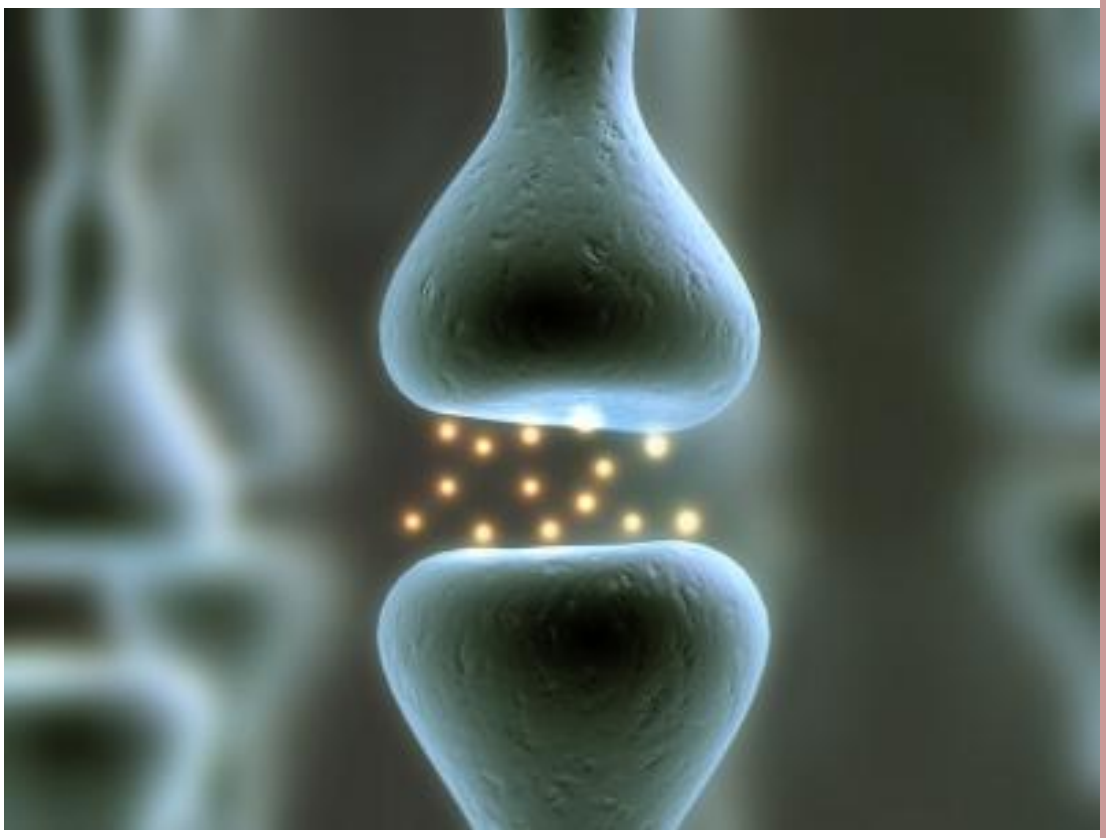
Practical Biochemistry of the CNS



Biochemical aspects of CSF

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How to be safe in Biochemistry lab? *Just go through it once in case a question came about it *

- Learn emergency procedures and be familiar with the location of the fire exits, fire extinguishers, blankets, water shower, eye fountains and first aid.
- Report any and all signs and symptoms of exposure to your supervisor
- Report all accident, injuries and spills to your supervisor
- If doubt, please ask.

Introduction:

- A chemical lab is a potentially hazardous environment as it contains hazardous samples, chemicals, materials, and equipments.
- Accidents and injury CAN happen any time.
- It is the responsibility of each individual to work safely, so as not to endanger himself, others, or property.
- Everyone using the lab should comply with prescribed safety standars, practices, and procedures.
- Chemicals may cause 2 different hazards:
    - Health hazards
    - Physical hazards

Safe work procedures:

The cardinal safety rules of the clinical laboratory to be developed, use common sense, and practice the following:

1- Good personal behavior/habits:

- ✓ Always use appropriate personal protective equipments (lab coat, gloves, masks, goggles, apron, boots, face shield, no open shoes, no eye lenses, and no cosmetics).
- ✓ Practice good hygiene when using chemicals:
    - wash your hands frequently.
    - Do not eat, drink, or smoke in the lab.
    - tie back long hair and use head cover.

