HEMATOLOGY
I- Blood picture
(Complete blood count)

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Hematology may be defined as the scientific study of the structure and function of the blood in health and disease. Hematology therefore is a laboratory science in which we quantitatively and qualitatively observe the different components of blood in order to diagnose a great variety of diseases.

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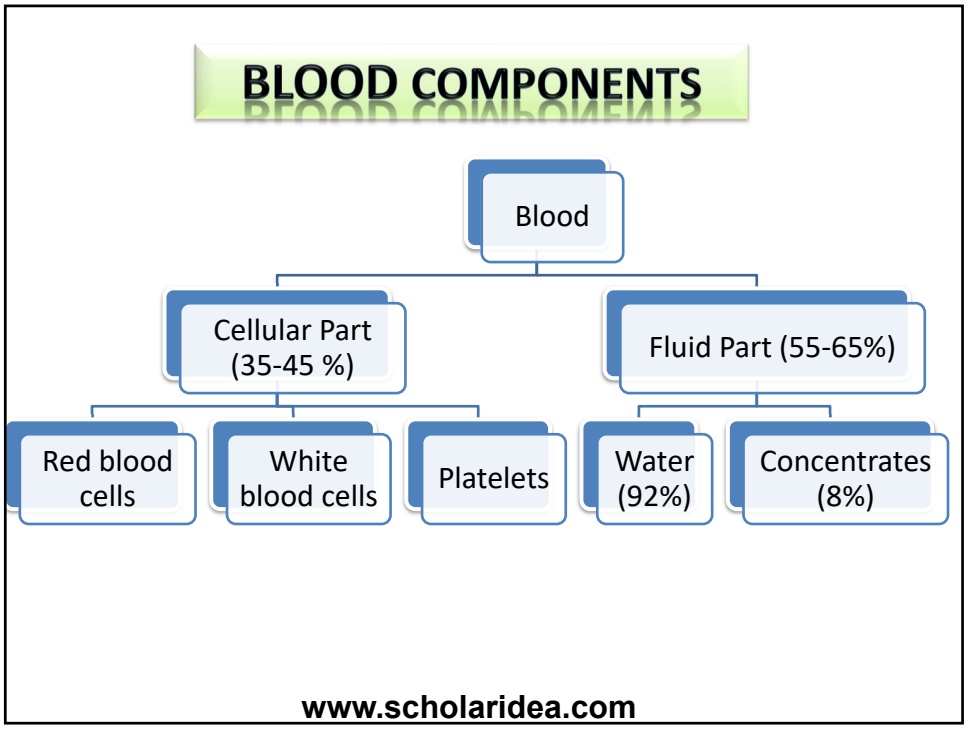

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Blood Picture
Complete Blood Count (CBC)
Hemogram

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Component	Your Value	Standard Range	Units	Flag
White Blood Cell Count	5.4	4.0 - 11.0	K/uL	
Red Blood Cell Count	5.20	4.40 - 6.00	M/uL	
Hemoglobin	16.0	13.5 - 18.0	g/dL	
Hematocrit	47.2	40.0 - 52.0	%	
MCV	91	80 - 100	fL	
MCH	30.8	27.0 - 33.0	pg	
MCHC	33.9	31.0 - 36.0	g/dL	
RDW	12.7	<16.4 -	%	
Platelet Count	149	150 - 400	K/uL	L
Differential Type	Automated			
Neutrophil %	56	49.0 - 74.0	%	
Lymphocyte %	23	26.0 - 46.0	%	L
Monocyte %	13	2.0 - 12.0	%	H
Eosinophil %	7	0.0 - 5.0	%	H
Basophil %	1	0.0 - 2.0	%	
Abs. Neutrophil	3.1	2.0 - 8.0	K/uL	
Abs. Lymphocyte	1.2	1.0 - 5.1	K/uL	
Abs. Monocyte	0.7	0.0 - 0.8	K/uL	
Abs. Eosinophil	0.4	0.0 - 0.5	K/uL	
Abs. Basophil	0.0	0.0 - 0.2	K/uL	

