



# Cerebrospinal Fluid Analysis

Year: 2025-2026

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# Lumbar Puncture

- **Definition:** A sterile procedure to obtain cerebrospinal fluid from the subarachnoid space at the lumbar level for **diagnostic or therapeutic** purposes.
- **Indications:**
  - **Diagnostic:** Suspected meningitis/encephalitis, subarachnoid haemorrhage, CNS malignancy
  - **Therapeutic:** Intrathecal drug administration, reduce ICP in idiopathic intracranial hypertension



# Lumbar Puncture: Contraindications

## **Absolute:**

- Signs of raised intracranial pressure (risk of brain herniation): papilledema, focal neurological deficits, altered consciousness, seizures
- Infection at puncture site
- Spinal cord mass lesion

## **Relative:**

- Coagulopathy

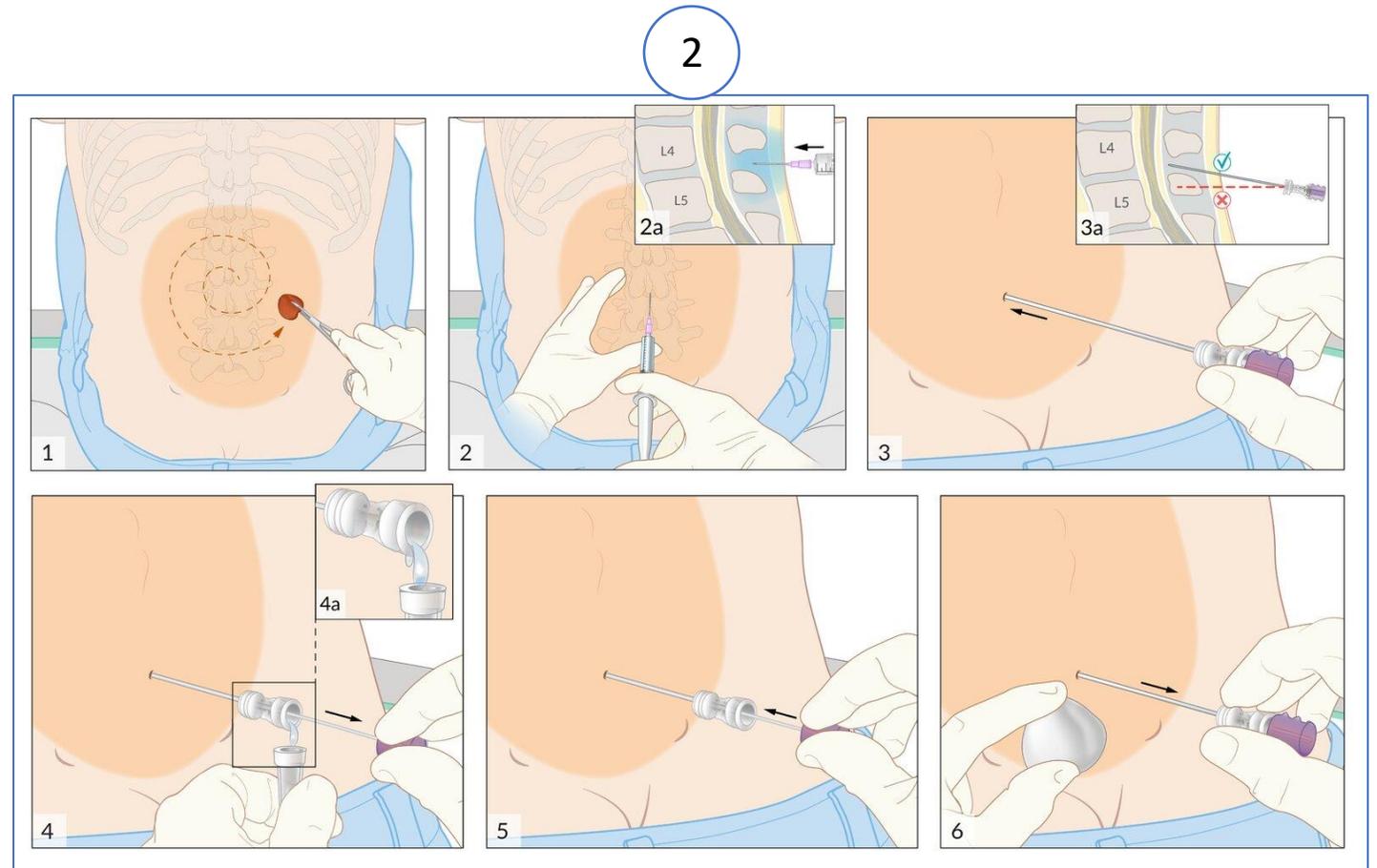
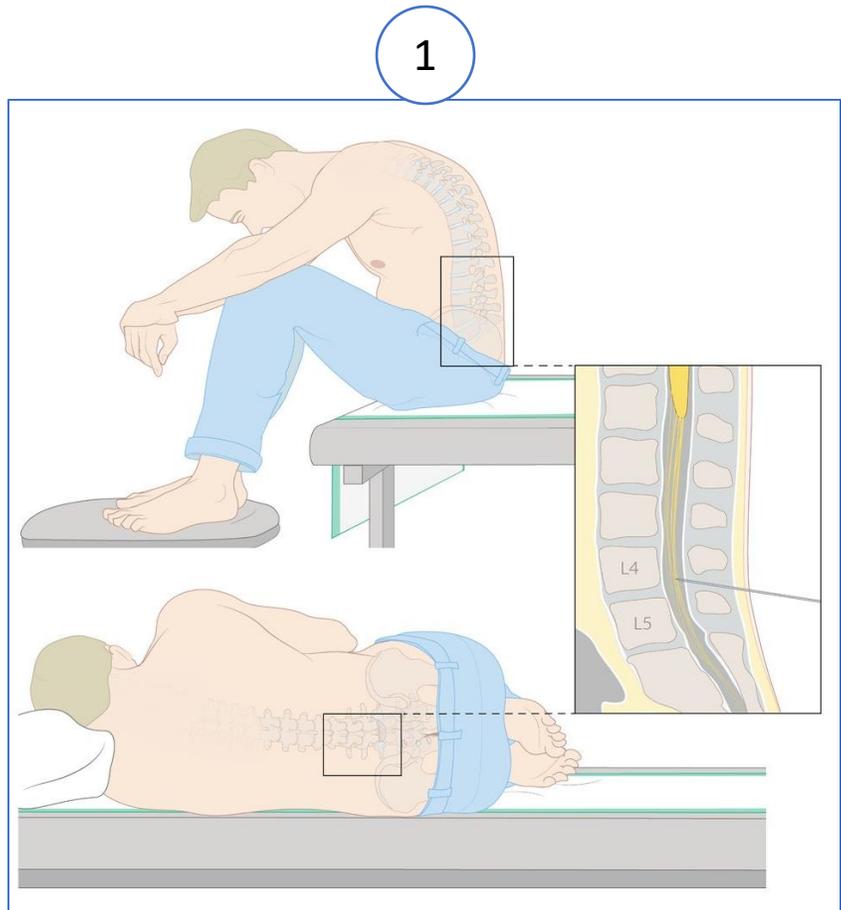


