

BLOOD PRACTICAL

CBC & ESR

BY

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Aims of the Practical

1. Counting Red blood cells.
2. Counting White blood cells.
3. Determination of hemoglobin concentration.
4. Determination of packed cell volume (PCV) hematocrit.
5. Calculation of red blood cell indices.
6. Determination of ESR



Objectives (CBC)

At the end of this lesson the student should be able to:

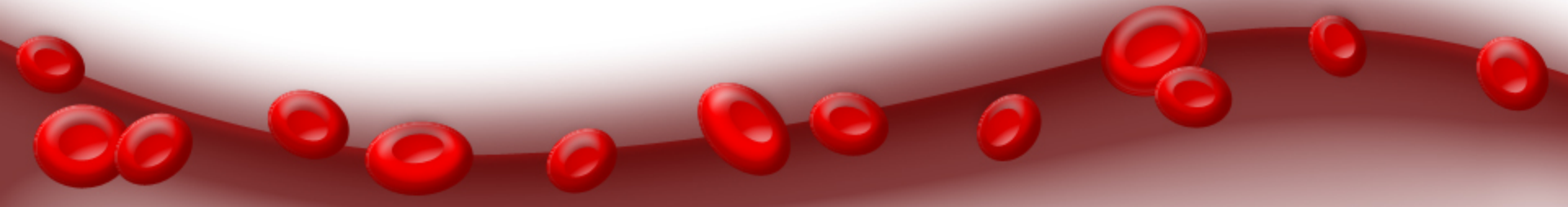
1. Recognize the method used to measure the different hematological values, and compare it with the normal values.
2. Do the calculation of indices, their normal values and their importance in diagnosis of different types of anemia.



Objectives (ESR)

At the end of this lesson the student should be able to:

1. To know how to measure the erythrocyte sedimentation rate.
2. To recognize what is the clinical value of these measurements.



Complete Blood Count (CBC)

It is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood.



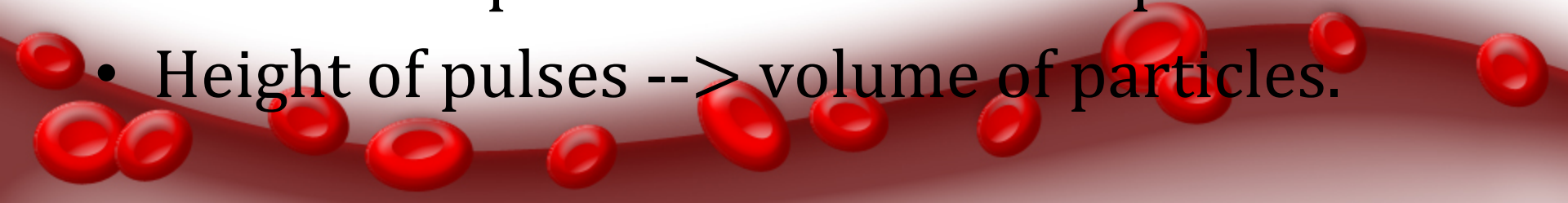
TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
CBC With Differential/Platelet					
WBC	5.7		x10E3/uL	4.0-10.5	01
RBC	5.27		x10E6/uL	4.10-5.60	01
Hemoglobin	15.4		g/dL	12.5-17.0	01
Hematocrit	44.1		%	36.0-50.0	01
MCV	84		fL	80-98	01
MCH	29.2		pg	27.0-34.0	01
MCHC	34.9		g/dL	32.0-36.0	01
RDW	13.7		%	11.7-15.0	01
Platelets	268		x10E3/uL	140-415	01
Neutrophils	47		%	40-74	01
Lymphs	46		%	14-46	01
Monocytes	6		%	4-13	01
Eos	1		%	0-7	01
Basos	0		%	0-3	01
Neutrophils (Absolute)	2.6		x10E3/uL	1.8-7.8	01
Lymphs (Absolute)	2.6		x10E3/uL	0.7-4.5	01
Monocytes (Absolute)	0.4		x10E3/uL	0.1-1.0	01
Eos (Absolute)	0.1		x10E3/uL	0.0-0.4	01
Baso (Absolute)	0.0		x10E3/uL	0.0-0.2	01
Immature Granulocytes	0		%	0-1	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01

Coulter Counter



Coulter Counter Principle

- It count and measure the size of the cells by **detecting and measuring electrical resistance when a liquid pass through aperture.**
- While passing the aperture, the cells impedes the current and causes a measurable pulse.
- Number of pulses --> number of particles.
- Height of pulses --> volume of particles.



