Hb (Sahli's Method):

Hb + HCl ⟶ Acid hematin $\xrightarrow[\text{match colour}]{\text{diluted to}}$ Hb reading on tube

Requirements: N/10 HCl, Stirrer, distilled water

→ Take N/10 HCl upto 2 marks
→ Suck blood into Hb pipette till 20 cu mm.
→ Blood in pipette transfered to tube with N/10 HCl.
→ Leave for 10 min
→ Add distilled water drop-by-drop
(Reading: lower meniscus)
→ 3 readings

Oxygen Carrying Capacity:

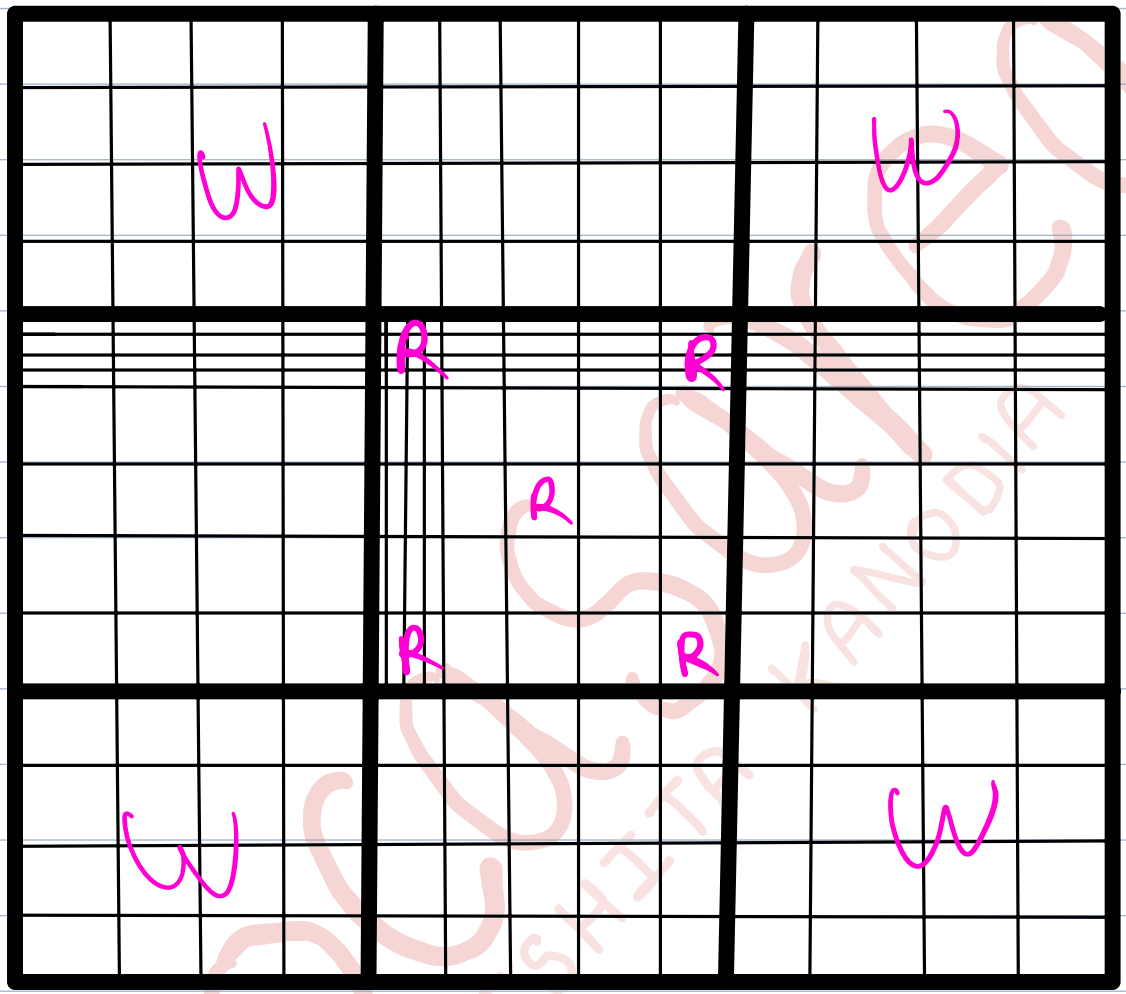
1 g Hb ⟶ 1.34 mL O_2

12 g ⟶ 12 × 1.34 mL O_2 / dL

- Other methods – gasometric
  – spectrophotometric
- Most accurate method ⇒ cyanometh-haemoglobin.

Haemocytometer:

Counting Chamber = 3mm x 3mm



RBC Count:

→ RBC pipette to be filled with blood till 0.5
→ Diluting fluid upto 101.

Principle: number of red cells is very high ∴ blood is diluted 200 times

Normal Values: Adult male: 4.5 – 6 million / mm^3
Adult female: 4 – 5.5 million / mm^3
Newborns ⇒ 6–8 million/mm^3

Hayem's Fluid:

NaCl – 0.5 g
Na_2SO_4 – 2.5 g
} maintain isotonicity & prevent roleaux formation

Mercuric chloride – 0.25 g ⇒ prevents microbial growth

Distilled water (100 mL) ⇒ solvent media

Calculation:

- Volume of central RBC square = $1mm \times 1mm \times \frac{1}{10} mm = \frac{1}{10} mm^3$
- Volume of medium square = $\frac{1}{5} mm \times \frac{1}{5} mm \times \frac{1}{10} mm = \frac{1}{250} mm^3$.
- Volume of 1 small square = $\frac{1}{20} mm \times \frac{1}{20} mm \times \frac{1}{10} mm = \frac{1}{4000} mm^3$
- Volume of 5 medium squares = $5 \times \frac{1}{250} = \frac{1}{50} mm^3$
- Total no. of cells = N
  [N cells in $\frac{1}{50} mm^3$ of diluted blood]

In $1mm^3$ of diluted blood, no. of cells = 50 N

Dilution = 1 : 200

∴ No. of red cells in $1mm^3$ of undiluted blood = $N \times 50 \times 200$
= 10,000 N.

