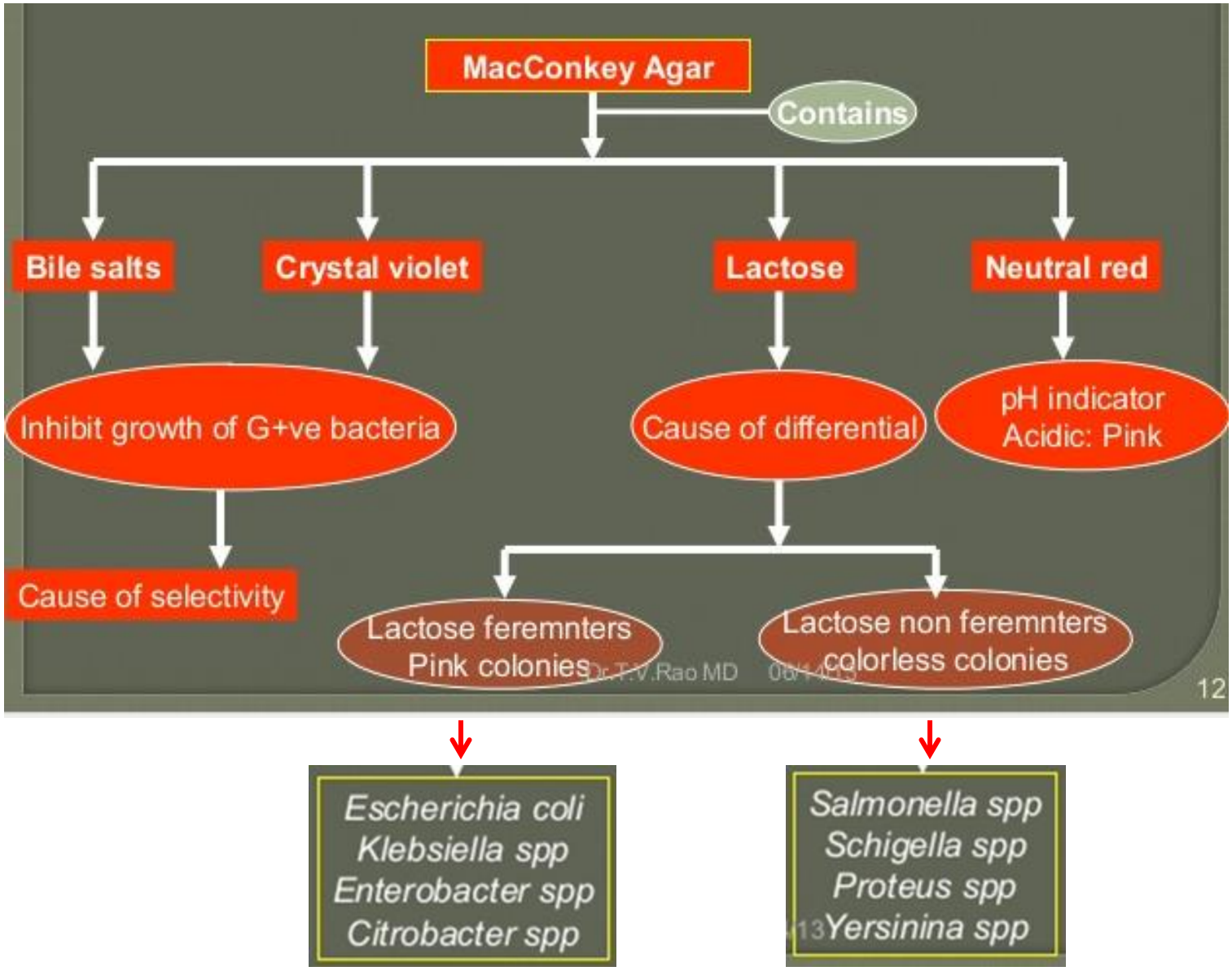
Microbiological Analysis of Urine Specimens

			Stapylococcus aureus *Streptococcus pyogenes*
Bacteria	**Gram positive**	Enterococci	*Streptococcus facalis* *Streptococcus faecium*
	Gram negative	*Escherichia coli* *Pseudomonas aeruginosa* *Proteus vulgaris* *Klebsiella pneumoniae*	
Viruses_____	**Venereal Disease** _____ __	*Treponema pallidum* *Neisseria gonorrhoeae* *Hemophillus ducreyi* *Calymnatobacterium granulomatis* *_Herpes hominus (type 11)*	
Fungi	*Candida albicans* *Blastomyces dermatitidis* *Coccidioides bancrofti*		
Protozoa	*Trichomonas vaginalis* *Entameoba histolytica*		

