وَقُل رَّبِّ زِدْنِي عِلْمًا

النادي الطبي

PERIPHERAL NERVOUS SYSTEM

SUBJECT : Micro Lab

LEC NO. :

DONE BY : Salsabeel almtour

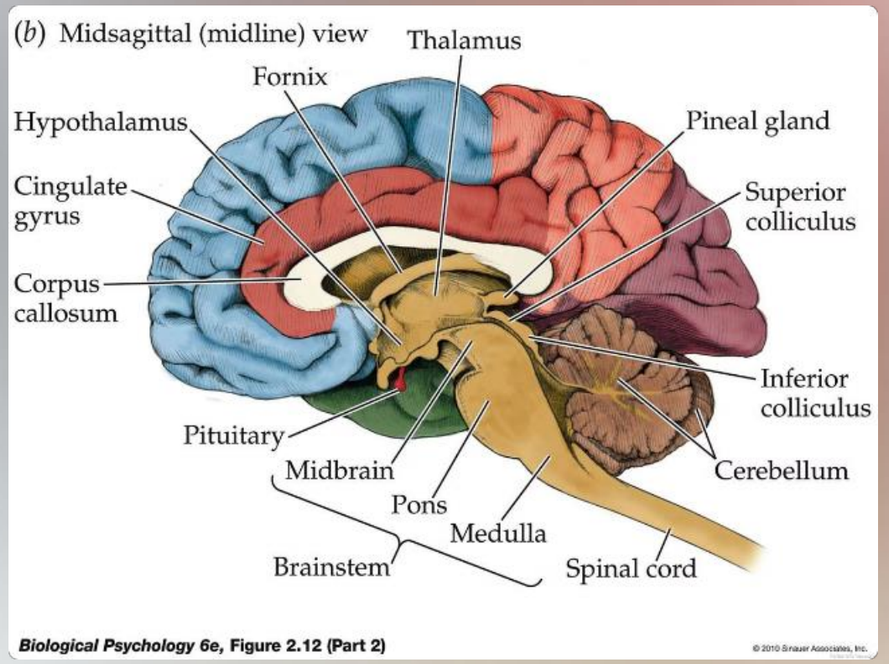
اي اشي عليه هايلايت فهو الاشي اللي قرأه
الدكتور اما الكلام الي مش محدد بهايلايت فالدكتور ما ذكره
+ركزو على كل النوت اللي ضفتها لانو الدكتور بالغالب حيترك
كل السلايدات و يجيب من كلامه 😊

ويلا نبدأ.....

CSF lab examination

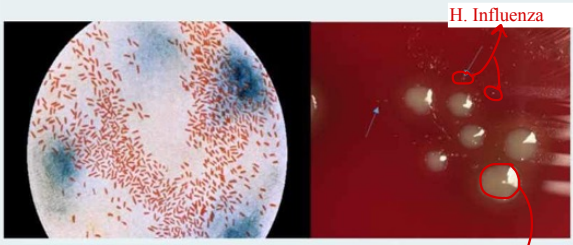
The central nervous system (CNS) is susceptible to a variety of infectious pathogens, including bacteria, viruses, fungi, and parasites. Identifying these CNS pathogens through cerebrospinal fluid (CSF) analysis is crucial for effective diagnosis and treatment.

Dr. Ashraf Khasawneh



سؤال الدكتور بلش فيه اللاب

A 45-year-old male presents to the emergency department with a sudden onset of severe headache, fever, photophobia, and confusion. On physical examination, he has nuchal rigidity and positive Kernig's sign. A lumbar puncture is performed, and cerebrospinal fluid (CSF) analysis reveals an elevated white blood cell count with a predominance of neutrophils, elevated protein levels, and decreased glucose levels. Gram stain of the CSF shows Gram-negative bacilli. *



- Haemophilus influenzae type b
- Streptococcus pneumoniae
- Neisseria meningitidis
- Group B Streptococcus
- E. coli

بنقدر نعرف انو meningitis

بتدل على Bacterial infection

Gram negative bacilli:

✓E.coli

✓Hemophilus

✓p.aeruginosa

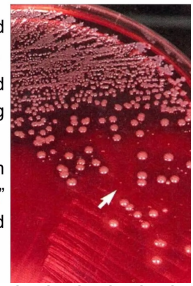
هلاء الجواب الاخير حيكون من خلال الصوره

بنلاحظ في اسهم على نقط صغيره و اللي هي ال Haemophilus

H. influenzae (culture)

Blood agar (satellitism):

- *H. influenzae* can grow on blood agar in the presence of *S. aureus*.
- *S. aureus* produces V factor and releases X factor by hemolysing blood.
- *Haemophilus* colonies will form small colonies called "**satellites**" in the hemolytic zone around *Staphylococcus* colonies.