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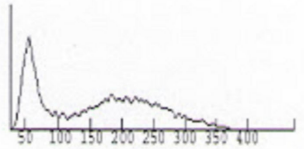
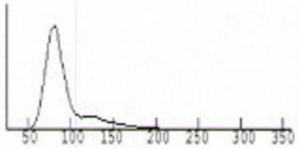
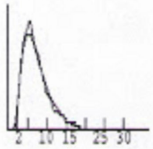
			Patient Limits 3	
WBC	7.2	x10^3/uL	4.5	10.5
LY	33.4	%	20.5	51.1
MO	6.4 3	%	1.7	9.3
GR	60.2 3	%	42.2	75.2
LY#	2.4	x10^3/uL	1.2	3.4
MO#	0.5 3	x10^3/uL	0.1	0.6
GR#	4.3 3	x10^3/uL	1.4	6.5
RBC	5.73	x10^6/uL	4.00	6.00
Hgb	15.0	g/dL	11.0	18.0
Hct	47.2	%	35.0	60.0
MCV	82.5	fL	80.0	99.9
MCH	26.2 L	pg	27.0	31.0
MCHC	31.8 L	g/dL	33.0	37.0
RDW	13.3	%	11.6	13.7
Plt	246.	x10^3/uL	150.	450.
MPV	7.0 L	fL	7.8	11.0



WBC HISTOGRAM



RBC HISTOGRAM



PLT HISTOGRAM

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Types of blood samples

Whole blood —Sample→ Blood + Anticoagulant

Serum —Sample→ Blood without Anticoagulant

Plasma —Sample→ Whole blood

Blood smear —Sample→ Whole blood or drop of blood

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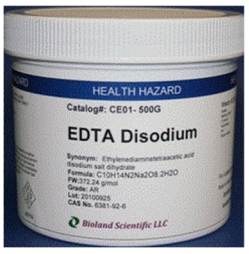
Anticoagulants

Ethylene Diamine Tetra-acetic acid (EDTA)

Dose: 1mg/ml blood



Mode: Binding ionized calcium

Advantages:

- ✓ Hematological analysis.
- ✓ No effect on leukocyte staining affinity.
- ✓ Preserve the blood sample for 24 hours.

Disadvantage

- ❖ Higher concentration of salt withdraws water from red cells and reduces PCV values.

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Haemolysis

It means the breakdown of the RBCs.

- **Using wet needle or syringe.**
- **Collection of the blood sample directly to the bottom of the tube.**
- **Vigorous mixing of the blood sample.**
- **Excessive negative pressure when collecting sample with a syringe will rupture cells and collapse the vein.**
- **Failure to remove the needle from the syringe, when transferring blood from a syringe to a container.**
- **Extreme heat or cold.**

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Clotting of blood samples

It means formation of clots in the whole blood sample.

- **Delay in mixing the blood sample with the anticoagulant.**
- **The amount of the collected blood sample is larger than the concentration of the anticoagulant.**
- **Taking long time in collection of the blood sample.**

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Evaluation of the red blood cells (RBCs)
Erythrocytes picture

Erythrocytes morphology

RBCs count

Mean corpuscular values

Packed cell volume

Hemoglobin concentration

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Evaluation of the White blood cells (RBCs)
WBCs picture

Total WBCs count

Differential leucocytes count

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Evaluation of Platelets (RBCs)
Platelets picture

Platelets (Thrombocytes) count
Mean platelet volume (MPV)
Plateletcrit (PCT)
Platelet distribution width (PDW)

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Evaluation of the red blood cells (Erythrocyte picture)

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Test description	Observed value	Unit	Reference range
Erythrocytes			
Total count	4.21	$\times 10^6/\mu L$	3.8–5.4
Hemoglobin	9.6	g/dL	10.5–14.0
PCV (hematocrit)	30.1	%	32–42
MCV	71.5	fL	72–88
MCH	22.8	pg	24–30
MCHC	31.9	g/dL	32–36
Leucocytes			
Total leucocyte count	11,700	%	4400–11,300
Neutrophils	31	%	45–74
Lymphocytes	66	%	22–50
Basophils	00	%	0–1
Eosinophils	02	%	0–4
Monocytes	01	%	1–8
Platelets			
Total count	840	$\times 10^3/\mu L$	10–400
MPV	7.7	fL	8–12
PDW	8.8	fL	9–14

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Blood Picture
Complete blood count (CBC)

