Microbiology

Lab 4

Blood agar ⇒ Gram + cocci

Manitol salt sugar ⇒ staph

MacConkey agar ⇒ Gram - bacilli

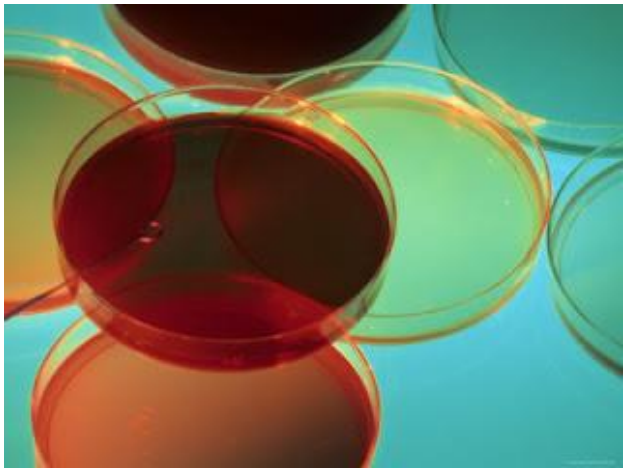
SS agar ⇒ Salmonella - Shigella.

TCBS agar ⇒ Cholera.

LJ medium ⇒ mycobacterium

Chocolate agar ⇒ Haemophilus influenzae.

Anaerobic gars ⇒ anaerobic bacteria



General Microbiology Lab

Types of Culture Media
2021-2022
Lab 4

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Department of Microbiology and Pathology
Faculty of Medicine, Mutah 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

Not all bacteria need the same growth requirement

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

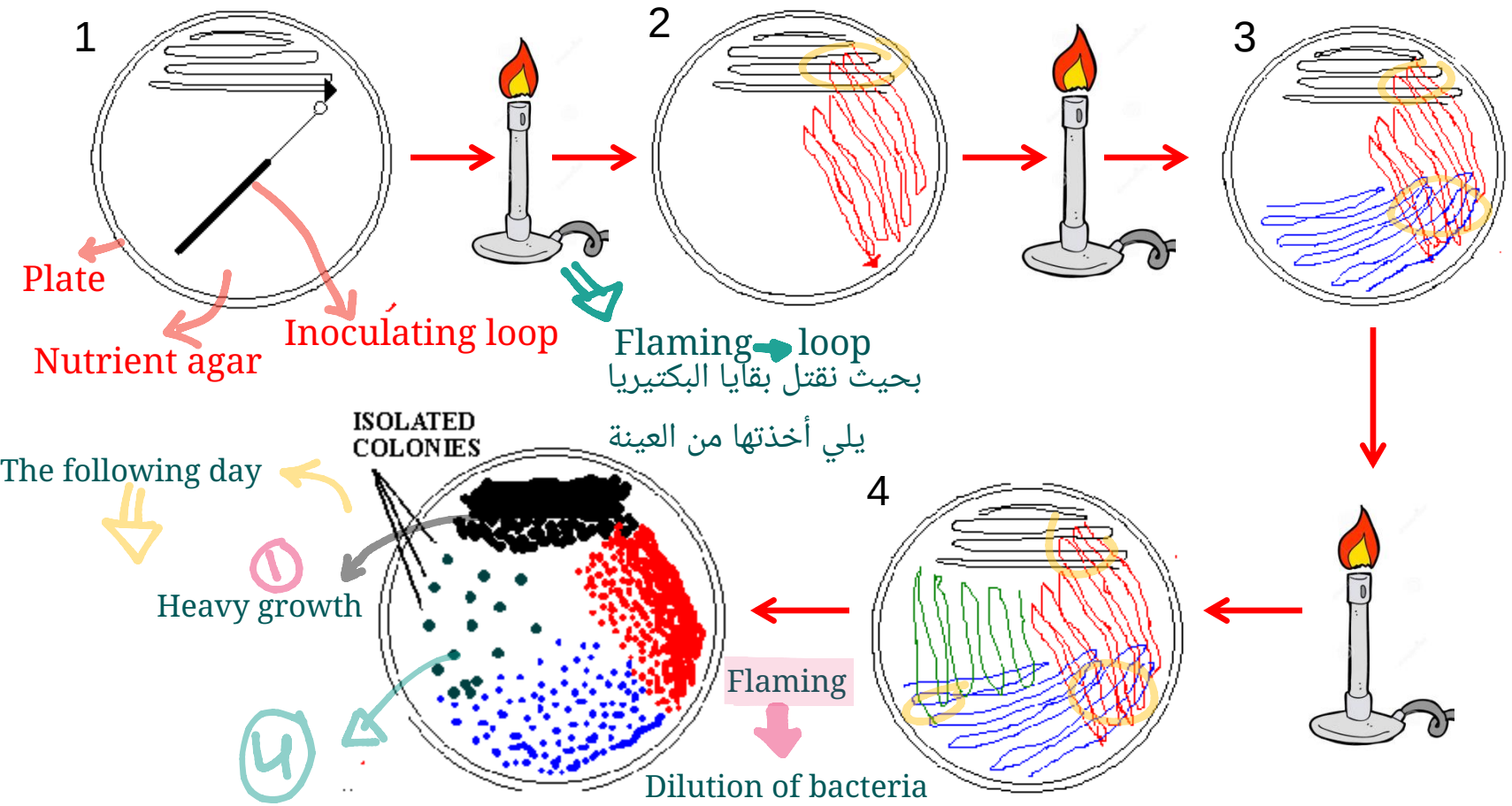
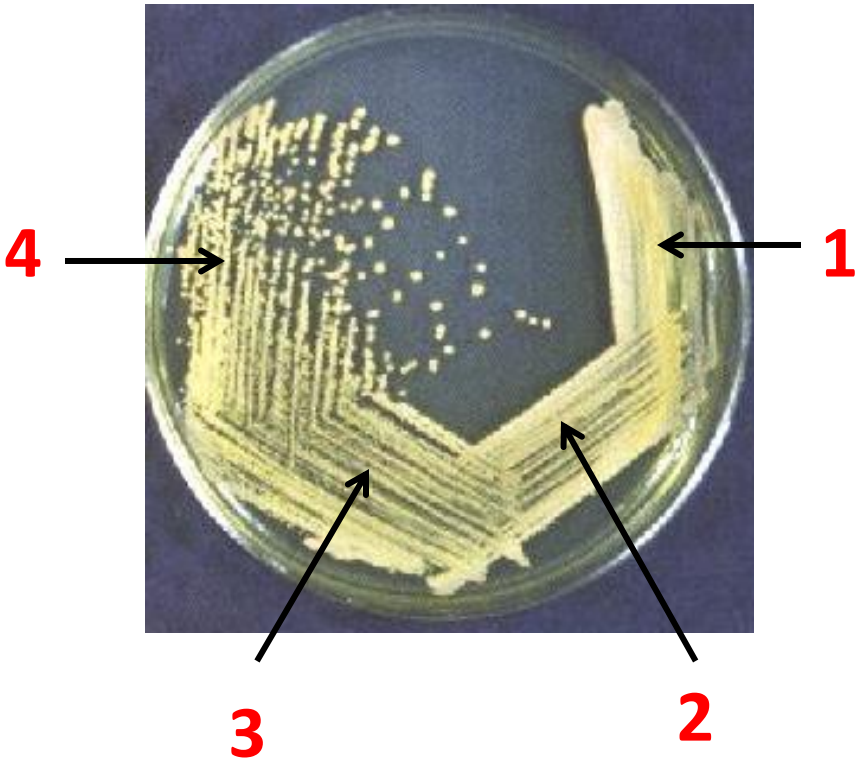


