

Objectives:

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

We highly recommend you to study the theoretical lecture “Bodyfluids: CSF” before studying this lecture .

Exam will include:

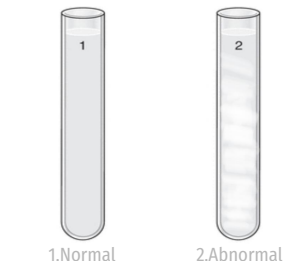
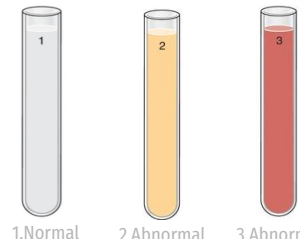
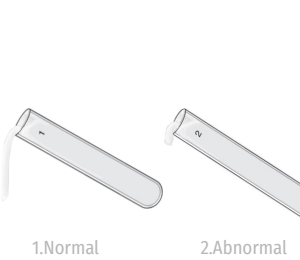
Dr. Sumbul:

- One station combined with microbiology.
- The station may include 3 questions from microbiology and 2-3 questions from biochemistry.
- If the station is separated from microbiology station then we will ask you about the calculation but if it is combined with microbiology then we will not ask you to calculate anything. 😊
- Know what are the **routinely performed tests**.
- Know the **indication and contraindications of CSF sample**.
- We may give you a case and in the case **for example** the glucose will be decreased, we will ask you **what is the cause** of decreased glucose.

CSF Sample

- ▶ The specimen should be delivered to the laboratory immediately after collection (100-150 ml)
- ▶ Glucose and protein estimations should be performed as soon as possible after drawing the CSF specimen
- ▶ If testing is to be delayed, the specimen should be frozen at – 20 C.

1- Physical examination [1]

Parameter	Normal how will you describe it ?	Abnormal how will you describe it ?	Pictures Extra
Turbidity	Clear - normal	Cloudy / turbid may indicate the presence of white, or red blood cells, microorganisms, or an increase in protein level	 <p>1.Normal 2.Abnormal</p>
Color	Colorless - normal	Yellow, orange-brown, or red may indicate the presence of blood [2]	 <p>1.Normal 2.Abnormal 3.Abnormal</p>
Viscosity [3]	Same consistency as water	Thicker CSF may be seen in patients with certain types of cancers or meningitis .	 <p>1.Normal 2.Abnormal</p>

2- chemical examination

Glucose	Protein	Lactate	Lactate dehydrogenase	Glutamine and acid-base parameters
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[1] We may give you the color, turbidity or viscosity then we will ask you what is the reason for the turbidity...etc

[2] it could be due to traumatic tap or subarachnoid hemorrhage

[3] viscosity = density

Remember !

- 1- Before any analysis, the fluid should be centrifuged to avoid contamination by cellular elements
- 2- CSF is the most precious biological material. Often, only small volumes of CSF are available for analysis due to difficulty in collection; hence handle this with care.
- 3- The specimen may contain virulent organisms, so strict safety precautions should be followed.

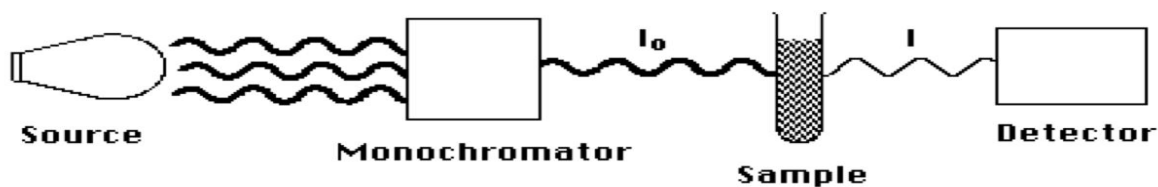
CSF Protein Assay

- ▶ Protein present in the CSF is detected by a kit based on Biuret method. [1]
- ▶ Biuret reagent when interacts with the peptide bonds in the protein give a blue coloured product
- ▶ The intensity of the colour is proportional the amount of protein in CSF
- ▶ Color intensity is determined by measuring the absorbance by the colored solution at a wavelength of 546nm [2]
- ▶ Absorbance is measured by an instrument, spectrophotometer

Spectrophotometer

Most of visible spectrophotometers are composed of:

- Light source which works with visible wavelengths (400-700 nm)
- Monochromator filter for choosing desired wavelength
- Sample holder (cuvette)
- Detector
- Meter or recorder



[1] biuret method is the most commonly used chemical test in laboratories, basically it's a reagent interacts with the peptides bonds of the protein and then will give you blue color product so the more protein you'll have in your sample the intensity of the blue color will be more and more, that means the concentration of your protein it's directly proportional to the intensity of the color produced.

[2] don't memorize the wave length, its dependent on the sample's color.

