Culture Media

BY:

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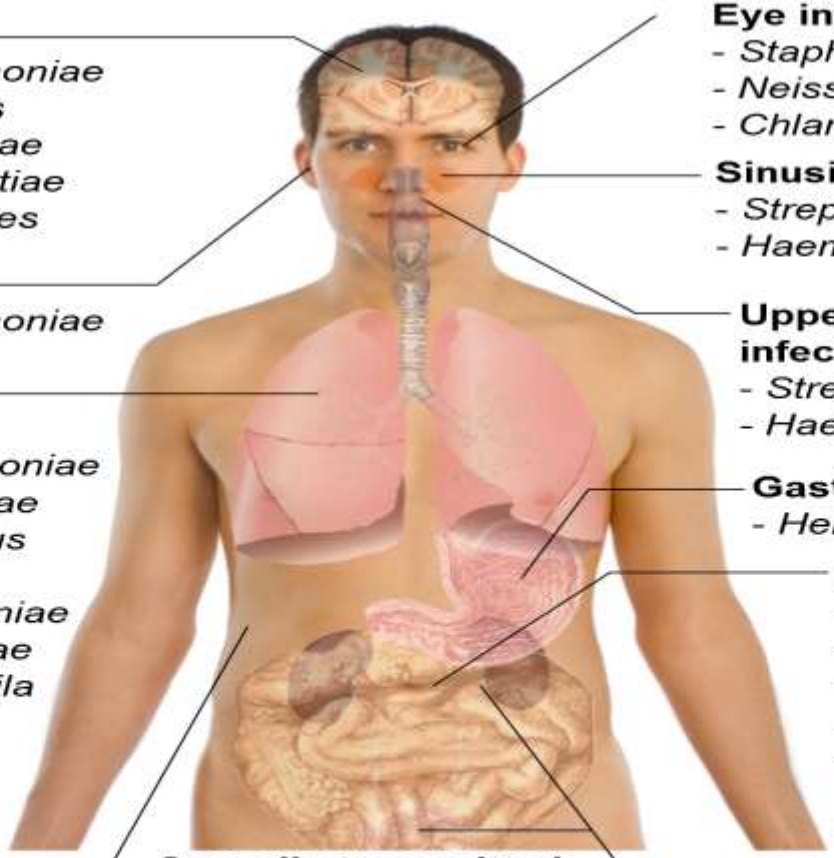
Overview of bacterial infections



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*

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*

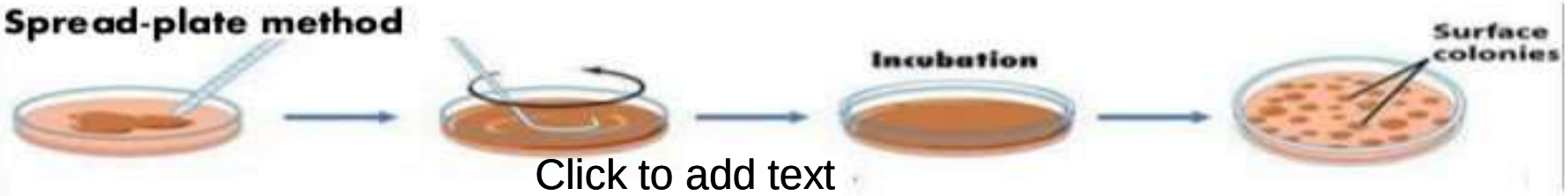
- اذا اجى مريض وبدك تاخد منه عينه من مكان معين لازم تكون عارف شو هي الmicroorganism الي رح تدور عليها
- في هذه الحاله رح اختار على اساس الmajor pathogen بختار الculture media) الموجوده في هذا المكان)

- بالاضافه الى ذلك بعرف الgrowth requirment) الي بتحتاجها في الmedia
- الي من خلالها بقدر اعمل (featuresال)ورح اكون عارف
- Recognition for these major pathogen.
- + هل المكان الي رح اخذ منه العينه فيه normal flora او لا
- + بعرف الcost

- **<u>Growth media are used to cultivate bacteria because it contains essential:</u>**
  - Necessary nutrients => يعني فيها ال growth requirment الي بتحتاجها ال microorganism
  - Moisture (humidity) we must provide it to micro organisms because microorganisms will die
  - pH to support microbial growth => very important to cultivate some microorganism need pH (alkaline) as colera

