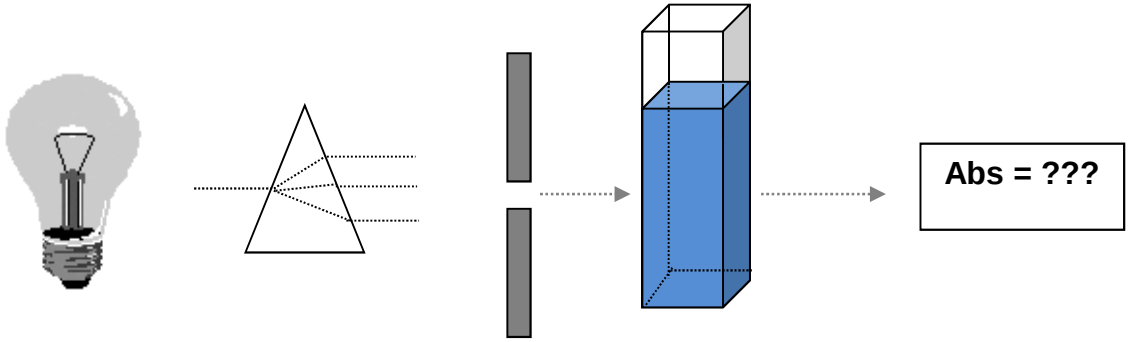
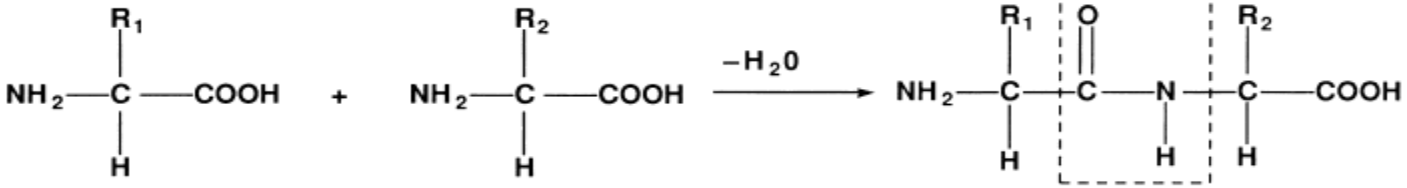




Assays for Total Protein

Yoseph Cherinet (BSc, MSc Asst. Prof)

Biochemistry II Lab

Rationale

- Every function in the living system depends on proteins.
- Various proteins constitute the bulk of the total amount of proteins present in blood.
- Total protein determination is often done to diagnose nutritional problems, liver disease, kidney disease etc

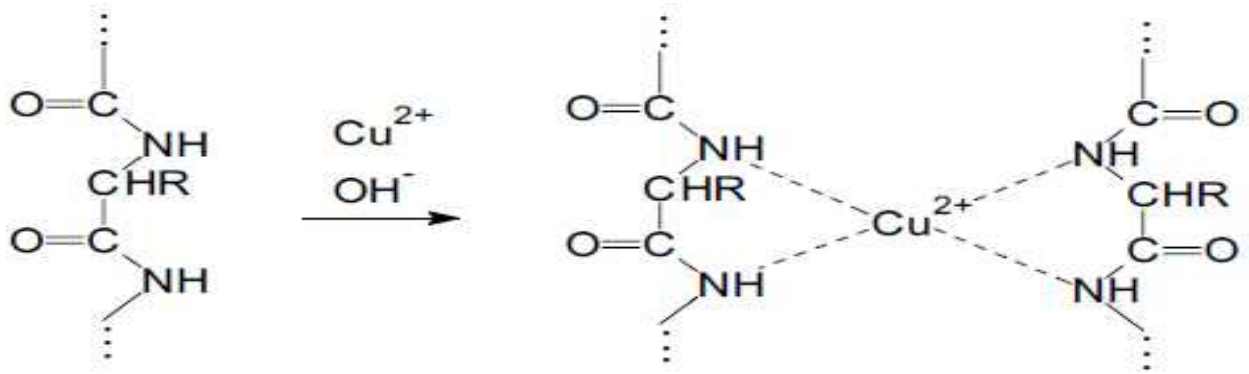
Methods for total protein determination.