# Lumbar Puncture

- **Site:** L3-L4 or L4-L5 intervertebral space – minimizes risk of cord injury
- **Patient position:** Lateral decubitus (fetal position) or sitting – flexed spine opens intervertebral spaces
- CSF sample is collected under sterile conditions into 3-4 sterile tubes.



# Lumbar puncture



<https://www.youtube.com/watch?v=7tcrSd5lLoc>



# Lumbar Puncture: CSF Sample Collection

**Tube 1:** Biochemistry (protein, glucose) and immunology studies

- these tests are **the least affected** by blood cells or skin bacteria that may be introduced during the initial needle insertion.

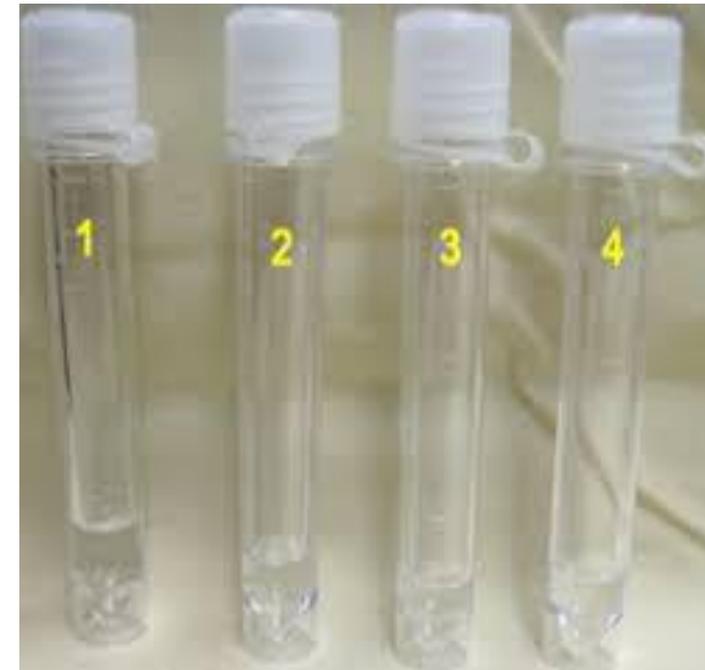
**Tube 2:** Microbiology (Gram stain, culture)

- Sterile handling critical

**Tube 3:** Cell count & differential

- Least likely to be contaminated

**Tube 4** (*if collected*): Special tests (cytology, viral PCR)



# Lumbar Puncture: CSF Sample Collection



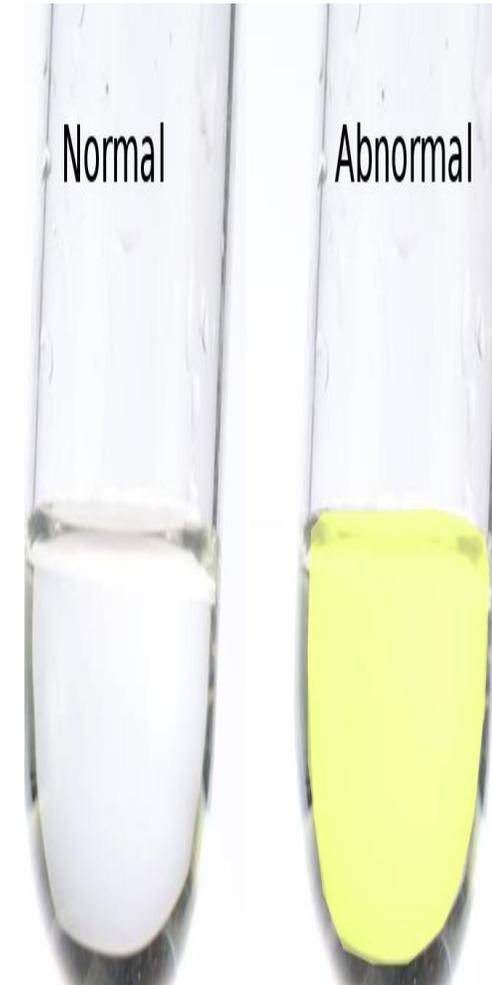
SAH

artificial bleeding



# CSF – Normal Characteristics

- **Appearance:** Clear, colourless → Any turbidity or colour is abnormal
- **Opening pressure:** 10-20 cmH<sub>2</sub>O (80-200 mmH<sub>2</sub>O)
  - Measured in lateral decubitus position
- **WBC count:** 0-5 cells/mm<sup>3</sup>
- **RBC count:** 0 cells/mm<sup>3</sup> → Any RBCs suggest haemorrhage or traumatic tap
- **Protein:** 15-45 mg/dL (0.15-0.45 g/L)
  - Higher in neonates and elderly
- **Glucose:** 50-80 mg/dL (2.8-4.4 mmol/L) Or **60-70% of serum glucose**
- **CSF: Serum glucose ratio:** ≥0.6 → More reliable than absolute value



# CSF Analysis in CNS Infections

## **Systematic Approach to CSF Analysis**

- **Macroscopic examination** – Appearance, colour, clarity
- **Opening pressure** – Elevated in most infections
- **Cell count & differential** – Neutrophils vs lymphocytes
- **Biochemistry** – Protein, glucose, lactate
- **Microbiological studies** – Gram stain, culture, special tests



