CLS 291 Clinical Hematology 1

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Lecture 2

Haemoglobinometery

Outlines

I. Difference between Manual and Automated procedures.
II. Hemoglobin protein overview.
III. Haemoglobinometry: haemiglobincyanide.
IV. Spectrophotometry.
V. Using the Micropipette.
VI. The purpose of using a blank
VII. Result calculation and interpretation.

Manual VS Automated Analysis

The Hemoglobin Protein

- Hemoglobin (Hb) = globin (protein) + haemochromogen (Fe (II)) complex).
- Function:
  - The primary function of Hb is to transport oxygen (O2) from the lung to tissues, binding and releasing O2 in a cooperative manner.
- Structure:
  - Normal adult hemoglobin A (Hb A) consists of four polypeptide chains, α2β2, each with its own haem group.
- When is the Hb test ordered for a patient? If anemia is suspected.
- Normal ranges:
  - Men 13-17 g/dl
  - Women 12-15 g/dl

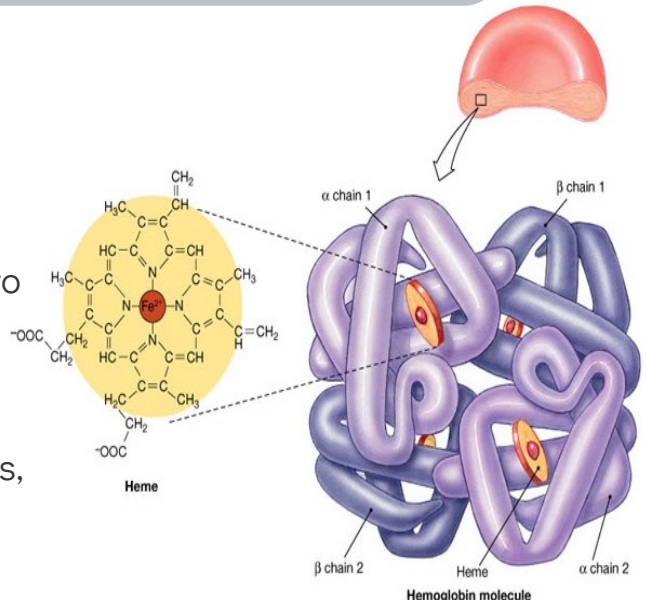


Table 2.3 Normal haemoglobins in adult blood.

	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

Haemoglobinometery

- The hemoglobin concentration (Hb) of a solution may be estimated by measurement of:

  1. It's color.
  2. Determination of its power of combining with oxygen or carbon monoxide.
  3. Analysis of its iron content.

- Two methods are used to measure hemoglobin concentration using a spectrometer:

  1. **Haemiglobincyanide (HiCN; cyanmethaemoglobin) method.**
     - The most commonly used method currently.

  2. **Oxyhaemoglobin (HbO2) method.**
     - Used in point-of-care instruments

  They are color or light-intensity matching techniques.

HAEMIGLOBINCYANIDE (CYANMETHAEMOGLOBIN) METHOD

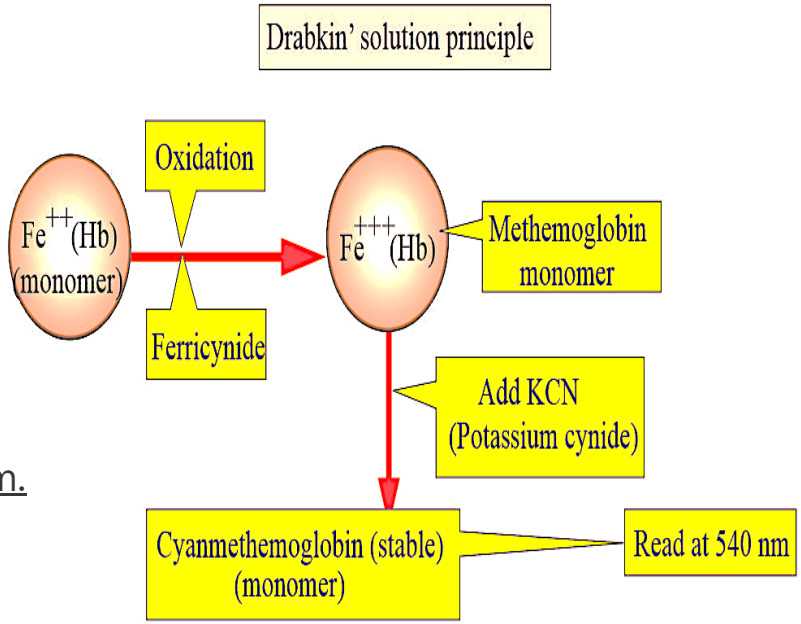
- The haemiglobincyanide (cyanmethaemoglobin) method is the internationally recommended method for determining the haemoglobin concentration of blood.
- The basis of the method is diluting the blood in **Drapkin's solution, which** contains potassium cyanide and potassium ferricyanide.
- The absorbance of the converted cyanhaemiglobin (HiCN) in the solution is then measured in a spectrometer at a wavelength of 540 nm.