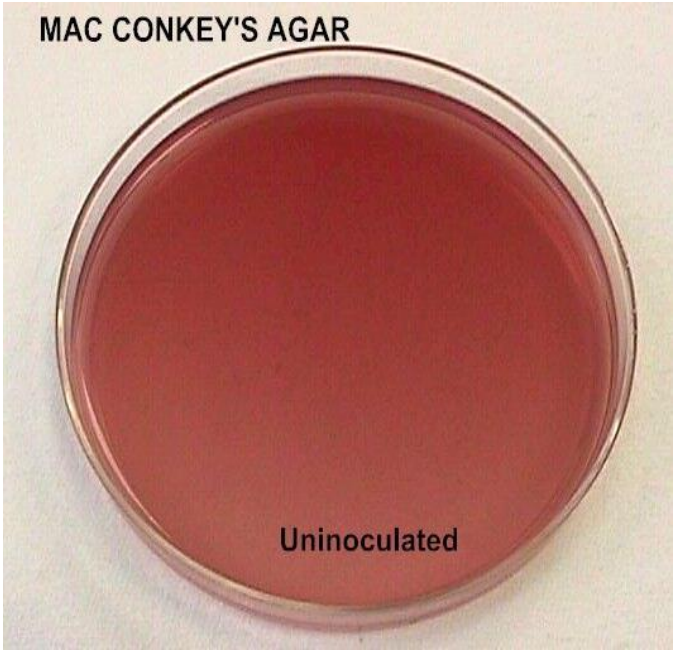
Urine analysis

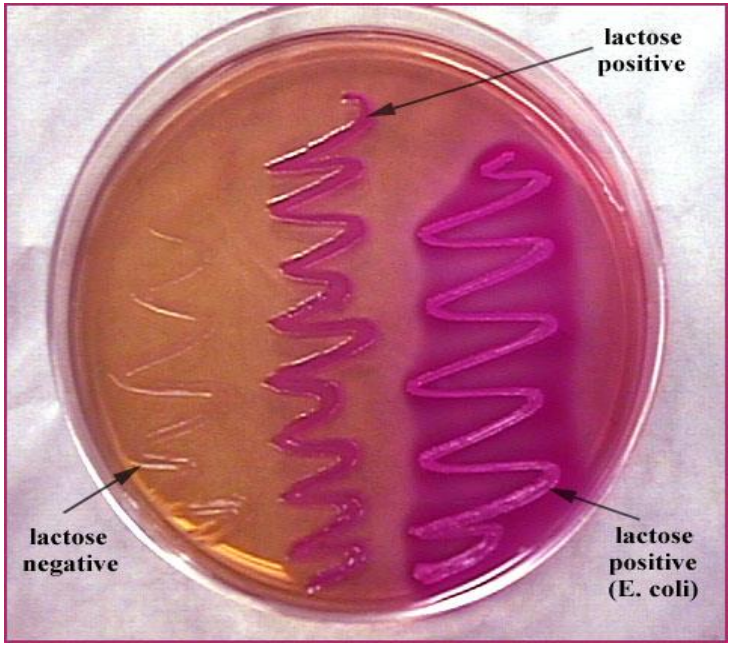


MacConkey agar is a selective and differential media for Enterobacteriaceae



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

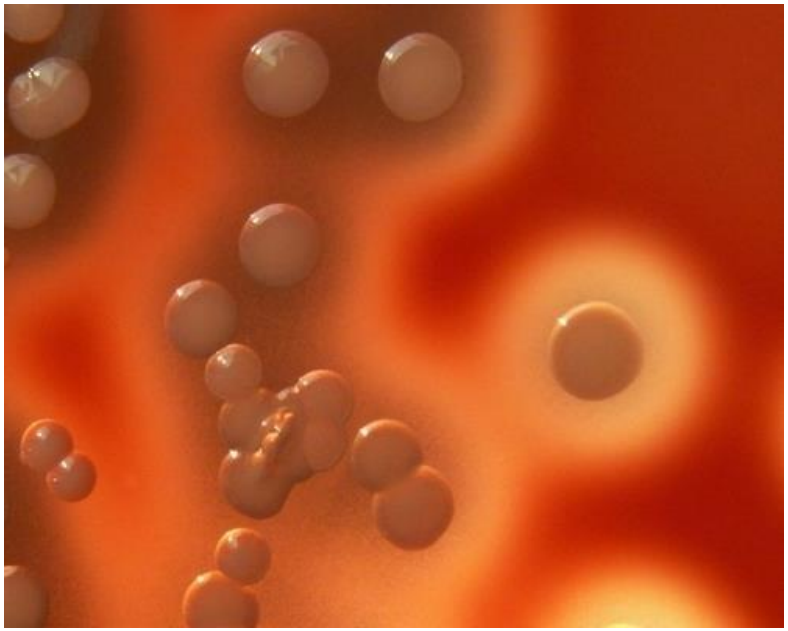
Growth on BA differentiates between the three groups of Bacteria:

1- Alpha hemolytic bacteria

2- Gamma hemolytic bacteria

3- Beta hemolytic bacteria

Beta hemolysis = Complete hemolysis



Alpha hemolysis

Hemoglobin containing Fe^{2+} (ferrous)

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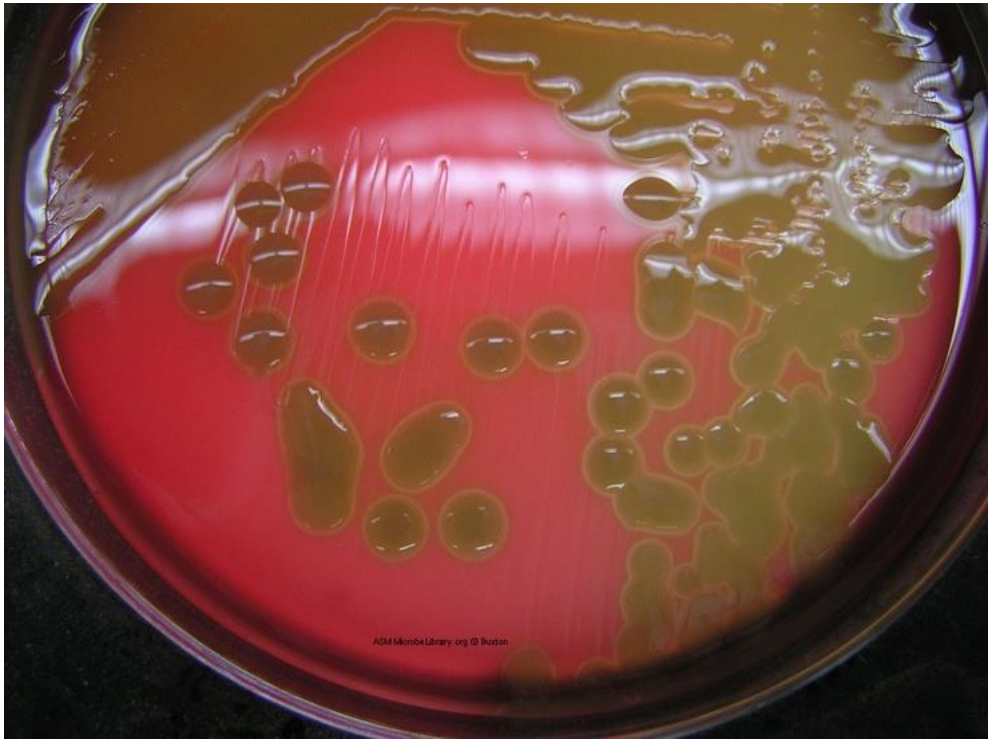
hydrogen peroxide produced by the bacterium