# Macroscopic Examination



**Normal CSF**



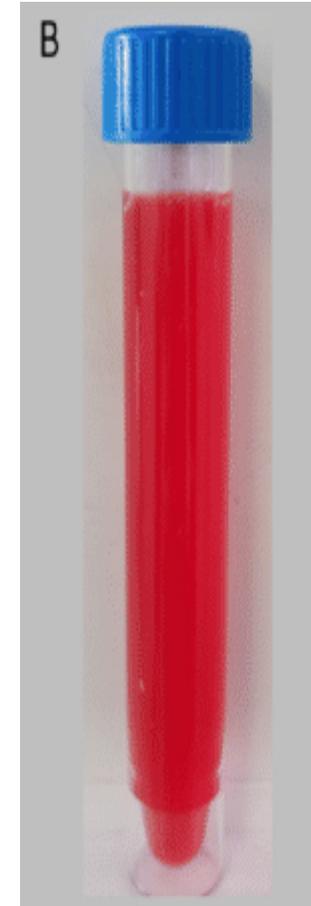
**Cloudy**



**Purulent**



**Pus**



**Haemorrhagic**



**Xanthochromia**



# Macroscopic Examination

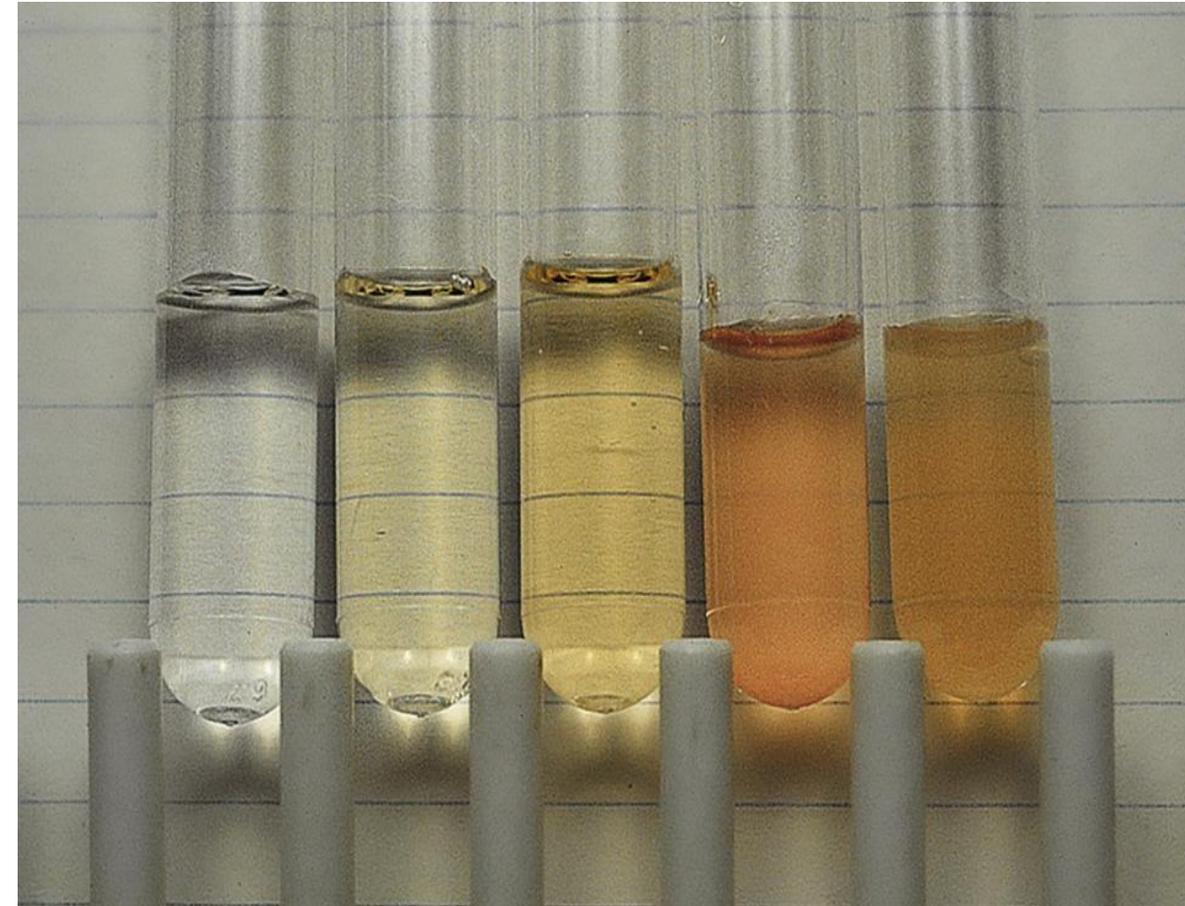
Appearance	Interpretation	Think of...
Crystal clear	Normal OR early/viral infection	Normal, viral meningitis
Turbid/Cloudy	High WBC or protein (>200 cells/mm <sup>3</sup> or protein >100 mg/dL)	Bacterial meningitis
Purulent (yellow-green)	Severe bacterial infection	Pyogenic meningitis
Xanthochromia (yellow)	Bilirubin from RBC breakdown	Subarachnoid haemorrhage, TB, high protein
Hemorrhagic (red/pink)	Blood in CSF	SAH or traumatic tap