هاي صوره خارجيه للتذكير بال culture

Specimen Collection: Lumbar Puncture

Preparation

The patient is positioned, the puncture site is sterilized, and local anesthetic is administered.

1

Needle Insertion

A thin, hollow needle is carefully inserted between the vertebrae to access the CSF space.

2

ال needle size بختلف من طفل ل adult وهكذا

CSF Collection

The CSF is collected in sterile containers for analysis and further testing.

3

بنجمع اكثر من عينه لاكثر من فحص مثلاً
لل chemistry و لل culture و لل
latex agglutination test و للهستو

Indications and Contraindications for Lumbar Puncture

امور بعمل فيها LP

Indications

- Suspected meningitis or encephalitis
- Evaluation of neurological disorders
- Suspected subarachnoid hemorrhage

امور ازا شفتها ممنوع اعمل فيها LP

Contraindications

- Increased intracranial pressure
- Spinal cord compression
- Bleeding disorders
- Skin infection at the puncture site

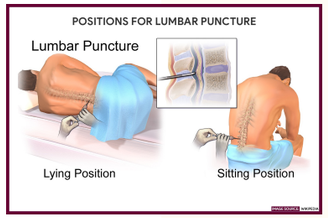
We do CT to know the reason for increased ICP

Lumbar Puncture Procedure
Procedure and Equipment
Equipment

- Spinal needle
  - Less than 1 yr: 1.5in
  - 1yr to middle childhood: 2.5in
  - Older children and adults: 3.5in
- Three-way stopcock
- Manometer
- 4 specimen tubes
- Local anesthesia
- Drapes
- Betadine

الدكتور ما فصّل بال equipment بس ذكر بشكل عام انو حجم ال needle زي ما حكينا بختلف من طفل ل adult و انو بنحتاج 4 عينات CSF

صوره خارجيه للتوضيح:



Patient Positioning

اما بحكيلوا يجلس و يعمل lean forward او lying
و يعمل flexion لل hips و spine on the side

The patient is typically positioned on their side or sitting upright, with the back curved to open the spaces between the vertebrae.

+عشان يرجع ال CSF لفوق و اقلل ال risk for injury

Sterile Technique

The clinician uses sterile gloves, drapes, and a local anesthetic to ensure a safe and comfortable procedure.

Specialized Equipment

The procedure requires a lumbar puncture needle, manometer, and sterile containers for collecting the CSF sample.

بشكل عام ال procedure تاعت ال CSF sample مش
سهله و بتلاقي الاهل و المريض كلهم خايفين منها

بالتالي ال CSF sample تعتبر one of the
very precious samples

Specimen Transportation and Storage

Transport

The collected CSF sample should be transported to the laboratory as soon as possible in a sealed, leak-proof container.

Storage

If immediate analysis is not possible, the sample should be refrigerated or frozen to preserve the integrity of the specimen.

بنحاول نبعد عن تجميد الsample و الافضل
انو نعمل ال test straight away

Time Sensitivity

Timely processing of the CSF sample is crucial, as delays can affect the accuracy of the test results.

مهم انو ننقل العينه ب the quickest and best way

Complications of Lumbar Puncture

1

Post-Dural Puncture Headache The most common one

Leakage of CSF through the puncture site can cause a severe, persistent headache.

2

Bleeding and Infection

Rare complications include bleeding at the puncture site or introduction of pathogens, leading to infections.

3

Nerve Damage Rare

In rare cases, the needle may inadvertently damage a nerve, causing temporary or permanent neurological symptoms.

ما قرأ الدكتور هاد الجدول

Complication	Description	Prevention
Headache	Caused by persistent CSF leak. Begins 24-48h post-procedure. Described as a throbbing headache worse when standing.	1) Use thinner needle 2) Use atraumatic needle 3) Replace stylet 4) Insert needle with bevel parallel to dural fibers
Back Pain	Transient, electrical type pain. Typically resolves without intervention.	Limit number of attempts
Infection	Local skin infection or spinal fluid infection	Practice aseptic technique
Bleeding	Spinal hematoma may compress spinal cord	Avoid LP in patients at high risk for bleeding
Herniation	Brainstem compression due to pressure changes	Avoid in high risk patients
Epidermoid Tumor	Due to epidermoid tissue transplanted into spinal canal during procedure	Use a stylet when inserting needle

Microbial Culture and Identification

Culture Media	Pathogenic Organisms
Blood Agar	Streptococcus pneumoniae, Neisseria meningitidis
Chocolate Agar	Haemophilus influenzae (Fastidious microorganism)
Sabouraud Dextrose Agar For fungal infection	Candida species, Cryptococcus neoformans
Thioglycollate Broth	Anaerobic bacteria

هلاء هون الدكتور ضاف حكي زياده عن موضوع ال thio broth :