↓

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

↓

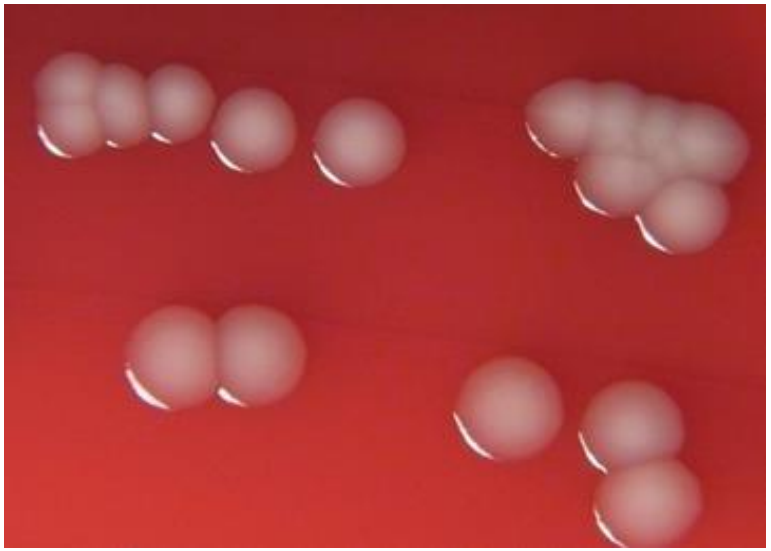
Hemoglobin converted into methemoglobin (greenish color)



Incomplete (partial) lysis of RBCs

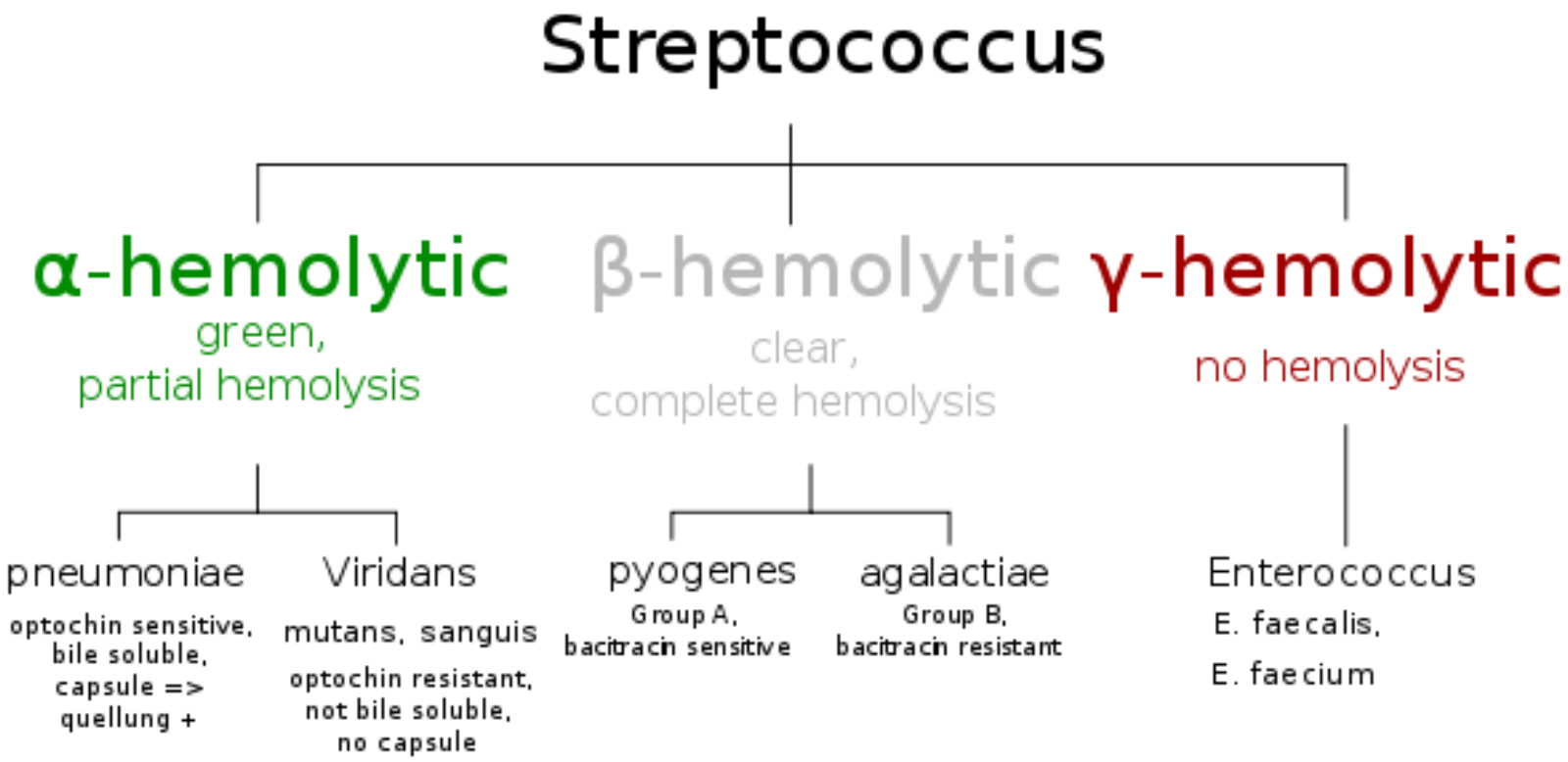
Gamma hemolysis

No hemolysis, and no change in the medium



The three types of hemolysis

The hemolytic pattern of different Streptococci



Mannitol salt agar

Selective and Differential for Staphylococci

- **Selective agent: 7.5% NaCl**

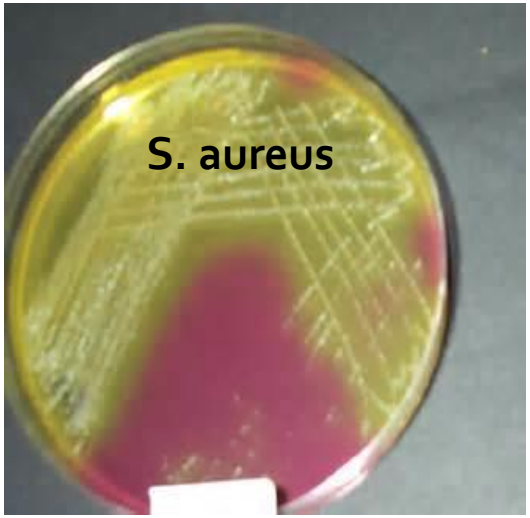
- **Differential agent:** mannitol to differentiate between mannitol Fermenters and non-fermenters