Why we use cultivate ?
1.for counting microorganisms
2.isolating microorganisms in pure cultures

Method of isolating pure cultures



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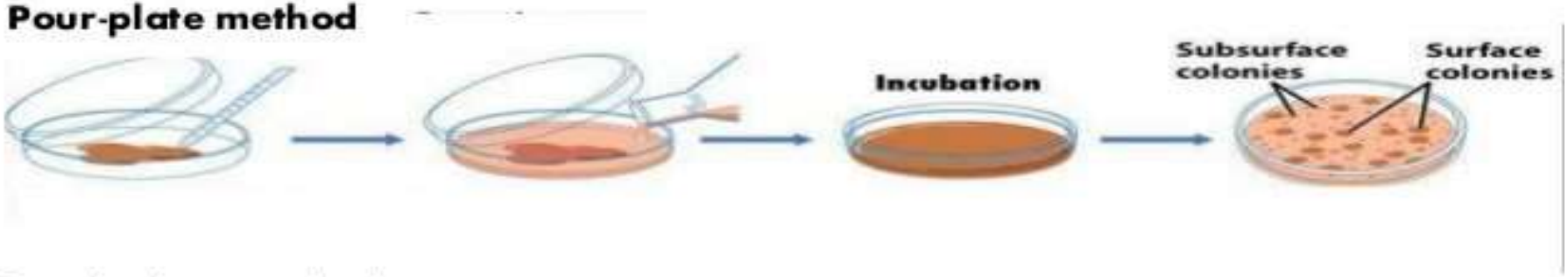
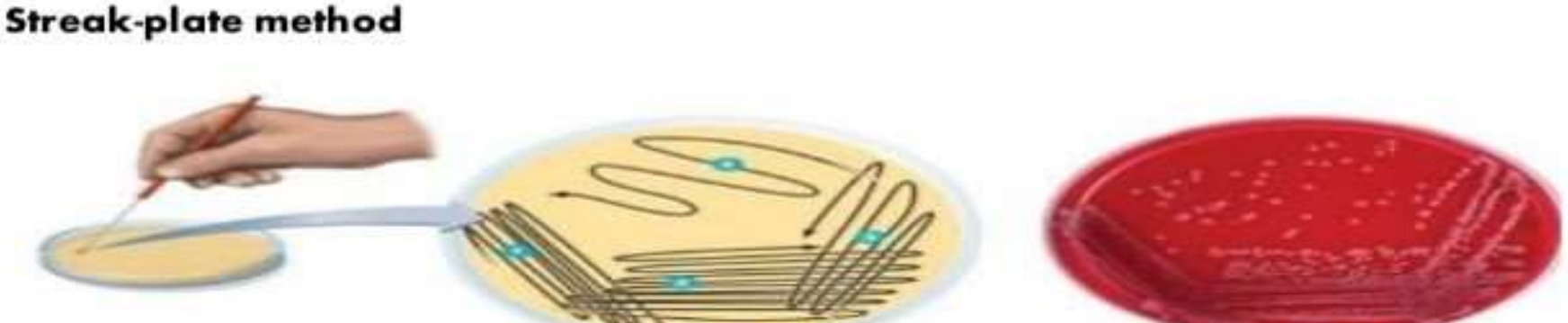




Method of isolating pure cultures

1.spread-plate-method

The culture media is in (petri dish)=>pull the sample by using pipet then put it on the surface of media +spread it by (glass rod)+intubation =>surface colonies

2.pour plate method

No media in plate=>I bring the sample and do pipeting in petri dish+ pouring the media on it

3.streak-plate method (use for any sample)

It is done by using streak loop which is wire loop that I use it in microbiology(flaming بعقمه عن طريق) then doing streaking for plate in the surface

*streak plate technique => a. flambing for wire loop befor starting cultivate the sample in media

B.to tack a sample from the tube we must do stelization

C streaking on the surface of the media (initial inoculum)

