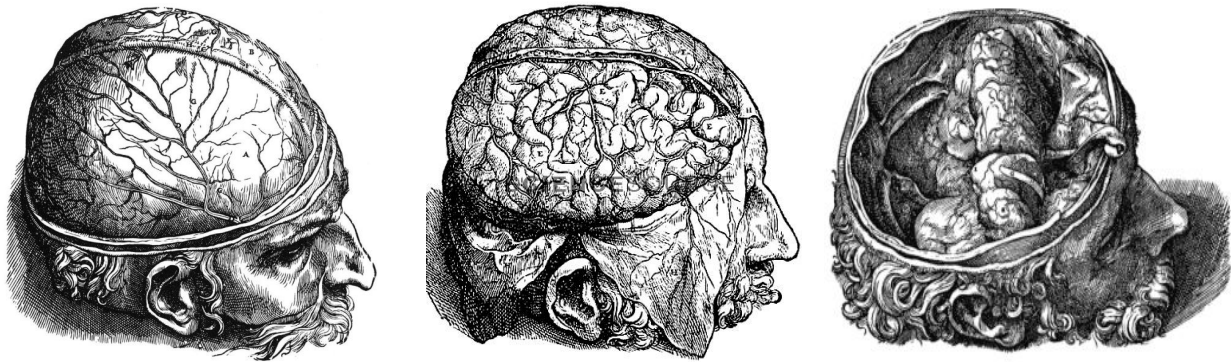


# Practical Microbiology

## CSF Examinations of Meningitis

435's Teamwork

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This work has been produced with the help of our tutors' notes. Please remember that the cases usually change in the exam, therefore, avoid pure memorization and do not skip a statement unless 100% understood. Do not hesitate to contact us whenever needed. Best of luck, fellows.

**Sara Alenezy & Ali Alzahrani**

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According to the onset pattern of the disease, its etiological factors and CSF results, **Meningitis is divided into three types with common scenarios as following:**

### 1. Acute Pyogenic Meningitis<sup>1</sup>

**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 cmH<sub>2</sub>O. Microscopy of the cerebrospinal fluid showed gram -ve cocci. The patient showed complete recovery after administration of ceftriaxone for 10 days.

CSF	Normal range	Patient's results	Net Result
Appearance	Clear	Turbid	Changed
WBCs (cells/mm <sup>3</sup> )	Few (<5 )	8320 Mainly polymorphonuclear cells (84%)	↑
Protein (g/L)	0.1 - 0.4	0.7	↑
Glucose (mmol/L)	3.0 - 4.5	1.3	↓

### 2. Viral (Aseptic/Lymphocytic) Meningitis<sup>2</sup>

**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. He was diagnosed with meningitis.

CSF	Normal range	Patient's results	Net Result
Appearance	Clear	Clear	Unchanged
WBCs (cells/mm <sup>3</sup> )	Few (<5 )	1200 Mainly Lymphocytes (80%)	↑
Protein (g/L)	0.1 - 0.4	0.4	Unchanged
Glucose (mmol/L)	3.0 - 4.5	3.0	Unchanged

### 3. Chronic Meningitis<sup>3</sup>

**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.

CSF	Normal range	Patient's results	Net Result
Appearance	Clear	Turbid	Changed
WBCs (cells/mm <sup>3</sup> )	Few (<5 )	300 Mainly Lymphocytes (80%)	↑
Protein (g/L)	0.1 - 0.4	0.8	↑
Glucose (mmol/L)	3.0 - 4.5	2.0	↓

<sup>1</sup> Acute onset + Symptoms of meningitis + Turbid CSF + High protein + Low glucose + Polymorphs = **Acute Pyogenic Meningitis**.

<sup>2</sup> Acute onset + Symptoms of meningitis + Clear CSF + Unchanged/high protein + Unchanged/low glucose + Lymphocytes = **Viral Meningitis**.

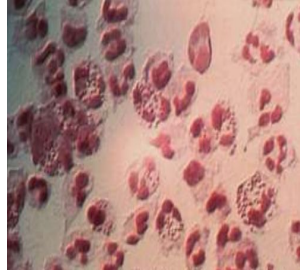
<sup>3</sup> Chronic onset + Symptoms of meningitis + Turbid CSF + High protein + Low glucose + Lymphocytes = **Chronic Meningitis**.

# Acute Pyogenic Meningitis

## 1. *Neisseria Meningitidis*<sup>4</sup>



**Clinical Presentation**<sup>5</sup>



**Gram Stain**



**Thayer-Martin Agar**

**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 negative intracellular bean-shaped diplococci with many pus cells.

**Q4: Name the media used for growing such organism?**

Thayer-Martin agar<sup>6</sup> or Chocolate agar.

**Q5: Describe the organism's morphology?**

Medium sized, greyish, convex, moist, circular with entire edge colony.

