MICROBIOLOGY PRACTICAL TEAMWORK 437

Examination of CSF

Objectives:

1. Identify the functions of CSF.
2. Recognize the normal and abnormal constituents of CSF.
3. Understand the role of CSF in diagnosis of different diseases of CNS.
4. Interpret the microbiological investigation results of CSF.
5. Hands-on the procedure for estimation of total protein in CSF.

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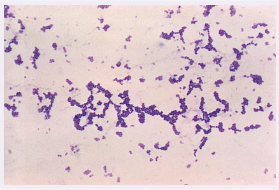
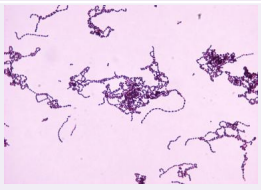
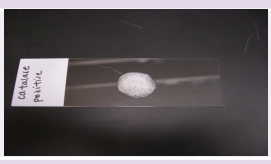
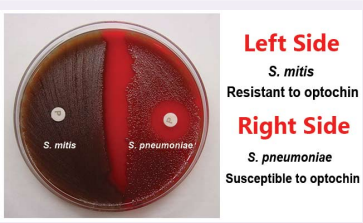
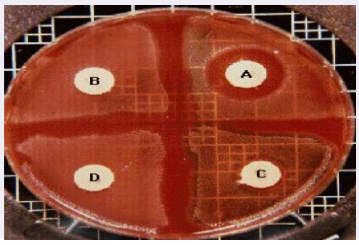
Special thanks to team 436 💜

How can I know if the bacteria is Gram positive or Gram negative? GRAM STAIN IS THE ONLY WAY	
+ve	-ve
Purple / Blue	Red / Pink
Coccus - Bacillus	Coccus - Bacillus

Gram +ve Cocci	
Staphylococcus (in clusters)	Streptococcus (in chains or pairs)
	
We differentiate between the two by using the “Catalase test”	
	
Bubbles → Catalase Positive	No reaction → Catalase Negative

Streptococcus		
Alpha Hemolytic (α)		
Species	Further test	Result
Streptococcus <u>pneumoniae</u> Streptococcus <u>viridans</u>	We differentiate between them using “Optochin Test”	Pneumoniae = Sensitive Viridans = Resistant
		
Beta Hemolytic (β)		
Species	Further test	Result
Group A (Pyogens) Group B (Agalactiae) Group C	We differentiate between them using “Bacitracin Test”	Group A = Sensitive Group B and C = Resistant
		

Key information
Acute Pyogenic Meningitis
Acute onset (sudden)+ pus cells+ fever, headache, stiff neck, other meningitis symptoms + Turbid CSF + High protein + Low glucose + Polymorphs.
Viral (Aseptic/Lymphocytic) Meningitis
Acute onset (sudden)+ Symptoms of meningitis +Clear CSF + Unchanged/high protein + Unchanged/low glucose + Lymphocytes.
Chronic Meningitis
Chronic onset + chronic headache,facial weakness, double vision, other meningitis symptoms + Turbid CSF + High protein + Low glucose + Lymphocytes.

causes of Acute Pyogenic Meningitis
it differs based on the age group and all are capsulated

Neisseria meningitidis	Streptococcus pneumoniae	Haemophilus influenzae	Escherichia coli
• Adults&Infants / Children. • Gram • -ve diplococci.(kidney shaped) • Glucose & maltose fermenter . • Its capsule: 1-Produce endotoxin (LPS). Causes skin rash and septic shock. 2-Resists phagocytosis.	• Adults&Infants / Children. • Gram +ve diplococci. • Optochin sensitive. • The most invasive pathogen, high mortality rate>30% • Its capsule: produce pnemolysin → immunogenic and induce immuneresponse.	• Infants / Children. • Gram -ve coccobacilli / pleomorphic. • Need blood for optimal growth, Hematin (factor X) & NAD (factor V). • Type B is invasive and capsulated. • The capsule is used as a conjugate vaccine	• Newborns. • Gram -ve bacilli / Lactose fermenter. • K1 sialic acid capsule → invasion of brain microvascular endothelial cells.