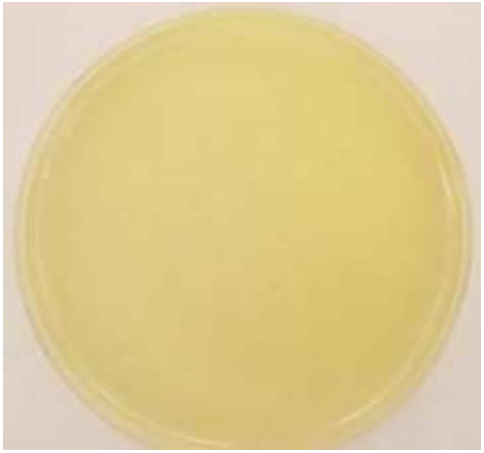
Solid media =>colonies تظهر بصورة
Fluid media =>turbidity or subsurface

BASIC MEDIA

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

  Nutrient agar

  Nutrient broth



Nutrient agar

Nutrient broth

Simi-solid => use 1.transport media
2.storag
3.molility (اذا بدنا نعرف انه الmicroorganism بنحرك او لا

Basic media : simple media , it has the least nutrienial requirment

محتويات هذه الميديا:

1.peptone =>common ingredient (basic substance)=>it is a water soluble product

Hydrolysis to animal or plant protein يتم الحصول عليه عن طريق

Peptone is a protein that will give a nitrogen to M.O or mineral and Vit.

2.water :deionize water or sterly water

3. agar :poly saccharide extract from aglea (طحالب) =>red seaweed

Agar is solidetided agent/ like gel

-enriched media =>we give M.O mor thing as: growth factor(blood, serum ,extra peptone and vitamin)

Blood agar => ينمو عليه معظم البكتيريا

Blood agar:

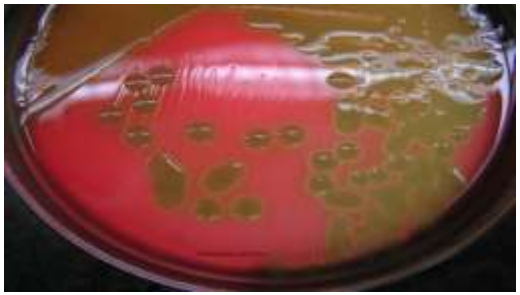
- **Enriched medium: containing peptones, yeast extracts, liver or heart extracts (depending on the medium), and blood.**
- **Differential medium: containing blood**
- **Some bacteria produce an enzyme called hemolysin that is able to lyse RBCs (hemolysis)**

Alpha hemolysis

Hemoglobin containing Fe^{2+} (ferrous) → free radical عباره عن hydrogen peroxide produced by the bacterium → **Oxidation of Fe^{2+} into Fe^{3+} (ferric) state** → Hemoglobin converted into methemoglobin (greenish color)

Growth on BA differentiates between the three groups of Bacteria:

Alpha hemolytic bacteria



Incomplete (partial) lysis of RBCs

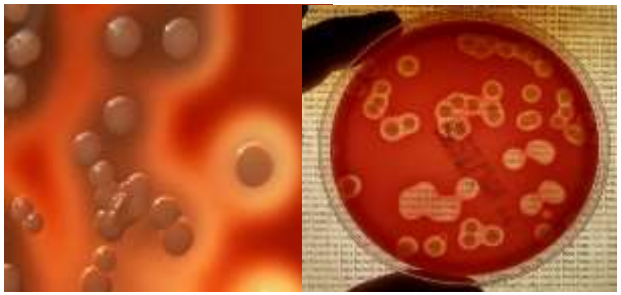
Viridans streptococci

Streptococcus pneumoniae

اللون الاخضر نتيجه ال

Formation of methemoglobin

Beta hemolytic bacteria

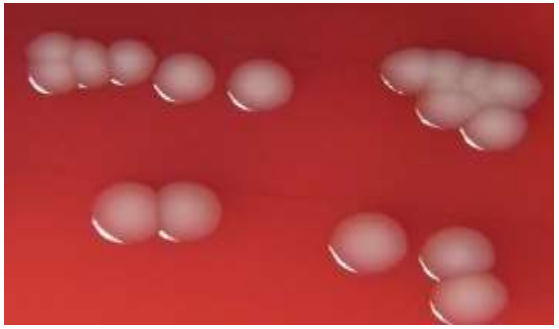


Complete hemolysis

Streptococcus pyogenes

Staphylococcus aureus

Gamma hemolytic bacteria



No hemolysis, and no change in the medium

Enterococcus faecalis

اسماء الmicroorganisms مهمه

Enrichment media

* اذا جاء مريض يحتاج تحليل(stool sample)