Procedure

- 1-Mix and incubate for 15 minutes at room temperature
- 2- Measure absorbance at 546 nm

	Test	standard	Blank
Reagent	2 ml	2ml	2ml
CSF sample	40 ul	-	-
Standard (60 mg/dL)	-	40 ul	-
Dist.H2O	-	-	40 ul

Calculation

$$\text{Protein concentration (g/L)} = \frac{\text{Absorbance of sample "test"}}{\text{Absorbance of standard}} \times \text{Conc. of standard (60 mg/dL)}$$

(note: you must convert from mg/dL TO g/L) , divide on 100

Normal Range

Normal reference values for CSF protein:

15 - 45 mg/dL (0.1 - 0.4 g/L)

Memorize the normal range

★ Very very important

Abnormal findings of CSF in some pathological conditions

Parameters		Proteins	Glucose	Chloride
Condition	Bacterial meningitis (progenic)	↑↑	↓↓	↓↓
	Tuberculous meningitis	↑↑	↓↓	↓↓
	Viral meningitis <small>Usually normal findings</small>	Normal	Normal or slightly ↓	Normal or ↓
	Brain tumor	↑	↓	Normal or ↓

CSF Examination Report

▶ Physical examination:

- Volume
- Color
- Appearance
- Viscosity

▶ Chemical examination

- CSF protein concentration (g/L)

▶ Group number & Student names



Sample T1

Parameter	Result	Normal
Appearance	Clear	Clear
Color	Colorless	Colorless

- Absorbance of protein standard- 0.349
- Absorbance of sample T1- 0.241
- Conc. Of sample = $(0.241/0.349) \times 60\text{mg/dL} = 41.43 \text{ mg/dL} = 0.4 \text{ g/L}$

Sample T2

Parameter	Result	Normal
Appearance	Yellow	Clear
Color	Turbid (with precipitate)	Colorless

- Absorbance of protein standard- 0.349
- Absorbance of sample T2- 0.295
- Conc. Of sample = $(0.295/0.349) \times 60\text{mg/dL} = 50.72 \text{ mg/dL} = 0.5 \text{ g/L}$

Case scenario 1 (bio+micro)

A 12-years old child was brought to the emergency department in kkuh by his mother. She said that her child has been suffering from fever and headache symptoms for the last two weeks, and developed A CSF sample was drawn from the patient and send to the microbiology and biochemistry labs.

The microbiology results: the culture revels the growth of Ect.

Parameter	Result	Normal
Predominant cell	Neutrophils	Nil
Protein	100 mg/dl	15-45 mg/dl
Glucose	35 mg/dl	50-80 mg/dl

Q1: what is the most likely diagnosis?

Bacterial meningitis

Q2: what other relevant finding is expected to be seen such condition?

Decrease cl levels

Case scenario 2

A 50 years old male presented to the er with excruciating headache. He said "it is the worst headache i have ever had in my life". The diagnosis of subarachnoid hemorrhage was suspected, the GP ordered a sample of the cerebrospinal fluid (CSF) to be drawn for examination.

The result of physical examination are:

Parameter	Result	Normal
Appearance	Cloudy	Clear
Color	Yellow	Colorless
Viscosity	As water	As water

Q1:the yellowish CSF color is referred as ? What is the pathogenesis underlying it ?

Xanthochromia . due to hemoglobin pigment breakdown and RBC lysis.

Q2: what i the level of spinal cord in which CSF sample can be drawn?

The interspace L3-L4

Indications for laboratory investigation of CSF

- ▶ **CNS infection.**
- ▶ **Demyelinating disease**
- ▶ **CNS Malignancy**
- ▶ **Hemorrhage in CNS**

Contraindications for performing lumbar puncture

Bleeding diathesis

Increased intracranial pressure

Infection at site of needle insertion

Examination of CSF

Physical examination		Biochemical examination
Normal CSF	<ul style="list-style-type: none"> - Colorless - Clear - Free of clots - Free of blood 	
Cloudy (turbid) → perform microscopic examination	<ul style="list-style-type: none"> - is usually due to leukocytes - May be due to microorganisms 	<ul style="list-style-type: none"> ● Glucose ● Protein: <ul style="list-style-type: none"> - Total - Specific: Albumin, Immunoglobulin, others (e.g. myelin basic protein; MBP) ● Glucose & protein are the most reliable diagnostically & accessible analytically
Blood	<ul style="list-style-type: none"> ● Traumatic tap <ul style="list-style-type: none"> - Bright red color - RBCs in decreasing number as the fluid is sampled ● Subarachnoid hemorrhage (SAH) <ul style="list-style-type: none"> - Xanthochromia (hemoglobin breakdown pigments) = RBCs lysis & metabolism previously occurred (at least 2 hr earlier) ● When would Xanthochromia indicate hemorrhage? <ul style="list-style-type: none"> If you exclude: <ul style="list-style-type: none"> - Prior traumatic tap - Hyperbilirubinemia (bilirubin > 20 mg/dL) 	<ul style="list-style-type: none"> ● Lactate

This file was done by team leaders



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**Special thanks to
Shatha Aldhohair**



[Handout](#)



[Practical Video](#)



[Extra video](#)