مبدئياً خلينا نتفق انو ال thio broth بنستخدمه ك اشي extra جنب ال culture بحيث ال broth هاد بنمو عليه مختلف انواع البكتيريا و بساعد بزيادة ال growth تاع البكتيريا بشكل non specific و اكتر اشي لل anaerobic bacteria على عكس ال culture العاديه فهي ع الاغلب بتكون اكثر specific ف من هون بنقدر نعرف ليه ال thio تعتبر اشي بجانب ال culture

طيب هلاء دايماً اي swap sample بتوصل لمختبر المايكرو بروحوا بزرعوها ب culture و برضو بحطوها ب thio broth يعني العينه بتتوزع ع التنين

ف تاعت ال thio بنحطها بال incubator ل 24 ساعه و تعتبر زي اشي احتياطي بحيث انا مرجعي الاساسي ال culture ف ازا ال culture ما كان فيها growth بشيك ع ال thio كمان ازا هي برضو صار عليها growth او لاء طبعاً عينة ال thio لو بعد ال 24 ساعه ما كان في نمو عادي برجع مره تانيه بتركها 24 ساعه كمان لانو ممكن البكتيريا كان بدها اكثر وقت لتتكاثر او كان عددها جداً قليل بالعينه ف لازم اعطيها فرصتها بال thio

هلاء ممكن انو انا ما اعمل ع طول culture و thio مع بعض ف هون مثلاً بكون عامله بس culture بدون ال thio و بعد ما تركت ال culture ل 48 ساعه و لسا ما لقيت growth فهون بروح اعمل subculture الها بال thio و بتركها 24 ساعه بال thio و بشوف ف ازا طلع growth بتكون هاي بكتيريا كانت بدها امور تانيه لتنمو و هيك طلع positive او انو ما بطلع فعلاً negative و بكون في growth

هلاء في حالات ما بنعمللها thio broth زي ال vaginal sample و
CSF sample ال و sputum sample ال

طيب ليه ؟

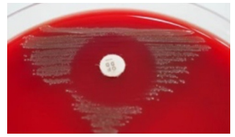
بالعاده ال sputum sample المفروض تتاخد بشكل دايركت من الرئه واللي بتكون sterile و ع طول we should cough it up directly from the lung into the cup بس هاد الاشي فعلياً صعب نقدر نعمله ف بالعاده المريض بعمل cough up من الرئه لل oral cavity و بعدها spit it out فهون المشكله بتكون انو ال oral cavity مليان microorganism فتخيلو اروح ازرعها على ال thio فهون حيصير growth لاشكال الوان من ال organisms ف ع الفاضي ما حميز ازا البكتيريا اللي عامله المشكله موجوده او لاء فهون الافضل انو ع culture تكون specific لحتى ما اعمل growth لل microorganisms التانيه اللي ما بتهمني

طيب كيف اعرف متى بقدر ازرع ال sputum ومتى لاء؟
بفحص عدد ال wbcs ازا طلع اكثرمن 25 و ال Epithelial cells اقل من 10 هون عادي بعملو culture بس غير عن هيك ما بزرع لانو بكون جاي من ال oral cavity

اما بالنسبه لل CSF برضو ما بنعمللها thio لانو عينة ال CSF بتكون highly oxygenated و ال thio حكينا اكتر اشي بتفيد بال anaerobic ف مش كتير حتفيد بالاضافه انو لما اوصل لمرحله اخد عينة CSF من مريض فهون اكيد حيكون عندي manifestation ظاهره و واضحه انو في بكتيريا ف الاغلب بكون عارف شو هي و ما بحتاج ال thio ك اشي زياده ، عشان هك عينة ال CSF بنزرعها على الblood or chocolate or macconkey ل 48 ساعه وبعدها ازا ما في growth تعتبر negative results

S. pneumoniae

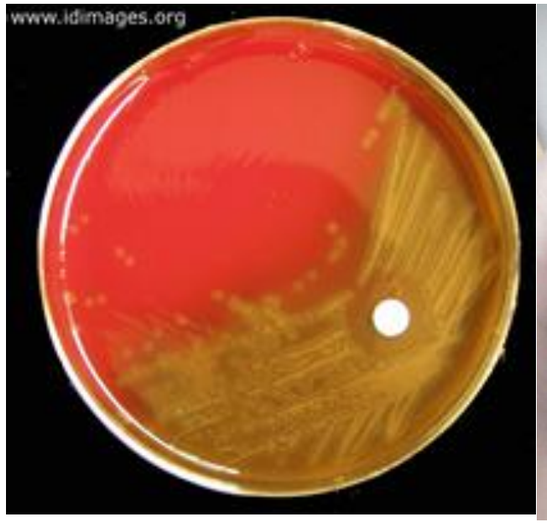
✓Gram-positive
✓Diplococci
✓a-hemolytic
✓optochin positive