- RBCs count (/mm^3 or T/l)
- Hemoglobin concentration (g/dl or g/l)
- Packed cell volume (PCV) or Hematocrit (%)
- Erythrocytes morphology
- Determination of Mean corpuscular values (MCV, MCH, MCHC)
- Total WBCs count (/mm^3 or G/l)
- Differential leucocytes count
- Platelets (Thrombocytes) count (/mm^3)

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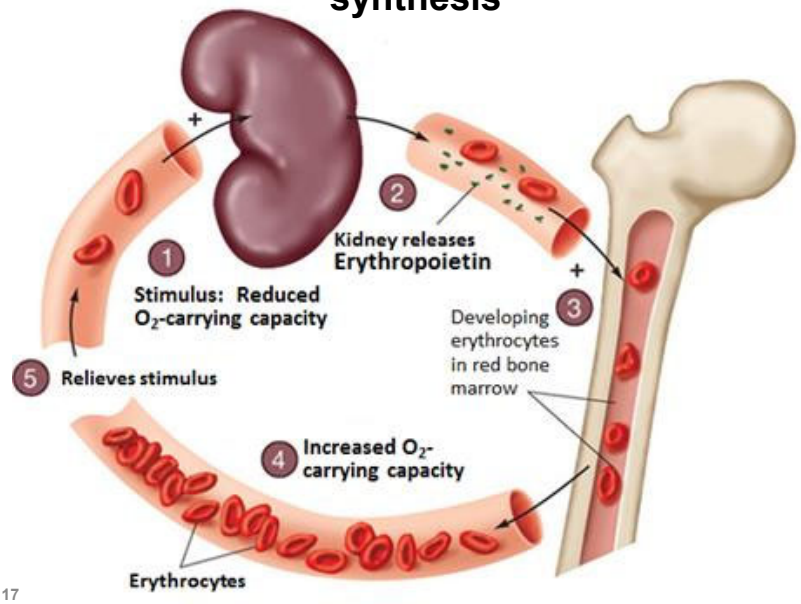
Mechanism of stimulation of Red blood cells synthesis



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Total erythrocytes counts

- Manual
  - Hemocytometer
- Automatic
  - Blood Cells Counter

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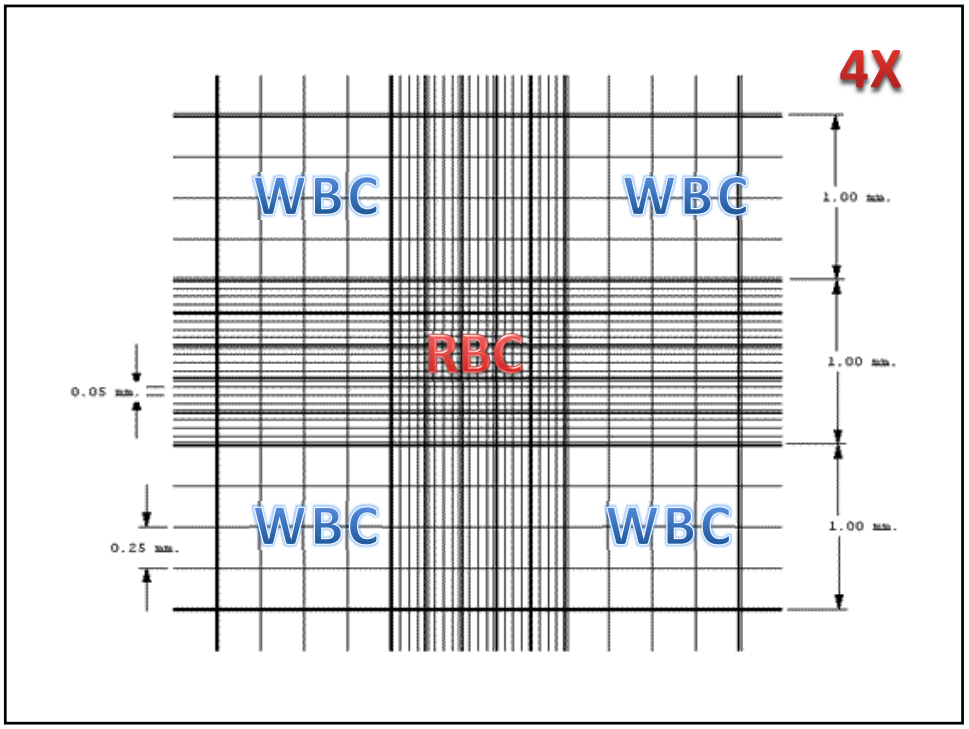