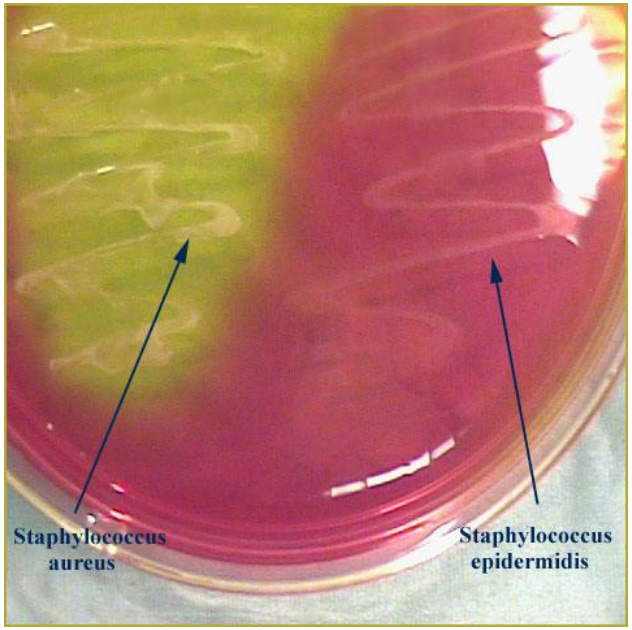
- pH indicator: Phenol red

Mannitol salt agar

MSA

Non-cultured

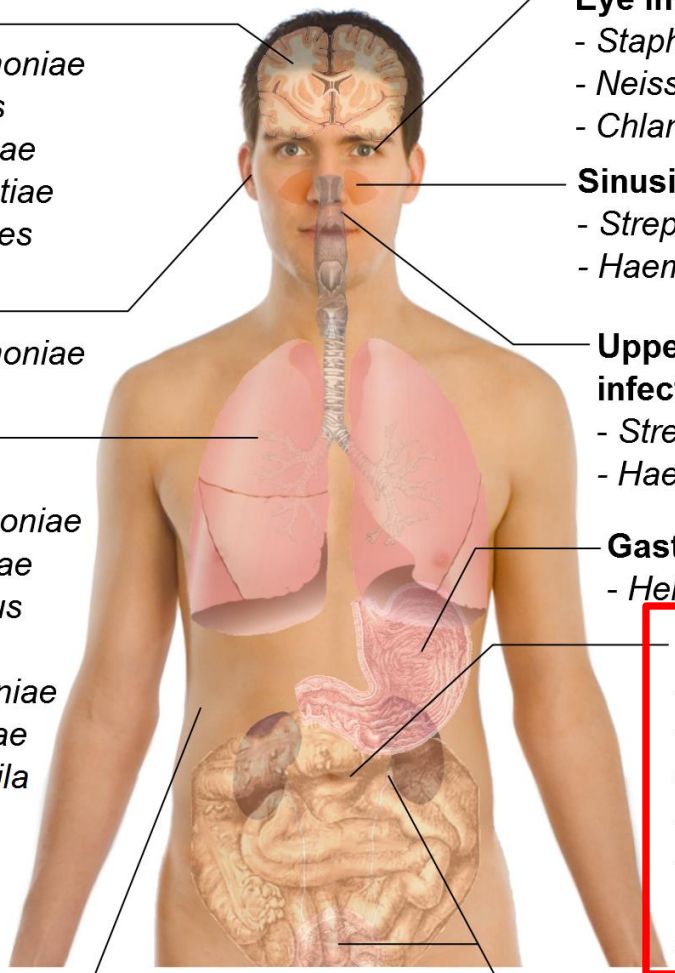




Blood agar

Beta hemolytic Staphylococci

Overview of bacterial infections



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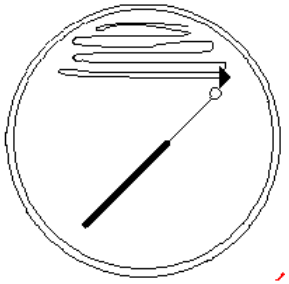
Processing of stool samples

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*

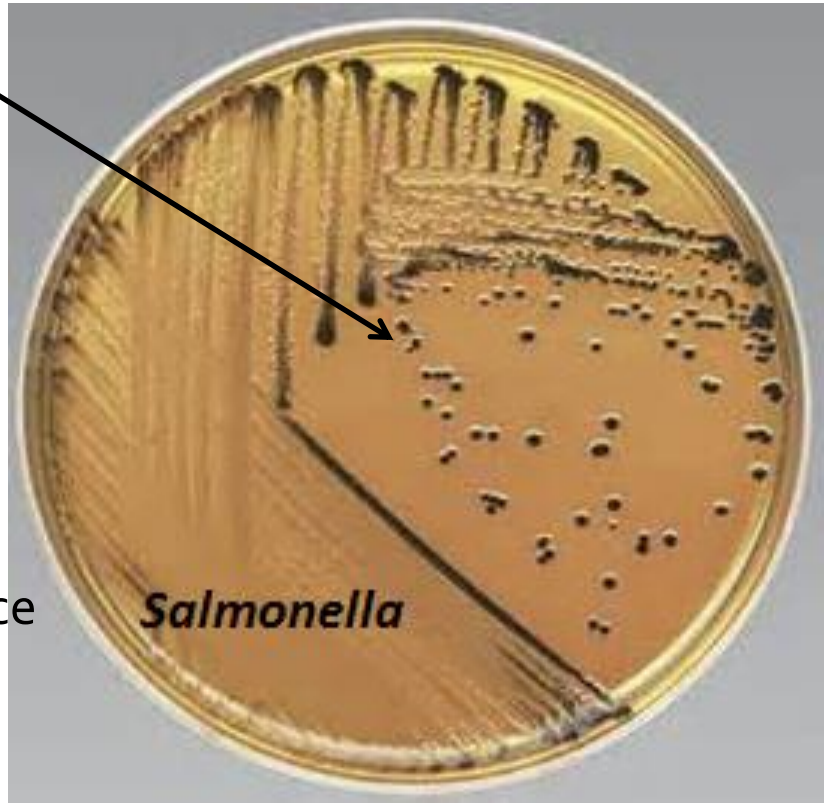
Components

- ✓ the presence of **brilliant green, sodium citrate and bile salts** which completely inhibit the growth of Gram-positive bacteria and partially inhibit the growth of Enterobacteriaceae and Proteus.
- ✓ **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** ($Na_2S_2O_3$): sulfur source
- **Fe^{3+}** (ferric) H2S indicator

↓

$Na_2S_2O_3$ + thiosulfate reductase ⟶ sulfite + H2S

H2S + Fe+3 ⟶ FeS (black precipitate presented by black-centered colonies)

Salmonella Shigella agar (SS agar)