Management
Treatment (10-14 days): Neonates Treatment : cefotaxime + gentamicin + ampicillin Adults : cefotaxime + Vancomycin If age is above 50Add ampicillin

Causes of Chronic Meningitis

Tuberculosis "Mycobacterium tuberculosis"	Brucellosism "Br.melitensis"
• Microscopically: Ziehl-Neelsen stain → acid fast bacilli. • Tests: Mantoux test, Tuberculin skin test (TST) • Treatment: Start with 4 drugs "for 2 months" Rifampicin + Isonized (INH) + Ethambutol + Pyrazinamide. Then: Rifampicin + Isonized (INH) "for 4-6 months"	• Affect people who are in contact with domestic animals "Sheep" or those who consume raw milk and milk products. • Can rarely be transmitted sexually & by inhalation.(lab. Acquired) Treatment: Rifampicin + Cotrimoxazole .

CASE 1

Acute Pyogenic Meningitis

1- Neisseria meningitidis

Scenario: A 15-year-old healthy male visited the ER presenting with fever, headache, vomiting and drowsiness. Physical examination showed decreased level of consciousness, neck stiffness and high temperature of 38℃. Cerebrospinal fluid (CSF) examination revealed opening pressure of 201 cm H2O.

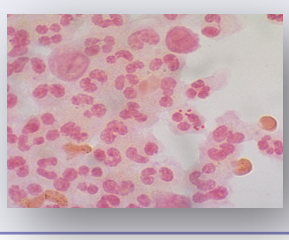
Microscopy of the cerebrospinal fluid showed gram –ve diplococci. The patient showed complete recovery after administration of ceftriaxone for 10 days. (purpura is classic sign for Neisseria)

The doctor in the emergency department takes a detailed history and conducts a clinical examination. Because of clinical findings, he decides to do a lumber puncture. The results of the lumber puncture are shown below:

Clinical Presentation

CSF	Patient's results	Normal range
Appearance	Turbid (cloudy) 	Clear 
WBCs (cells/mm³)	8,320 Mainly polymorphonuclear leucocytes (84%)	Few (<5)
Protein (g/L)	5.0	0.1-0.4
Glucose (mmol/L)	1.3	3.0-4.5
Chloride(mmol/L)	110	115-130

Acute Pyogenic Meningitis
1- Neisseria meningitidis



Microscopic Appearance

Culture on Thayer-Martin agar
Specific for Neisseria

CASE 1

Acute Pyogenic Meningitis

1- Neisseria meningitidis

Q1: What is your diagnosis?
Acute Pyogenic Meningitis.

Q2: What is the most likely infection responsible?
Bacterial infection. More serious than viral infections.

Q3: What is your justification for your answer to question two?
↑ WBCs + ↑ Protein + ↓ Glucose + there is Polymorphs.

Q4: Describe the microorganism's appearance under microscope?
Gram negative intracellular bean-shaped diplococci + many pus cells.
Oxidase and catalase tests are (positive)

Q5: Name the media used for growing such organism?
Thayer-Martin agar or Chocolate agar.

Q6: What further investigation would you like to do at this stage?
CSF culture and smear, Blood culture, CBC (complete blood count) , Protein and glucose levels, PCR (polymerase chain reaction) (DNA detection), Serology (Antigen detection). antigen = capsule)

-NOTE from doctor Fawzia about when we use PCR in bacteria:

لما نعطيه انتيبيوتك ونبي نعرف ايش الاورقانزم بعد ما قضينا عليها

Q7: Mention two recommended empirical antibiotics that can be used in such a case?
Ceftriaxone with Vancomycin.

Extra Questions :
-if the patient received the required vaccination before his travel to Hajj, how would you explain his infection despite vaccination?
A: he might take the vaccination for 2 serotypes (A&C) and he may be infected by W135.
B: he should take the vaccination before traveling by tow weeks at least.
C: The vaccine might be deactivated due to inadequate storage or expiry.