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Blood Cells Counter or Hematology Analyzer

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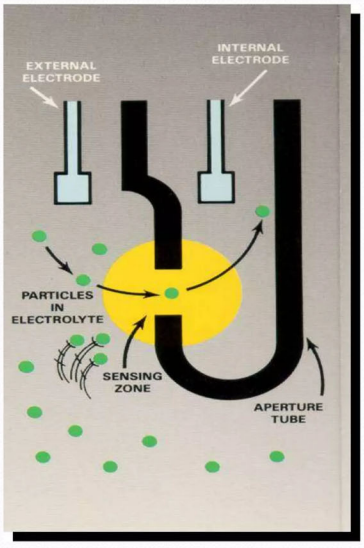
Electrical Impedance

- Diluant displacement causes potential difference
- Voltage pulse displayed on an osciloscope
- No. of impulse = No. of cells
- Height = vol. of cells
- Freq dist curve & size dist histograms
- Requisite – High dilution



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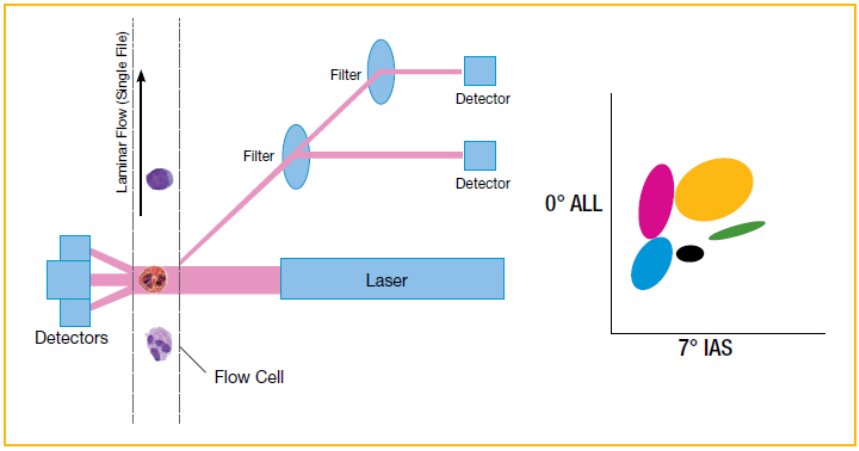
Light Scatter



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3-Part differential	5-part differential	7-part differential
Granulocytes (large cells)	Neutrophils	5 plus
Lymphocytes (small cells)	Eosinophils	Large immature cells (blasts, immature granulocytes) Atypical lymphocytes (including blasts)
Monocytes (mid)	Basophils	
	Lymphocytes	
	Monocytes	
	Large unstained cells (atypical, abnormal cells)	

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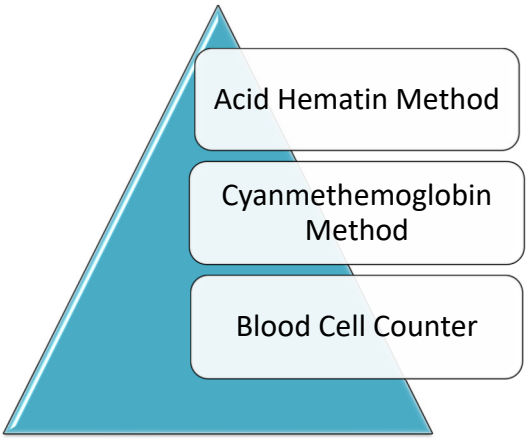
Hemoglobin concentration



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Hemoglobin concentration

Increase haemoglobin concentration

- Chronic carbon monoxide poisoning.
- Polycythemia.
- Cardiac diseases.
- Pulmonary diseases.

Decrease hemoglobin concentration

- Anemia.
- Amyloid nephrosis.
- Leukaemia.
- Malignant tumors.