# Macroscopic Examination

Left to right:

- 1- Normal CSF.
- 2- Mildly xanthochromic CSF.
- 3- Moderately xanthochromic CSF.
- 4- Red-tinged turbid CSF caused by haemorrhage
- 5- Cloudy red-tinged fluid with bacterial meningitis

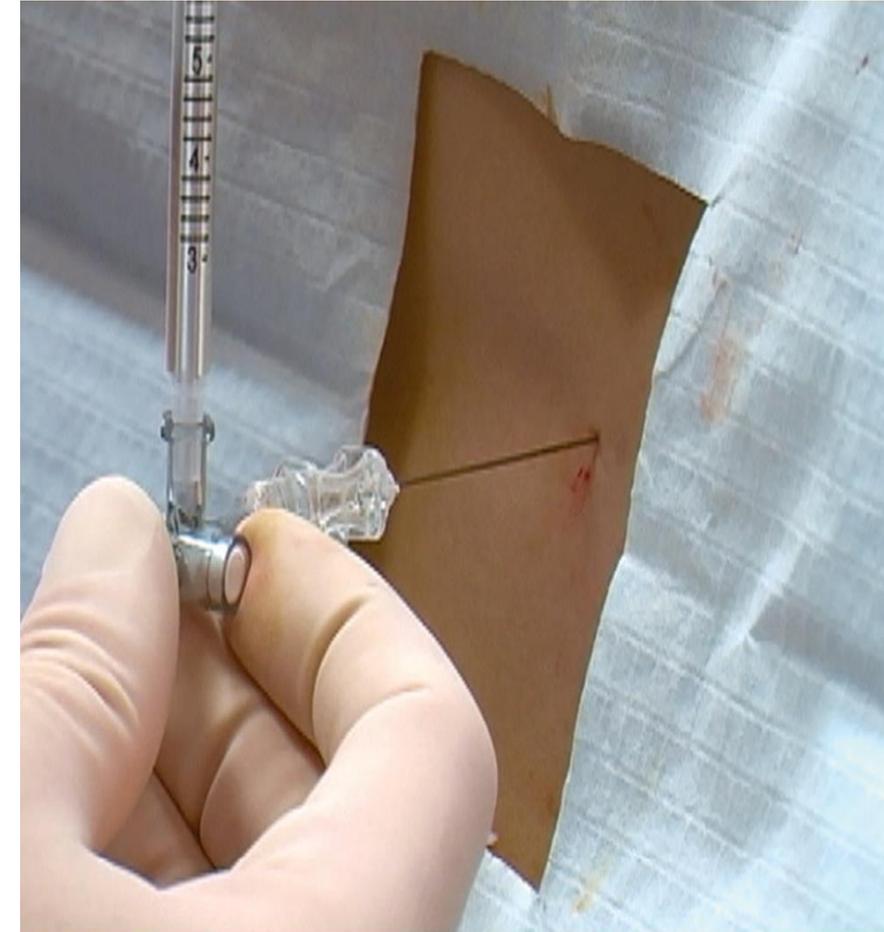


# Opening Pressure

- **Opening Pressure** is the **pressure of cerebrospinal fluid** measured at the moment the spinal needle enters the subarachnoid space, **before any CSF is withdrawn**.
- It reflects the **intracranial pressure (ICP)** and is measured using a manometer in the lateral decubitus position.
- **Normal range:** 10–20 cmH<sub>2</sub>O