Plate streaking technique

Streaking Microbial Cultures On Agar Plates

Plate streaking technique



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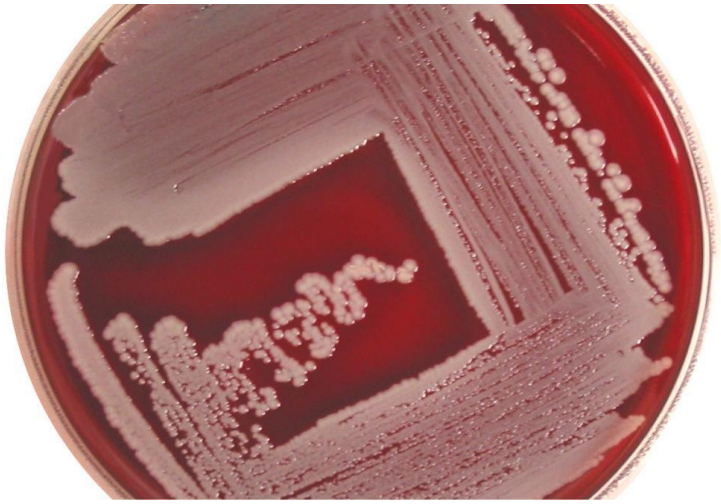
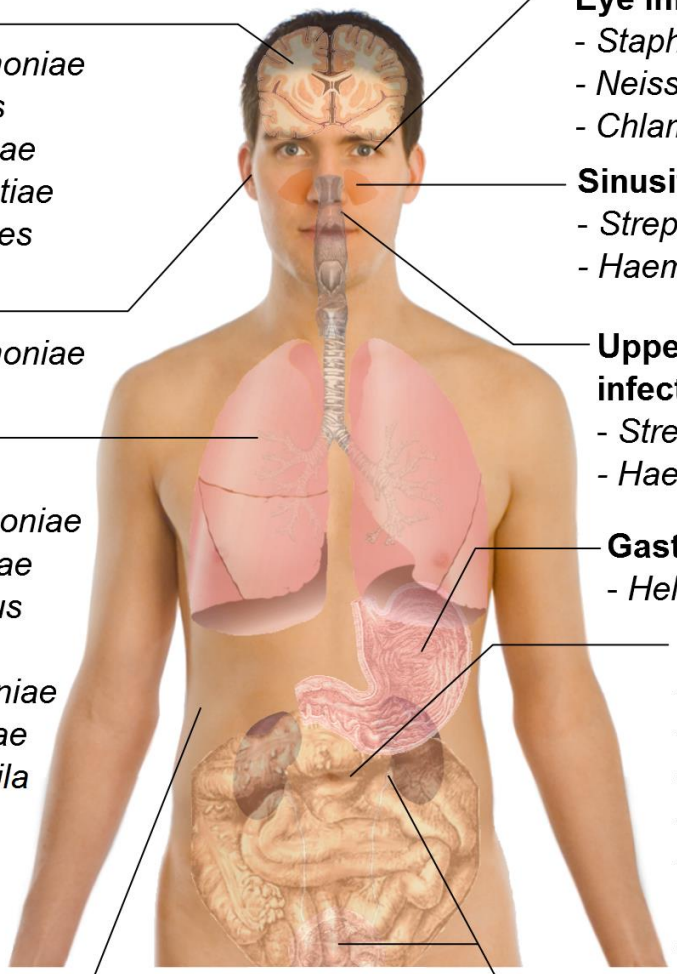


Overview of bacterial infections



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- *Streptococcus pneumoniae*
- *Neisseria meningitidis*
- *Haemophilus influenzae*
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Otitis media
- *Streptococcus pneumoniae*

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Community-acquired:
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Urinary tract infections
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- *Staphylococcus saprophyticus*
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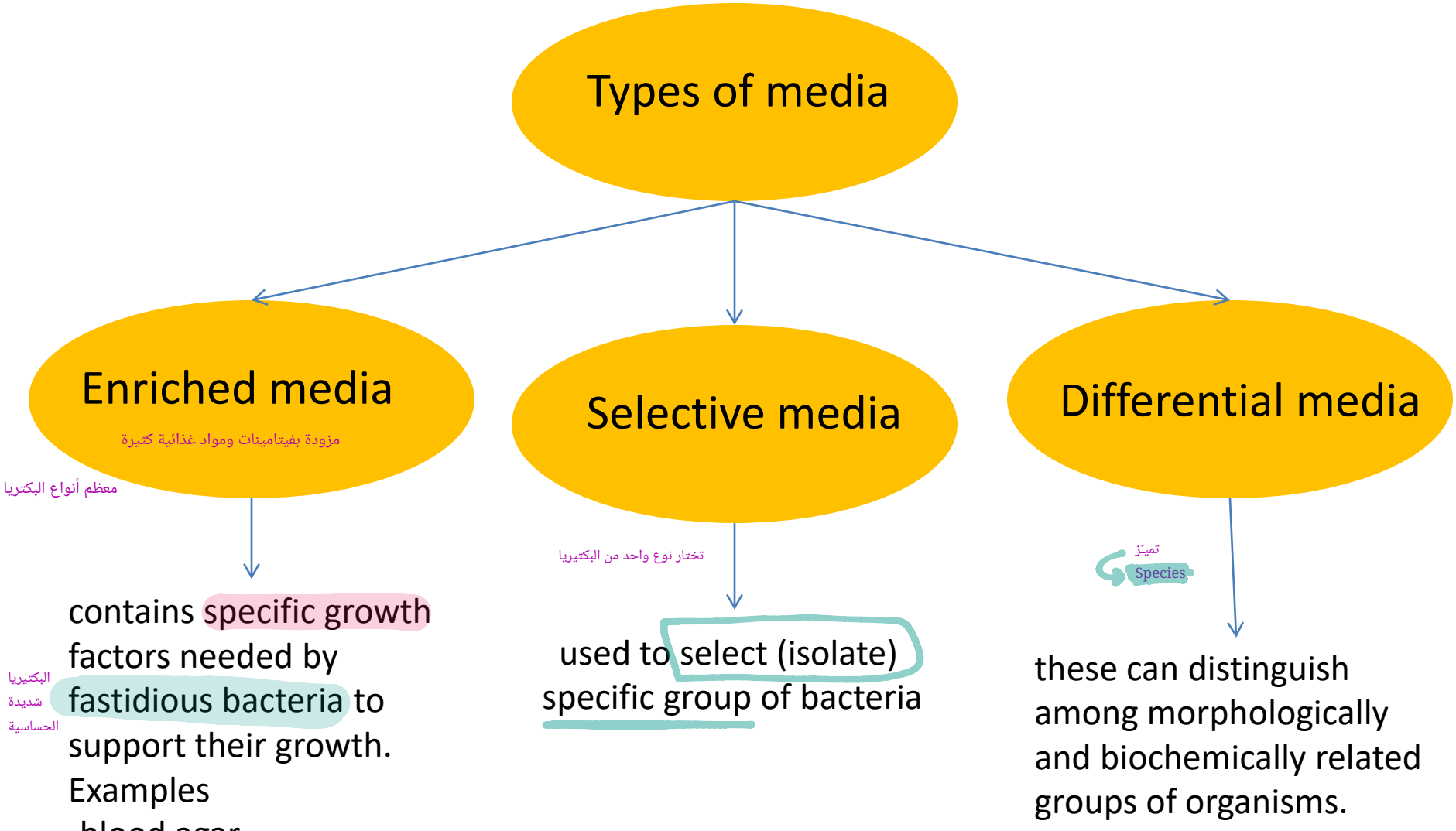
Types of media