Materials and methods

- Coulter analyzer
- Diluent reagents
- Lytic reagent
- Calibrator kit
- EDTA anticoagulant blood



Diluent – Reagent

Is an isotonic electrolyte solution that:

- Dilute the whole blood sample
- Stabilize cell membrane for accurate counting and size
- Conduct aperture current
- Rinse instrument components between analysis
- Prevent duplicate cell counts

Lytic reagent

Lysis RBCs for WBCs count and hemoglobin measurements

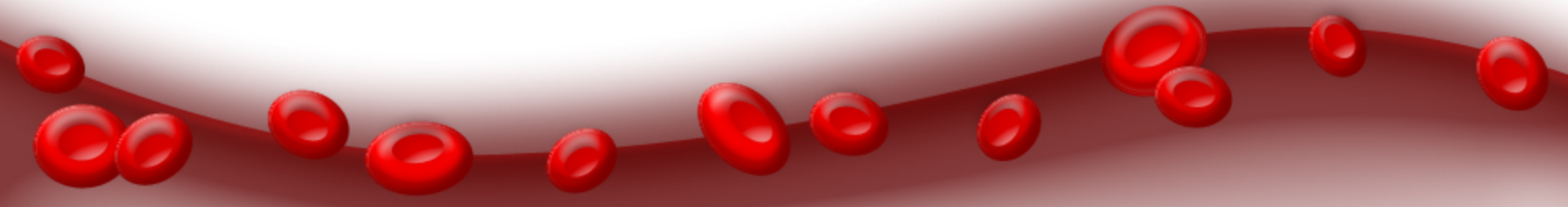
Calibrator Kit

Is an alternative to to the whole blood reference method for calibration



RBC, WBC cell count & HB

- 5ml of venous blood will be drawn in EDTA anticoagulant tube.
- Diluted by the reagent I and used to count **RBC**.
- Lysing RBC using reagent II and used for counting **WBC** and **Hb**.



Normal values

	Male	Female	Average
RBC	4.5-6.5 x10 ⁶ / μl	3.8-5.8 x10 ⁶ / μl	4.7-6.5 x10 ⁶ /μl
WBC	4 – 11 x10 ³ / μl	4 – 11 x10 ³ / μl	4 – 11 x10 ³ / μl
HB	13-18 g/dl	11.5-16.5 g/ dl	13 –18 g/dl
Platelet	150-400x10 ³ /μl	150-400x10 ³ /μl	150-400x10 ³ /μl

Clinical terms

1. ↓ RBC = aneamia
2. ↑ RBC = polycythemia
3. ↓ WBC = leucopenia
4. ↑ WBC = leucocytosis
5. ↓ Platelets = thrombocytopenia
6. ↑ Platelets = thrombocytosis



Clinical applications

Low numbers of RBCs may indicate:

- Blood loss:
 - Anemia (various types).
 - Hemorrhage.
- Bone marrow failure (for example, from radiation, toxin, fibrosis, tumor).
- Erythropoietin deficiency (secondary to renal disease).
- Hemolysis (RBC destruction).



High numbers of RBCs may indicate:

- Low oxygen tension in the blood
 - Congenital heart disease
 - Cor pulmonale
 - Pulmonary fibrosis
- Polycythemia vera.
- Dehydration (such as from severe diarrhea).
- Renal (kidney) disease with high erythropoietin production.



High numbers of WBCs (Leucocytosis) may indicate:

- Infectious diseases.
- Inflammatory disease (such as rheumatoid arthritis or allergy).
- Leukemia.
- Severe emotional or physical stress.
- Tissue damage (burns).



Low numbers of WBCs (leukopenia) may indicate:

- Bone marrow failure (for example, due to infection, tumor or fibrosis).
- Presence of cytotoxic substance.
- Autoimmune/collagen-vascular diseases (such as lupus erythematosus).
- Disease of the liver or spleen.
- Radiation exposure.



Platelet count:

Thrombocytosis :

- Chronic myeloid leukaemia.

