

# BIOCHEMISTRY PRACTICAL:

## Cerebrospinal fluid (CSF) analysis for total protein

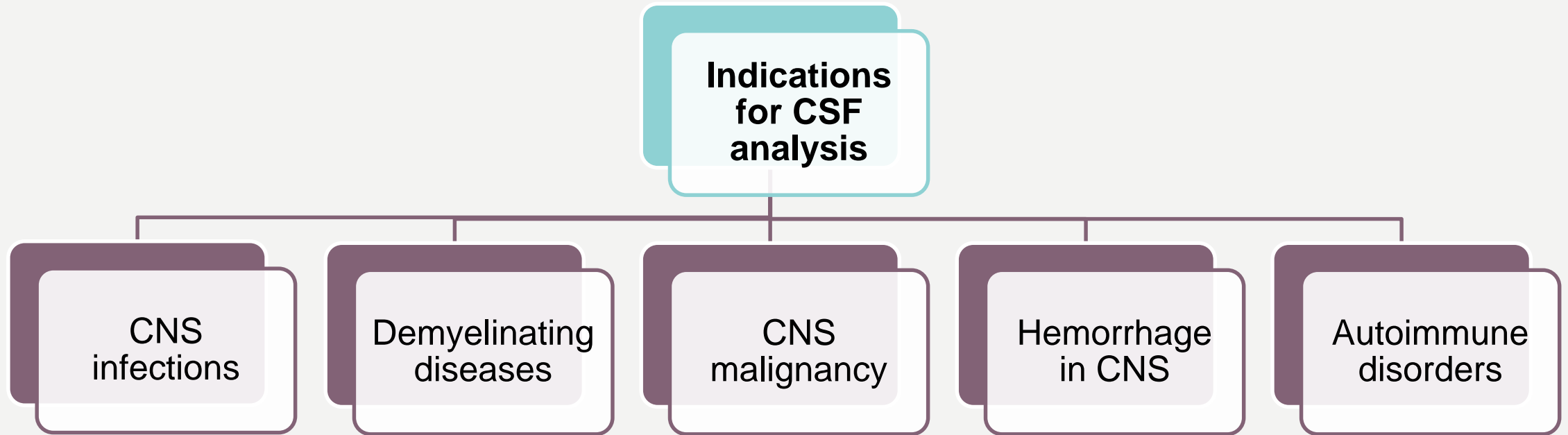
Color index:

- **Important**
- Extra explanation

# CSF Examination Report:

1. Physical examination:
  - Color
  - Appearance
  - Viscosity
2. Chemical examination:
  - CSF protein concentration (g/L)
3. Group number & Student names.

# CSF examination



The following examinations performed for CNS samples:



# specimen collection and storage

- Only a physician or a specially trained nurse must collect the specimen.
- CSF is withdrawn by spinal tap (lumbar puncture) which is a process involving aseptic insertion of a long needle into the subarachnoid space between L3 and L4.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 – 200 C.**

\*EXTRA\*

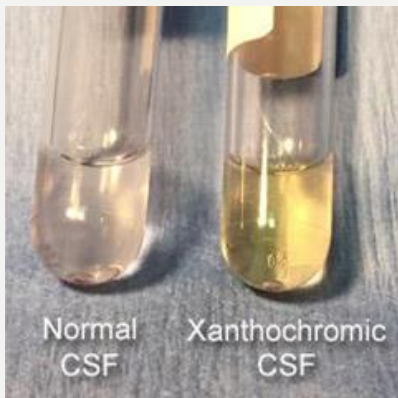


[Lumbar Puncture](#)