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Hematocrit
Packed cell volume
(PCV)

Packed cell volume (PCV) or Hematocrit, is defined as the percentage of blood occupied by RBCs, or simply you can define PCV as the percentage of RBCS in whole blood.

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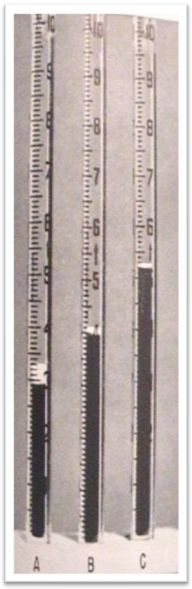
Packed cell volume (PCV)

- Wintrobe's Method
- Microhematocrit Method
- Blood Cell Counter



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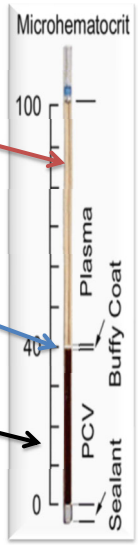
Hematocrit
Packed cell volume (PCV)

Plasma layer

Buffy coat.

PCV layer.



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Advantages of the microhematocrit method:

- The amount of blood required is considerably less.
- Time required for the entire procedure is less.
- accurate.

Disadvantages of the microhematocrit method

- Special reader is required for reading.
- It is impossible to determine E.S.R. in such small tubes.
- It is difficult to evaluate the depth of the buffy coat.

Interpretation of packed cells volume

a. PCV layer

An elevated PCV layer occurs in cases of:

1- Physiological causes.

2- Hemoconcentration following dehydration.

3- Chronic obstructive pulmonary disease.

4- Pulmonary diseases that associated with hypoxia.

5- Congestive heart failure.

6- Polycythemia

Lowered PCV layer occurs in cases of:

- **Oligocythemia (Erythrocytopenia).**
- **Hemodilution.**
- **Renal failure, because of decreased secretion of erythropoietin.**
- **Malignant tumors.**
- **Leukemia.**

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2. Buffy coat

In blood from normal animal, the buffy coat consists of a white to gray layer 0.5 to 1.2 mm. In size occurring immediately above the PCV layer.

For routine clinical application, a buffy coat of less than 0.5 mm would suggest leucopenia, while above 1.5 mm indicate Leucocytosis

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3. Plasma layer

The color of the plasma can help with :

a) Dark yellow color is an indicator for hemolytic, hepatic and obstructive jaundice.

b) The pink color of plasma is indicative for hemolysis of erythrocytes, which associate some diseases as bacillary hemoglobinuria and blood parasites as Babesia species .

c) Milky color of plasma indicates the increase of lipids in blood.

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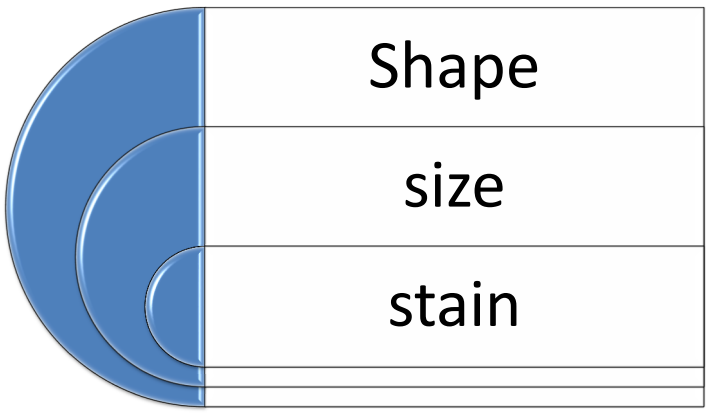
Determination of erythrocytes morphology



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Morphological examination of erythrocytes

All parameters of the erythrocyte picture can be determined using a blood cell counter, except assessing the morphology of the RBCs, which must be evaluated using a blood smear. This means, that both electronic blood cell count and blood smear are required to perform the erythrocyte picture.

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Morphological examination of erythrocytes

Morphological examination of erythrocytes is carried out by:

- Examination of a stained blood film (Giemsa stain) under the light microscope and by using the oil immersion lens (X100), the RBCs are examined for shape, size, and stain.
- Calculating and interpreting the mean corpuscular values (indices).