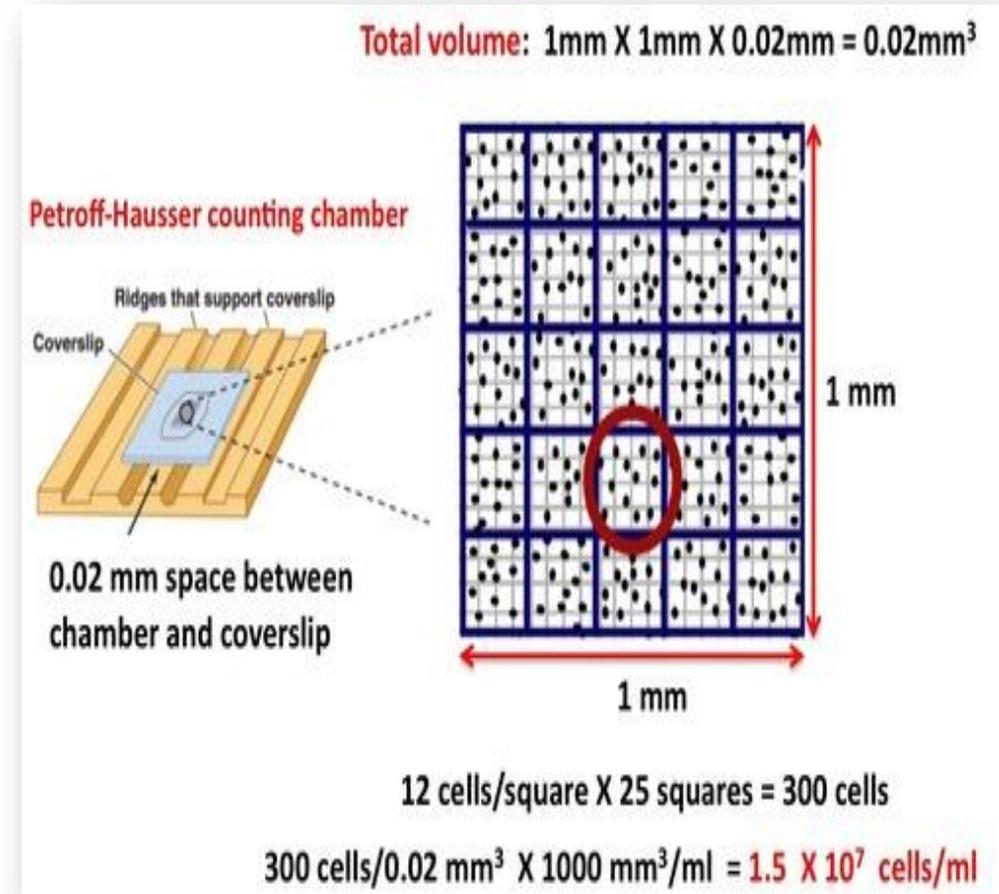
## Interpretation:

- Normal to mildly elevated in Viral meningitis
- **Elevated** (20-50 cmH<sub>2</sub>O):
  - **Infections: Bacterial meningitis**, TB meningitis, Cryptococcal meningitis
  - haemorrhage, tumour
  - idiopathic intracranial hypertension



# Cell Count

- All specimens should be examined microscopically
- Manual count using standard hemacytometer counting chamber
- Normal CSF results:
  - RBCs: no cells regardless of age
  - WBCs: 0 - 5 white blood cells



# Cell Count

Parameter	Bacterial	Viral	TB	Fungal
<b>WBC count</b>	↑↑↑ (1,000-10,000+)	↑ (50-500)	↑ (50-500)	↑ (50-500)
<b>Predominant cell</b>	<b>Neutrophils (&gt;80%)</b>	<b>Lymphocytes</b>	<b>Lymphocytes</b>	<b>Lymphocytes</b>
Typical % neutrophils	>80%	<20%	<50%*	<50%



# Biochemistry: Protein & Glucose

<b>Cerebrospinal fluid analysis</b>				
<b>Diagnosis</b>	<b>WBC count (mm<sup>3</sup>)</b>	<b>Glucose (mg/dL)</b>	<b>CSF: Glucose Serum</b>	<b>Protein (mg/dL)</b>
<b>Normal</b>	0-5	40-70	$\geq 0.6$	<40
<b>Bacterial meningitis</b>	>1,000	<40	<0.4	>250
<b>Viral meningitis</b>	10-500	40-70	$\geq 0.6$	<150