**Q6: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

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

Ceftriaxone with Vancomycin.

**Q8: If the patient presented with meningitis had previously been vaccinated, yet, still had meningitis, what would explain that?**

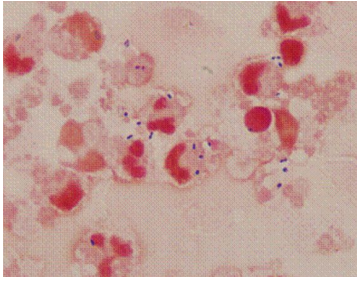
1. The vaccine might be deactivated due to inadequate storage or expiry.
2. The patient's immune system might be impaired.
3. The vaccine might not be meant to cover *Neisseria Meningitidis* (e.g. HIB vaccine), and even if it covers it, it might not match the serotype which initiated the infection.

<sup>4</sup> The infection is usually associated with patients performing Hajj.

<sup>5</sup> *Neisseria* lipopolysaccharides typically affects vascular structures causing coagulopathy, therefore, skin rash appears.

<sup>6</sup> Modified Chocolate agar that is selective for *Neisseria*; it prevents other organisms from overgrowing.

## 2. *Streptococcus Pneumoniae*



**Gram Stain**



**Blood Agar**



**Optochin Test**

**Q1: What is your diagnosis?**

Acute Pyogenic Meningitis.

**Q2: What is the most likely infection responsible?**

Pneumococcal bacterial infection.

**Q4: Describe the microorganism's appearance under microscope?**

Gram positive diplococci with many pus cells.

**Q5: Name the media used for growing such organism?**

Blood agar.

**Q6: Describe the microorganism's morphology?**

Alpha hemolytic streptococci.

**Q7: Describe the microorganism's reactivity towards the Optochin test?**

Optochin sensitive.

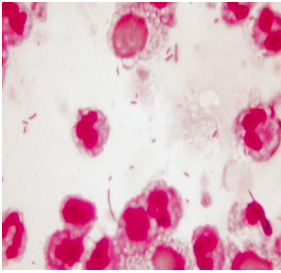
**Q8: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q9: Describe the organism's pathogenicity?**

Has a high mortality rate (up to 20%) and severe neurologic sequelae once associated with the CNS due to the antiphagocytic feature of its capsule.

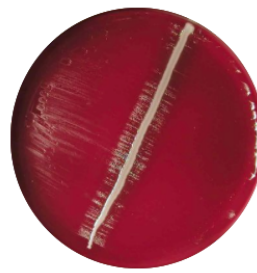
### 3. *Haemophilus Influenzae*



**Gram Stain**



**Nutrient Agar**



**Blood Agar**



**Chocolate Agar**

**Q1: What is your diagnosis?**

Acute Pyogenic Meningitis.

**Q2: What is the most likely infection responsible?**

Bacterial infection.

**Q4: What is the most probable Pathogen isolated?**

*Haemophilus Influenzae*.

**Q5: Describe the microorganism's appearance under microscope?**

Gram negative pleomorphic bacilli.

**Q6: Name the media used for growing such organism?<sup>7</sup>**

Chocolate agar<sup>8</sup>, Blood agar and Nutrient agar<sup>9</sup>.

**Q7: Describe the microorganism's morphology on Chocolate Agar?**

Grey mucoid colonies of *Haemophilus Influenzae* due to the presence of X and V factors.

**Q8: Describe the microorganism's morphology on Blood Agar?**

Normal blood agar has the X factor only, *Staphylococcus Aureus* provides the V, hence, *Haemophilus Influenzae* will only grow around a streak of *Staphylococcus Aureus* on the blood agar, a process that is called "Satellitism".<sup>10</sup>

**Q9: Describe the microorganism's morphology on Nutrient Agar?**

Only grown around the paper disc that has been saturated with X and V factors.

**Q10: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q11: Mention two recommended empirical antibiotics that can be used in such a case?**

Ceftriaxone with Vancomycin.

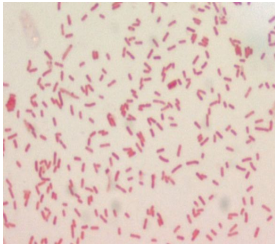
<sup>7</sup> In order for *Haemophilus Influenzae* to grow, it needs a medium that has both X and Y factors.

<sup>8</sup> The case scenario might say that the organism grows ONLY on chocolate agar because heating the blood releases X and V factors.

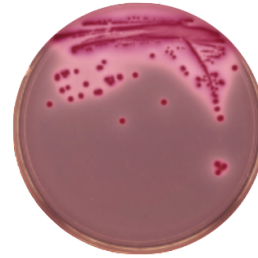
<sup>9</sup> They put three paper discs 1. X factor 2. V factor 3. X&V factors. The organism should grow around the third.