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Evaluating the shape of the RBCs

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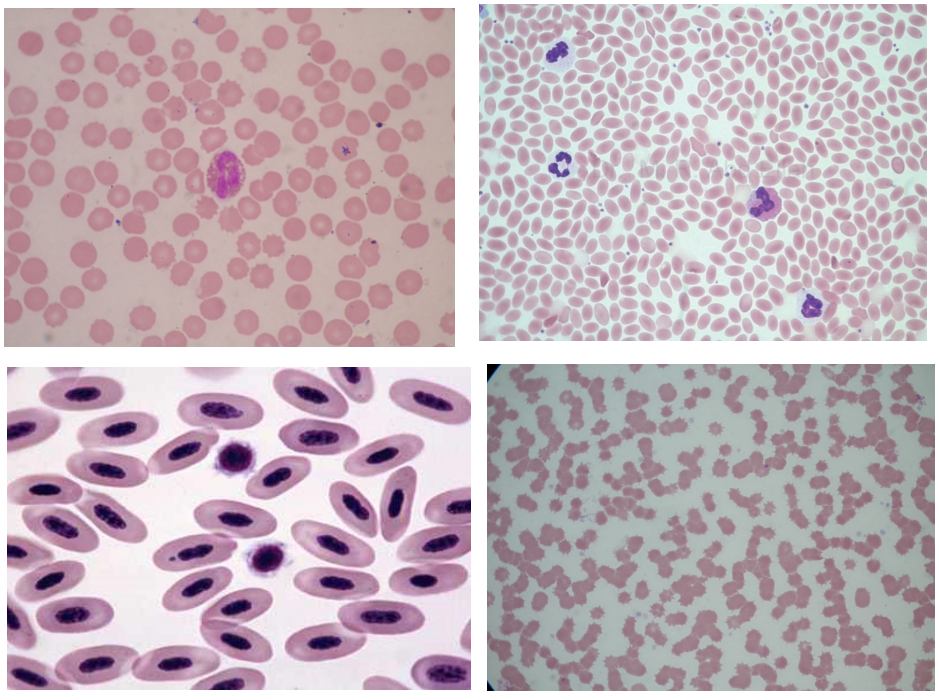

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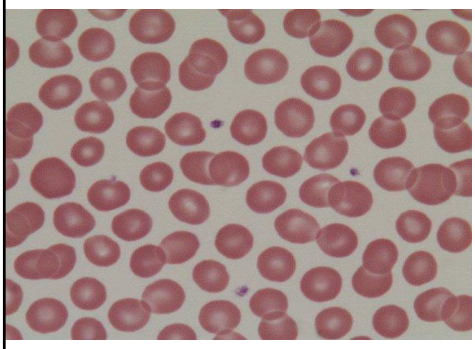
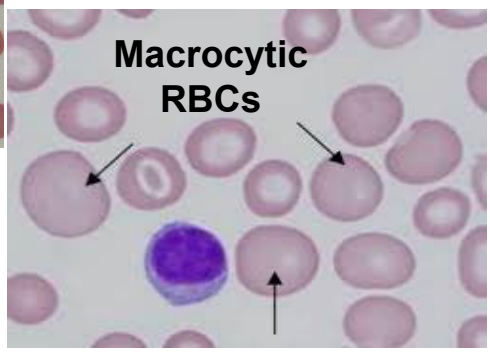
Evaluation of the Size of the RBCS



Normal RBCs



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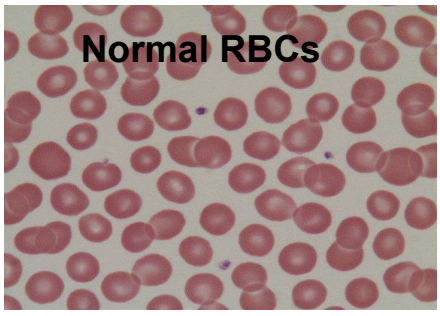
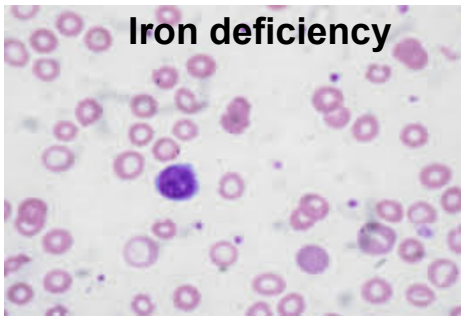
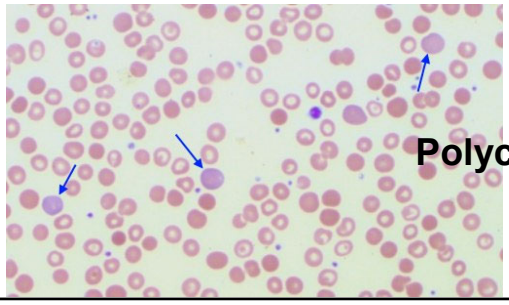
Evaluation of the stain of the RBCS