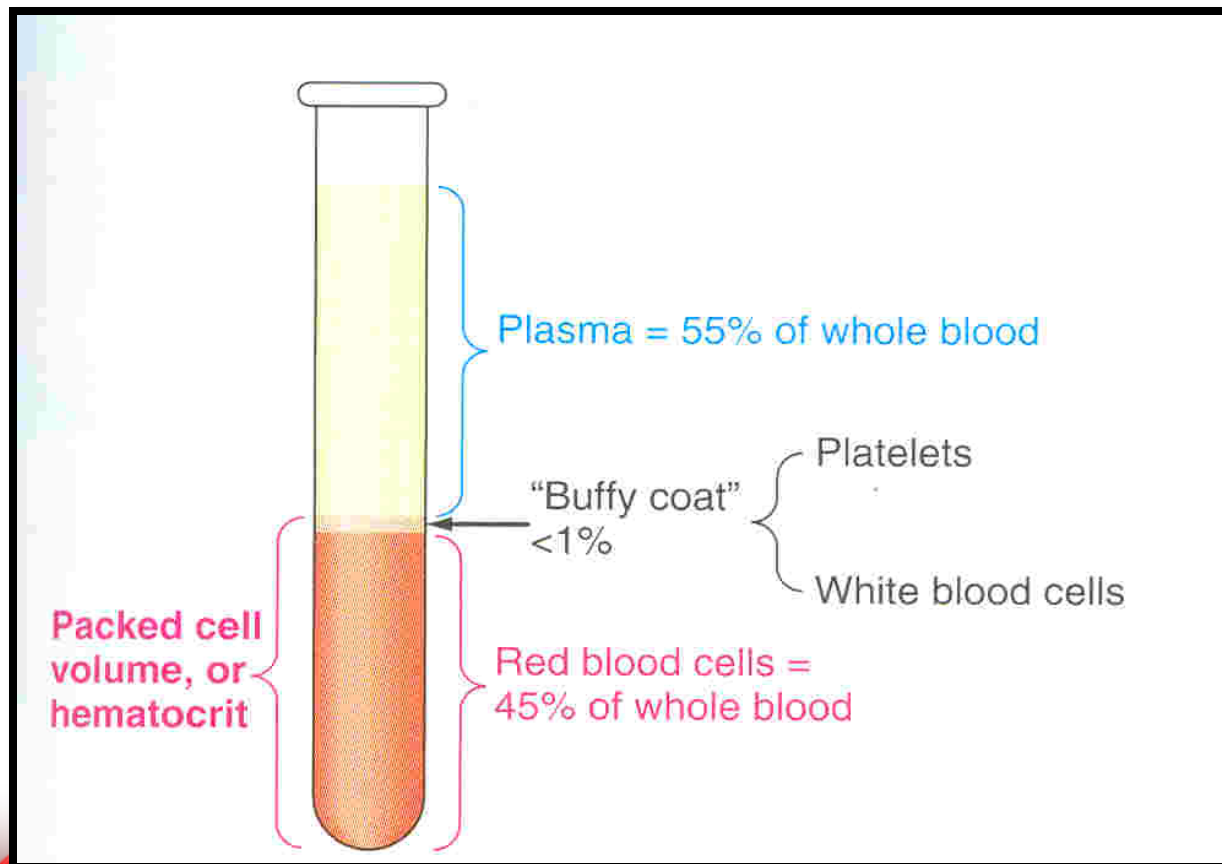
Thrombocytopenia :

- A plastic anemia.
- Chemotherapy.



Packed Cell Volume (PCV) Hematocrit

The ratio of packed blood cells volume to plasma.