Results

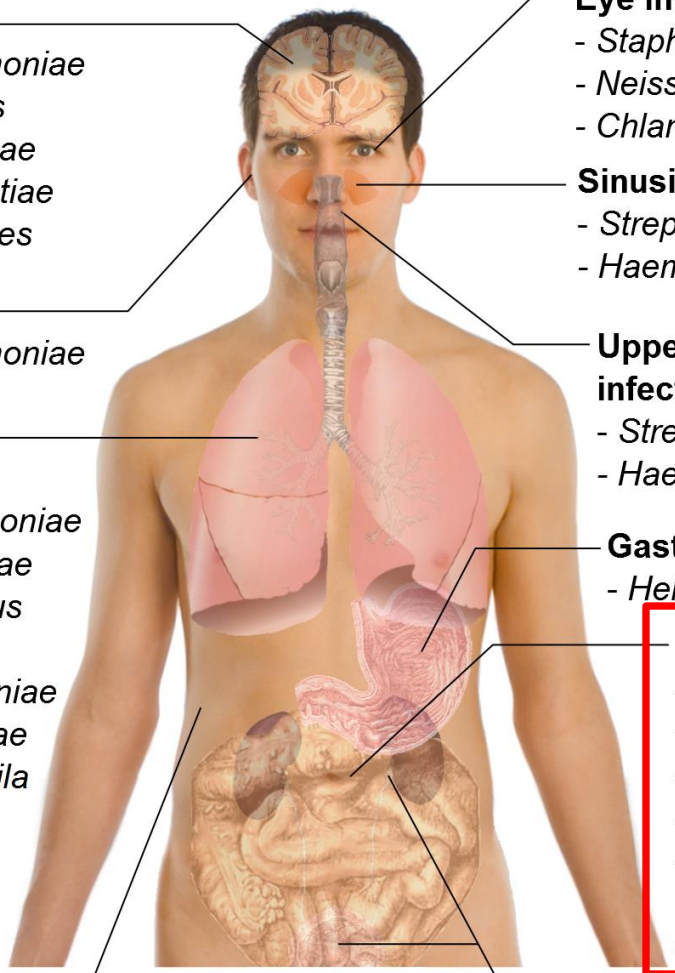
- Lactose fermenters: pink to red colonies (few can grow)
- Non lactose fermenters: translucent, colorless colonies with or without black centers

***Salmonella*:** colorless colonies with black centers

***Shigella*:** colorless colonies without black centers

Lactose fermenter flora: pink to red colonies

Overview of bacterial infections



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- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*