Polychromacia

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Mean corpuscular values

1. Mean Corpuscular Volume (MCV)

Mean corpuscular volume (MCV, fl or femitoliter) is a measure of average size of RBC and represents the volume of a single RBC.

This value used to classify red cells as:

- Normocytic red blood cells are of normal size.
- Microcytic red blood cells are smaller than normal.
- Macrocytic red blood cells are larger than normal.

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2. Mean Corpuscular Hemoglobin (MCH)

Mean corpuscular hemoglobin (MCH, pg or pictogram) is average weight hemoglobin of erythrocyte in a population of erythrocytes.

3. Mean Corpuscular Hemoglobin concenration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC, g/dl) is the average percent of hemoglobin occupied by the erythrocyte (g/dl)

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Mean corpuscular values

1. Mean Corpuscular Volume (MCV)

$$\text{MCV (fl)} = \frac{\text{PCV (\%) x10}}{\text{RBC count x } 10^6\text{/ul}}$$

2. Mean Corpuscular Hemoglobin (MCH)

$$\text{MCH (pg)} = \frac{\text{Hb. g/dl x10}}{\text{RBC count x } 10^6\text{/ul}}$$

3. Mean Corpuscular Hemoglobin concentration (MCHC)

$$\text{MCHC (g/dl)} = \frac{\text{Hb. g/dl X100}}{\text{PCV (\%)}}$$

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Based on MCH and MCHC red blood cells may be:

- Normochromic red cells with normal Hb concentration.
- Hypochromic red cells with lowered Hb concentration.
- Hyperchromic red cells with elevated Hb concentration.

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Complete Blood Count:

	Patient Value	Normal Range 2 years – 6 years
WBC	8.4 x 10^9 / L	(5.0 – 17.0)
RBC	2.77 x 10^{12} / L	(3.90 – 5.30)
Hgb	7.5 g/dl	(11.5 – 13.5)
Hct	21.8 %	(34.0 – 40.0)
MCV	78.6 fl	(75.0 – 87.0)
MCH	26.9 pg	(25.0 – 31.0)
MCHC	34.2 gm/dl	(31.0 – 36.0)
RDW	17.3 %	(11.5 – 15.0)
PLT	192 x 10^9 / L	(150 – 450)

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COMPLETE BLOOD COUNT

TEST		RESULT	UNIT	REFERENCE RANGE
White Blood Cells	H	12.3	10^3/uL	4.5 - 11.5
Red Blood Cells		4.9	10^6/uL	4.00 - 5.40
Hemoglobin	L	9.3	g/dL	12.0 - 15.0
Hematocrit	L	31.9	%	35.0 - 49.0
MCV	L	65.1	fL	80.0 - 96.0
MCH	L	19.1	pg	27.0 - 33.0
MCHC	L	29.3	g/dL	32.0 - 36.0
RDW	H	19.3	%	11.5 - 14.0
MPV		9.0	fL	6.8 - 10.2
DIFFERENTIAL COUNT				
Neutrophils	H	73.1	%	50.0 - 70.0
Lymphocyte	L	16.8	%	18.0 - 42.0
Monocyte		8.7	%	2.0 - 11.0
Eosinophil	L	0.8	%	1.0 - 3.0
Basophil		0.6	%	0.0 - 2.0
Platelet Count	H	460	10^9/L	150 - 450

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Erythrocytes sedimentation rate (ESR)

It is defined as the rate of sedimentation of erythrocytes in a column of anti-coagulated blood at certain time.

