Cerebrospinal Fluid (CSF) Analysis for total protein

CSF sample

- The specimen should be delivered to the laboratory immediately after collection
- 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 – 20^0 C.

Physical Examination

- **Turbidity**
  - Clear- normal
  - Cloudy/ turbid- may indicate the presence of white, or red blood cells, microorganisms, or an increase in protein level

Physical Examination

- **Color**
  - Colorless- normal
  - Yellow, orange-brown, or red- may indicate the presence blood

Physical Examination

- **Viscosity**
  - Normal CSF should have the same consistency as water
  - Thicker CSF may be seen in patients with certain types of cancers or meningitis.

Chemical Analysis

- Routinely performed biochemical tests in CSF are:
  - glucose
  - protein (total and specific)
  - lactate
  - lactate dehydrogenase
  - glutamine and acid-base parameters

Remember !!

- Before any analysis, the fluid should be centrifuged to avoid contamination by cellular elements
- 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
- 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.
- 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

CSF Protein Assay

- Color intensity is determined by measuring the absorbance by the colored solution at a wavelength of 546nm
- 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

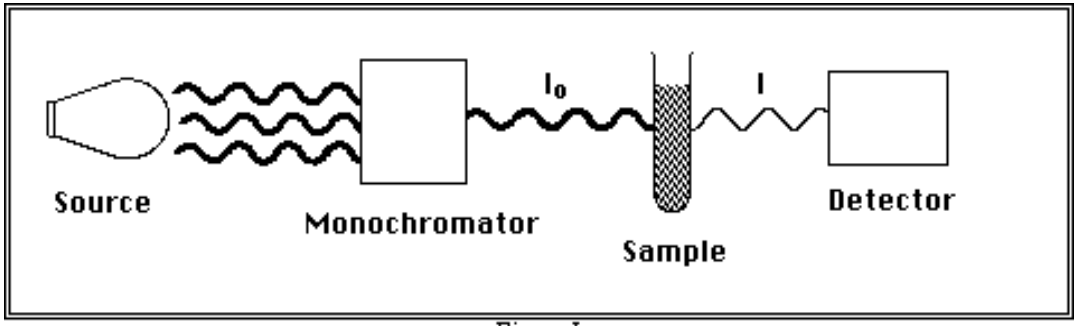


Figure I

Procedure

	Test	Standard	Blank
Reagent	2 ml	2 ml	2 ml
CSF sample	40 µl	-	-
Standard (60mg/dL)	-	40 µl	-
H_2O	-	-	40µl

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

Calculation

- Protein conc (mg/dL) =

$$\frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc of standard (60mg/dL)}$$

- To convert from mg/dL to g/L, divide the concentration by 100

Normal Range

Normal reference values for CSF protein:

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

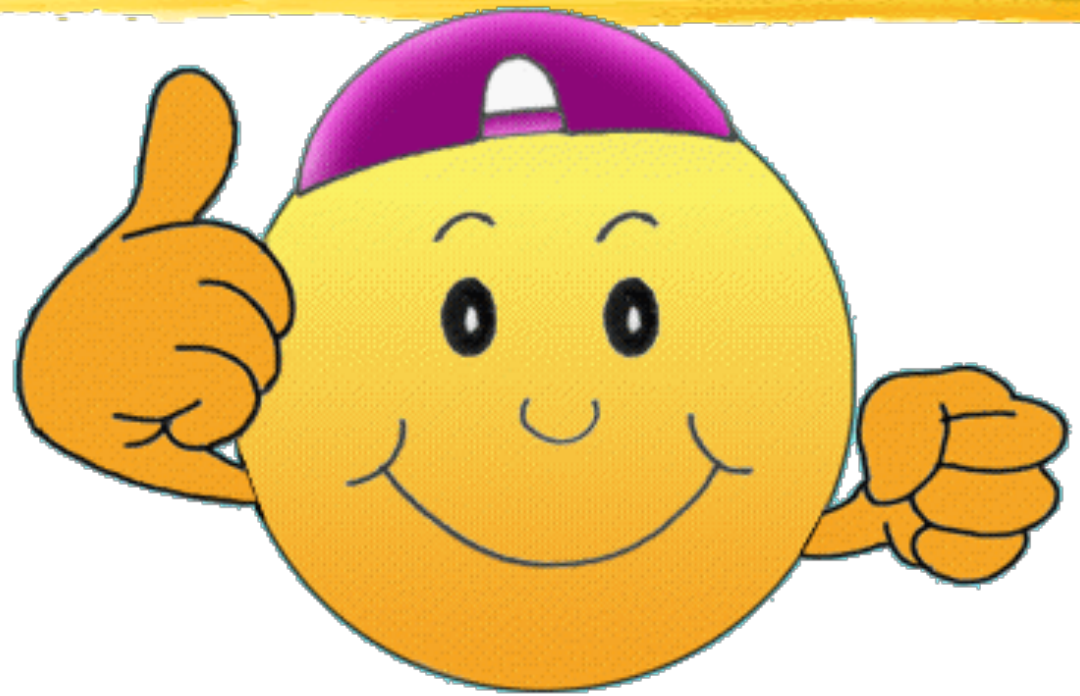
CSF Examination Report

- **Physical examination**
  - **Volume**
  - Color
  - Appearance
  - Viscosity
- **Chemical examination**
  - CSF protein concentration (g/L)
- **Group number&Student names**

Abnormal findings of CSF in some pathological conditions

Parameter	Condition			
	Bacterial Meningitis	Tuberculous Meningitis	Viral Meningitis	Brain Tumor
Protein	↑ ↑	↑ ↑	Normal	↑
Glucose	↓ ↓	↓ ↓	Normal or slightly ↓	↓
Chlorides	↓ ↓	↓ ↓	Normal or ↓	Normal or ↓

GO FOR IT !



GOOD LUCK !

Sample T1

- Colour- Colourless
- Appearance- Clear

- Absorbance of protein standard- 0.349
- Absorbance of sample T1- 0.241
- Conc. Of sample = (0.241/0.349) * 60mg/dL
  =41.43 mg/dL
  = 0.4 g/L

Sample T2

- Colour- Yellow
- Appearance- turbid (with precipitate)

- Absorbance of protein standard- 0.349
- Absorbance of sample T2- 0.295
- Conc. Of sample = (0.295/0.349) * 60mg/dL
  =50.72 mg/dL
  = 0.5 g/L