-If they die what is mostly the cause ? Septic shock

-The characteristic : skin rash (purpura) of meningococcal septicemia, caused by *Neisseria meningitidis*

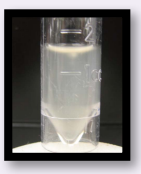
CASE 1

Acute Pyogenic Meningitis

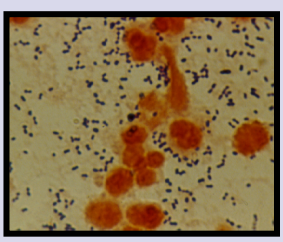
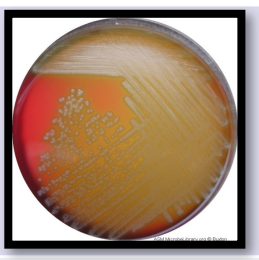
2-Streptococcus Pneumoniae

Scenario: A **59** year-old male farmer with sudden onset of **fever**, **headache**, **neck stiffness** and **confusion**

The results of the lumber puncture are shown below:

CSF	Patient's results	Normal range
Appearance	Turbid (cloudy) 	Clear 
WBCs (cells/mm³)	**3520 Neutrophils(100%)**	**Few (<5)**
Protein (g/L)	**3.68**	**0.1-0.4**
Glucose (mmol/L)	**0.5**	**3.0-4.5**

2-Streptococcus Pneumoniae

		
Microscopic Appearance	blood agar	Optochin Test

Q1: What is your diagnosis?
Acute Pyogenic Meningitis

Q2: What is the most likely infection responsible?
bacterial infection.

Q3: Describe the microorganism's appearance under microscope?
gram-positive diplococci with lanceolate shape and polymorphneoclear leucocyte (many pus cells)

Q4:Name the media used for growing such organism?
Blood agar.

Q5: Describe the microorganism's morphology on blood agar ?
Alpha hemolytic streptococci.

Q6: Describe the microorganism's reactivity towards the Optochin test?
Optochin sensitive.

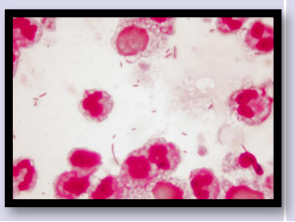
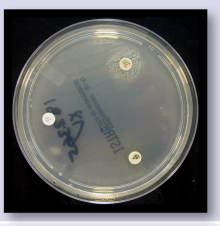
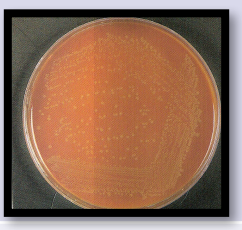
Q7: What further investigation would you like to do at this stage?
CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR

Q8: Mention two of the recommended antibiotics that can be used as empiric treatment in such a case?
Vancomycin + ceftriaxone + ampiciiln

CASE 1

Acute Pyogenic Meningitis
3- Haemophilus Influenzae

3- Haemophilus Influenzae

			
Microscopic Appearance	Nutrient agar	chocolate agar	Blood agar

Q1: What is your diagnosis?
Acute Pyogenic Meningitis.

Q2: What is the most likely infection responsible?
Bacterial infection.

Q3: What is the most probable Pathogen isolated?
Haemophilus Influenzae.

Q4: Describe the microorganism's appearance under microscope?
Gram-Negative pleomorphic coccobacilli with many polymorphneuclear leukocyte.

Q5: Name the media used for growing such organism?
Chocolate agar , Blood agar (please read Q8 For a better understanding) and Nutrient agar.

Q6: Describe the microorganism's morphology on Chocolate Agar?
Grey mucoid colonies of ***Haemophilus Influenzae*** due to the presence of X and V factors.

Q7:Describe the microorganism's morphology on Nutrient Agar?
H. influenzae :Growth around XV factors (requires both factors XV)
no growth around X or V alone the optimum growth temperature is (35°C - 37°C in 5% CO2).
In this culture ***haemophilus*** has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor.

Q8:Describe the microorganism's morphology on Blood Agar?
Growth on blood agar showing **satellitisim** adjacent to a streak of ***S.aureus.***
S.aureus producing V factor (hemophilus require both x and v) increasing growth of adjacent ***H.influenzae.***
satellitism is the pattern of arrangement of ***heamophillus influenzae*** on blood agar strearked with ***staphylococcus aureus*** in the centre.