A whole blood sample is allowed to stand for a certain time in a perpendicular tube, The erythrocytes are settling down, leaving a clear layer of plasma at the top of the tube. The length of the plasma layer in mm represents a measure of the ESR and expressed as mm/time.

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Theories of sedimentation:

- **Rouleaux formation theory:**
  The red cells of healthy horses has a natural tendency to form chain like arrangement and thus results in rapid settling of red cells.
- **Electrical theory.**
- **Plasma protein theory.**

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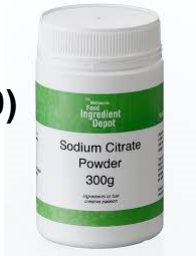
Methods of estimations:

- Wintrobe method.
- Westergreen method.

Anticoagulant

The type of the anticoagulant used for ESR is different according to the method used. EDTA is used for ESR determined by wintrobe method. However, Sodium Citrate (3.8%) is used for ESR that determined using the westergreen method, sodium citrate is used in the ratio of 1:4.

Sodium citrate

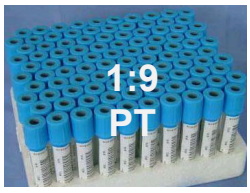


Dose: Sodium citrate 3.8% (1:4 or 1:9)

Mode of action: Binding ionized calcium.

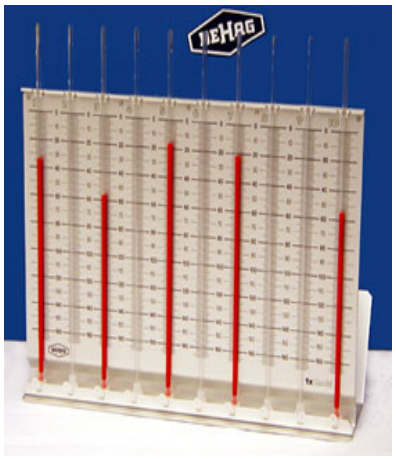
Advantages

- ✓ Blood transfusion.
- ✓ ESR (1:4).
- ✓ Prothrombin time (1:9)
- ✓Bacteriological culture.

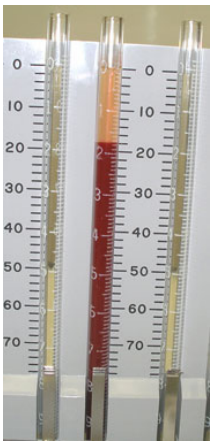


Advantages

- Not suitable for hematological analysis.



Westergreen method



Wintrobe method

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Erythrocytes sedimentation rate

Suitable time for ESR:

Equine: 10 minutes and 20 minutes

Cattle: after 24 hrs

Dogs: 1hour and 2hour

Sheep: 7hrs and 24hrs

Buffaloes: 1 hour

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Factors influence the ESR:

1. Physical factors: Temperature, change in size of RBCs and Rouleux formation
2. Chemical factors: Anticoagulant.
3. Physiological factors: Sex, age, pregnancy, exercise altitude and digestion.
4. Technical factors: Position of the tube (Sedimentation is rapid in an inclined position)

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5. Pathological factors

Accelerated ESR observed in cases of:

1. All acute generalized infection.
2. Acute localized inflammatory process of serous membranes such as pleura, pericardium and peritoneum.
3. Chronic localized infection.
4. Toxemia.
5. Severe anemia.
6. Malignant neoplasia.
7. At the beginning of abscess formation.
8. Inflammation of draining cavities such as uterus and head sinuses.

Retarded ESR in cases of:

1- Hemoconcentration following dehydration.

2- Chronic obstructive pulmonary disease.

3- Pulmonary diseases that associated with hypoxia.

4- Congestive heart failure.

5- Polycythemia.

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DIPHASIC SEDIMENTATION

Occasionally in ESR determination there is no definite line between the settled erythrocytes and the plasma. Reticulocytes and immature erythrocytes exhibit less tendency to form clumps or chains (rouleaux) than mature erythrocytes; thus ESR is retarded or exhibits a diphasic pattern.

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DIPHASIC SEDIMENTATION

This phenomenon occurs as a result for the presence of reticulocytes or other young form of erythrocytes which having abnormal shape. This trailing out of erythrocytes occurs because these cells are larger and don't actively participate in rouleux formation.

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