Haemiglobincyanide Principle

1. When **blood** is mixed with **Drapkin's solution**, the **potassium ferricyanide** oxidizes the **iron** to form methemoglobin.

2. The **potassium cyanide** then combines with methemoglobin → to form cyanmethemoglobin.

   - **Cyanmethemoglobin** is a stable color pigment read photometrically at a wavelength of 540 nm.

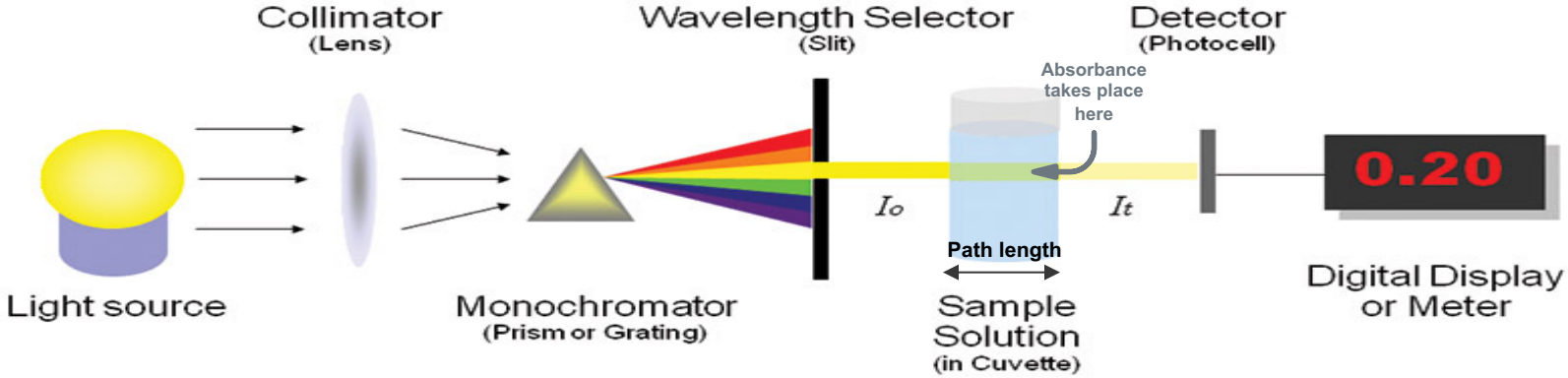


Spectrophotometry

- **A spectrophotometer** is a device used to measure light intensity and can measure either the absorbance or transmittance of light.

Principle:

- A small beam of light with a specific wavelength is emitted from the spectrophotometer, which goes through the sample in a small container called a **cuvette.**
- The spectrophotometer measures how much light is absorbed by the sample or how much of the light passes through the sample, which is transmittance.



Haemiglobincyanide Material

- Material:
  - 10 or 5 ml graduated pipette.
  - P20 or P100 micropipette.
  - Tips.
  - Plastic tube.
  - Rack.
- Sample
  - EDTA whole blood sample.
- Reagent:
  - Diluent of Drapkin's solution

DRABKIN-TYPE REAGENT

Reagent	Amount
Potassium ferricyanide (0.607 mmol/l)	200 mg
Potassium cyanide (0.768 mmol/l)	50 mg
Potassium dihydrogen phosphate (1.029 mmol/l)	140 mg
Nonionic detergent*	1 ml
Distilled or deionised water	To 1 litre

Haemiglobincyanide Procedure

Procedure:

1. In a plastic tube, add 4 ml of the diluent.
2. Invert the whole EDTA blood several times.
3. Aspirate 20 ul (by Micropipette) of the blood sample and add it to the 4 ml of diluent, making the dilution 1/201.
4. Close the tube and mix by inverting the tube 3 times.
5. incubate at room temperature for 5 min. (why do we need to incubate the mixture?)
6. Pour the mixture into a cuvette and read the absorbance using a spectrometer at 540 nm against Drabkin's solution blank.