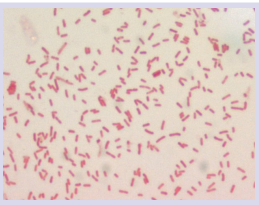
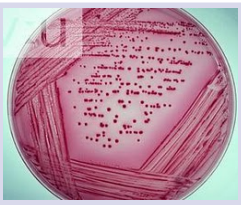
Q9: What further investigation would you like to do at this stage?
CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

Q10: Mention two recommended empirical antibiotics that can be used in such a case?
Ceftriaxone with Vancomycin.

CASE 1

Acute Pyogenic Meningitis

4. Escherichia Coli

4. Escherichia Coli	
	
Microscopic Appearance	MacConkey agar

Q1: What is your diagnosis?
Acute Pyogenic Meningitis.

Q2: What is the most likely infection responsible?
Bacterial infection.

Q3: What is the most probable Pathogen isolated?
Escherichia Coli.

Q4: Describe the microorganism's appearance under microscope?
Gram negative bacilli (rods).

Q5: Name the media used for growing such organism?
MacConkey's agar.

Q6: Describe the microorganism's morphology on MacConkey Agar?
Lactose fermenter (pink colonies).

Q7: What further investigation would you like to do at this stage?
CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

Q8: Mention two recommended empirical antibiotics that can be used in such a case?
Children: Ceftriaxone with Vancomycin.
Neonates: Ampicillin +gentamicin +cefotaxime

CASE 2

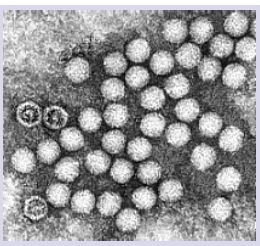
Viral (Aseptic/Lymphocytic) Meningitis

Scenario: A 10-year-old boy is brought to the emergency department at KKUH accompanied by his mother. He has fever, headache, and vomiting for the last 2 days. Clinical examination confirmed that he has meningeal irritation.

The results of the lumber puncture are shown below:

CSF	Patient's results	Normal range
Appearance	**Clear**	**Clear**
WBCs (cells/mm^3)	**1200** **Mainly lymphocytes (80%)**	**Few (<5)**
Protein (g/L)	**0.5**	**0.1-0.4**
Glucose (mmol/L)	**2.7**	**3.0-4.5**
Chloride (mmol/L)	**115**	**115-130**

Electron Micrograph of Enterovirus



CSF Molecular testing is **positive**

Q1: What is your diagnosis?
Aseptic (Lymphocytic) Meningitis.

Q2: What is the most likely infection responsible?
Viral Infection.

Q3: What is your justification for your answer to question two?
↑ WBCs + moderate ↑ Protein + normal Glucose + high Lymphocytes.

Q4: What further investigation would you like to do at this stage?
CSF culture and smear, Blood culture, CBC, Protein and glucose levels, **PCR(**RT.PCR**).**

CASE 3

Chronic Meningitis

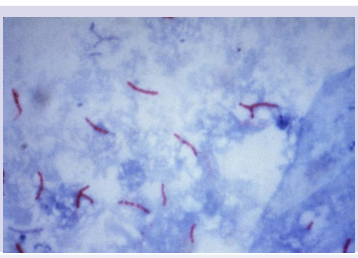
Mycobacterium Tuberculosis

Scenario: A 65-year-old is referred from a general practitioner because of headache, fever, excessive sweating at night, and weight loss over the last 4-5 months. He has lost his appetite for food. On examination, there is neck rigidity. Laboratory tests including blood count, serum and electrolytes, blood urea, creatinine and blood culture are all normal.

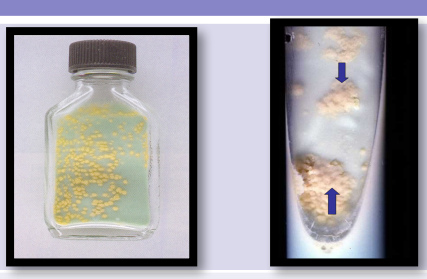
The results of the lumber puncture are shown below:

CSF	Patient's results	Normal range
Appearance	Turbid 	**Clear** 
WBCs (cells/mm^3)	**300** **Mainly lymphocytes (80%)**	**Few (<5)**
Protein (g/L)	**0.8**	**0.1-0.4**
Glucose (mmol/L)	**2.0**	**3.0-4.5**
Chloride (mmol/L)	**110**	**115-130**

Chronic Meningitis
Mycobacterium Tuberculosis



Microscopic Appearance



Lowenstein-Jensen Medium

CASE 3

Chronic Meningitis
Mycobacterium Tuberculosis

Q1: What is your diagnosis?
Chronic Bacterial Meningitis.