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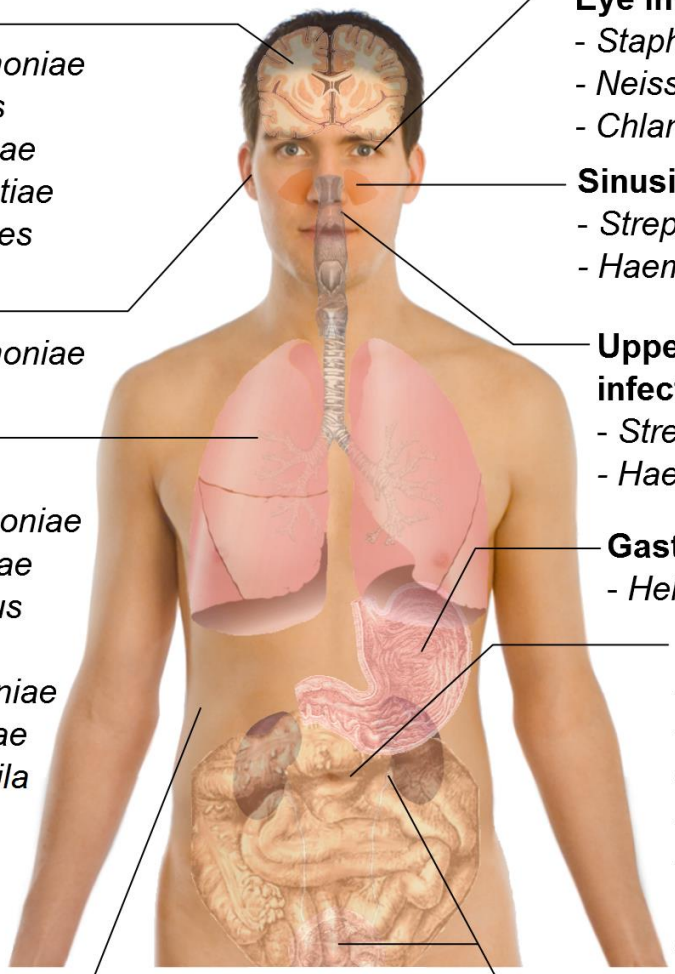