Cholera identification

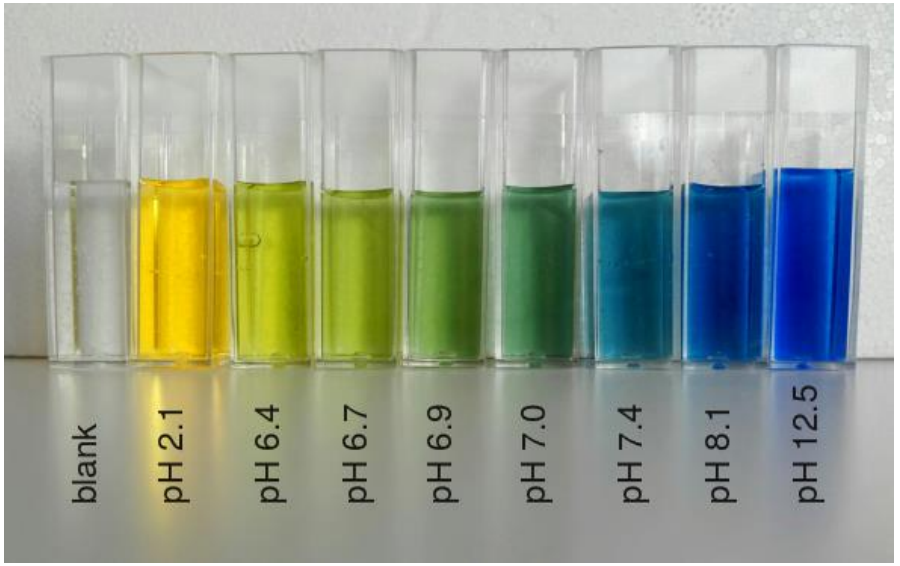
Identification

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

Important components

- ✓ Sucrose: sugar source
- ✓ Bromothymol blue: pH indicator

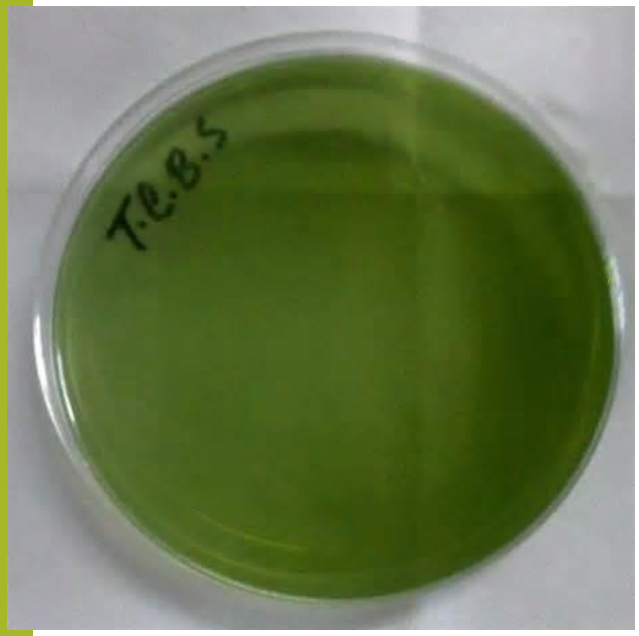
  - pH<6.0 - yellow
  - pH>7.6 –blue



Cholera identification

Results

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

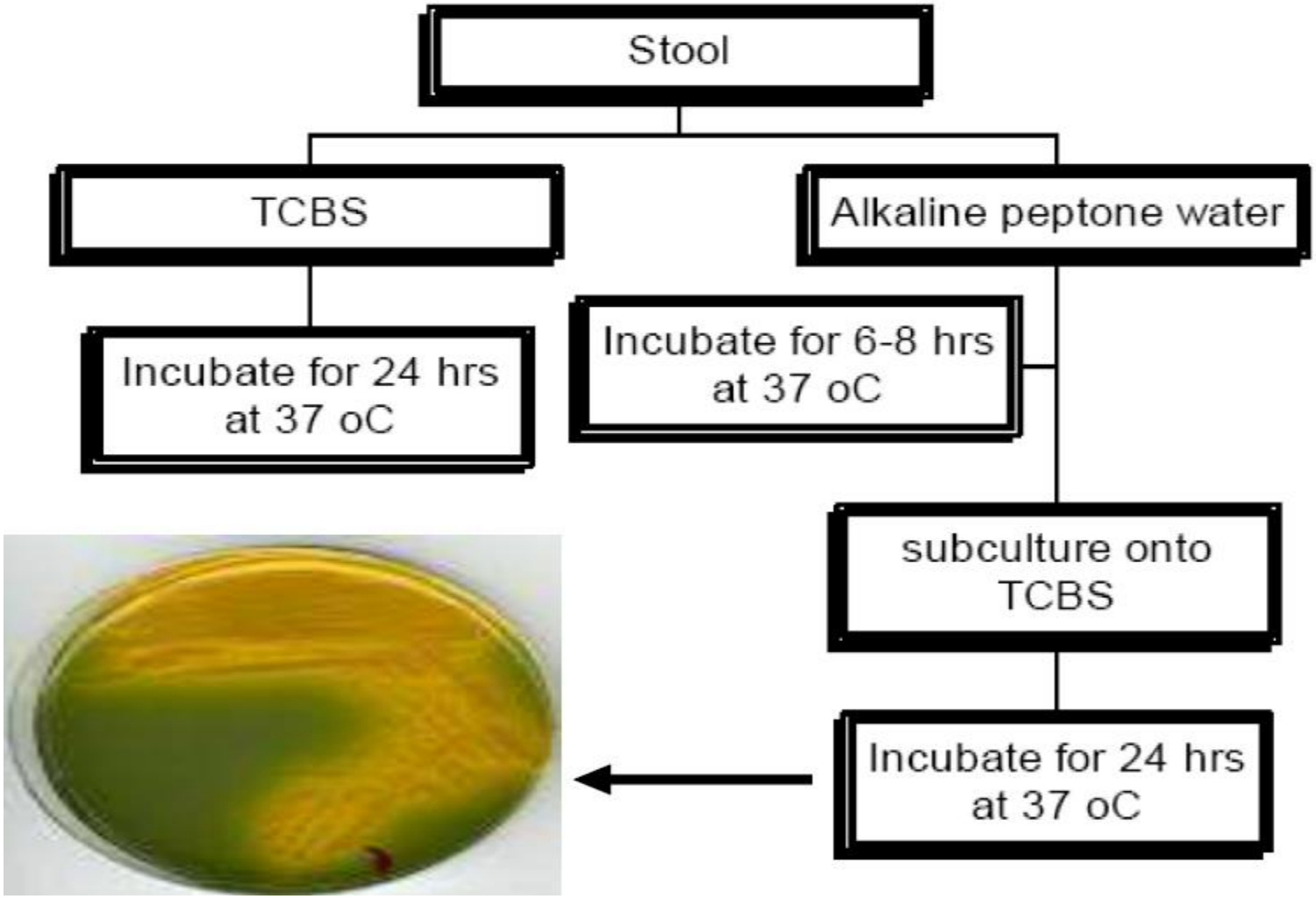


TCBS media

V. cholera

V. parahemolyticus

Cholera identification



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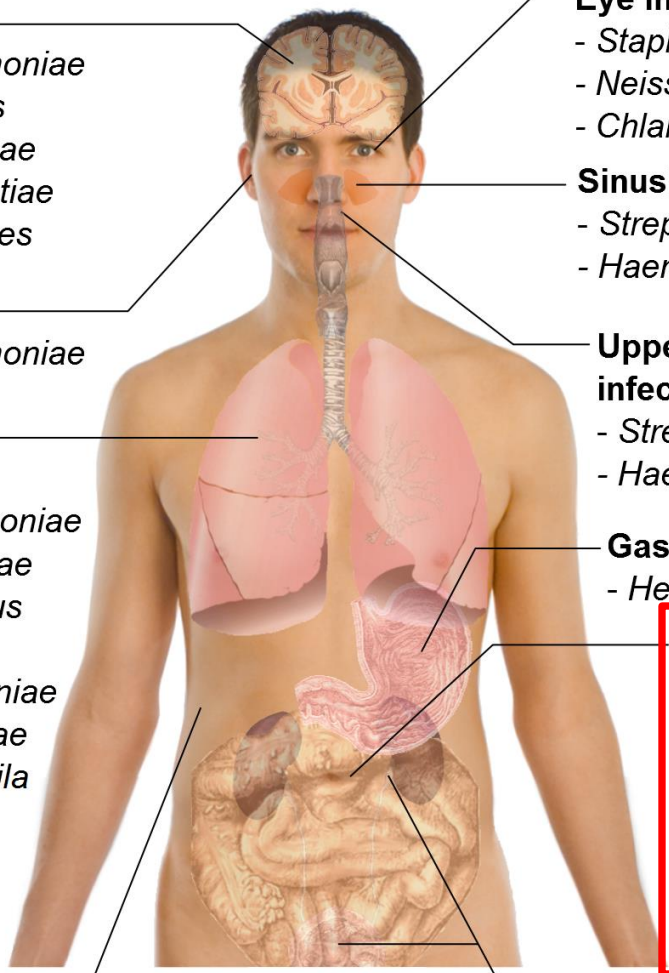

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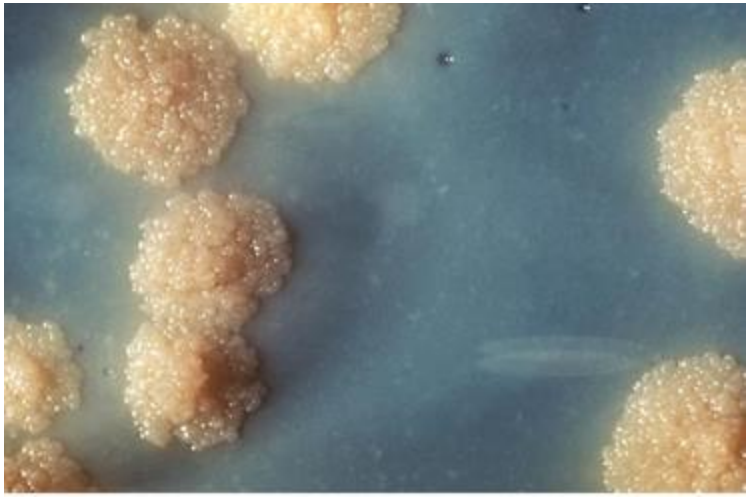
Löwenstein–Jensen (LJ) medium