Q2: What is the most likely infection responsible?
Mycobacterial infection.

Q3: What is your justification for your answer to question two?
↑ WBCs + ↑ Protein + ↓ Glucose + present of Lymphocytes + ↓ chloride
Extra from doctor fawzia : symptoms of TB like weight loss and sweating

Q4: What is the most probable Pathogen isolated?
Mycobacterium Tuberculosis.

Q5: What is the stain used to identify such organism?
Ziehl-Neelsen (ZN) stain for Acid Fast Bacilli (AFB).

Q6: Describe the microorganism's appearance under microscope?
Acid Fast Bacilli (AFB) with a blue background.

Q7: Name the media used for growing such organism?
Lowenstein-Jensen (LJ) media.

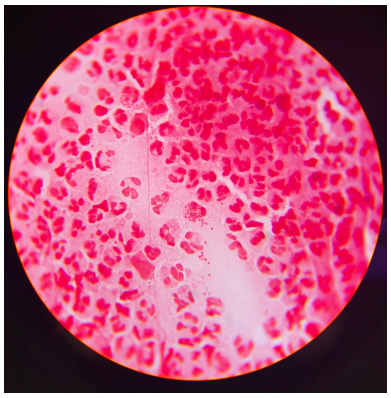
Q8: describe the culture on Lowenstein-Jensen?
Colonies or growth is rough, tough and buff.

Q9: what further investigation would like to do at this stage?
CSF culture (on LJ media for 2 to 3 weeks) , PCR, CBC, Tuberculin skin test, chest X-ray.

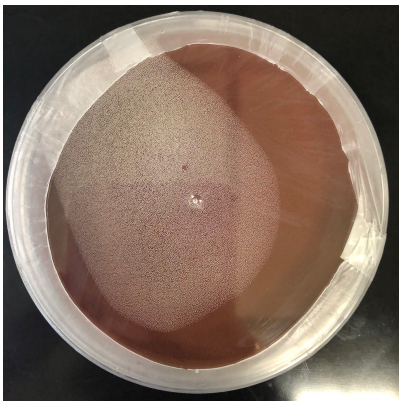
Q10: Name the drug used to treat such infections?
For the first 2 months: Rifampicin + Isoniazid (INH) + Ethambutol + Pyrazinamide. Then, for 4-6 months: Rifampicin + Isoniazid (INH).