**WBC** = white blood cell.

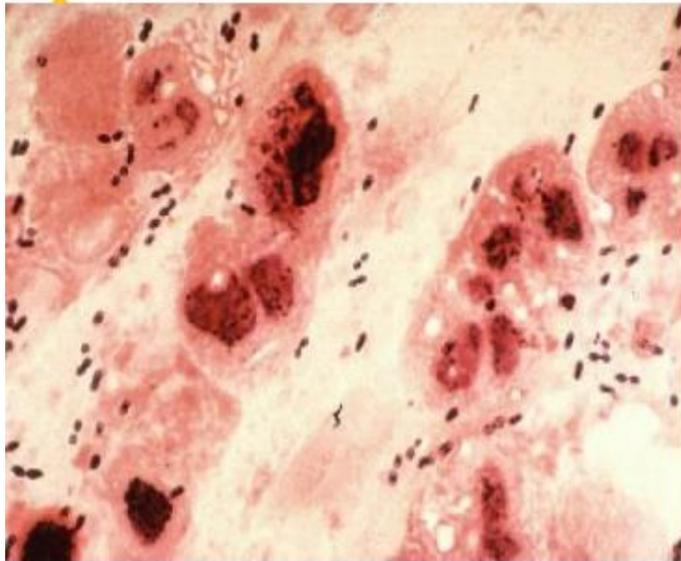


# Microbiology Studies: Gram Stain

<b>Gram Stain Finding</b>	<b>Morphology</b>	<b>Most Likely Organism</b>
<b>Gram-positive cocci in pairs</b>	Lancet-shaped diplococci	<i>Streptococcus pneumoniae</i>
<b>Gram-positive cocci in clusters</b>	Clusters	<i>Staphylococcus aureus</i> (post-neurosurgery)
<b>Gram-negative diplococci</b>	Kidney bean pairs (intracellular)	<i>Neisseria meningitidis</i>
<b>Gram-positive bacilli</b>	Short rods, may show tumbling motility	<i>Listeria monocytogenes</i>
<b>Gram-negative bacilli</b>	Rods	<i>E. coli</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>
<b>Gram-negative coccobacilli</b>	Pleomorphic, small	<i>Haemophilus influenzae</i>