2- Good laboratory techniques:

- ✓ Until you have received enough training do not operate new or unfamiliar equipment.
- ✓ Read labels and instructions carefully.
- ✓ Use chemicals properly:
    - Have knowledge of the chemicals you work with
    - Never mouth pipette
    - Handle, use, and dispose-off chemicals and biological waste properly
    - Be careful and avoid any spills of chemicals and biological waste properly
    - In case of any spills, or broken glassware, ask for help of your supervisor
    - Use volatile and flammable compounds only in a fume hood
    - Never smell a solvent, read the label on the solvent bottle to identify its contents

- All blood samples and other body fluids are considered as potentially infectious, so should be collected transported, handled, and processed using strict precautions
- For disposable contaminated waste, use containers with yellow plastic garbage bags and for regular waste like paper use containers with black plastic bags
- All sharp objects such as needles, scalpels, and even broken glassware go in the yellow-red sharps container.

Cerebrospinal fluid:

Indication for CSF analysis:

To diagnose some disease affecting the CNS such as infection, hemorrhage, malignancy, demylinating disease, or autoimmune disorder.

Specimen collection and storage:

- ✓ Only physician or a specially trained nurse must collect the specimen
- ✓ CSF is withdrawn by spinal tab (lumbar puncture) which is a process involving aseptic insertion of a long needle into the subarachnoid space between L3 and L4
- ✓ 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°C**

The following examinations are performed for CSF sample:

1. Physical examination
2. Chemical examination
3. Microscopic examination
4. Microbiological test

Physical examination:

A. Volume
(Normal CSF volume is about 150ml or about 8% of the total CNS cavity volume)

B. Turbidity
(Normal CSF is clear cloudy or turbid CSF may indicate the presence of white or red blood cells, microorganisms, or an increase in protein level)

C. Color
(Normal CSF is colorless and free from blood or clots, changes in color may point additional substances in the fluid)

Yellow, Orange to Brown or red colors may indicate blood

- The two most common reasons for blood and hemoglobin pigments in CSF are:
  -Traumatic tap "color is bright red and erythrocyte number is decreased"
  -subarachnoid hemorrhage "SAH" xanthochromia is present.

D. 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

The following biochemical test are routinely performed in clinical practice for CSF samples:

1. Glucose
2. Protein (total and specific)
3. Lactate
4. Lactate dehydrogenase
5. Glutamine and acid-base parameters

* Most often used Glucose and Protein

* Before any analysis, the fluid should be centrifuged to avoid contamination by cellular elements (cells will settle at the bottom)

Remember:

- CSF is the most precious biological material. Often, only small volumes of CSF are available for analysis due to difficulty in collection; hence handle with care.
- The specimen may contain virulent organisms, so strict safety precautions should be followed.

Total CSF protein:

*Biuret reagent is going to be used in measuring the SCF protein (Biuret method).
*This reagent reacts with peptide bonds that adjoining the amino acids of the protein.
*This reaction gives a blue color product
* The more the intensity of the blue color, the more protein we have in the specimen.

Principle of the method:

Protein present in the CSF is detected by a series of enzymatic reactions that ultimately form a colored product. The intensity of color is proportional to the amount of protein in CSF. Color intensity is determined by measuring the absorbance by colored solution at a wavelength of 546nm using the spectrophotometer.