Acute Pyogenic Meningitis

1- Neisseria meningitidis



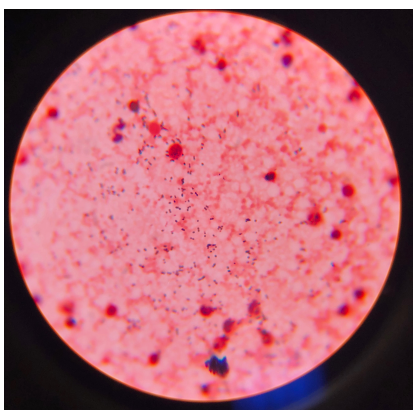
Microscopic Appearance



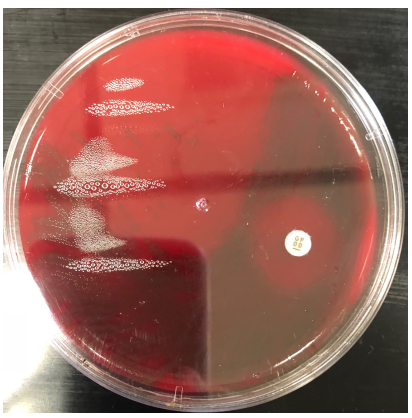
Thayer-Martin agar

Acute Pyogenic Meningitis

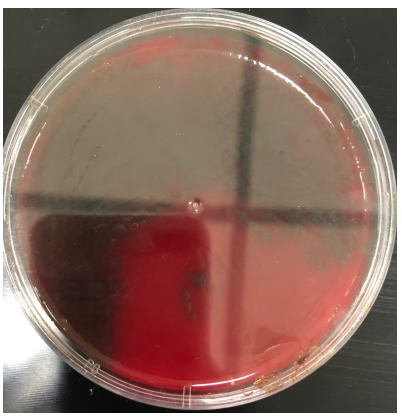
2-Streptococcus Pneumoniae



Microscopic Appearance



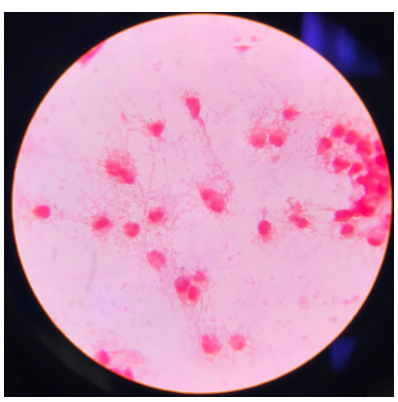
Optochin Test



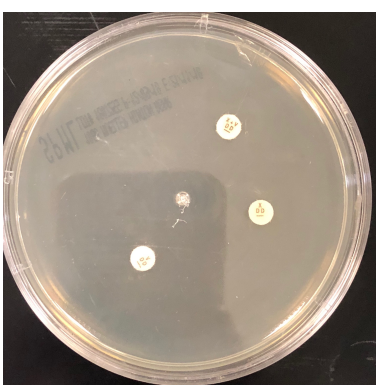
Alpha hemolytic blood agar

Acute Pyogenic Meningitis

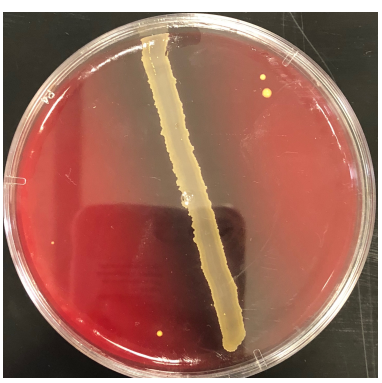
3- Haemophilus Influenzae



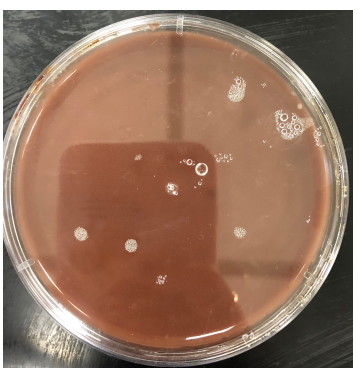
Microscopic Appearance



Nutrient agar



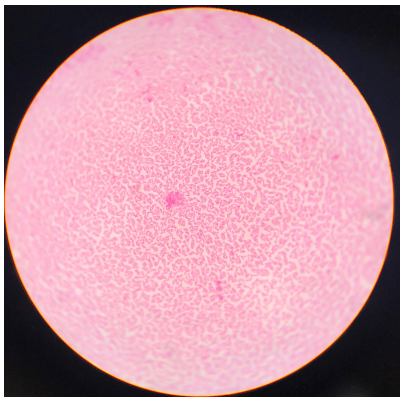
Blood agar showing **satellitisim**



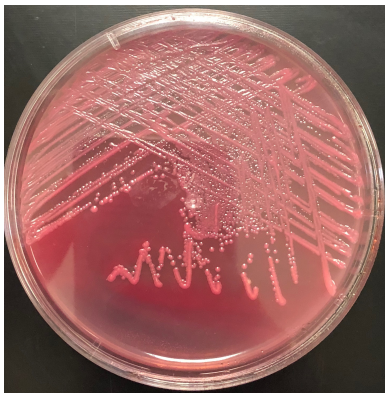
chocolate agar

Acute Pyogenic Meningitis

4. Escherichia Coli



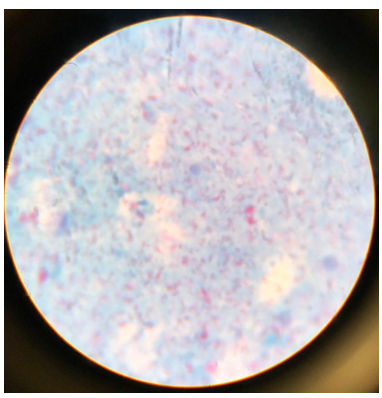
Microscopic Appearance



MacConkey agar

Chronic Meningitis

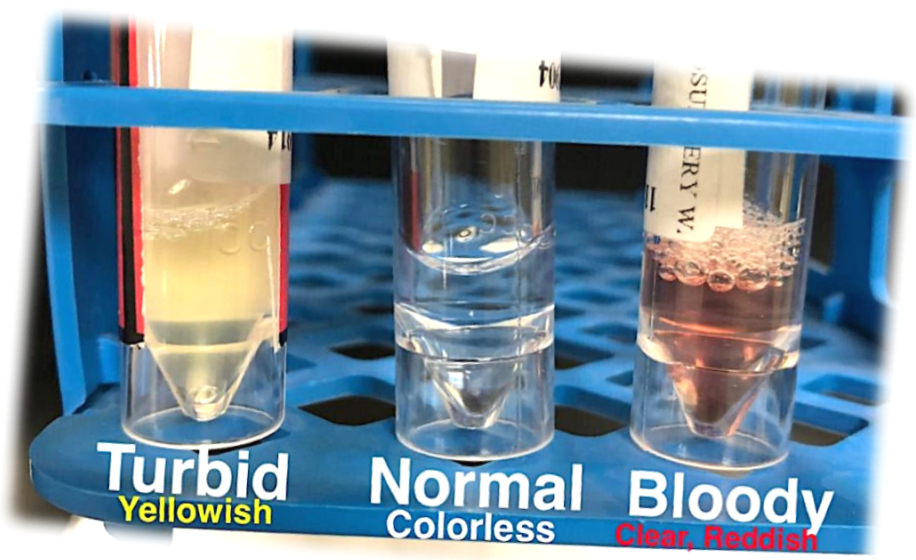
Mycobacterium Tuberculosis



Microscopic Appearance

Lowenstein-Jensen Medium

CSF Appearance



Dr.khalifa notes

Age, CSF analysis gram stain, culture findings Are important
Age:
The most common causative organism in neonates:
E. Coli Group B Listeria monocytogenes
Most common cause of Meningitis in children:
-H. Influenza -stept.pneumonia -N.Meningitidis
-Adults: stept.pneumonia N. Meningitidis
If age is more than 50 you should add listeria