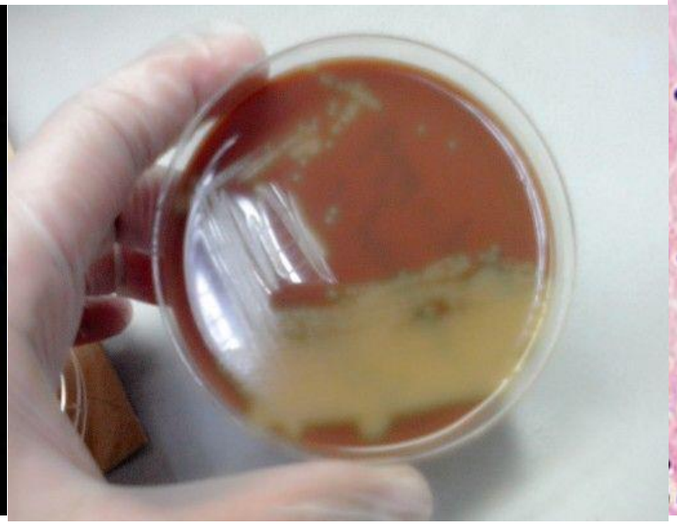


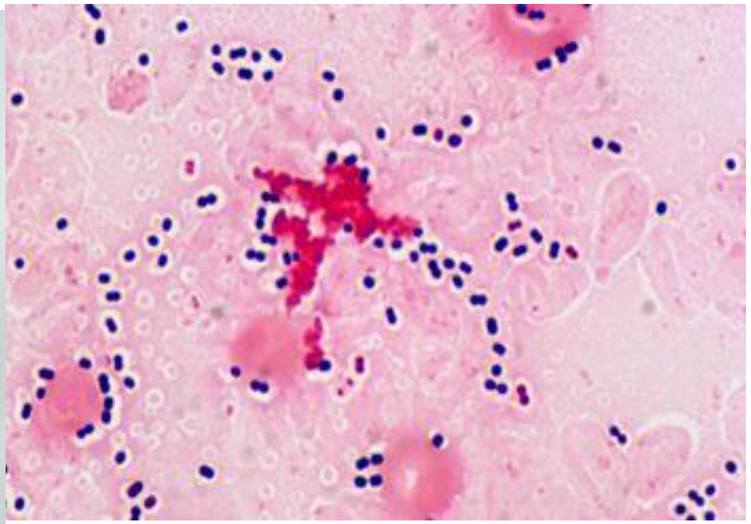
مهم تعرفوا كل بكتيريا مذكوره شو خصائصها فحكتبلكم اهم الخصائص اللي الدكتور حكاهم لكل وحده

predisposing conditions

- pneumococcal pneumonia
- acute or chronic pneumococcal sinusitis or otitis media
- Alcoholism
- diabetes
- Splenectomy
- Hypogammaglobulinemia
- complement deficiency
- head trauma with basilar skull fracture and CSF rhinorrhea.



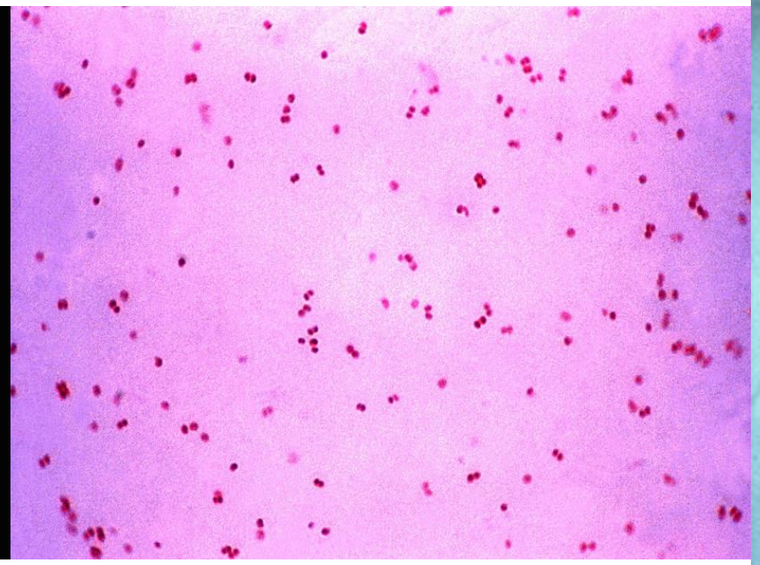




We also see rash in Rocky Mountain spotted fever

- Petechial or purpuric skin lesions are important clue for the *N. meningitis*.
- It is fulminant, progressing to death within hours.
- Initiated by nasopharyngeal colonization
- Individuals with deficiencies of any of the complement components, are highly susceptible.
- Oral sex is dangerous risk for this disease.