Spectrophotometer

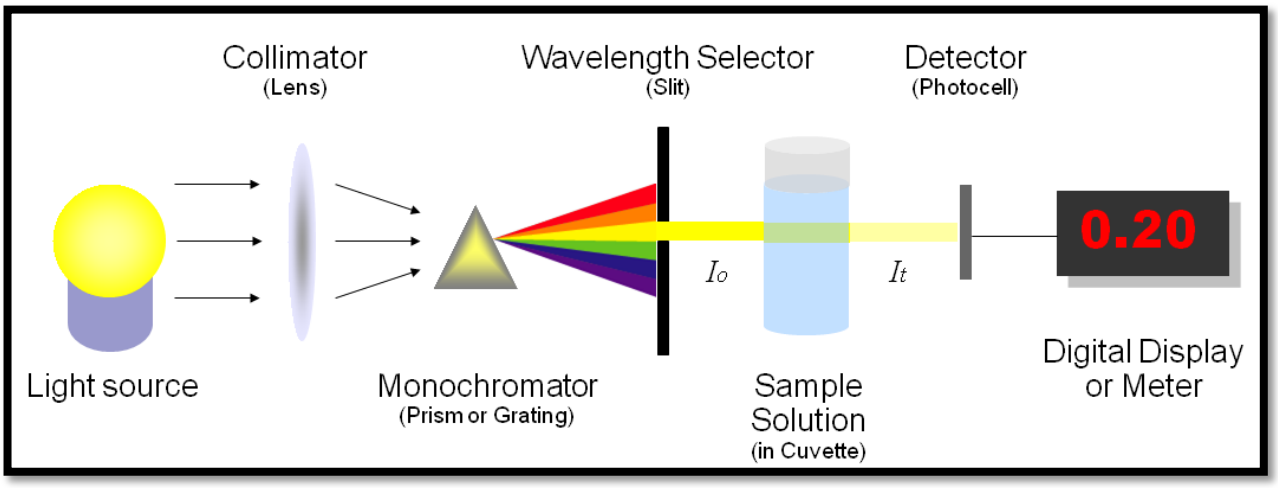
A spectrophotometer is a *photometer* (a device for measuring light intensity) that can measure intensity as a function of the light source wavelength.

Most of the visible spectrometers are composed of:

- Light source which is a visible wavelength in range (400-700nm)
- Monochromator filter for choosing desired wavelength
- Sample holder (cuvette)
- Detector
- Meter or recorder

In short, the sequence of events in the spectrophotometer is as follows:

1. The light source shines through a monochromator.
2. An output wavelength is selected and beamed at the sample.
3. A fraction of the monochromatic light is transmitted through the sample and to the photo detector.



To understand: spectrophotometer is basically an instrument that has a light source; the light source will give light with all visible wavelengths which are 400 – 700nm. Then, a filter is placed to allow only the 546nm wavelength to pass and the rest will be filtered.

Some of the light will be absorbed when passing through the sample and this depends on the amount of protein in the sample. The rest will be transmitted and detected by a detector which will be then converted by a meter into a number.

Procedure

	Test	Standard	Blank
Reagent	2 ml	2ml	2ml
CSF sample	100µl	-	-
Standard	-	100 µl	-
H2O	-	-	100 µl

-Mix and incubate for 15 minutes at room temperature.

-Measure the absorbance (A) of the **test** and **standard** against **blank** at 546nm.

Calculation:

To simplify, In order to measure the amount of protein in a patient's sample, you will need a standard sample which is a sample of known protein concentration, place the standard sample in the spectrophotometer to get its absorbance. So you know at this protein concentration the absorbance is the number you got. Now place the patient's sample in the spectrophotometer to get its absorbance. After that, calculate back the patient's sample protein concentration

$$\frac{\text{Standard's protein conc. (Known)}}{\text{Standard's protein absorbance (known)}} = \frac{\text{patient's protein conc. (?)}}{\text{patient's protein absorbance (known)}}$$

Total protein conc. (g/L)= $\frac{\text{A test}}{\text{A standard}}$ **X conc. of standard (60 g/L)**

Normal reference value for CSF total protein: 0.1 – 0.4 g/L

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↓

***Why do we use "blank"?** Using biuret reagent to measure protein does not give an accurate reading because biuret reagent by itself absorbs some light. To get an accurate reading, this lights absorbed by the reagent must be subtracted. To do that, first measure the absorbance by the reagent and make it your zero, then carry on measuring absorbance in standard and the patient's sample.