Haemiglobincyanide Procedure

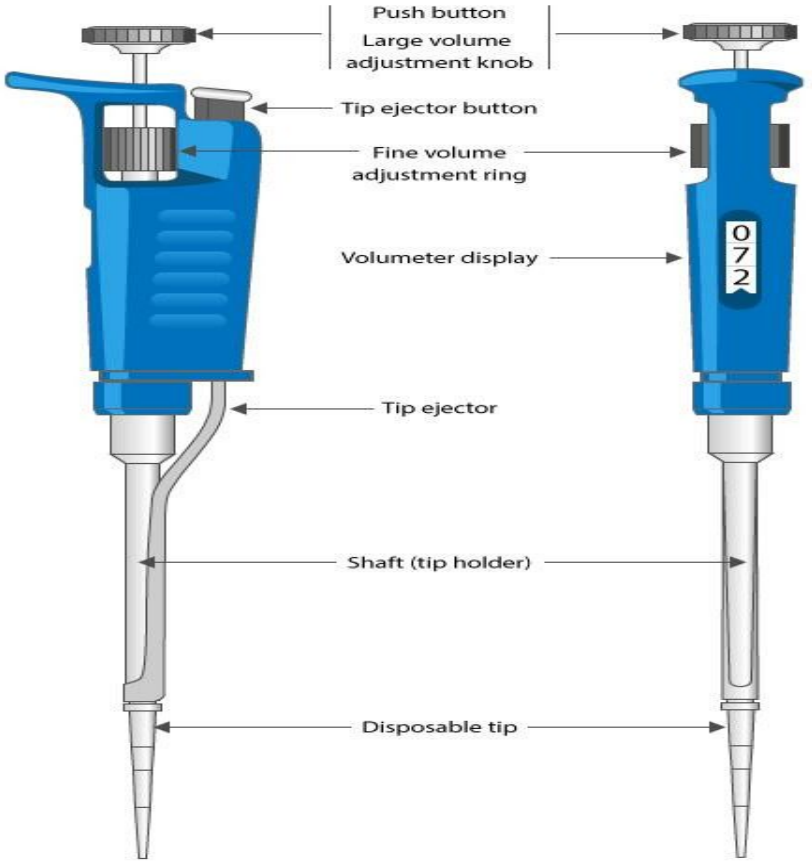
Spectrophotometry:

1. Set the wavelength at 540 nm.
2. Start by blanking using (setting the spectrophotometer set to zero absorbance at 540nm) by using Drabkin's solution as blank.
3. Read the absorbance of your sample and record it.
4. Do your calculation.
5. The optical density (or absorbance) is proportional to the concentration of hemoglobin.