- Biuret method
- Direct photometric method
- Dye-binding method
- Turbidimetric /Nephelometric
- Refractometery
- Reagent Strips

1. Biuret method

Principle

- Peptide bonds react with Cu^{2+} ions in alkaline solutions to form a violet colored product.
- The intensity of the color is proportional to the amount of protein present in the reaction. The reaction mixture is measured at 540nm.



Reaction of Cu^{2+} with peptide bonds of protein

Procedure : Sample either serum /plasma

	Blank	Standard	Sample
Biuret reagent	1000ul	1000ul	1000ul
Standard	-----	20ul	-----
Sample	-----	-----	20ul

Mix and incubate the tubes at 25^{O} for 10 minutes and read the absorbance against reagent blank. The color is stable for an hour.

Calculation

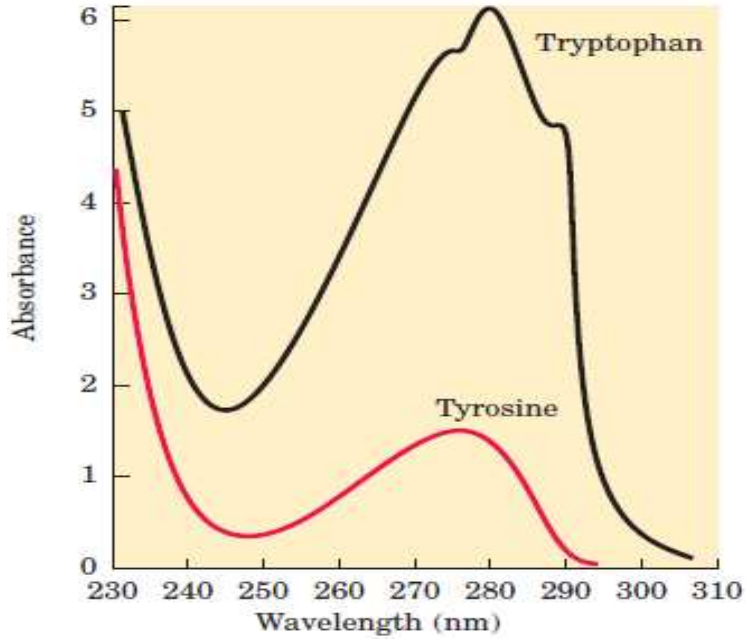
$$C_{test} = \frac{\text{A test X C std}}{\text{A std}}$$

Normal values in g/dl	Dog	Cat	Cow	Horse	Sheep	Goat	Man
	5.5-7.5	5.7-8.0	6.2-8.2	5.7-7.9	5.9-7.8	6.1-7.4	6.6-8.7

2. Direct photometric methods

Principle

- Test relies on direct photometric measurement of samples.
- Aromatic rings of tyrosine and tryptophan absorb UV light at 200-225 nm and 272-290 nm.