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UTI

Most common cause of UTI /80%

Microbiological Analysis of Urine Specimens

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

Urine analysis

Midstream urine sample

The second third of the urine. No contamination with the bacteria in urethrae

Sample inoculation

Blood agar

Non selective/enriched media

Significant growth

Gram stain

Gram positive cocci

catalase

positive

Staphylococci

Mannitol salt agar

Differential Media for staph

negative

Streptococci

MacConkey agar

Selective and differential meia

Abundant growth

Gram stain

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Gram negative bacilli

Escherichia coli, Pseudomonas aeruginosa

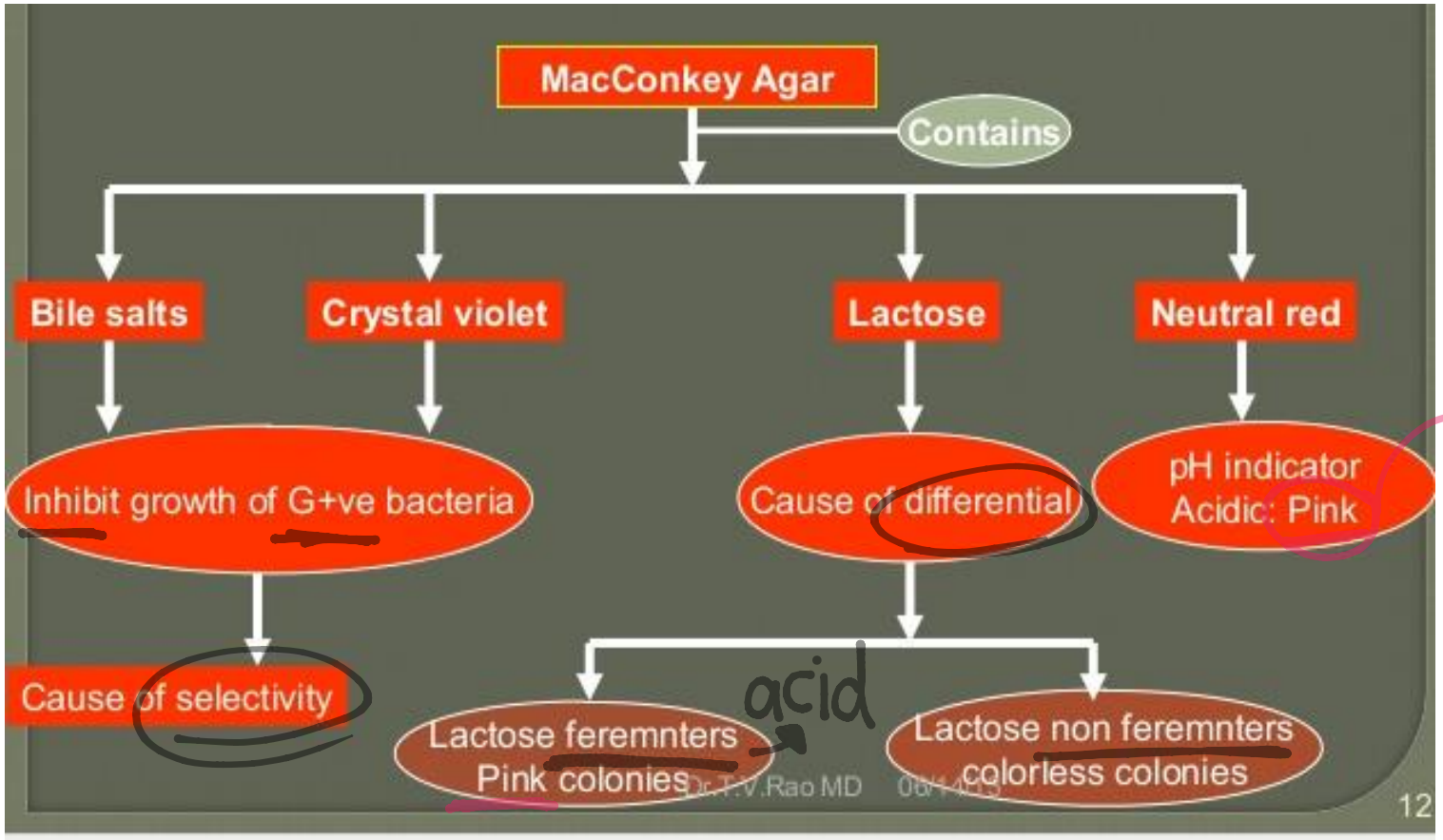
Proteus vulgaris, Klebsiella pneumoniae

Biochemical reactions

بعد اسبوع

بنحدد المسبب لل infection

MacConkey agar is a selective and differential media for Enterobacteriaceae

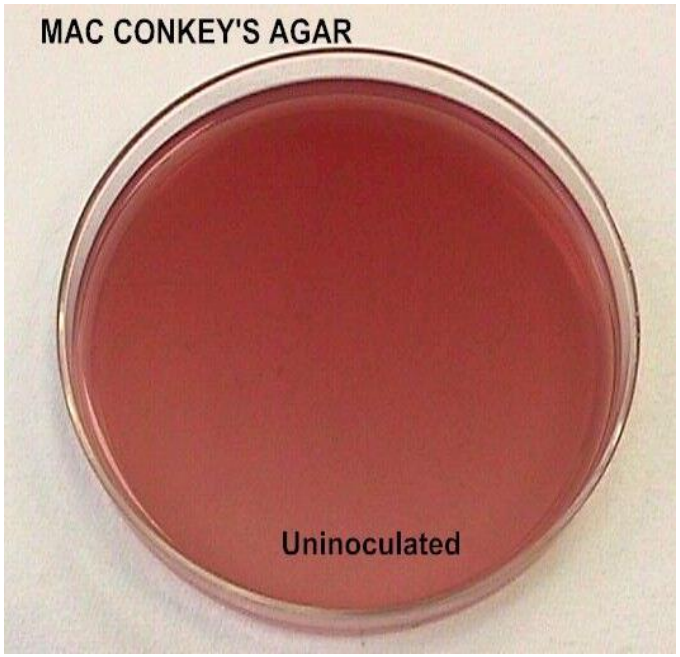


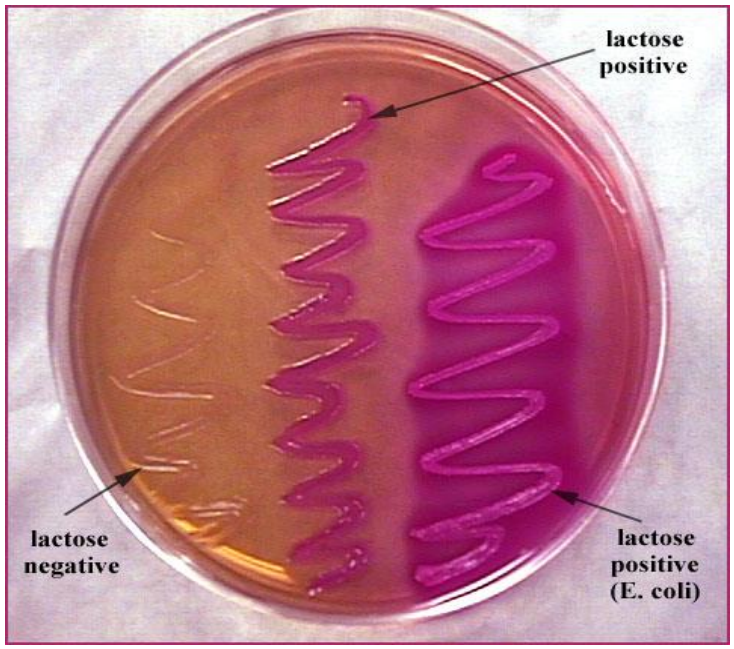
Gram negative lactose feremnter

Not enough! We need

Biochemical reaction

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 Partial hemolysis

2- Gamma hemolytic bacteria Complete hemolysis

3- Beta hemolytic bacteria No hemolysis

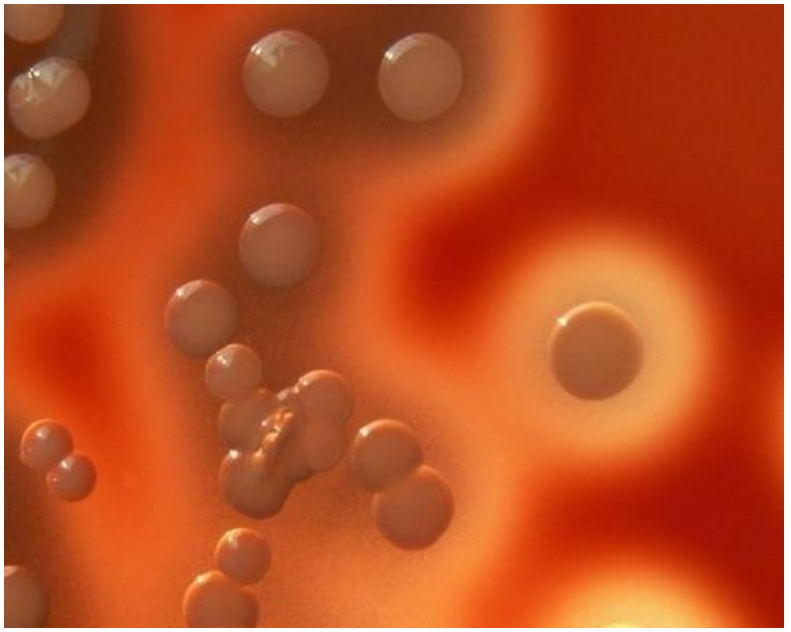
Notes in the previous slide

*A growth medium is considered differential if, when specific microbes are present, the medium or bacterial colonies themselves exhibit a color change that provides information about their identity.

*Blood agar (BAP) is a differential growth medium which microbiologists use to distinguish clinically significant bacteria from throat and sputum cultures. BAP contains 5% sheep blood. Certain bacteria produce exotoxins called hemolysins, which act on the red blood cells to lyse, or break them down.

Microbiologist use differential media to identify and isolate specific bacteria. An example of this is the bacteria Streptococcus pyogenes, which causes strep throat. You can grow these bacteria on a complex media such as nutrient agar, but if other bacteria are also growing on that agar, it is very difficult to distinguish one bacterial colony from another without the use of microscopic examination and special staining techniques. If you grow it on blood agar, though, it will destroy the red blood cells in a process called beta-hemolysis, and other cells will not cause this reaction, which makes identifying Streptococcus pyogenes much easier.

Beta hemolysis = Complete hemolysis



- Transparent hallows around bacterial growth

Alpha hemolysis

Greenish

Hemoglobin containing Fe^{2+} (ferrous)

↓

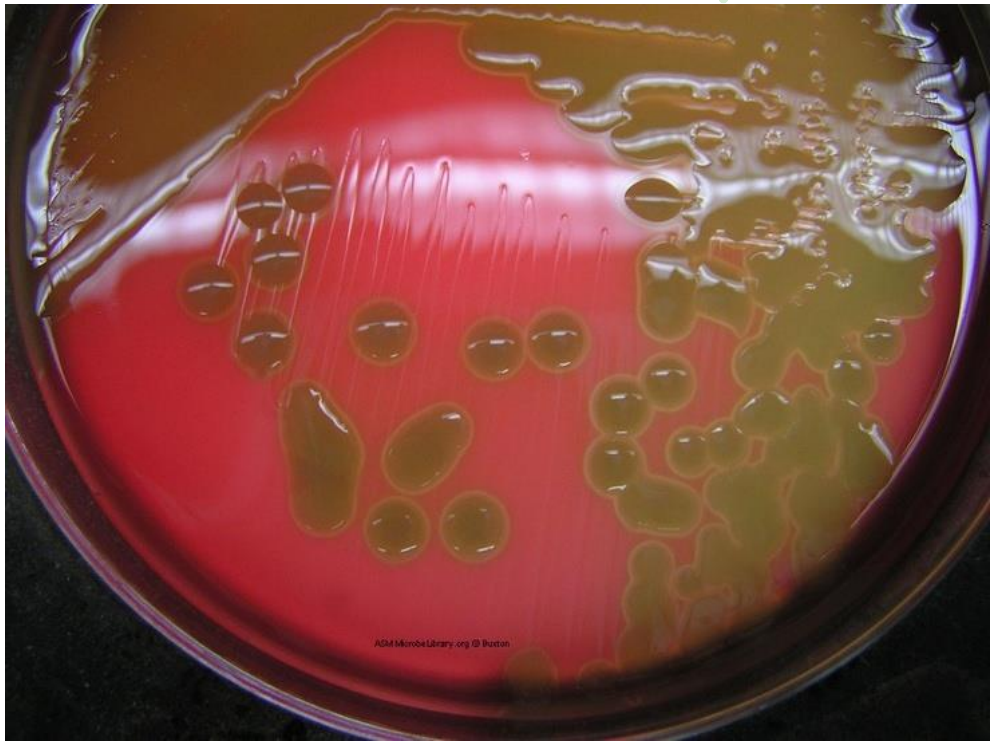
hydrogen peroxide produced by the bacterium

↓

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

↓

Hemoglobin converted into methemoglobin (greenish color)

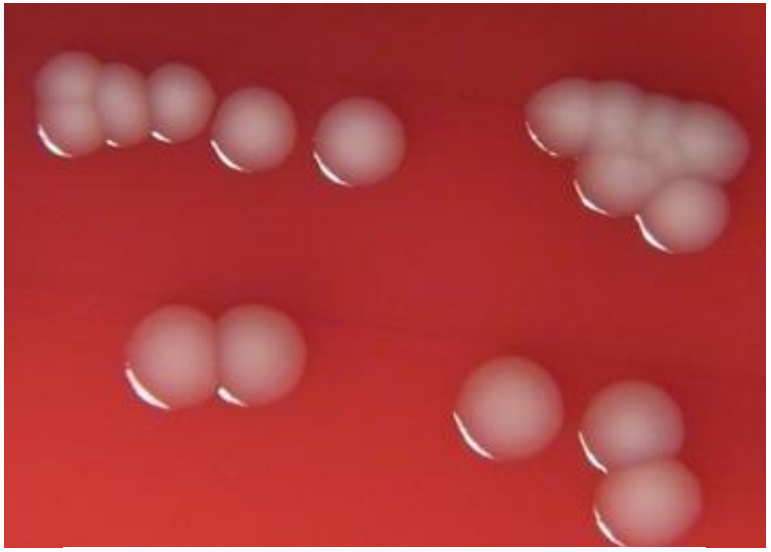


Incomplete (partial) lysis of RBCs

Methemoglobin (pronounced "met-hemoglobin") is a form of the oxygen-carrying metalloprotein hemoglobin, in which the iron in the heme group is in the Fe3+ (ferric) state, not the Fe2+ (ferrous) of normal hemoglobin. Methemoglobin cannot bind oxygen, unlike oxyhemoglobin. It is bluish chocolate-brown in color. In human blood a trace amount of methemoglobin is normally produced spontaneously. But when it is present in excess the blood becomes abnormally dark bluish brown. The NADH-dependent enzyme methemoglobin reductase (diaphorase I) is responsible for converting methemoglobin back to hemoglobin.