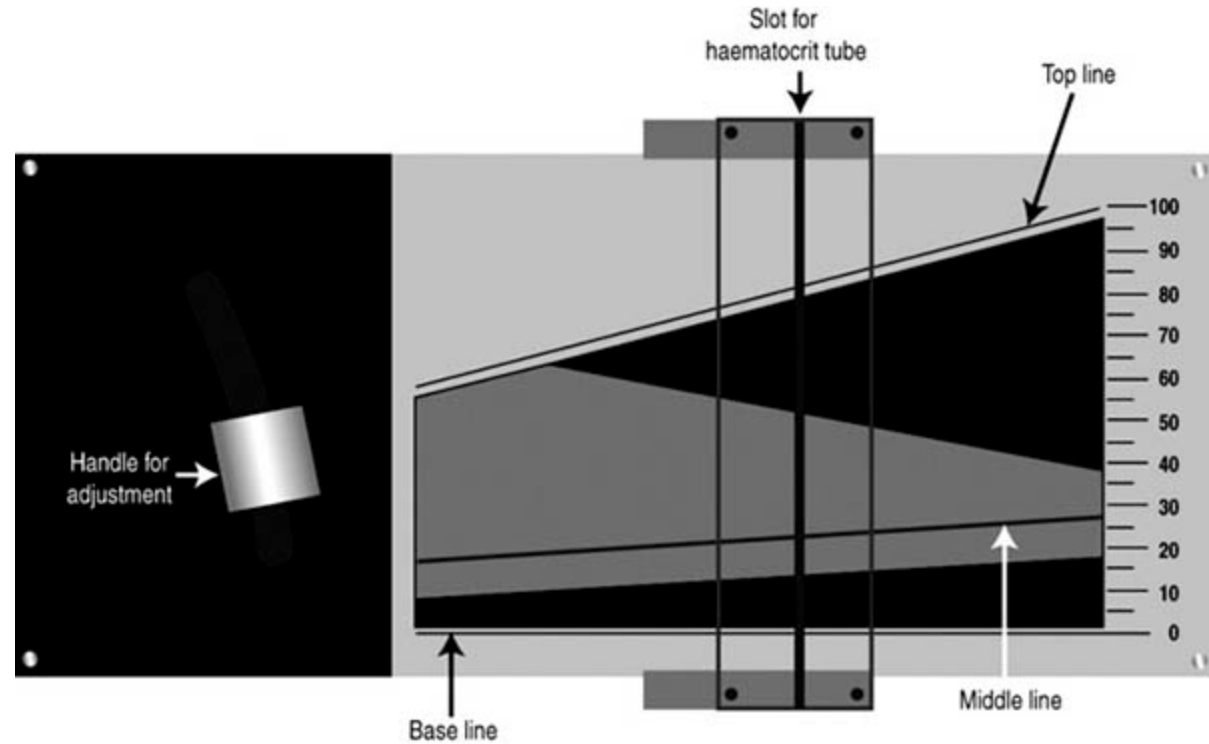


Procedure

- Capillary blood obtained from pricking finger tip after cleaning it with alcohol
- Fill a heparinised capillary tube, then seal one end by plasticine.
- Centrifuge for 5 minutes to packed the cells at one end of the tube leaving a clear plasma on top.
- Use the hematocrit reader to find the packed cell volume.



Haematocrit Reader



Normal value PCV

	Male	Female	Average
PCV %	40-54	35-47	35-54



Clinical applications

High hematocrit may indicate:

- Dehydration
 - Burns
 - Diarrhea
- Polycythemia vera.
- Low oxygen tension (smoking, congenital heart disease, living at high altitudes).



Low hematocrit may indicate:

- Anemia (various types).
- Blood loss (hemorrhage).
- Bone marrow failure (for example, due to radiation, toxin, fibrosis, tumor).
- Hemolysis (RBC destruction) related to transfusion reaction.
- Leukemia.



The calculation of Red Blood Indices

1. Mean cell volume (MCV)

- The average volume of red blood cell measured by femtoliters(fl)

- $$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count}} = 85 \pm 8 \mu\text{m}^3 \text{ (fl)}$$

- ↓ MVC : microcytes

- ↑ MCV : macrocytes

2. Mean cell hemoglobin (MCH)

– The average weight of Hb in red cells measured by picogram (pg)

$$\text{MCH} = \frac{\text{Hb} \times 10}{\text{RBC count}} = 29.5 \pm 2.5 \text{ pg}$$

-↓ MCH : hypochromic

-↑ MCH : hyperchromic

3. Mean cell Hb concentration (MCHC)

- Concentration of Hb per 100 ml of RBC
- $$\text{MCHC} = \frac{\text{Hb} \times 100}{\text{PCV}} = 33 \pm 3 \text{ g/dl}$$

Normal values

	Average
MCV	78-98 μm^3
MCH	27-32 pg
MCHC	30-35 g/dl

Types of Anemia

	Case A	Case B
RBC	Low	Low
HB	Low	Low
PCV	Low	Low
MCV	Low	high
MCH	Low	N/ high
MCHC	Low	N/low
Type of anemia	Microcytic Hypochromic	Macrocytic megaloblastic
Cause	Iron deficiency	Vit B12 or Folic deficiency

Erythrocyte Sedimentation Rate (ESR)

- Is the rate at which red blood cells sediment in a period of 1 hour.
- non-specific measure of inflammation.