✓Gram negative
✓diplococcus with a "kidney" shape facing each other
✓It grows better in chocolate agar



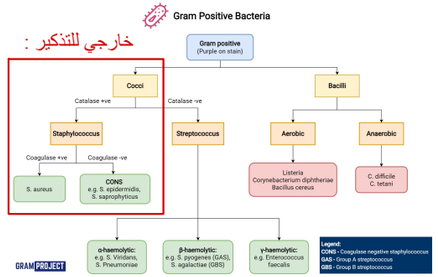
Staphylococcus aureus:

Important cause of meningitis that follows invasive neurosurgical procedures, particularly shunting procedures for hydrocephalus or after intrathecal chemotherapy.

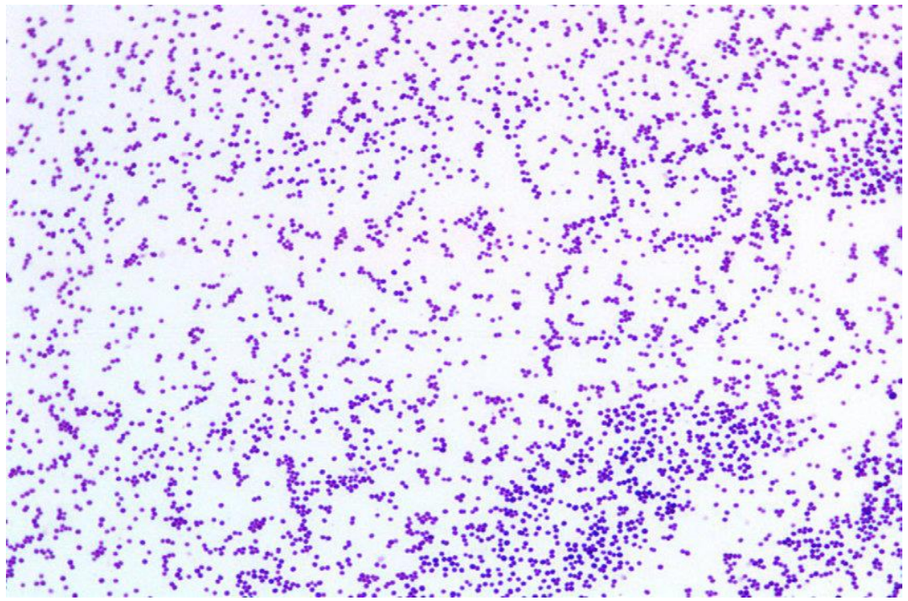
✓Gram positive

Blood Agar	Beta haemolysis
Mannitol salt Agar (MSA)	It is a selective medium for S. *aureus* produces yellow colored colonies due to fermentation of mannitol

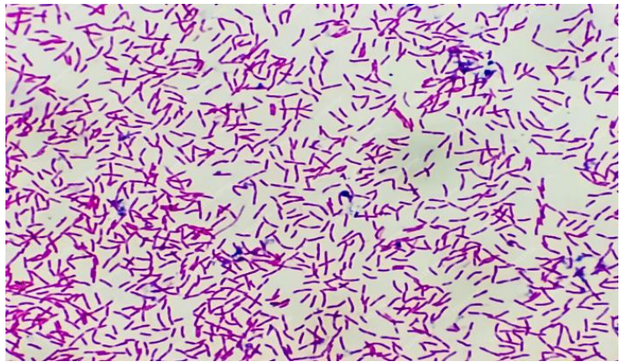
✓Coagulase positive



لازم ال mannitol fermentation مع ال
عشان احكي انو coagulase positive
اما S. A هاي mannitol لحالو ما بزبط



Gram-negative meningitis can complicate neurosurgical procedures, particularly craniotomy.

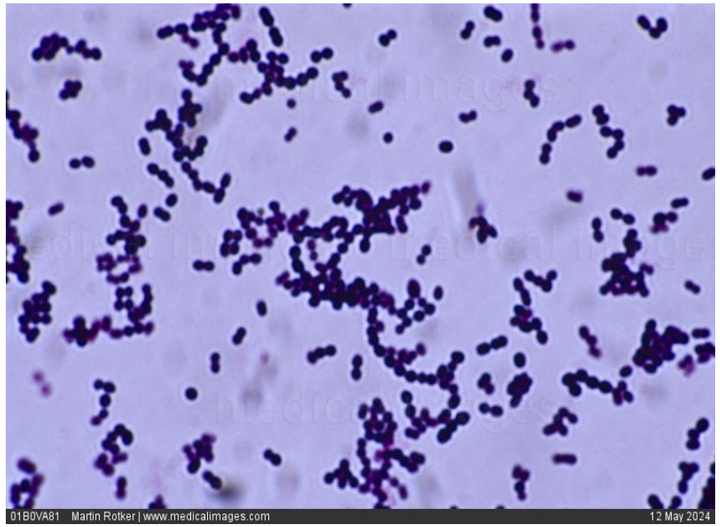


الها نوت بالسلايد البعدو

Group B streptococcus

in neonates

in individuals ≥ 50 years of age..