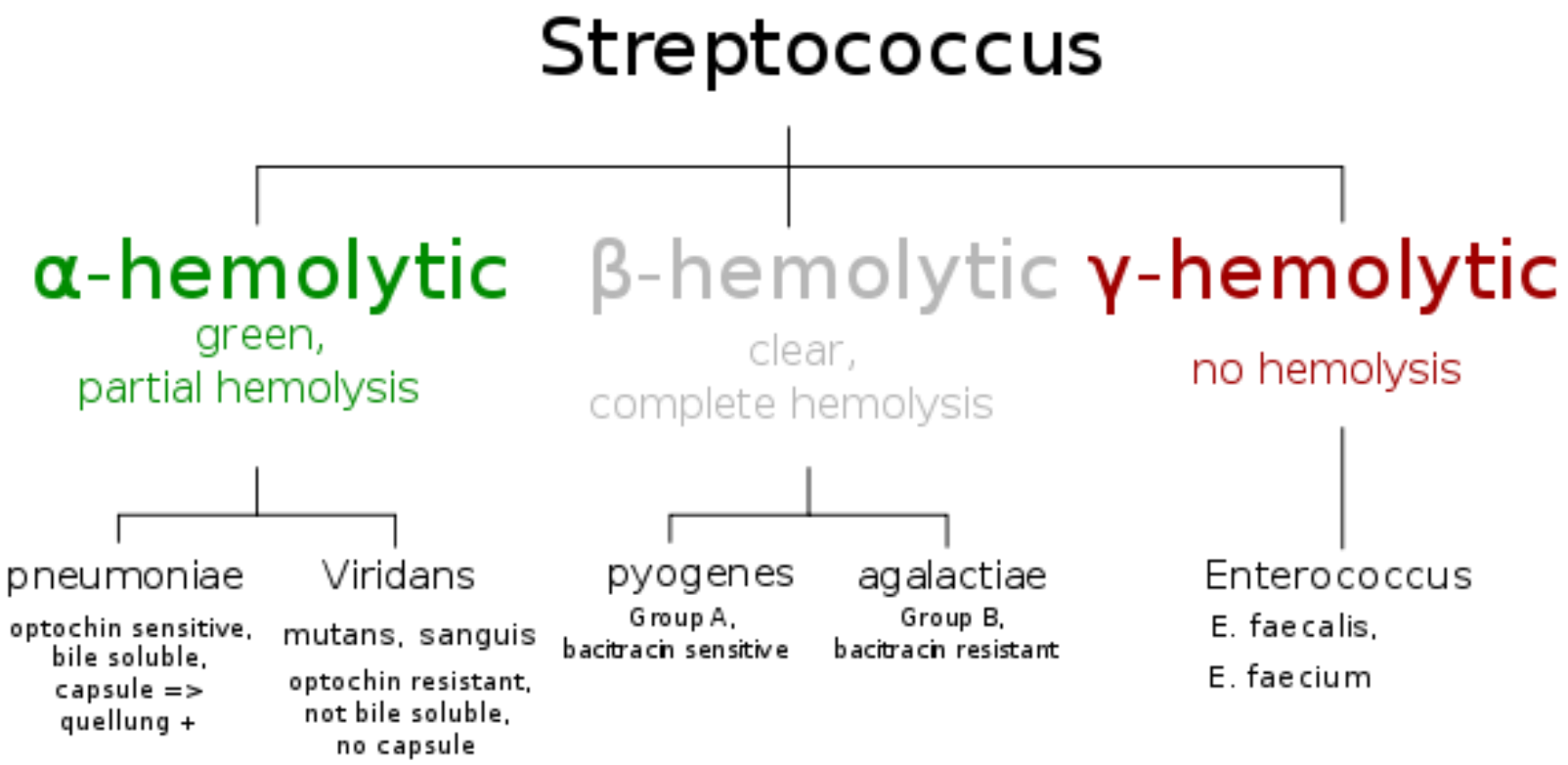
Gamma hemolysis

No hemolysis, and no change in the medium

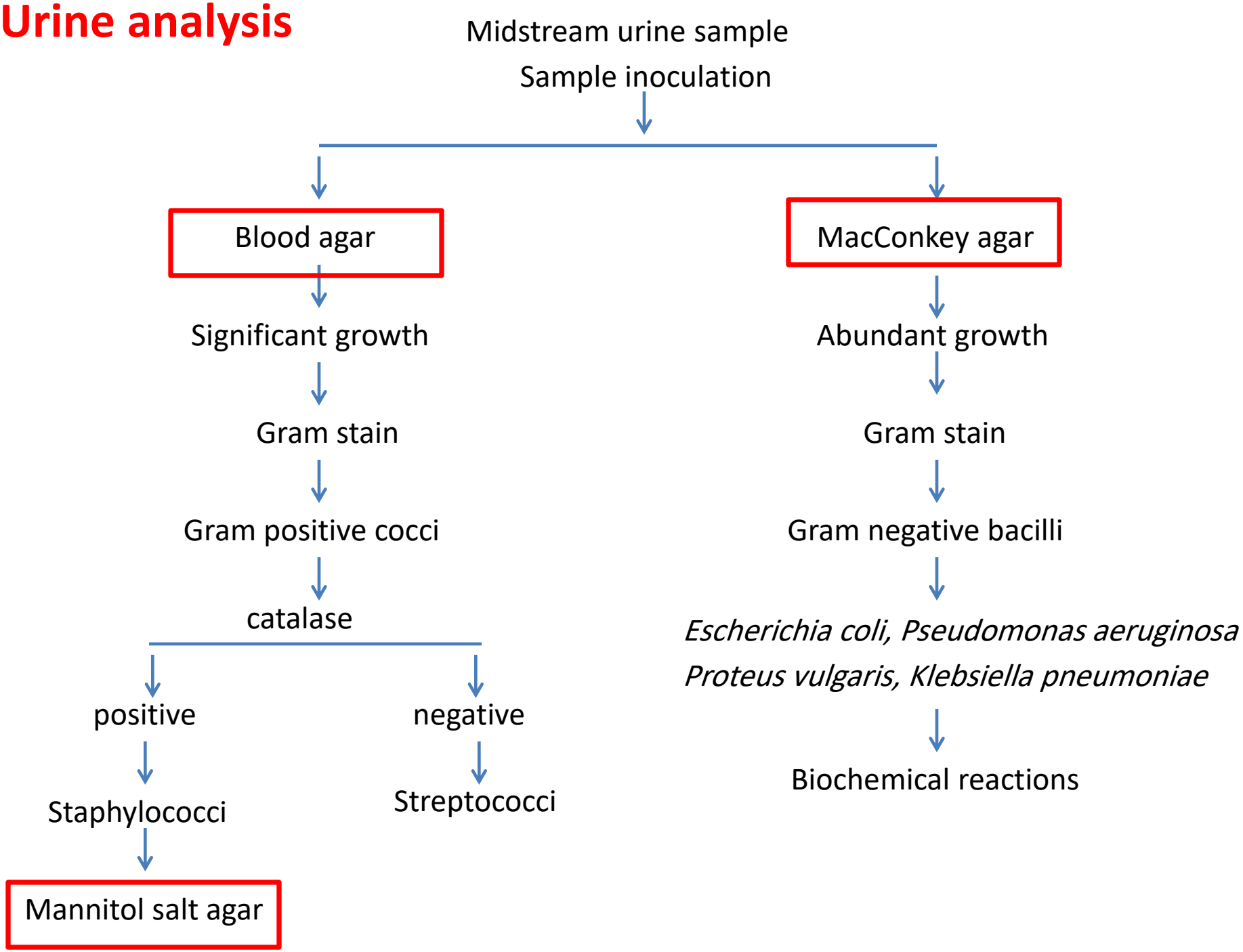


The three types of hemolysis

The hemolytic pattern of different Streptococci



Urine analysis



Mannitol salt agar

Selective and Differential for Staphylococci

- **Selective agent: 7.5% NaCl**

- **Differential agent:** mannitol to differentiate between mannitol Fermenters and nonfermenters

