



**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  $-20^{\circ}\text{C}$ .

# 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

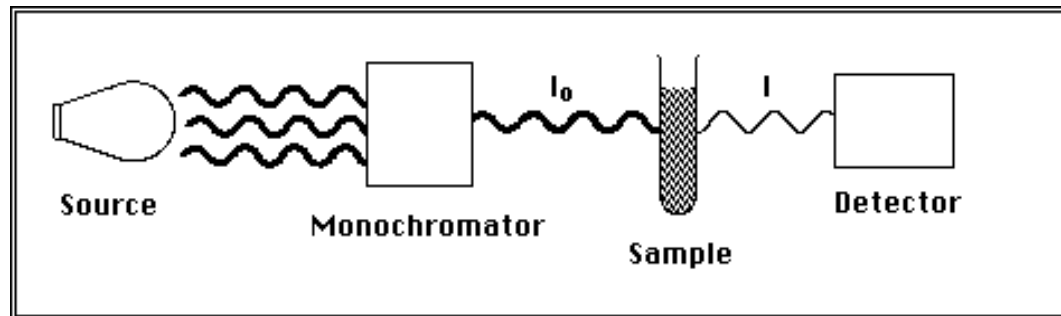


Figure 1

# Procedure

|                       | Test       | Standard   | Blank      |
|-----------------------|------------|------------|------------|
| Reagent               | 2 ml       | 2 ml       | 2 ml       |
| CSF sample            | 40 $\mu$ l | -          | -          |
| Standard<br>(60mg/dL) | -          | 40 $\mu$ l | -          |
| H <sub>2</sub> O      | -          | -          | 40 $\mu$ l |

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

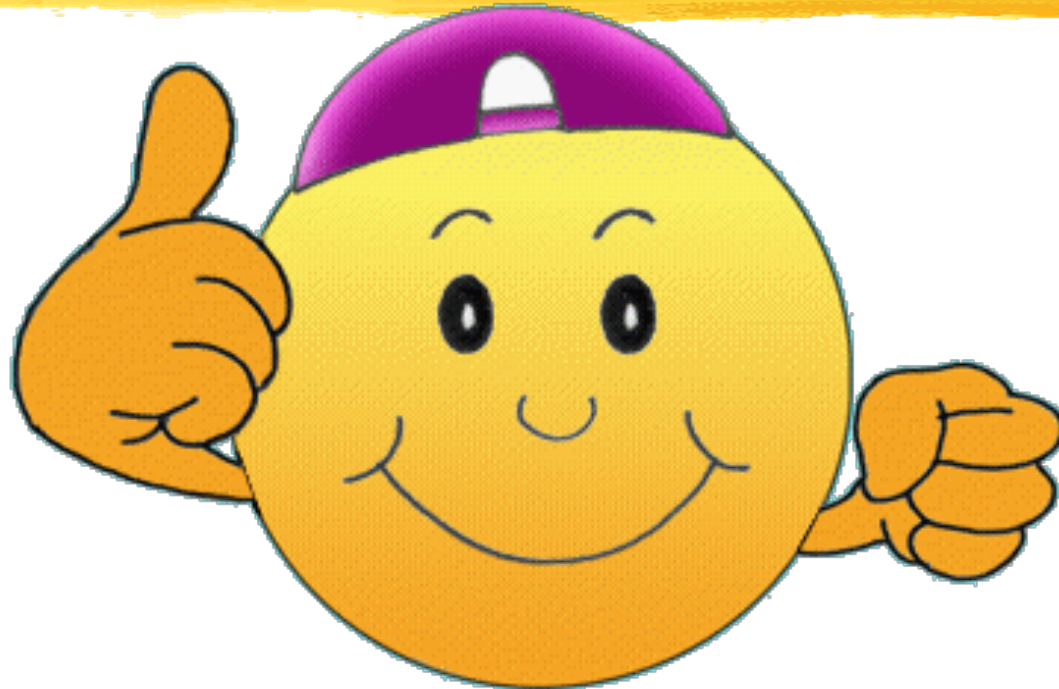


- Physical examination
  - Volume
  - Color
  - Appearance
  - Viscosity
- Chemical examination
  - CSF protein concentration (g/L)
- Group number & Student names

# 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 =  $(0.241/0.349) * 60\text{mg/dL}$   
= 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 =  $(0.295/0.349) * 60\text{mg/dL}$   
= 50.72 mg/dL  
= 0.5 g/L