Empiric therapy:
Neonates Treatment : cefotaxime + gentamicin + ampicillin
Adults : cefotaxime + Vancomycin
If age is above 50Add ampicillin (know the empiric Treatment, you don't have to know the specific bacterial treatment)

Symptoms :stiff neck Fever, headache....(Skin rash usually indicates severe infection Caused either by N. Meningitidis\stept.pneumonia
CSF in acute bacterial(pyogenic) Meningitis: -WBC count over thousand not just hundreds, Low glucose ,Low chloride ,High protein
(he noted :viral CSF Normal chloride, While TB chloride is low)

Most common viral cause is enterovirus, How to diagnose enterovirus? RT.PCR
Chronic Meningitis causes? TB. Brucella How to know if its TB from CSF findings? Fibrin web High protein Low glucose Low chloride Increased WBC (mainly lymphocytes)How to confirm? Acid fact stain(Bacteria in red with blue background)
Culture in L.J. Media(Yellowish colonies)

اسئلة سأل عنها في المحاضرة
How to confirm N.Meningitidis? Sugar utilization (glucose, maltose)
How to confirm H.infleunza? Chocolate agar, grows around X-V factors
Describe E.coli growth in MacConkey ager? Lactose fermenting colonies

And he Sayed it the end: The case will be very short (age, symptom)
ايش ممكن يجي مع الكيس؟
Gram stain, CSF findings +CSF normal ranges will be given

-We advise you to read meningitis lecture before the exam