Staphylococcus aureus

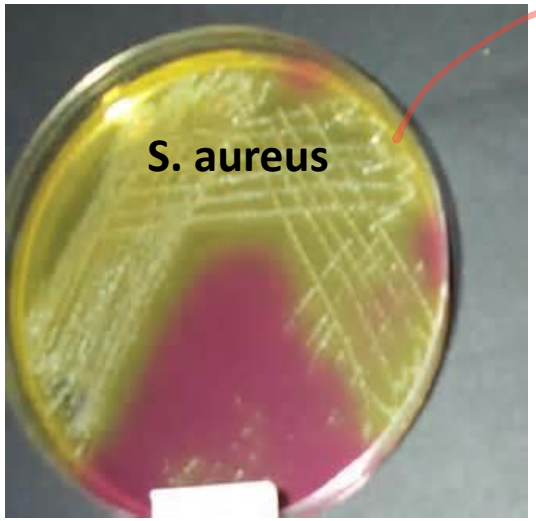
Other staphylococcus

- pH indicator: Phenol red

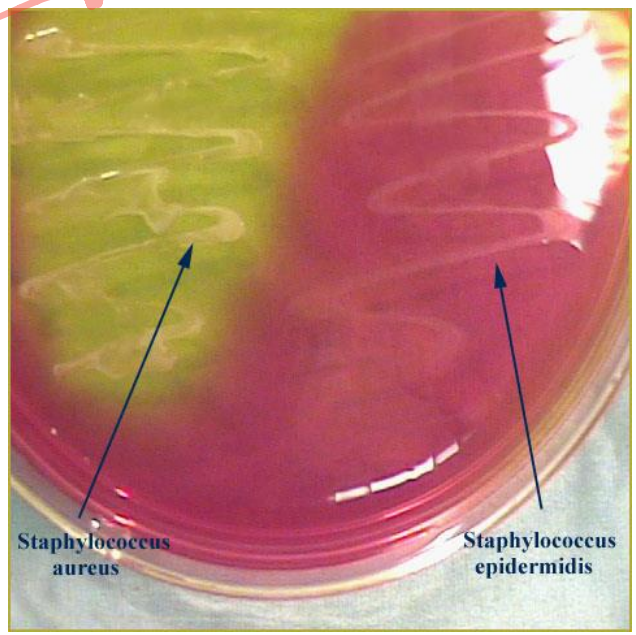
Mannitol salt agar

MSA

Non-cultured



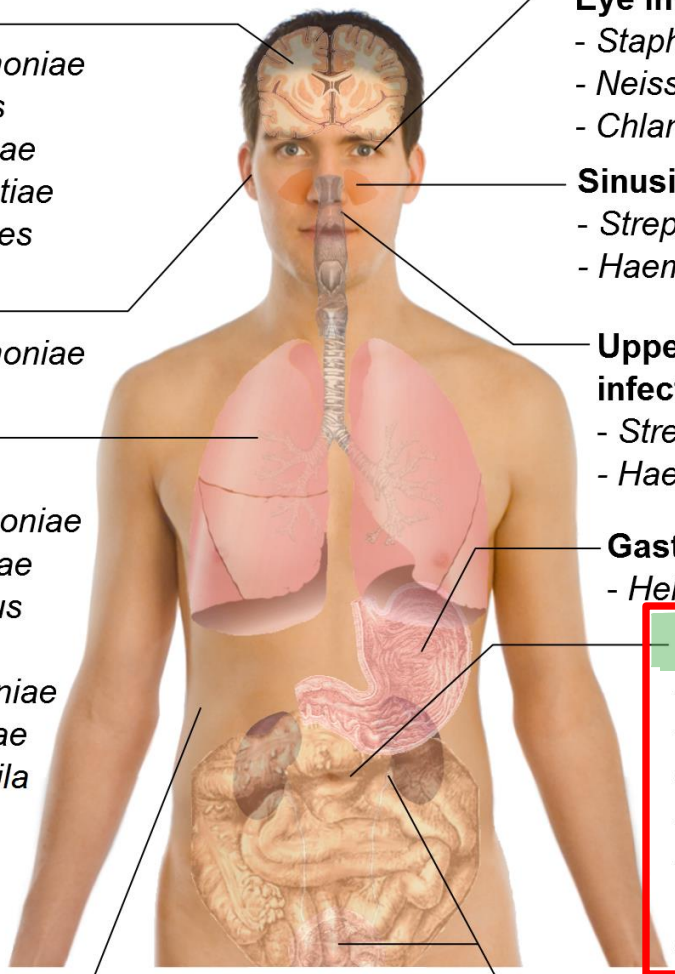
Halophilic



Blood agar

Beta hemolytic Staphylococci

Overview of bacterial infections



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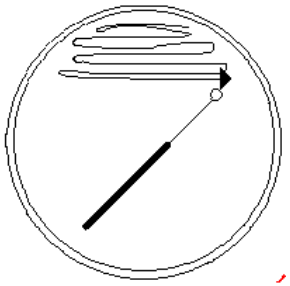
Processing of stool samples

Patient with diarrhea

Stool sample received in the lab



Culture for bacteria

Microscopy for parasites

Parasitology

Salmonella -Shigella agar (SS agar)

Purpose

For isolation and differentiation of *Salmonella & Shigella*

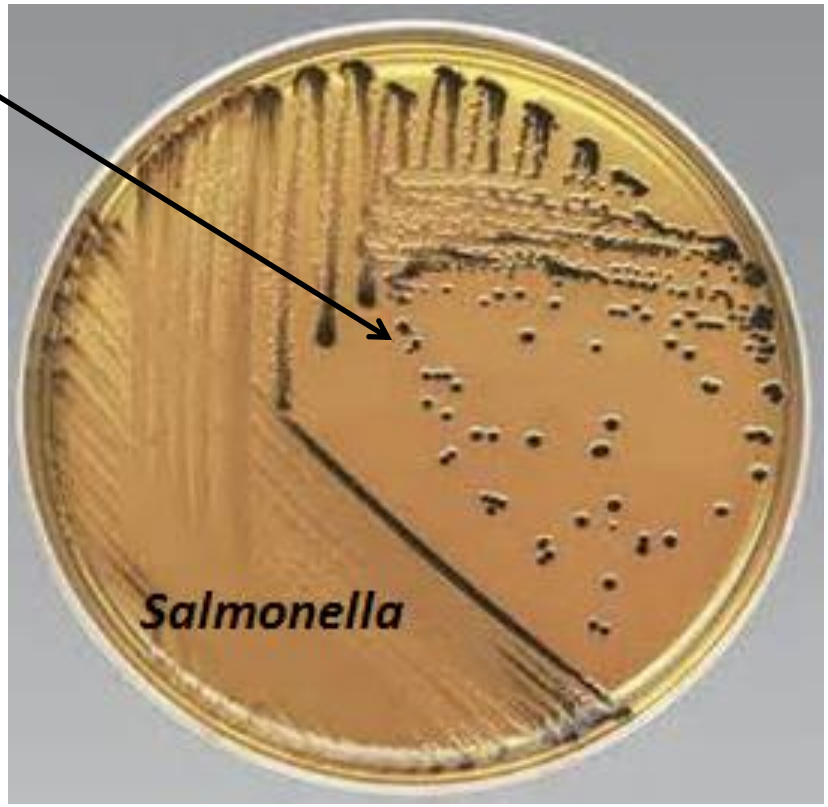
Components

- ✓ 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?
Salmonella

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)