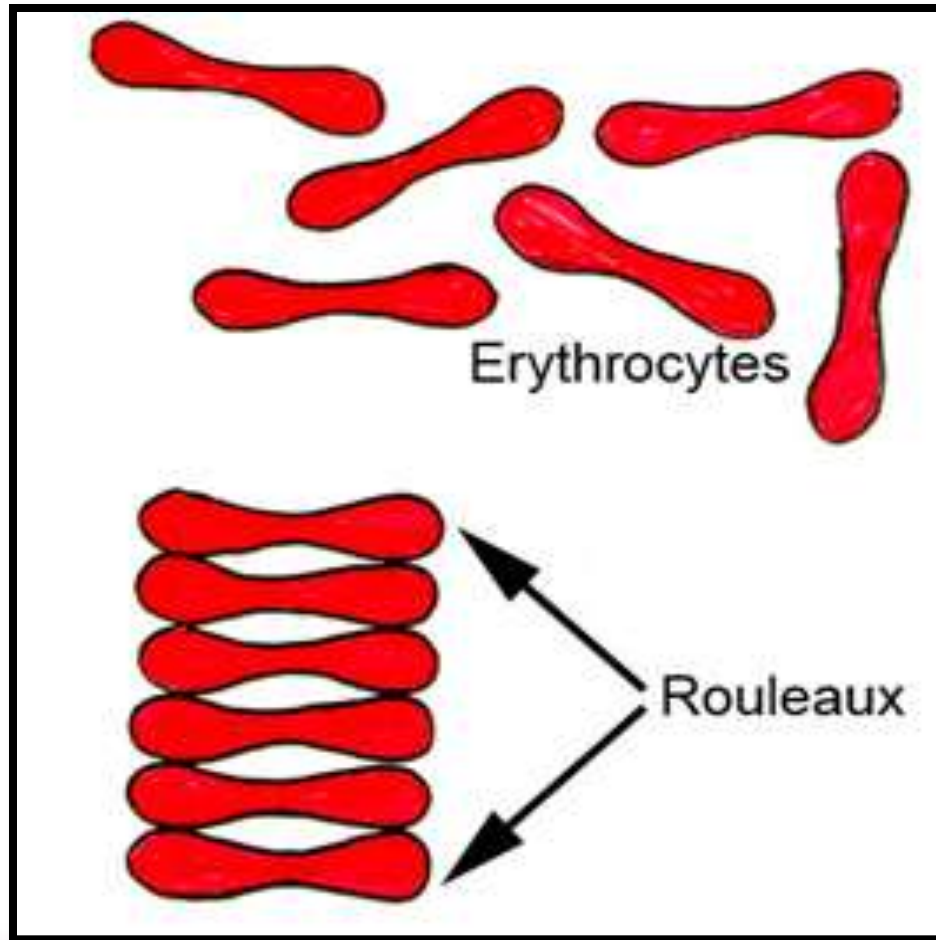


RBC Sedimentation

- Is controlled by the balance between plasma protein fibrinogen, and the negative charge of the erythrocytes.
- In inflammatory, the high fibrinogen level causes RBCs to stick to each other to form stacks (rouleaux), which settle faster.



Rouleaux formation



Material and methods

- Westergren's sedimentation apparatus.
- Anticoagulant (EDTA).
- Disposable sterile syringes and needles.



Westergren apparatus



Procedure

1. Using a sterile syringe draw 1.6ml of blood from a suitable vein. Transfer it to a test tube containing EDTA and then draw up blood into a Westergren tube exactly to the zero mark.
2. Place the tube upright in the stand and leave undisturbed. The height of the column of clear plasma at the top of the tube is noted at the end of an hour and again at the end of 2nd hours.



ESR Results

- Normal ESR in male = 3-5 mm/ 1st hour and 7-15mm/2nd hour. In females values are slightly higher.
- Moderately elevated ESR occurs: infections, inflammation, anemia, malignancies, pregnancy, and old age.
- A very high ESR associated with **multiple myeloma, polymyalgia Rheumatic, temporal arteritis.**



Clinical application of ESR

- Nonspecific test.
- Prognostic not diagnostic.
- Monitor disease activity and response to therapy.
- ESR is a nonspecific marker of inflammation and is affected by other factors, ESR results must be used along with other clinical findings.



C-reactive protein & ESR

- C-reactive protein is an acute phase protein produced by the liver during an inflammatory reaction.
- Since C-reactive protein levels in the blood rise more quickly after the inflammatory or infective process begins, ESR is often replaced with C-reactive protein measurement.



Thank you ... 