# 1- Physical Examination

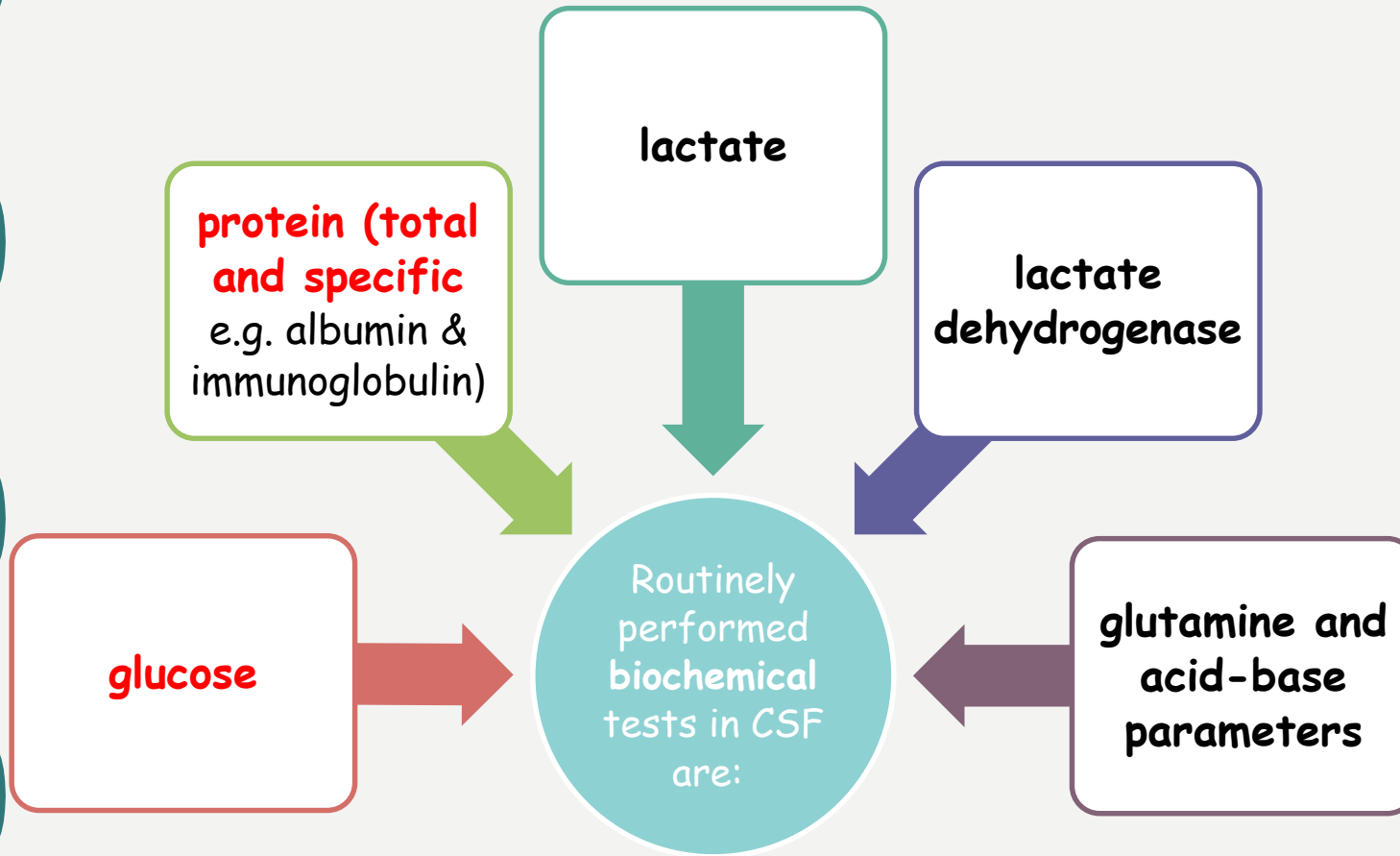
**\*important**

Parameter	Normal	abnormal
<b>Volume</b>	100-150 ml (8% of CNS cavity volume)	
<b>Turbidity</b>	Clear	Cloudy/turbid- may indicate the presence of: <b>white, or red blood cells, microorganisms, or an increase in protein level.</b>
<b>Color</b>	Colorless	<b>Yellow</b> (Xanthochromia) <sup>1</sup> , <b>orange-brown</b> or <b>red</b> may indicate the presence of <b>blood or hemoglobin pigments</b> <sup>2</sup> .
<b>Viscosity</b> <sup>3</sup>	same consistency as water	<b>Thicker CSF</b> may be seen in patients with certain types of <b>cancers or meningitis.</b>



- 1- Xanthochromia is the yellow discoloration indicating the presence of bilirubin in the (CSF).
- 2- The two most common reasons for blood and hemoglobin pigments in CSF are:
  - traumatic tap (color is bright and red and erythrocytes number is decreased.
  - subarachnoid hemorrhage (SAH) = xanthochromia is present.
- 3- you should shake the sample to inspect its viscosity.

# 2- Chemical Analysis



- protein is normally low in CSF: if increased suspect infection, but its decrease is not significant.
- lactate and lactate dehydrogenase increase in bacterial infection, but not in viral.
- glutamine increase in CSF due to liver disease

## 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 not be pre-tested for micro-organisms** ( may contain virulent organisms), so strict safety precautions should be followed.

Centrifuge:



# CSF Protein Assay

- **Protein** present in the CSF is detected by **a series of enzymatic reactions and give a blue colored product** (the **intensity** of the color is proportional to the **amount of protein in CSF**).