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



M.tuberculosis produces rough and tough colonies

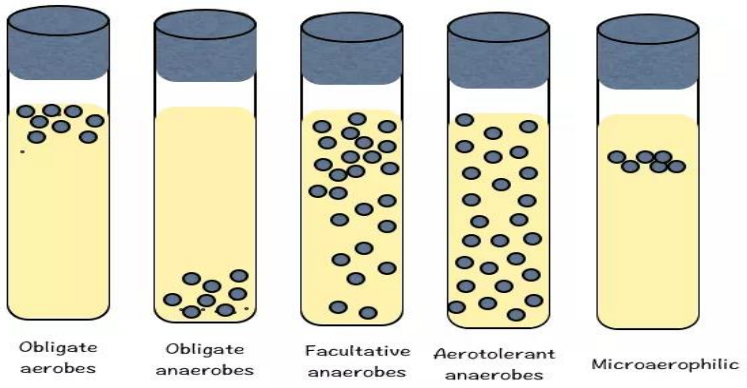
Löwenstein–Jensen (LJ) medium

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

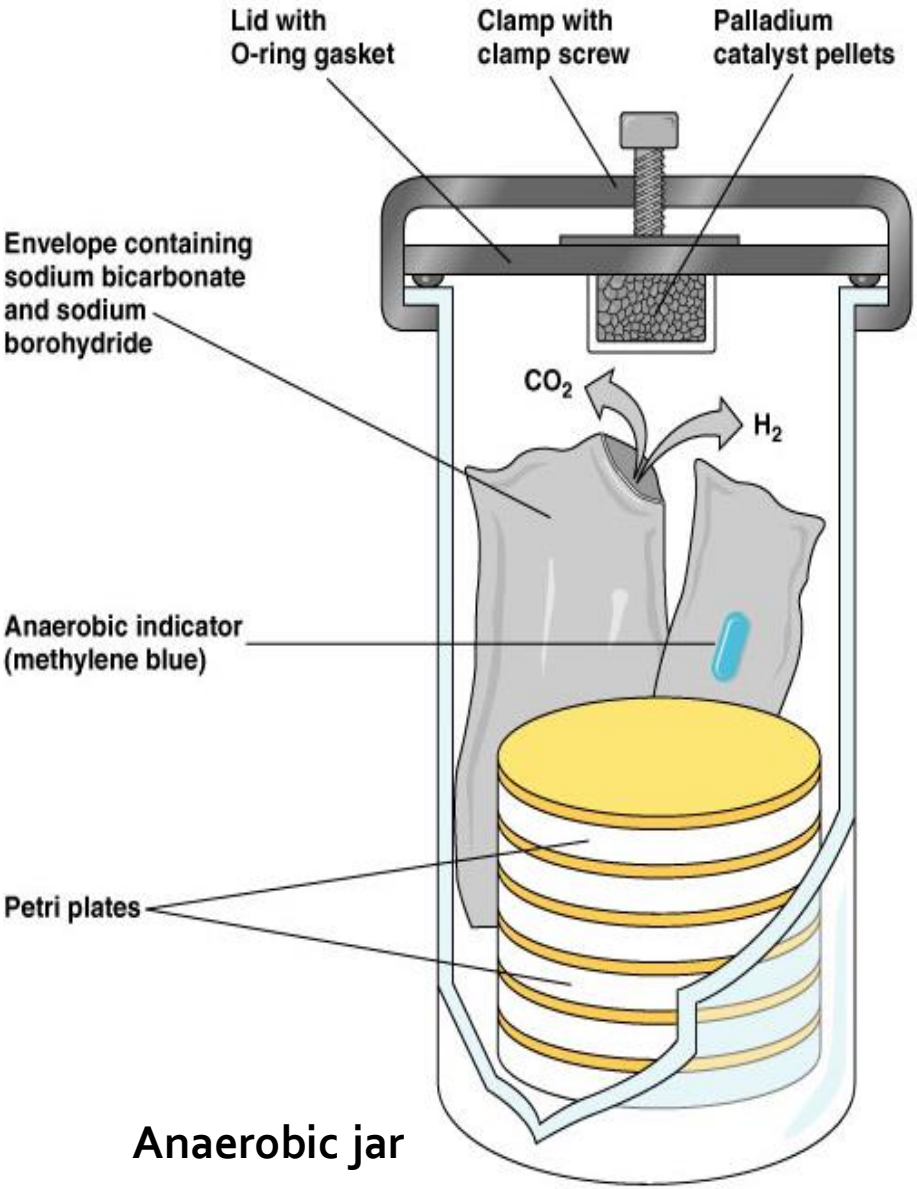
Composition	**Ingredients Gms / 600 ml**
L-Asparagine	**3.600**
Monopotassium phosphate	**2.400**
Magnesium sulphate	**0.240**
Magnesium citrate	**0.600**
Potato starch, soluble	**30.000**
Malachite green	**0.40**

Cultivation of Anaerobic Bacteria – Thioglycollate medium

Thioglycolate is a multipurpose, enriched, differential medium used primarily to determine the oxygen requirements of microorganisms. It acts as an enrichment broth which is most frequently used in diagnostic bacteriology. This broth supports the growth of anaerobes, aerobes, fucultative anaerobes microaerophilic, and aerotolerant microorganisms.



