



Practical Microbiology

Neuropsychiatry Block

- **Doctor's notes**
- Extra explanation
- **Answers**

"لا حول ولا قوة إلا بالله العلي العظيم" وتقال
هذه الجملة إذا داهم الإنسان أمر عظيم لا
يستطيعه ، أو يصعب عليه القيام به .

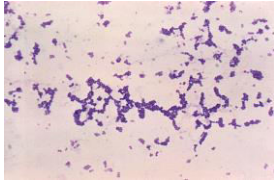
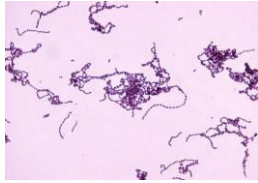
EXTRA

this slide is just review the basics of microbiology
If you are already familiar with these terms just skip it.

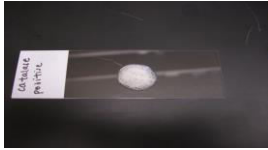
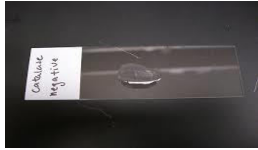
How can I know if the bacteria is **Gram positive** or **Gram negative**?
GRAM STAIN IS THE ONLY WAY

+ve		-ve	
Purple / Blue		Red / Pink	
Coccus	Bacillus	Coccus	Bacillus

Gram **+ve** Cocci

Staphylococcus (in clusters)	Streptococcus (in chains or pairs)
	

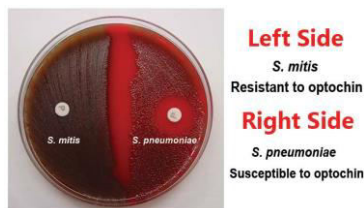
We differentiate between the two by using the **“Catalase test”**

	
Bubbles → Catalase Positive	No reaction → Catalase Negative

Streptococcus

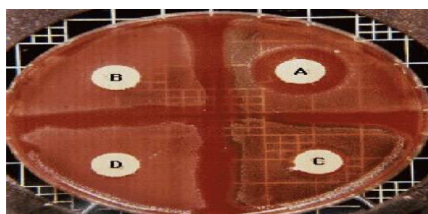
Alpha Hemolytic (α)

Species	Further test	Result
<i>Streptococcus pneumoniae</i> <i>Streptococcus viridans</i>	We differentiate between them using “Optochin Test”	<i>Pneumoniae</i> = Sensitive <i>Viridans</i> = Resistant



Beta Hemolytic (β)

Species	Further test	Result
Group A (Pyogen) Group B (Agalactiae) Group C	We differentiate between them using “Bacitracin Test”	Group A = Sensitive Group B and C = Resistant



EXTRA

Key words to help you answer cases' questions.

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			
<i>Neisseria meningitidis</i>	<i>Streptococcus pneumoniae</i>	<i>Haemophilus influenzae</i>	<i>Escherichia coli</i>
<ul style="list-style-type: none"> Adults&Infants / Children. Gram -ve diplococci. (kidney shaped) Glucose & maltose fermenter . Its capsule: <ol style="list-style-type: none"> 1-Produce endotoxin (LPS). Causes skin rash and septic shock. 2-Resists phagocytosis. 	<ul style="list-style-type: none"> Adults&Infants / Children. Gram +ve diplococci. Optochin sensitive. The most invasive pathogen, high mortality rate>30% Its capsule: produce pneumolysin → immunogenic and induce immuneresponse. 	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> Newborns. Gram -ve bacilli / Lactose fermenter. K1 sialic acid capsule → invasion of brain microvascular endothelial cells.

Management
Treatment (10-14 days): <ul style="list-style-type: none"> Children & Adults: Ceftriaxone + Vancomycin Neonates: Ampicillin + Cefotaxime

Causes of Chronic Meningitis	
<i>Tuberculosis "Mycobacterium tuberculosis"</i>	<i>Brucellosism "Br.melitensis"</i>
<ul style="list-style-type: none"> <u>Microscopically</u>: Ziehl–Neelsen stain → acid fast bacilli. <u>Tests</u>: Mantoux test, Tuberculin skin test (TST) <u>Treatment</u>: Start with 4 drugs "for 2 months" Rifampicin + Isonized (INH) + Ethambutol + Pyrazinamide. Then: Rifam Rifampicin + Isonized (INH) "for 4-6 months" 	<ul style="list-style-type: none"> 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) <u>Treatment</u>: Rifampicin + Cotrimoxazole .