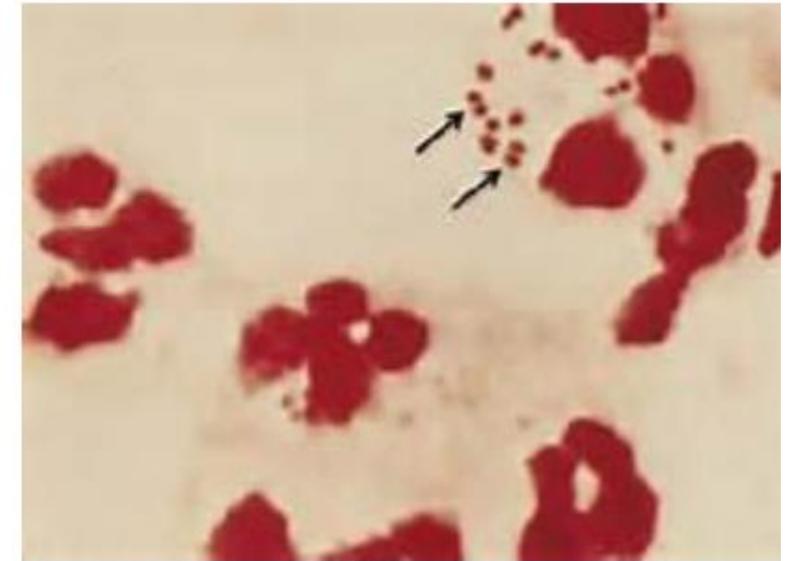
# Microbiology Studies: Gram Stain



Gram +ve diplococci  
*S. pneumoniae*



gram-positive, rod-shaped,

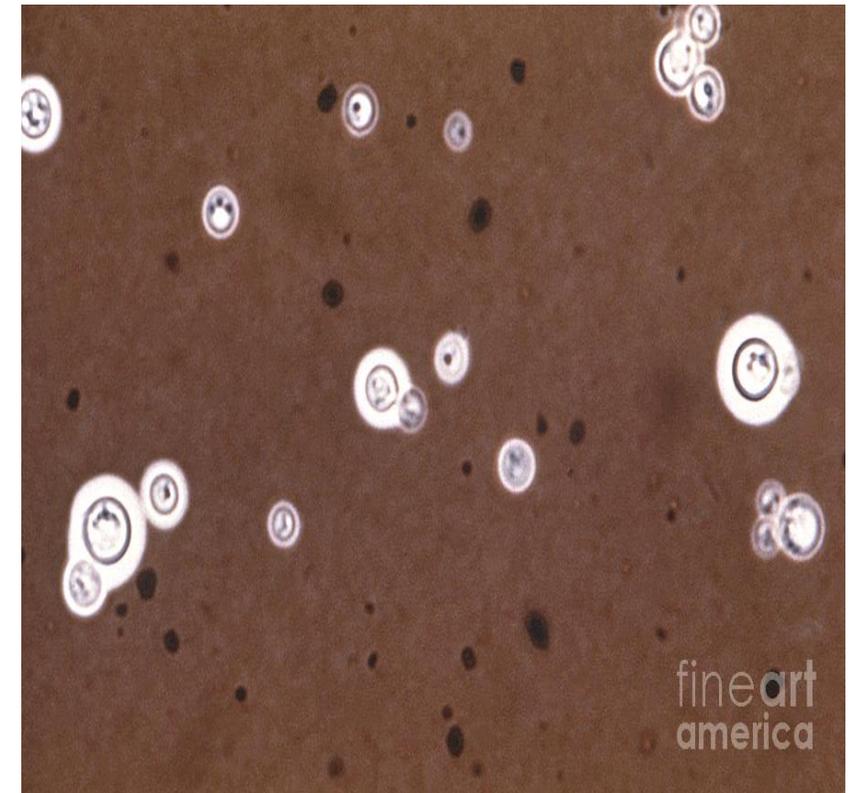


*N. Meningitidis*  
gram-negative, coffee-bean  
shaped diplococci.



# Microbiology Studies: India Ink Stain

- The large polysaccharide capsule of *Cryptococcus neoformans* allows these organisms to be visualized by the India ink stain.
- Latex agglutination testing for capsular antigen is more sensitive and specific.
- To perform the India ink preparation, a drop of CSF sediment is mixed with one third volume of India ink



# Microbiology Studies: Culture media

Medium

**Blood agar**

**Chocolate agar**

**MacConkey agar**

Purpose

General bacterial growth

Fastidious organisms

Gram-negative selection

Organisms

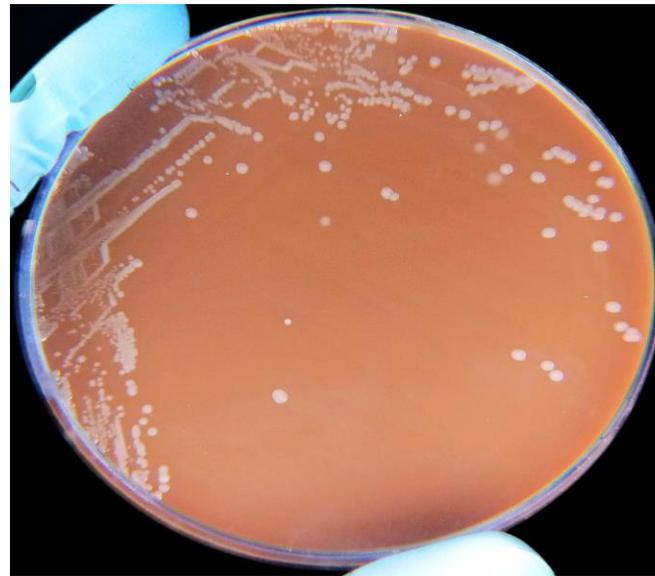
Most bacteria

*H. influenzae*, *N. meningitidis*

Enterobacteriaceae



*Streptococcus pneumoniae* on Blood agar



*H. influenzae* on a chocolate agar plate



*Neisseria meningitidis* on Blood agar



# Microbiology Studies: Other identification techniques

- Methods based on the detection of microorganism's genome by polymerase chain reaction (PCR)



## Common causes of bacterial meningitis