هذا ال stool يحتوي الكثير من ال m.o

اذا بدنا ال m.oبهاي الي سبب المشكله بنفس الوقت نسبتها قليله الحاله بنستخدم

Enriched media before the cultivate => to enriched this bacteria and multiply the pathogen and in the same time decrease the number of normal flora or unwanted bacteria

DIFFERENTIAL MEDIA

تستخدم للتمييز بين نوعين من البكتيريا

Media to which indicators, dyes, or other substances are added to differentiate microorganisms so these media can distinguish among morphologically and biochemically related groups of organisms.

SELECTIVE MEDIA

Media contain substances that prevent the growth of microorganisms other than the pathogens for which the media are intended.

Many culture media are both selective and differential.

MacConkey agar

MacConkey agar is a selective and differential media for Enterobacteriaceae

بعطوا ال

Selectivity

Cause of selectivity

- Inhibit growth of G+ bacteria
- Inhibit growth of G+ bacteria
- Bile salts
- Crystal violet
- Neutral red
- Lactose
- pH indicator: Acidic=pink
- Cause of differential

Neutral red in pink in acidic media

Lactose fermenters: Pink colonies

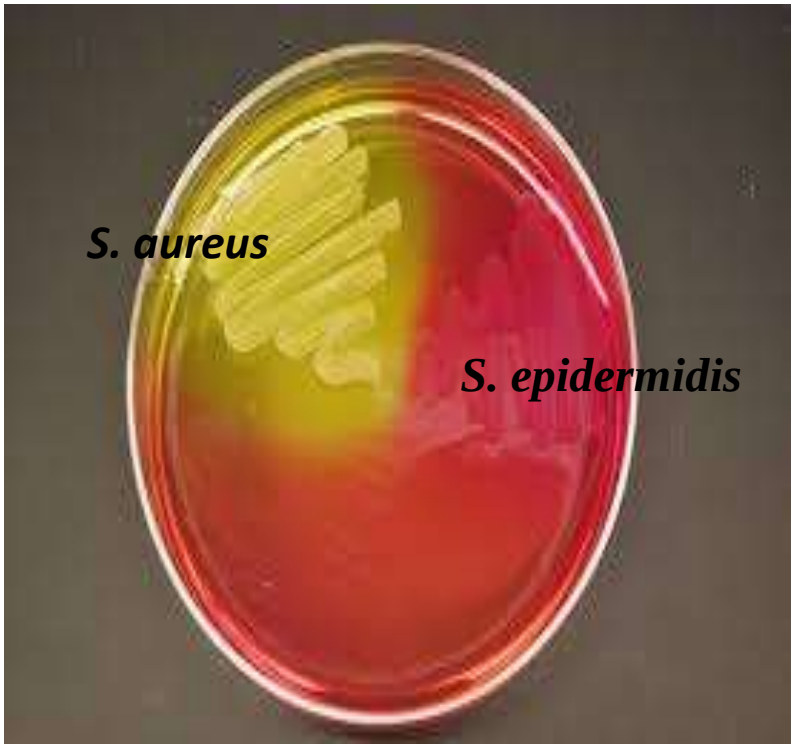
- *E. Coli*
- *Klebsiella spp*
- *Entrobacter spp*
- *Citrobacter spp.*

Lactose non fermenters: Colorless colonies

- *Salmonella spp.*
- *Shigella spp.*
- *Yersinia spp.*

S.aureus=>doing frementation for mannitol

يتغيير ال رحpH indicator from red to yallow in acidic media



staplyالي هم هنا سيتم التفريق بين انواع ال

1 aureus (coagulase +)

2 coagulase (-)

Thiosulfate citrate bile salt sucrose (TCBS) agar

Selective agent

The medium is alkaline (pH 8.6) which enhances the growth of Vibrio species

TCBS media

Important components:

Differential agent

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

نتيجه وجوده يكون اللون الاخضر للميديا

- pH<6.0 - yellow

- pH>7.6 – blue

Salmonella -Shigella agar (SS agar)

For isolation and differentiatio Selective +differential
of *Salmonella & Shigella*