Streptococcus agalactiae :

✓Gram positive

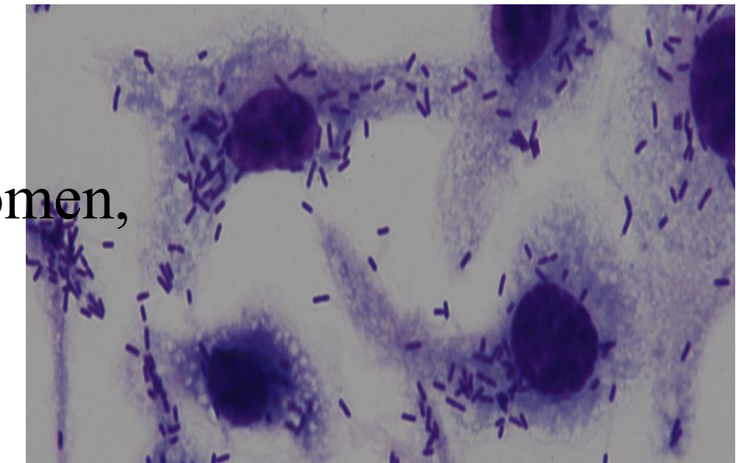
✓Cocci in chains

✓B hemolytic

L. monocytogenes

in infants (1st month of age), pregnant women,

individuals ≥ 60 years of age.

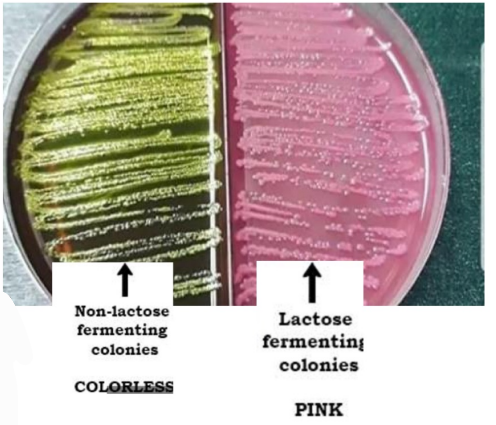


✓Gram positive

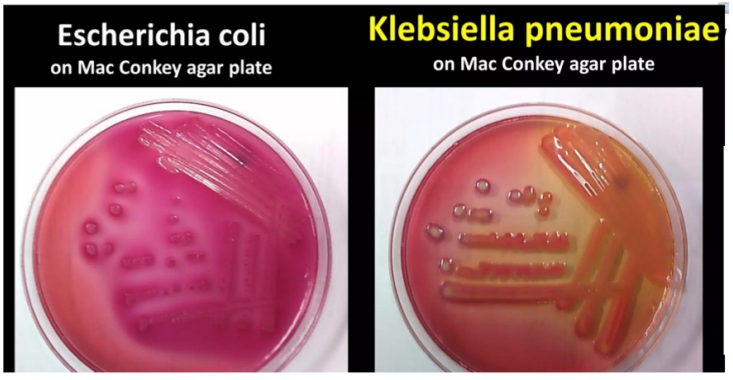
✓Cocci bacilli

Gram-negative

مثل ال E coli بتنمو على ال blood agar و
ال chocolate و ال macconkey agar
اللي تعتبر selective for gram negative



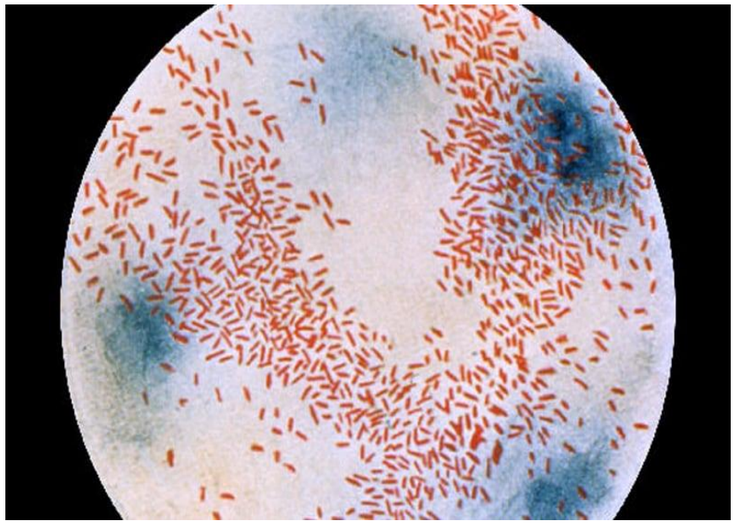
و كمان بنفرق بين ال E coli and klebsilla عن طريق ال Mac برضو بحيث :