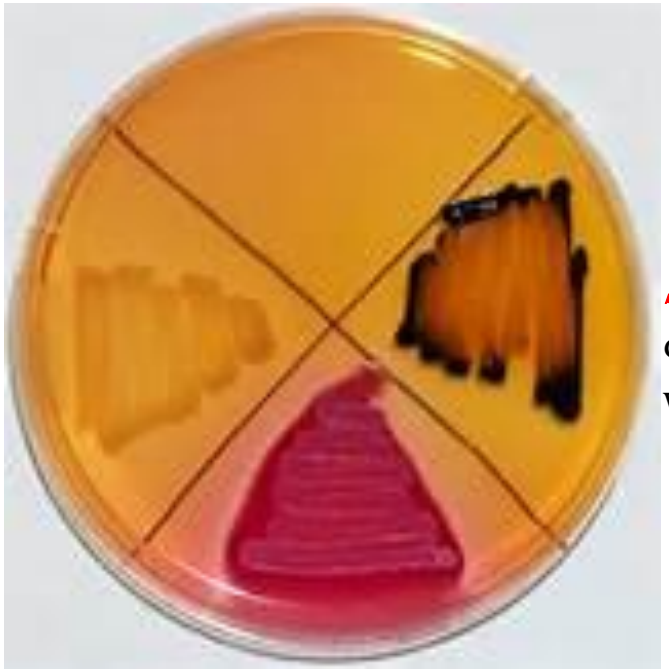
نستغل وجود هذا الا نزيم في ال salmonellaحتى نكتشفها

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

Shigella: colorless colonies without black centers

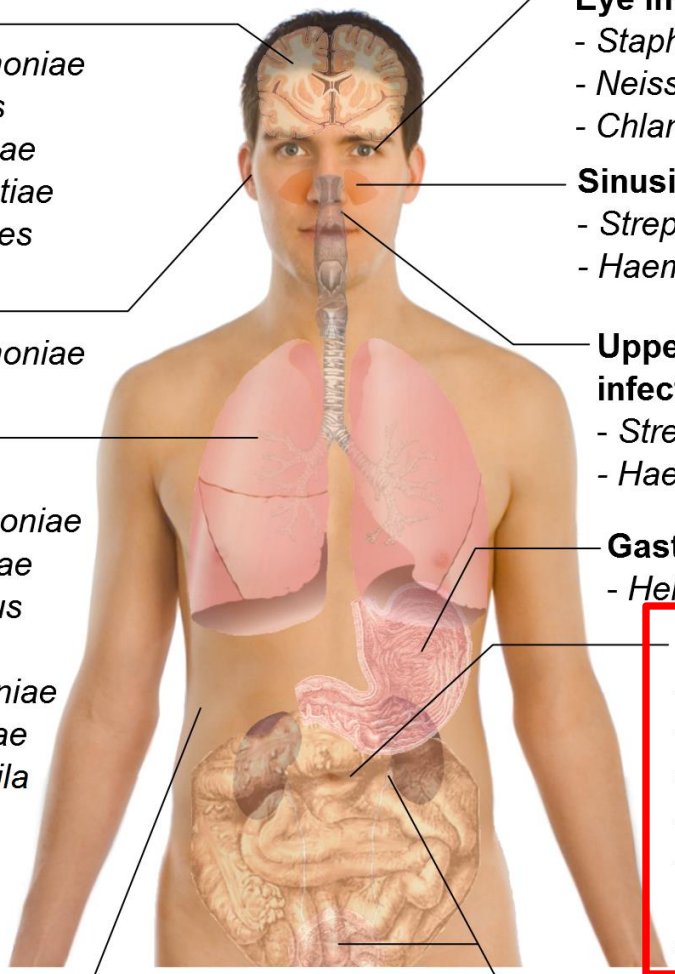


Lactose fermenter flora: pink to red colonies

Salmonella: colorless colonies with black centers

Owl eye

Overview of bacterial infections



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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

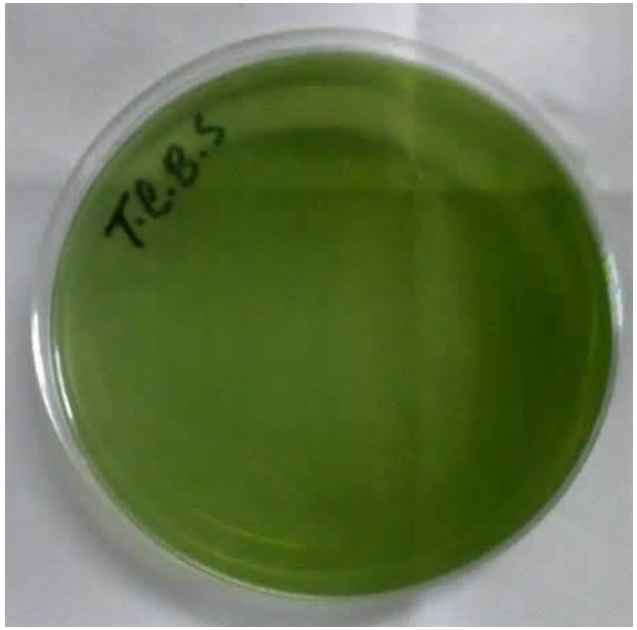
  - pH>7.6 –blue

Cholera identification

Results

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

لون ال media



TCBS media

V. cholera

V. parahemolyticus

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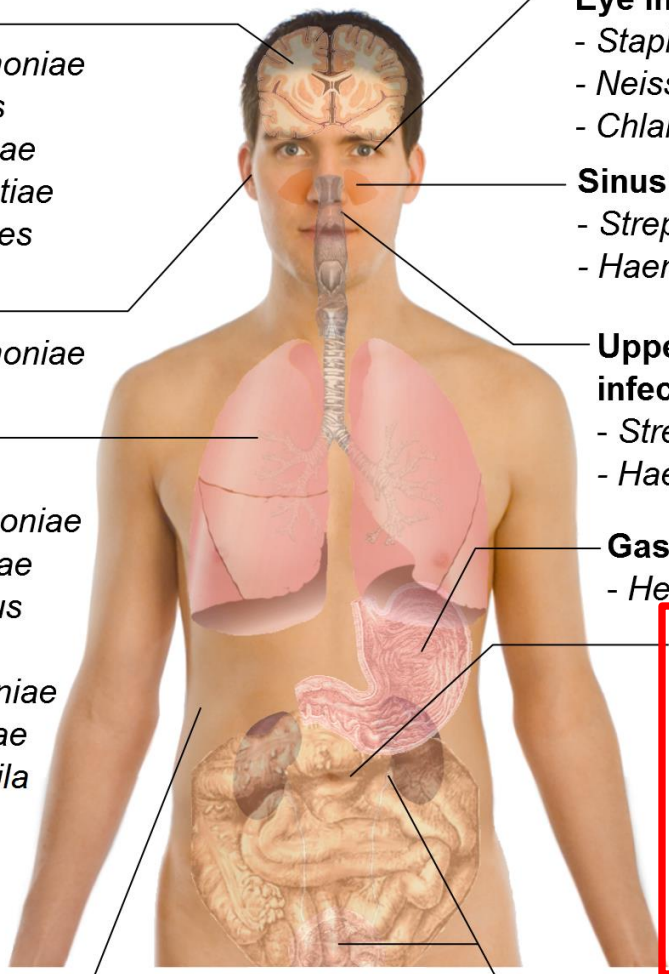