CASE 1

Acute Pyogenic Meningitis

Clinical Presentation

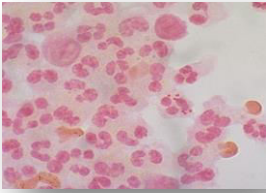


Scenario: A 15-year-old healthy male visited the ER presenting with fever, headache, vomiting and drowsiness for the past week. Physical examination showed decreased level of consciousness, neck stiffness and high temperature of 38°C. Cerebrospinal fluid (CSF) examination revealed opening pressure of 20 cmH₂O. 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 results of the lumbar puncture are shown below:

CSF	Patient's results	Normal range
Appearance	Turbid (cloudy)	Clear
WBCs (cells/mm ³)	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

Protect against meningitis caused by *Neisseria meningitidis*, by Meningococcal conjugate vaccine for people going to Hajj.

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)

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

Ceftriaxone with Vancomycin. Because they're bactericidal.

Extra Q: 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.

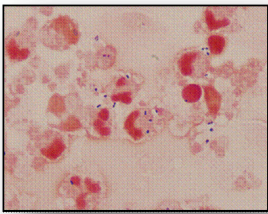
CASE

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

The results of the lumbar puncture are shown below:

CSF	Patient's results	Normal range
Appearance	Turbid	Clear
WBCs (cells/mm ³)	3520 Neutrophils(100%)	Few (<5)
Protein (g/L)	3.68	0.1-0.4
Glucose (mmol/L)	0.5	3.0-4.5

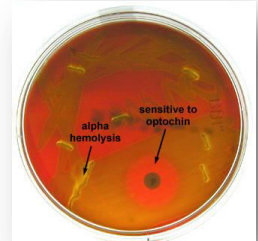
2-Streptococcus Pneumoniae



Microscopic Appearance



blood agar



Optochin Test

Q1: What is your diagnosis?

Acute Pyogenic Meningitis

Q2: What is the most likely infection responsible?

Pneumococcal bacterial infection.

Q3: Describe the microorganism's appearance under microscope?

gram-positive diplococci with lanceolate shape and polymorphneoclear leucocyte

Q4: Name the media used for growing such organism?

Blood agar.

Q5: Describe the microorganism's morphology?

Gray white, Alpha hemolytic streptococci.

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

Optochin sensitive.

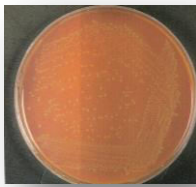

Q7: What further investigation would you like to do at this stage?

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?


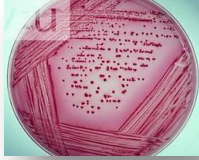
Vancomycin + ceftriaxone

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 leucocyte.
- Q5:** Name the media used for growing such organism?
Chocolate agar , Blood agar and Nutrient agar.
- Q6:** Describe the microorganism's morphology on Chocolate Agar?
Grey mucoïd 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 satellitism adjacent to a streak of S.aureus.
S.ureus producing surplus factor increasing growth of adjacent H.influenzae.
 satellitism is the pattern of arrangement of **heamophillus influenzae** on blood agar streaked 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.

4. Escherichia Coli

	
Microscopic Appearance	MacConkey agar

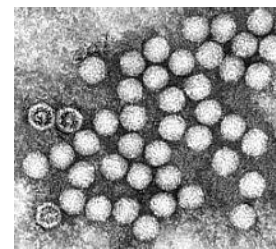
- Q1:** What is your diagnosis?
Acute Pyogenic Meningitis.
- Q2:** What is the most likely infection responsible?
Bacterial infection.
- Q3:** What is the most probable Pathogen isolated?
Escherichia Coli.
- Q4:** Describe the microorganism's appearance under microscope?
Gram negative bacilli (rods).
- 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?
Child: Ceftriaxone with Vancomycin.
Neonate: Ampicillin with Gentamicin.

CASE 2

Viral (Aseptic/Lymphocytic) Meningitis

Electron Micrograph of Enterovirus

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.



CSF Molecular testing is positive

The results of the lumbar 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)	100	115-130

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 + present of 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.

CASE 3

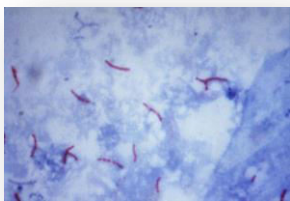
Chronic Meningitis

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 lumbar puncture are shown below:

CSF	Patient's results	Normal range
Appearance	Turbid	Clear
WBCs (cells/mm³)	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)	115	115-130

Chronic Meningitis *Mycobacterium Tuberculosis*



Microscopic Appearance



Lowenstein-Jensen Medium

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.

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, blood culture, 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).