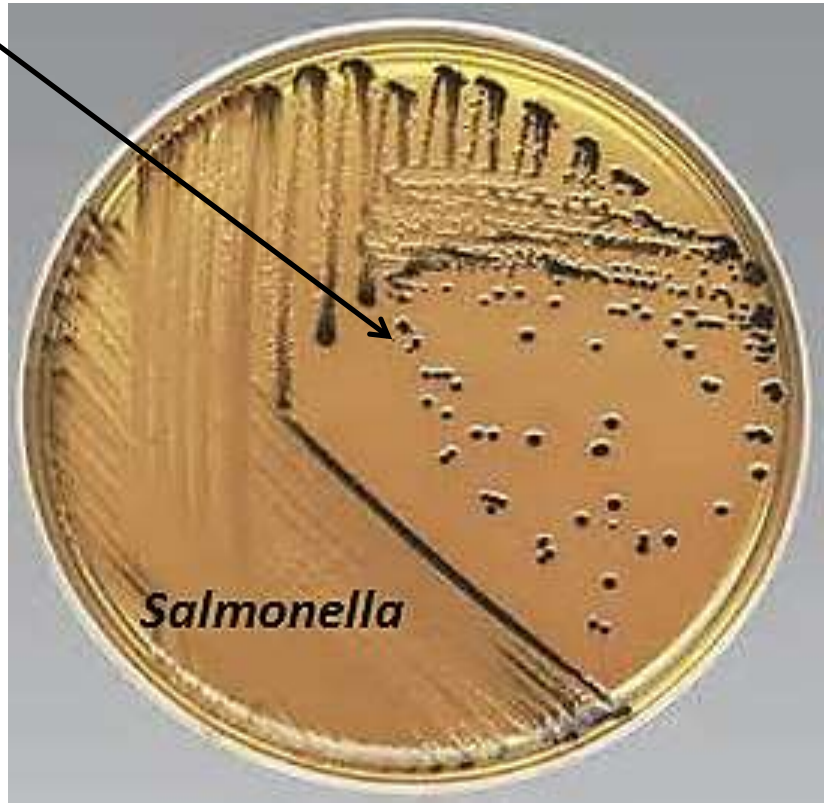
Components:

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

Macconkeg agar بشبه ال

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

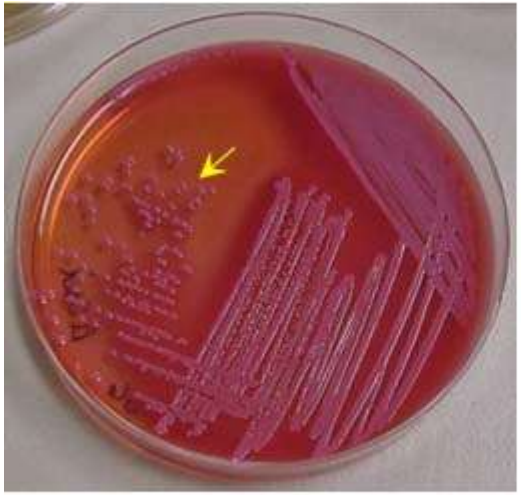
↓

عباره عن system indicate the M.O اذا بطلع h2s اولا

$Na_2S_2O_3$ + thiosulfate reductase ⟶ sulfite + H2S

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

- **Lactose fermenters: pink to red colonies**
- **Non lactose fermenters: translucent, colorless colonies with or without black centers**



Escherichia coli

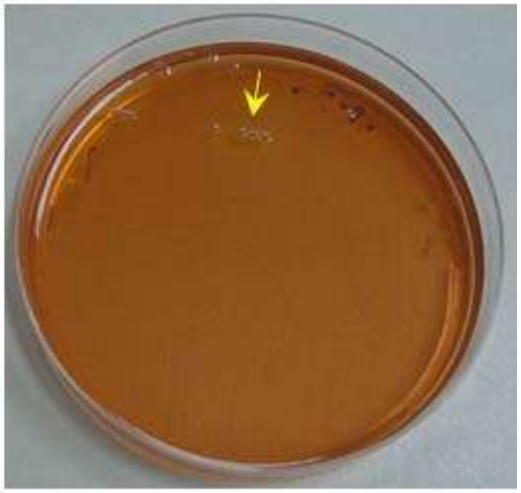
Lactose fermenter flora:
pink to red colonies