<sup>10</sup> *H. influenzae* will not grow outside the zone of *S. aureus* due to the lack of nutrients such as factor V in these areas.

#### 4. *Escherichia Coli*<sup>11</sup>



**Gram Stain**



**MacConkey's Agar**

**Q1: What is your diagnosis?**

Acute Pyogenic Meningitis.

**Q2: What is the most likely infection responsible?**

Bacterial infection.

**Q4: What is the most probable Pathogen isolated?**

*Escherichia Coli*.

**Q5: Describe the microorganism's appearance under microscope?**

Gram negative rods.

**Q6: Name the media used for growing such organism?**

MacConkey's agar and CLED agar.

**Q7: Describe the microorganism's morphology on MacConkey Agar?**

Lactose fermenter (pink colonies).

**Q8: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q9: Mention two recommended empirical antibiotics that can be used in such a case?**

**Child:** Ceftriaxone with Vancomycin.

**Neonate:** Ampicillin with Gentamicin.

**Q10: Describe the mode of transmission of such organism?**

*Escherichia Coli* is one of the microorganisms colonizing the female's perineum, it gets transmitted to the newborn through delivery and causes meningitis.

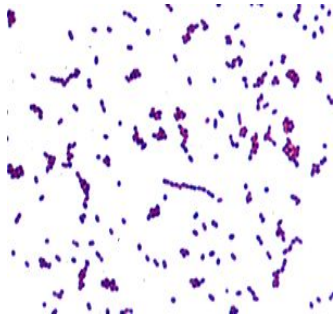
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<sup>11</sup> Usually infects neonates.



EXTRA - Just in case

5. Group B Streptococcus (*Streptococcus Agalactiae*)<sup>12</sup>



Gram Stain



Blood Agar

**Q1: What is your diagnosis?**

Acute Pyogenic Meningitis.

**Q2: What is the most likely infection responsible?**

Bacterial infection.

**Q4: What is the most probable Pathogen isolated?**

*Group B Streptococcus*.

**Q5: Describe the microorganism's appearance under microscope?**

Gram positive chains.

**Q6: Name the media used for growing such organism?**

Blood agar.

**Q7: Describe the microorganism's morphology on blood Agar?**

Beta hemolytic colonies.

**Q8: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q9: Mention two recommended empirical antibiotics that can be used in such a case?**

**Child:** Ceftriaxone with Vancomycin.

**Neonate:** Ampicillin with Gentamicin.

**Q10: Describe the mode of transmission of such organism?**

*Group B Streptococcus* is one of the microorganisms colonizing the female's perineum, it gets transmitted to the newborn through delivery and causes meningitis.

<sup>12</sup> Usually infects neonates.

**EXTRA - Just in case**

*6. Listeria Monocytogenes*

**Q1: What is your diagnosis?**

Acute Pyogenic Meningitis.

**Q2: What is the most likely infection responsible?**

Bacterial infection.

**Q4: What is the most probable Pathogen isolated?**

*Listeria monocytogenes.*

**Q8: What further investigation would you like to do at this stage?**

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q9: Mention two recommended empirical antibiotics that can be used in such a case?**

**Child:** Ceftriaxone with Vancomycin.

**Neonate:** Ampicillin with Gentamicin.

**Q10: Describe the mode of transmission of such organism?**

Spread to the fetus following hematogenous spread inside the mother.



## Viral (Aseptic/Lymphocytic) Meningitis

**Q1: What is your diagnosis?**

Aseptic (Lymphocytic) Meningitis.

**Q2: What is the most likely infection responsible?**

Viral Infection.

**Q3: What further investigation would you like to do at this stage?**

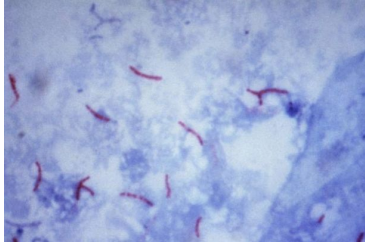
CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

**Q4: What justifies your answer from TB?**

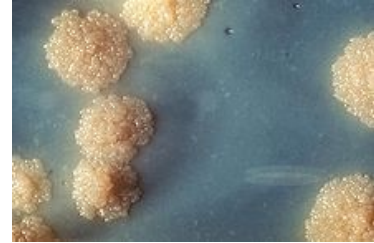
Protein and glucose levels are unchanged or slightly changed in comparison with TB or bacterial in general, and the CSF is clear, not turbid.

## Chronic Meningitis

### *Mycobacterium Tuberculosis*



**Ziehl-Neelsen Stain**



**Lowenstein-Jensen Medium**

**Q1: What is your diagnosis?**

Chronic Bacterial Meningitis.

**Q2: What is the most likely infection responsible?**

Mycobacterial infection.

**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 microorganism's morphology on LJ media?**

Buff, rough and tough colonies.

**Q9: 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).