The proteins present in CSF are detected by a kit based on **Biuret method**, in which Biuret reagent interacts with the peptide bonds in the protein .

- Color intensity is determined by measuring the **absorbance** by the colored solution at a wavelength of **546nm**, the **absorbance is measured by an instrument known as spectrophotometer**.
- Spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the light source wavelength.

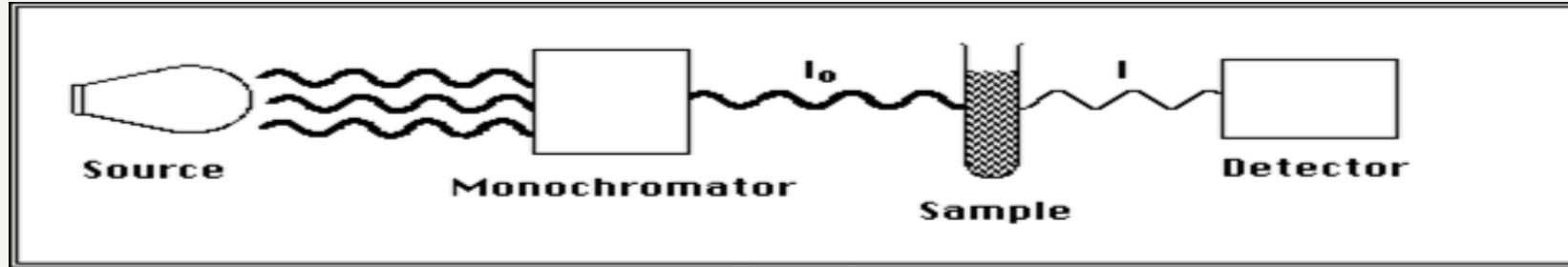


[How does a spectrophotometer work?](#)



# Spectrophotometer

Most of visible spectrophotometers are composed of:



Light source which works with visible wavelengths (400-700 nm)

Monochromator filter for choosing desired wavelength (546nm at this case)

*In greek: mono-, "single", and chroma, "colour"*

Sample holder (cuvette)



Detector of incident light

+ Meter or recorder

to convert light into a number

- In short, the sequence of events in a spectrophotometer is as follows:
  - The light source shines through a monochromator
  - An output wavelength is selected and beamed at the sample
  - A fraction of the monochromator light is transmitted through the sample and to the photodetector.



# Procedure

\*EXTRA\*



1



2



3



5



1- use 3 tubes (add 40 $\mu$ l in each) :

- blank (B) has water that subtracts absorbance of any molecule other than protein
- standard (S) has same substance (protein) of known concentration (60 g/L).
- test (T) is the CSF sample of which we want to measure protein (unknown concentration)

2- use 3 other tubes filled with 2 ml of biuret reagent.

3- add the reagent to each tube and mix the components.

4- incubate in room temperature for 15 minutes.

5- pour the solution of each tube into a Cuvette and then in the spectrophotometer to measure absorbance in each tube.

6- use the results (sample & standard absorbance) & the formula (next slide) to calculate protein concentration in the sample.

# Procedure

	Test	Standard	Blank
Reagent	2 ml	2 ml	2 ml
CSF sample	40 µl	-	-
Standard	-	40 µl	-
H <sub>2</sub> O	-	-	40 µl

1

- Mix and incubate for 15 minutes at room temperature.
- Measure absorbance (Abs) at 546 nm by Spectrophotometer.

2

- Calculation: Protein conc (g/L) =  $\frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc of standard (60g/L)}$

3

- Normal reference values for CSF protein:
  - 15-45 mg/dL (0.15 -0.45 g/L)

Important: you must convert from mg/dl to g/l (تقسما علی ۱۰۰).