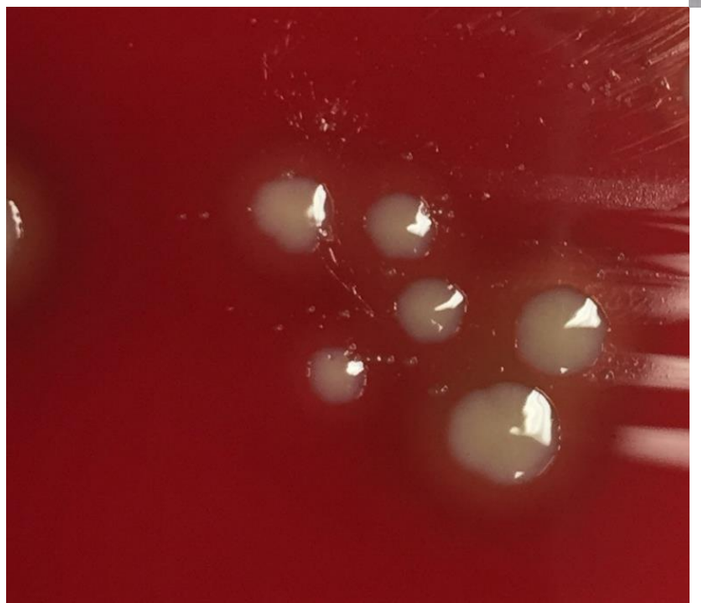


More pink-ish
Dry

More yellow-ish
Wet

H. influenzae





Latex Agglutination Tests for Meningitis

Meningitis

It is a guide for the empirical ttt then it can be confirmed by either a culture or pcr

1 Rapid Detection

Latex agglutination tests can quickly identify the presence of bacterial antigens antigens in CSF, enabling prompt diagnosis of meningitis.

2 Broad Spectrum

These tests can detect a variety of meningitis-causing pathogens, including including Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae.

+ E coli and group B streptococcus

3 Cost-Effective

Latex agglutination tests are relatively inexpensive, making them accessible for laboratories with limited resources.

Streptococcus group B, *Haemophilus influenzae* type b, *Streptococcus pneumoniae* (pneumococcus), *Neisseria meningitidis* (meningococcus) groups A, B, C, Y or W135 and *Escherichia coli* K1

Limitations and Challenges of New CSF Tests

1

Sample Collection

Proper collection and handling of CSF samples is crucial, as contamination or delays can compromise test results.

2

Interpretation

Interpreting CSF test results requires specialized expertise, as various factors can influence the interpretation of findings.

3

Cost Considerations

Advanced CSF testing methods, such as multiplex panels and ELISA kits, may be more expensive, limiting accessibility in certain healthcare settings.

الدكتور طلب واجب من احد الطلاب عن ال CSF analysis :

رح نحكي بالاول عن ال normal ويقسم الى اربع اقسام

Physical

لازم يكون clear وما turbidity في

Chemical

البروتين لازم يكون من 15-45

يشكل ال CSF 1% من البلازما لانو اصلاً ال CSF هو infiltrate من ال blood

ال glucose من 40-80

ال bilirubin لازم absent

ال chloride من 100 -120

Presence of microorganism

There must not be any microorganism

Microscopic findings (Cell count)

- In Adults: 0 - 5 cells/cumm
- In Neonates: 0 - 30 cells/cumm

WBCs

No RBC's should be present in normal CSF

هلاء ال abnormal :

البروتين اذا اعلى من 45
ال glucose اقل من 40

Viral

الجلوكوز normal
البروتين slightly elevated

Bacterial

الجلوكوز بكون low
البروتين بكون كتير elevated

Cerebrospinal Fluid (CSF) Analysis

ما انقرأ بس
هي مقدمه يعني

1 **Cell Count**

Evaluating the number and type of cells in the CSF can provide clues about the underlying infection.

2 **Protein Level**

Elevated protein levels in the CSF may indicate inflammation or an infectious process.

3 **Glucose Level**

A decrease in CSF glucose compared to blood glucose can suggest certain types of CNS infections.

4 **Gram Stain and Culture**

Direct visualization of microorganisms and growth in culture media can identify the specific pathogen.