- Physiological
  - Increase – high altitude – newborns – excessive sweating
  - Decrease – pregnancy – women < men
- Pathological
  - Increase – hypoxia
  - Decrease – anemia

WBC Count:

→ WBC pipette to be filled with blood till 0.5
→ Turk's fluid till 11.

Principle: WBC count is low compared to RBCs

∴ dilution factor = 20.

Turk's fluid:

- Glacial acetic acid (1%) ⇒ causes destruction of RBC & Swelling of WBC nuclei
- Gentian violet (0.3% w/v) ⇒ stains WBC nuclei
- Distilled water ⇒ solvent

Calculation:

Volume of each small WBC square $= \frac{1}{4}\text{ mm} \times \frac{1}{4}\text{ mm} \times \frac{1}{10}\text{ mm} = \frac{1}{160}\text{ mm}^3$.

Volume of 16×4 (64) such squares $= 64 \times \frac{1}{160} = \frac{2}{5}\text{ mm}^3$

Total WBC's in 64 squares = N

In 1mm^3 of diluted blood, no. of WBCs $= \frac{5N}{2}$

In 1mm^3 of undiluted blood, no. of WBCs $= \frac{5N}{2} \times 20 = \underline{\underline{50N}}$.

Normal count:

Adults: 4,000 – 11,000 cells/mm^3
Newborns: 10,000 – 25,000 cells/mm^3
Infants: 6,000 – 18,000 cells/mm^3

Physiological

- Increase – newborns, pregnancy, physical exercise
- Decrease – very rare (on exposure to severe cold)

Pathological Increase – bacterial infection, inflammation, tissue injury
Pathological Decrease – typhoid fever, starvation, aplastic anemia.

Differential Leukocyte Count:

Leishmann's Stain:

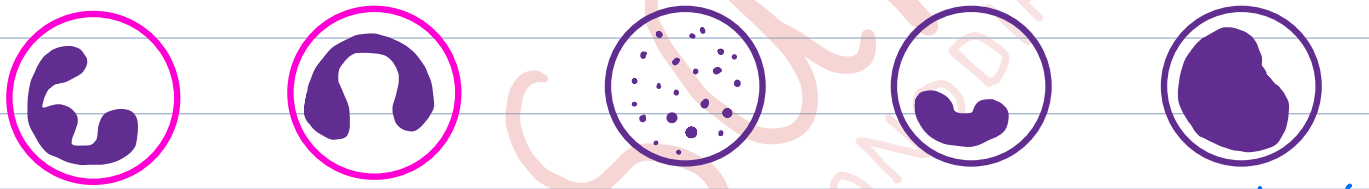
Eosin : acidic dye which stains basic part of cell

Methylene blue : basic dye which stains acidic part of cell

Acetone free methyl alcohol : fixative

Fixation time : 2 minutes

Staining time : 10 minutes



Neutrophil | Eosinophil | Basophil | Monocyte | Lymphocyte

Normal Leucocyte Count:

N : 60 - 70%

E : 1 - 4%

B : 0 - 1%

L : 25 - 30%

M : 5 - 10%

Neutrophilia	Neutropenia
Physiological: Pregnancy, Emotional Stress	Physiological: rare (on exposure to severe cold)
Pathological: Acute haemorrhage, Muscle trauma	Pathological: starvation, aplastic anemia

Eosinophilia	Eosinopenia	Basophilia	Basophilopenia
Allergy	ACTH therapy	CML	Aplastic anemia
Hodgkin's disease	Aplastic anemia	Polycythemia	
Parasitic infestation	Cushing's disease		

Lymphocytosis	Lymphocytopenia	Monocytosis	Monocytopenia
Chronic infections	ACTH therapy	Protozoan diseases	Bone marrow failure
Leukaemia	Hodgkin's disease	ACTH therapy	Aplastic anemia.
		Hodgkin's disease	

Blood Groups:

Landsteiner Law: It states that:

- If an agglutinogen is present on RBC membrane of an individual, the corresponding aglutinin must be absent in plasma
- If an agglutinogen is absent on RBC membrane of an individual, the corresponding aglutinin must be present in plasma.

(Applicable only to ABO blood group system).

Different Blood Group systems:

ABO system	Kell system	Ii system
Rh system	Duffy system	
MN system	Lewis system	

Bleeding Time: (Duke's Method) (other method = IVY method)

Normal Range = 1-5 minutes

Bleeding time = No. of drops on filter paper x 30 s.

Bleeding Time: time interval b/w onset of bleeding & spontaneous arrest of bleeding

Prolonged BT: thrombocytopenia
thrombasthenia
blood vessel fragility

(BT is an indicator of vasoconstriction & platelet plug formation)

Clotting Time:

- Capillary Tube method
- Lee-white method (venepuncture)

Break a piece after 2 mins first.
Thereafter, break after every 15 seconds.

Normal range = 2-8 mins

Clotting Time: time interval b/w onset of bleeding & clot formation

Hemophilia

A: deficiency of clotting factor VIII
B: " " " " IX
C: " " " " XI
D: " " " " V

CT is an indicator of formation of blood clot & fibrinolysis.

BT ↑ , CT ≈ ⇒ platelet ✗

BT ≈ , CT ↑ ⇒ clotting factor ✗

BT ↑ , CT ↑ ⇒ Von Willebrand factor ✗