\*important

# Abnormal findings of CSF in some pathological conditions

**\*important**

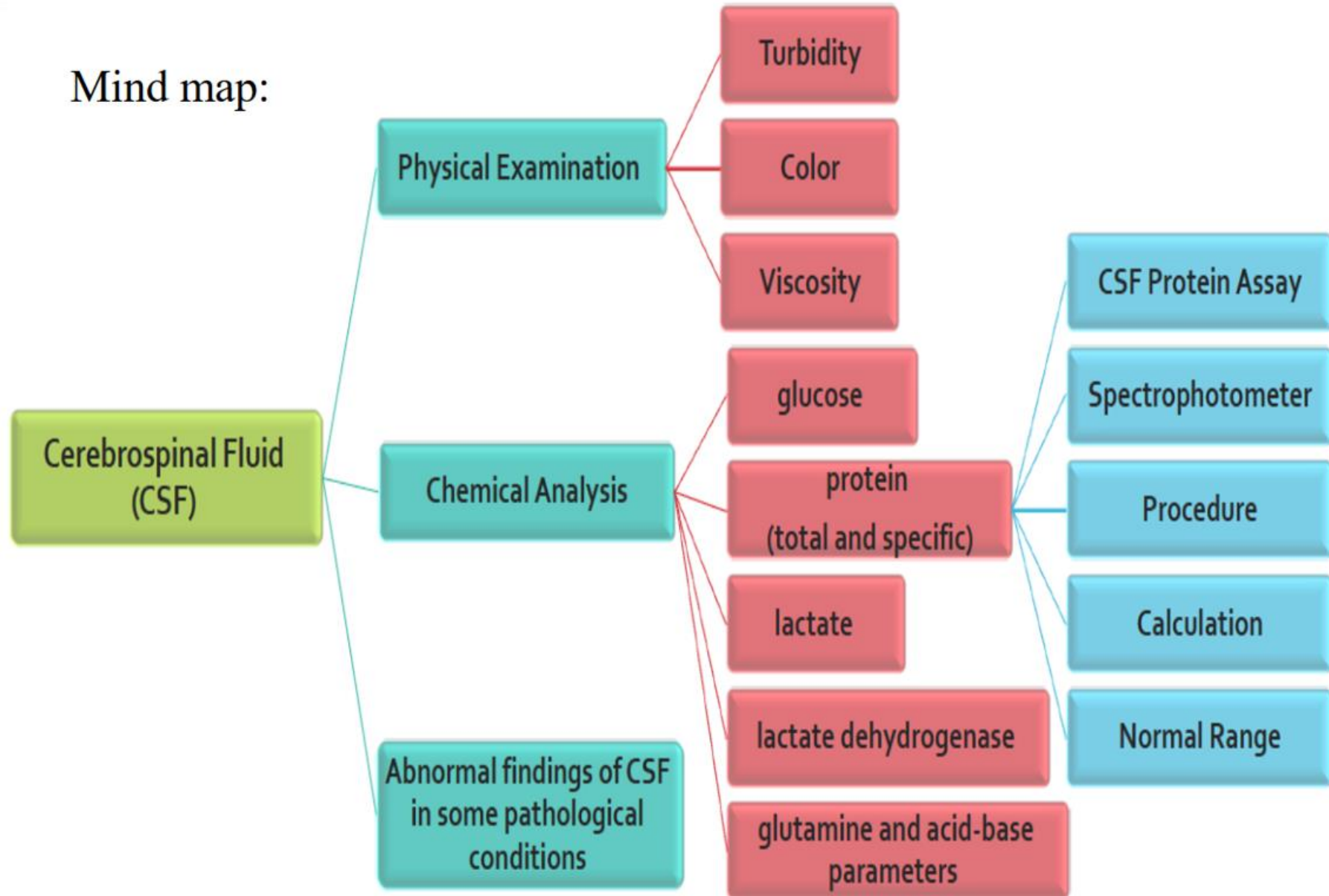
Parameter:	Condition			
	Bacterial Meningitis	Tuberculous Meningitis	Viral Meningitis	Brain Tumor
<b>Protein</b>	↑ ↑	↑ ↑	Normal	↑
<b>Glucose</b>	↓ ↓	↓ ↓	Normal or slightly ↓	↓
<b>Chlorides</b>	↓ ↓	↓ ↓	Normal or ↓	Normal or ↓

You can think of it this way:

- Bacteria needs glucose for nutrition and thus there will be decreased CSF glucose in bacterial meningitis, **increased protein** is due to inflammation. (TB, fungal infections and tumors also apply, same for Cl)
- Conversely, viruses are not living creatures and thus will not affect these parameters much.

# Mind map by team 434

Mind map:



# Case scenario 1 (Bio + Micro)

A 12-years old child was brought to the emergency department in KKHU by his mother. She said that her child has been suffering form fever and headache symptoms for the last two weeks, and developed a stiffness in the neck recently.

A CSF sample was drawn from the patient and sent to the microbiology and biochemistry labs.

The microbiology lab results: The culture reveals the growth of ..... Etc.

Biochemistry lab results for analysis of CSF:

Parameter	Result	Normal state
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 in such condition ?**

Decreased 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 shown below :

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 RBCs lysis.

**Q2) What is the level of spinal cord in which the CSF sample can be drawn ?**

The interspace L3-L4.

## Done by:

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## Resources:

- biochemistry practical slides.
- integrated practical handouts.

\*نستقبل اقتراحاتكم وملاحظاتكم على:



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