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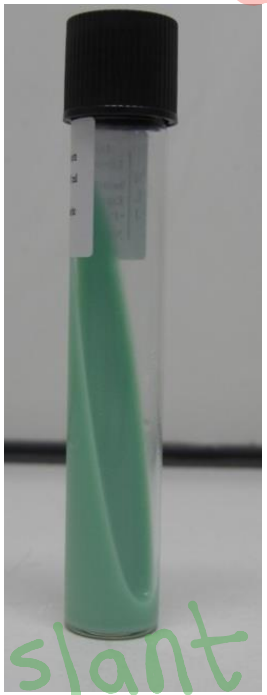
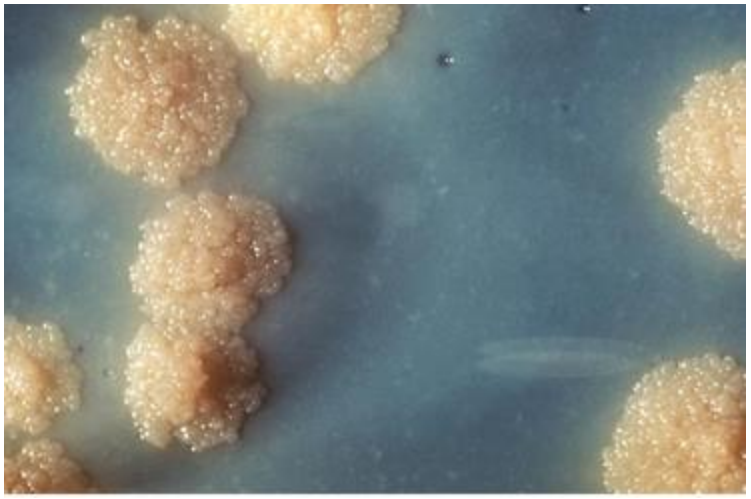
Löwenstein–Jensen (LJ) medium

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



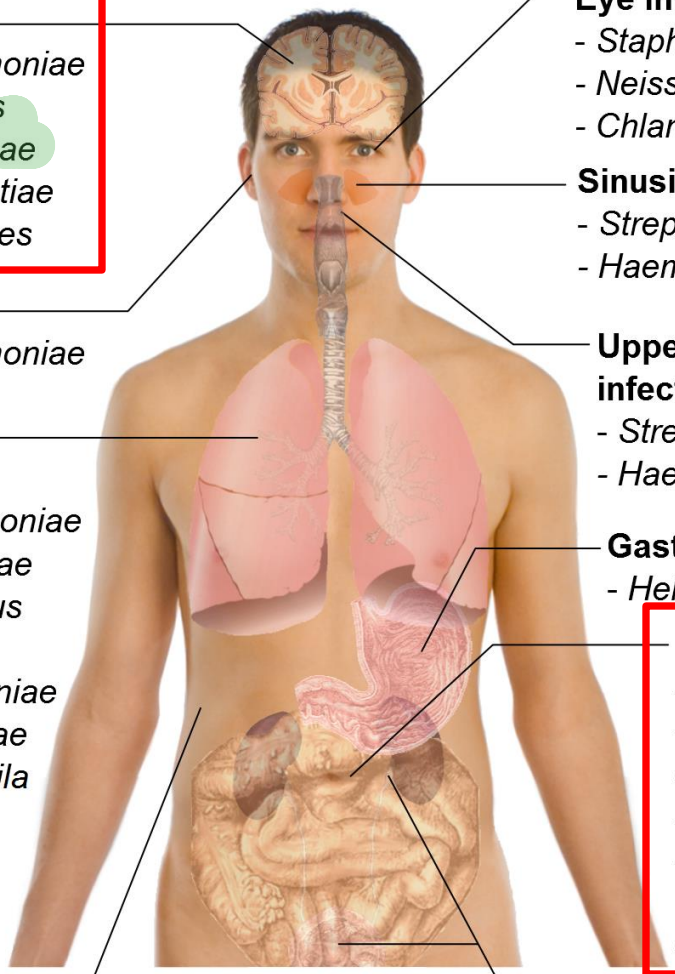


M.tuberculosis produces rough and tough colonies

Because of the micolic acid

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

Overview of bacterial infections



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- *Listeria monocytogenes*

Chocolate agar
يعيشوا على مواد داخل ال RBCs

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*
- *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*

Urinary tract infections
- *Escherichia coli*
- *Other Enterobacteriaceae*
- *Staphylococcus saprophyticus*
- *Pseudomonas aeruginosa*

CSF analysis

CSF

↓

Gram stain

↓

Sample inoculation

↓

Blood agar

↓

growth

↓

Gram stain

↓

Gram stain determines the next step
For the infection causative agent
Identification

Chocolate agar

نفس ال blood agar
بس تتم اضافة الblood على الخليط
المعقم على درجة حرارة ٦٠ °
بتكسر الخلا يا

↓

growth

↓

Gram stain

↓

gram-negative rods or coccobacilli

↓

Identification by

- ✓ Colony morphology
- ✓ Biochemical reactions for differentiation
- ✓ Serological identification (serotyping)
  (Slide agglutination test.)

Chocolate agar

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

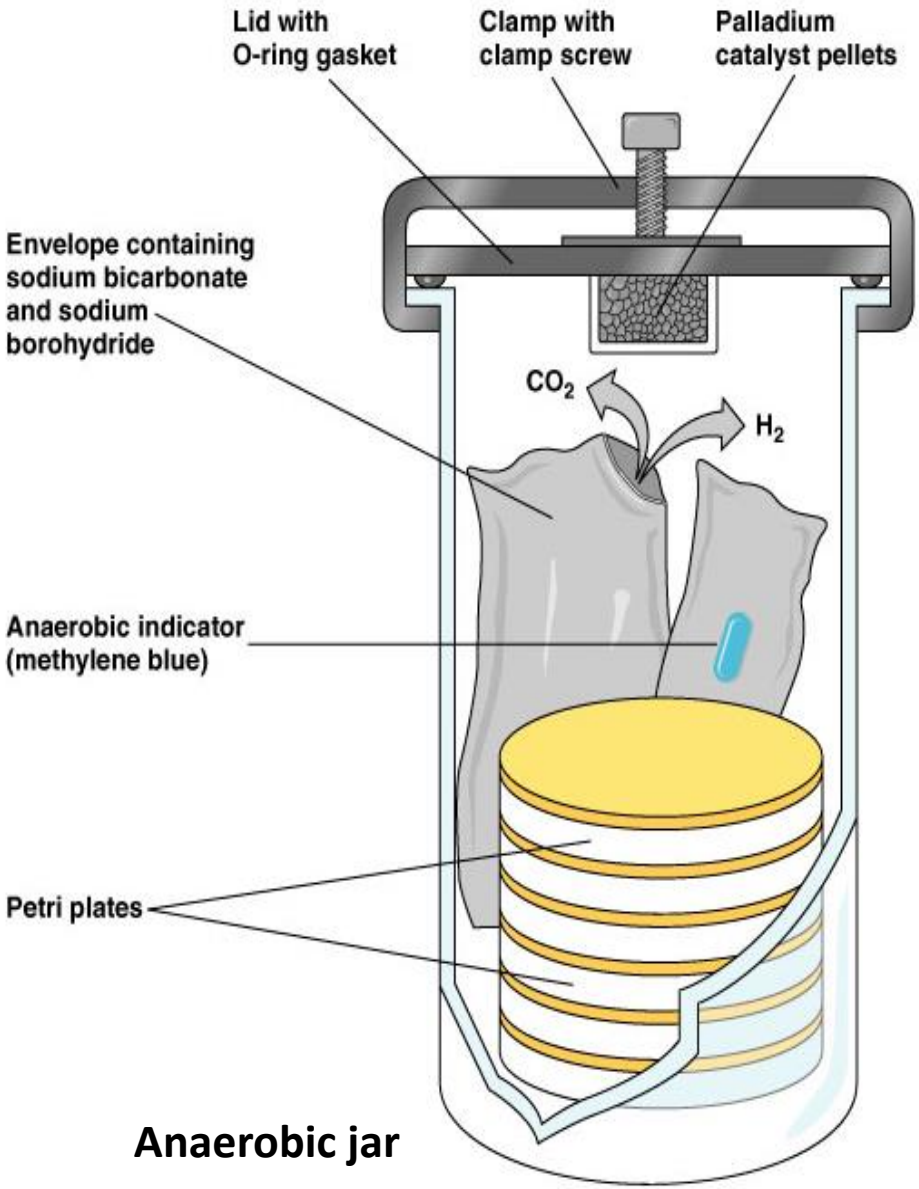
Chocolate agar

Haemophilus influenzae growth on Chocolate agar

Hemophilus requires both X factor (hematin, containing iron) and V factor (NAD). These should be relesed from bloood cells and therefore we use heating. Heating is also important to inactivate NADase

Anaerobic gars

بنزرع فيه ال
Anaerobic bacteria



Anaerobic jar

Anaerobic candle jar