Anaerobic jars

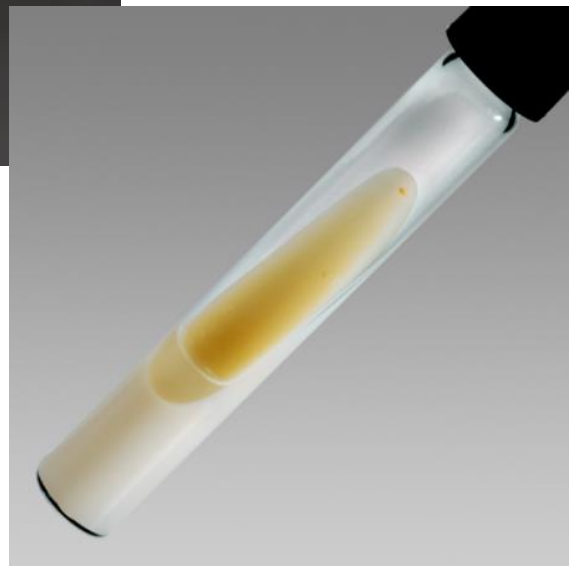


Anaerobic jar

Anaerobic candle jar

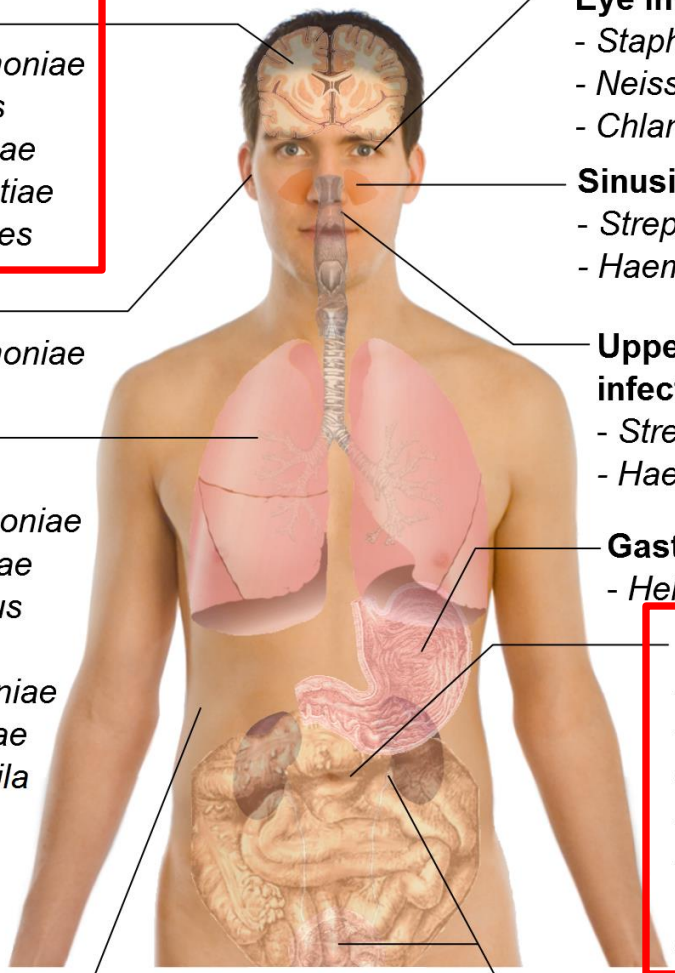
Löffler's medium

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)

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*

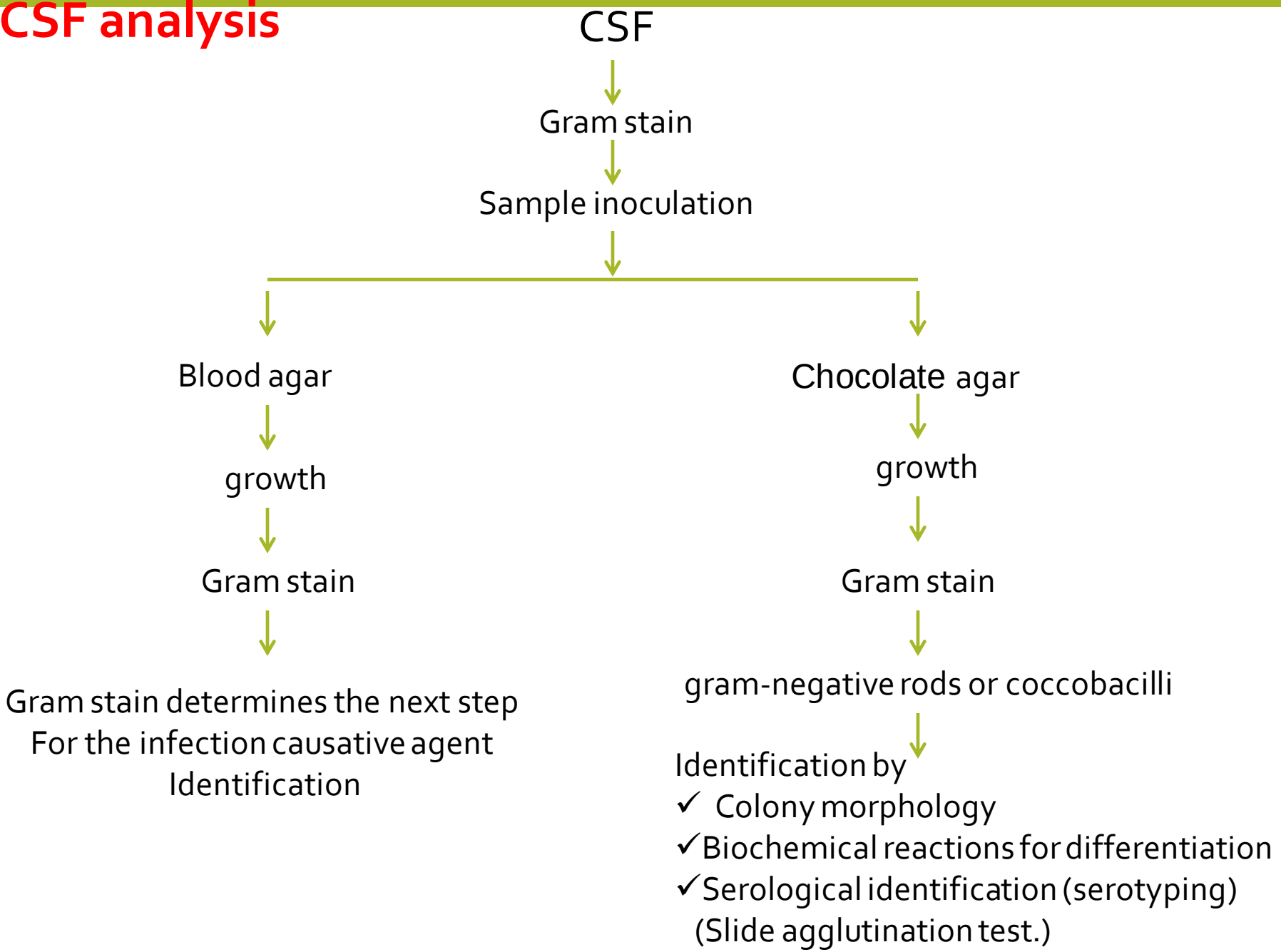
Gastritis
- *Helicobacter pylori*

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*

CSF analysis



Chocolate agar

- Used to isolate ***Haemophilus influenzae***
- Is a hemolysed blood either by heating blood to 80^{0}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


Thank you