3. Dye-binding method

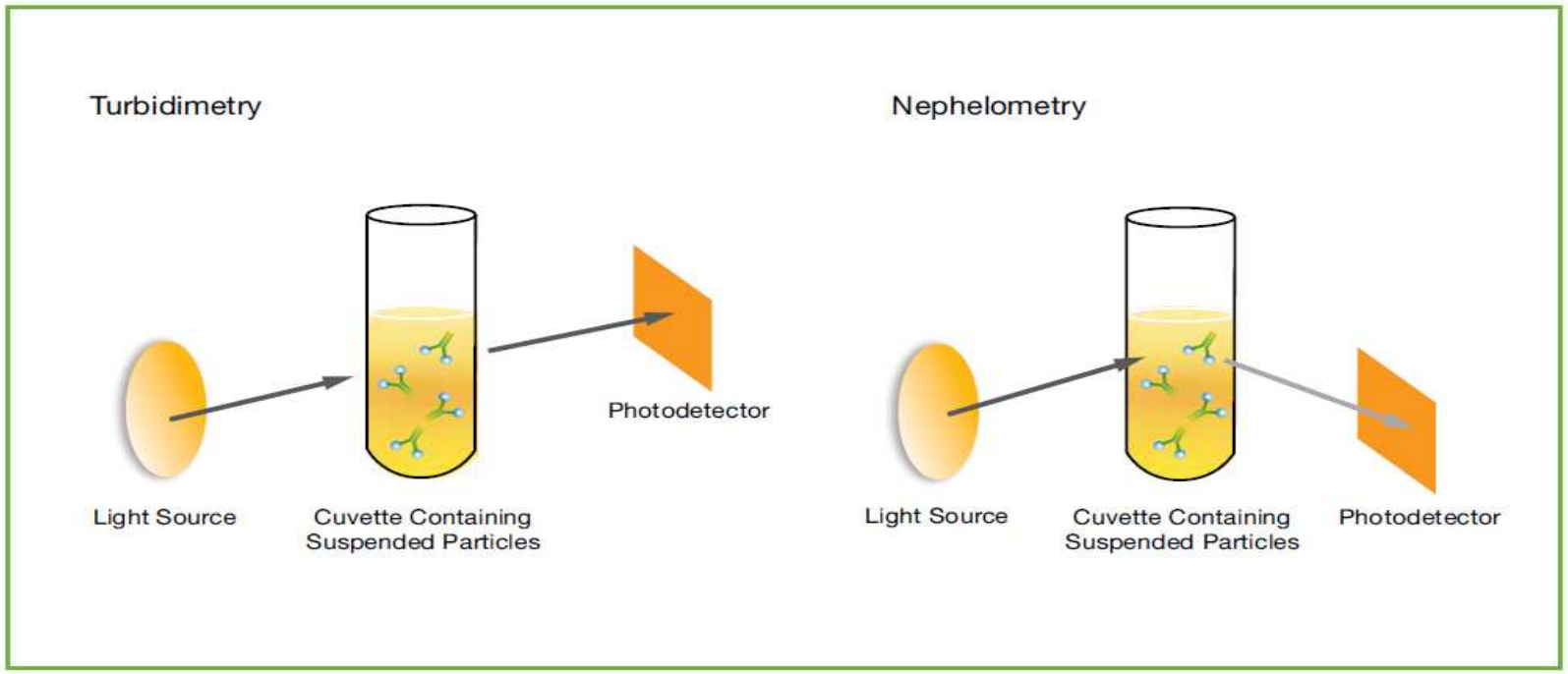
Principle

- Based on the ability of proteins to bind dyes such as amido black 10B and Coomassie Brilliant Blue.
- The color change produced when the dye binds to proteins provides a measure of total protein at 595 nm.

4. Turbidimetric and nephelometric methods

Principle:

- Protein in the sample is precipitated with addition of Sulfosalicylic acid or Trichloroacetic acid to produce turbidity. Degree of turbidity measured with Turbidometeric or nephelometric methods.



5. Refractometry

- This method uses an instrument called refractometer. It measures the refractive index of solutions.
- Refractive index of water is 1.330, if a solute is added the refractive index increases linearly.
- In refractometery, it is assumed that the concentration of other compounds do not vary appreciably from serum to serum & that difference in their refractive index reflects primarily differences in protein.

6. Reagent strips

- Reagent strip testing for protein uses the principle of the protein error of indicators to produce a visible colorimetric reaction.

$$\underset{\text{(Yellow)}}{\text{Indicator}} + \text{Protein} \xrightarrow{\text{pH 3.0}} \text{Protein} + \text{H}^+ \quad \underset{\text{(Blue-green)}}{\text{Indicator} - \text{H}^+}$$

- Proteins carries charge at physiologic PH.
- As the protein concentration increases, the color progresses through various shades of green to blue.