Interpreting CSF Test Results

	Leukocyte/ mm3	% PMN Polymorphonuclear neutrophils	Glucose % of blood	Protein (mg/dl)
Normal	0-5	0	≥ 60	≤ 30
Viral	2-2000 (80) Slightly elevated	≤ 50	≥ 60 Normal	30-80 Slightly elevated
Bacterial	5-5000 (800) Highly elevated	≥ 60 كتير Increased	≤ 45 Decreased	>60 Highly elevated
TB and fungal	5-2000 (100)	≤ 50	≤ 45	>60
N neonate	0-32 (8)	≤ 60	≥ 60	20-170 (90)

Meningitis Panels and their Capabilities

Multiplex Approach

Meningitis panels utilize multiplex assays to simultaneously detect and identify multiple pathogens in a single CSF sample.

Improved Accuracy

These panels combine various testing methods, such as real-time PCR and immunoassays, to provide more accurate and comprehensive results.

Expanded Coverage

Meningitis panels can detect a a broader range of infectious agents, including viruses and fungi, in addition to bacteria.

HSV
Enterovirus
cryptococcus

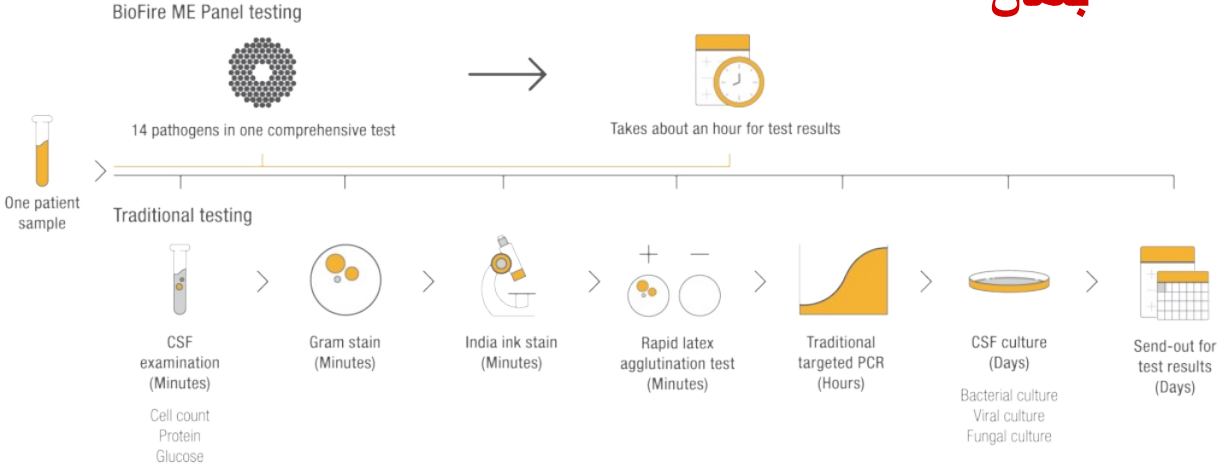
هاد السلايد اضافي مني
لتوضيح اهمية ال
meningitis panel
حالياً و كيف انو وفر
وقت جداً كبير كنا
بنحتاجه للتشخيص
علماً انو حالياً مش
موجود الا بمختبر واحد
بعمان

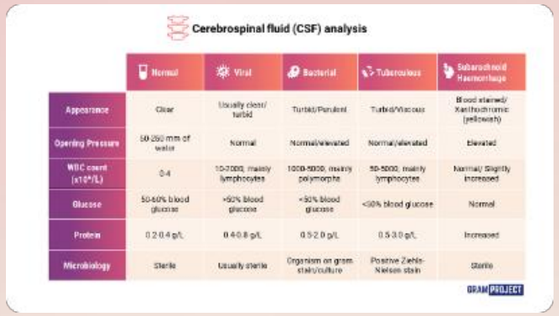
Every Minute Counts When it Comes to Meningitis.

24-48 hrs

Time to diagnosis is critical. Bacterial meningitis can be fatal in healthy people in 24 to 48 hours.[2] The right treatment depends on quick identification of the pathogen as bacterial, viral, or yeast.

Patients with suspected meningitis are treated empirically pending diagnostic results.[3,4] This can mean lengthy hospitalizations and unnecessary antimicrobial use, all of which add to the overall cost of care.





Future Directions in CSF Testing

1 Biomarker Discovery

Ongoing research aims to identify novel biomarkers in CSF that can aid in the early detection and monitoring of neurodegenerative diseases.

Not infection

2 Personalized Medicine

Advancements in CSF testing will enable more personalized treatment approaches, tailored to an individual's unique disease profile.

3 Point-of-Care Testing

على السريع اقدر اعملو ال test واعرف ال causative organism

The development of portable, rapid CSF testing devices could improve access to timely diagnosis and management of neurological conditions.

بتمنى اكون فدتكم وما تنسونا
من صالح دعائكم .... 🤍