Gram +

Salmonella

Salmonella:
colorless colonies with black centers

Non lactose fermenter
But it H2S produse



Shigella

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

H2s + non lactose ما بتطلع
fermenter

TRANSPORT MEDIA:

Black megia

- **Amies Transport Medium**

Contain charcoal
لونها اسود لانها بتحتوي الفحم الي بيعمل
Absorption for all inhibitory
substance الي ممكن تطلع اثناء
عمليه الtranspartation

- **Cary Blair Transport Medium**

For fecal pathogen

Löffler's medium:

Enriched media

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

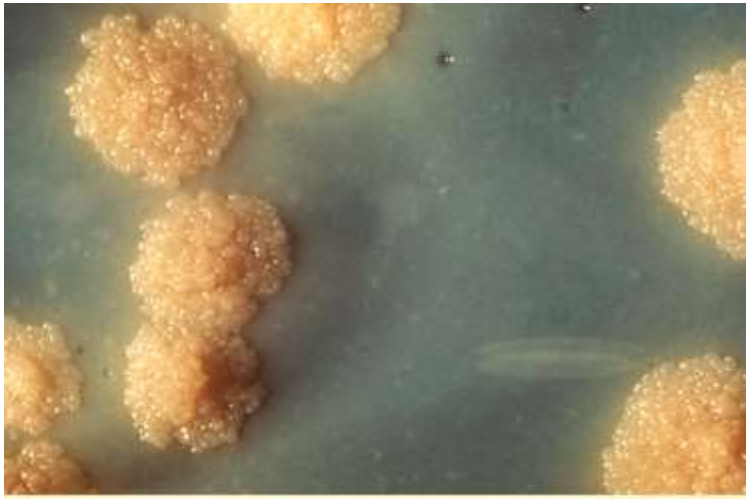
Gram-positive rod-shaped bacteria that are straight or slightly curved. The bacteria group together in a characteristic way (Chinese letters)

Löwenstein–Jensen (LJ) medium :