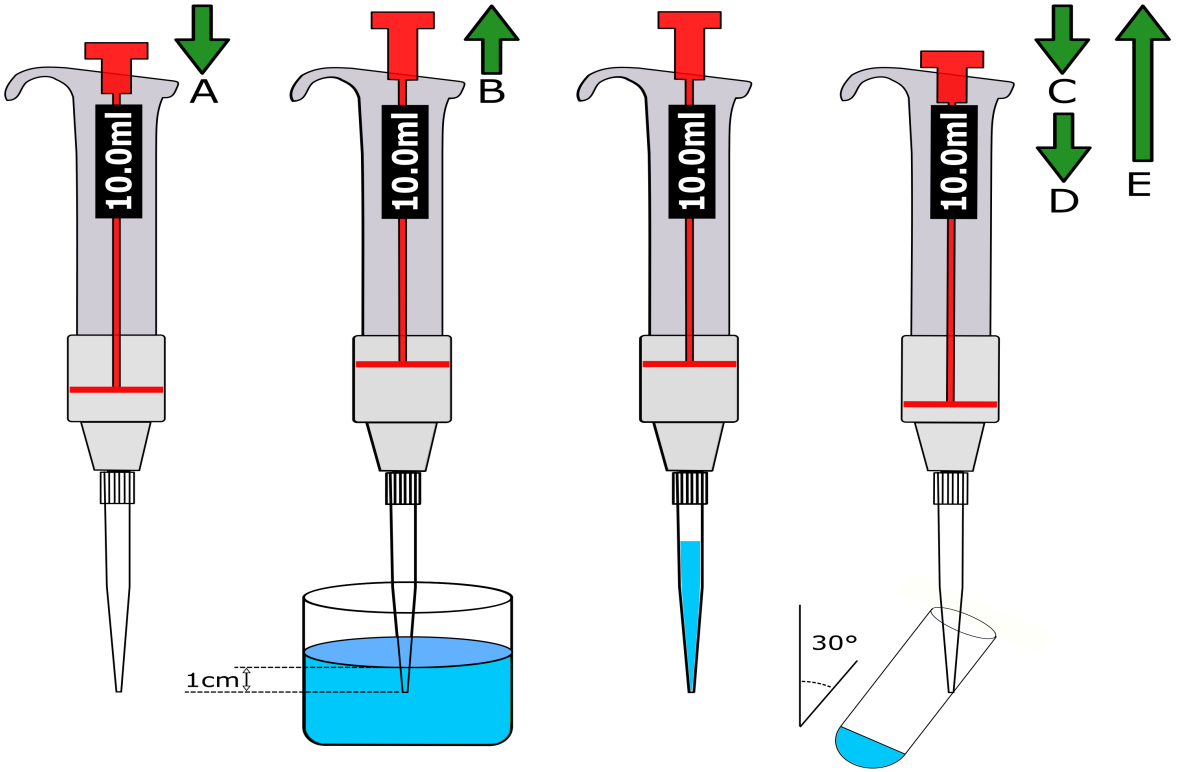
Using Micropipettes

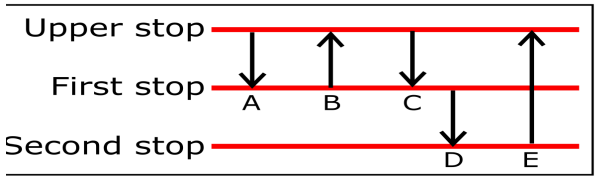
- Micropipettes allow you to measure and dispense small and accurate volumes of liquid solutions.
- A microliter is 1 millionth of a liter or 10^-6 L

Micropipette Model	Volume Range
P10	0.5 -10 ul
P20	2- 20 ul
P100	10 - 100 ul
P200	20 -200 ul
P1000	100 – 1000 ul



Using Micropipettes





Why do we need a Blank?

- In order to measure the absorbance of a particular substance in a reaction mixture, it is necessary to first "zero out" the spectrophotometer such that **only the absorbance of the substance of interest is measured.**

- This is done with a blank which is a cuvette that contains all the carrier solvents (reagents) **EXCEPT the substance of interest (analyte).**

Result calculation

- Use the following formula:

$$\text{Hb in g/dl} = \frac{Ax\ 64500\ x\ DIL}{44\ x\ D\ x\ 1000\ x\ 10}$$

- $= A \times 29.3$

- A = Reading of absorbance of Hb solution 64500 = Molecular wt. of Hb
- 44 = Millimolar coefficient extinction
- D = Thickness of cuvette = 1
- 1000 = Conversion factor of mg to g
- DIL = Dilution Factor = 201 (total volume (solvent+ solute)/ solute)
- 10= to convert from g/l to g/dl

Result Interpretation

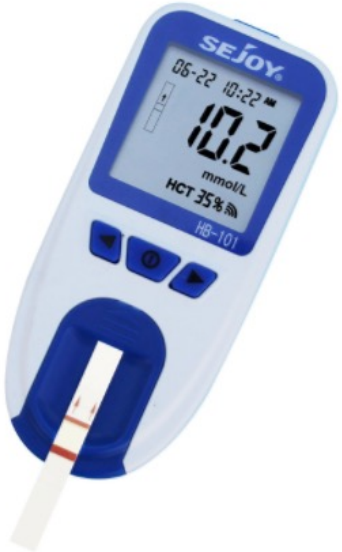
- The result is expressed as g/dl and should know if below, within, or above the normal range.
- Normal ranges:
  - Men 13-17 g/dl
  - Women 12-15 g/dl
- Low Hemoglobin Level indicates:
  - Anemia (of several causes).
- High Hemoglobin Level indicates:
  - Polycythemia.
  - Smoking.

Advantages and Disadvantages of Haemiglobincyanide

- Advantages:
  - Convenient for sample batching, as reading is not needed to be done immediately after dilution.
  - All forms of hemoglobin, except SHb, are readily converted to HiCN.

- Disadvantage:
  - The use of cyanide compounds which are considered hazardous.

Automated Hemoglobin Measurement



- Point of care testing

- Complete blood count (CBC)