Enriched (لانه فيها egg)+selective لانه فيها malachite green

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

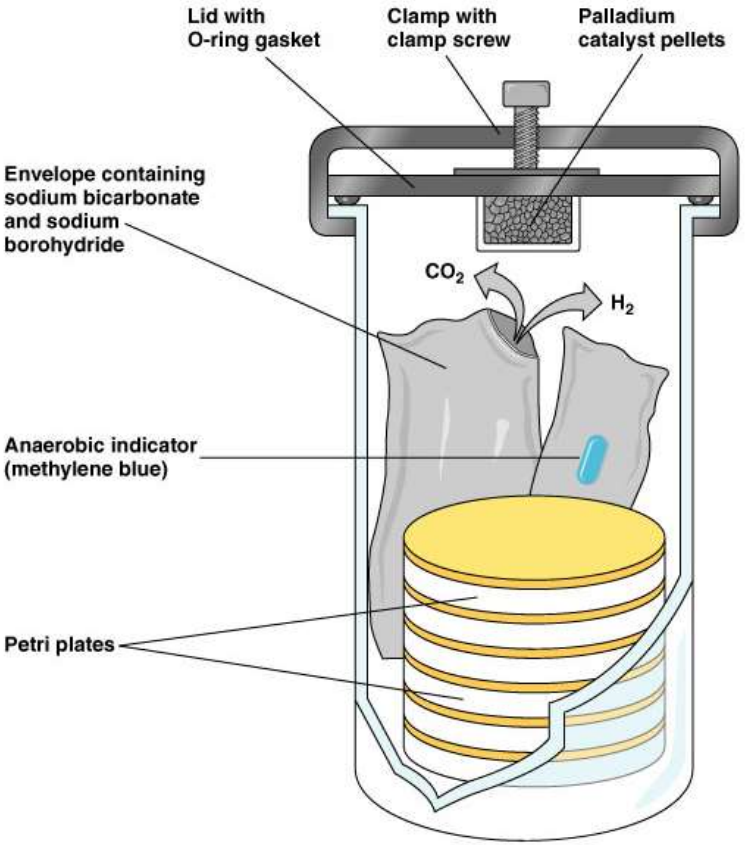
Selective to it



M. tuberculosis **produces rough and tough colonies**

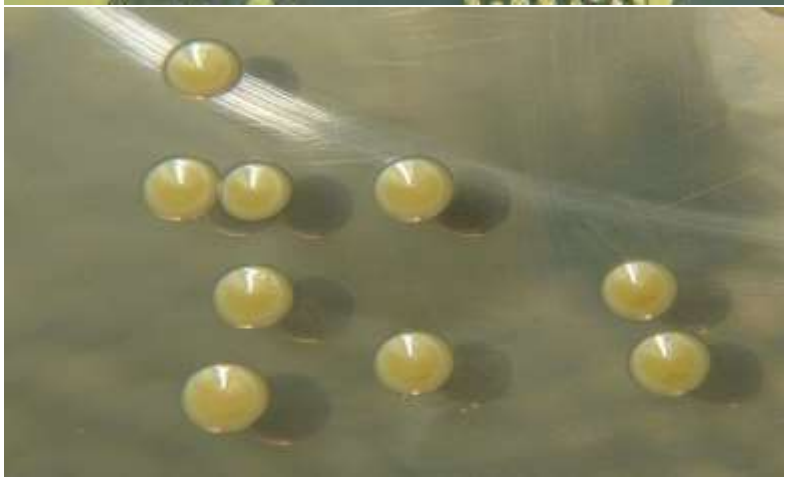
Anaerobic jars:

Have gas exchangative gates

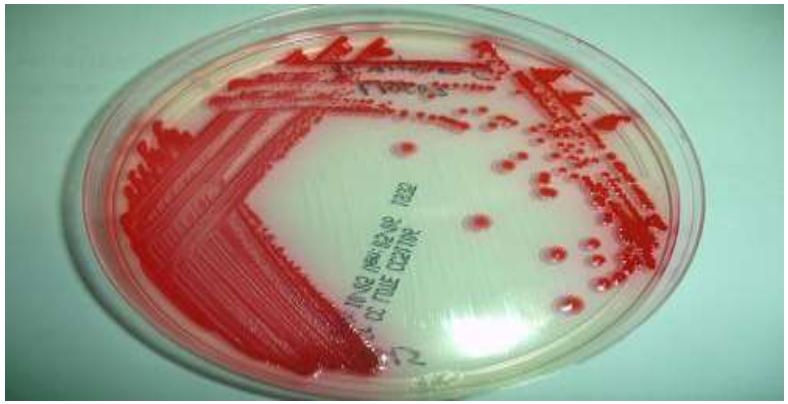


Anaerobic jar

Anaerobic candle jar



Golden yellow pigments of *Staphylococcus aureus*



Serratia marcescens

Upper respiratory track infection+ urinary track infection مشهوره لانها موجوده بالمستشفى

بعض انواع البكتيريا بتطلع pigments which help in identification

Endopigment

exopigment

لونها biocunine (blue)

Swarming growth of *Proteus*

Yellow green pigments of *Pseudomonas aeruginosa*

Exopigment

اذا حطيناها بدرجه حراره الغرفه بكون اللون اوضح لانه الحراره مش مناسبه الها بخايها تعيش pigmentبالتالي زياده ال

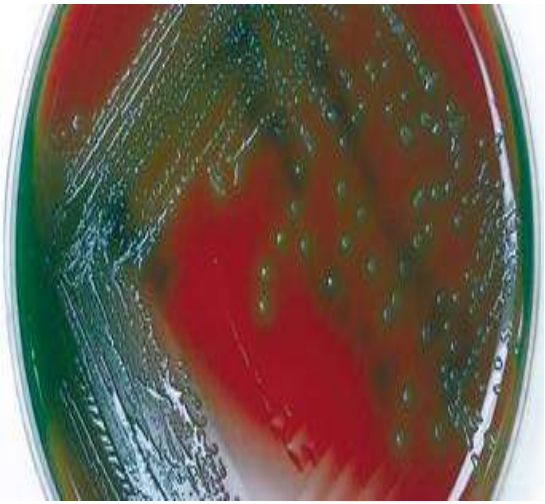
Blood agar

Macconkajagar

TCBS

Non sacanse

Blood agar complete hemolysis
Beta hemolysis



Partial hemolysis
Alpha hemolysis

- Done by :Safaa matar& Mai bani atta