HEMATOLOGY PART I

By

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Hematology includes a broad range of subjects, including hematopoiesis (production of all cell types in blood) and laboratory assessment of hematopoietic cells. There are marked species differences in hematopoietic cells. For example, birds, amphibians and reptiles have nucleated erythrocytes, which makes assessment of their blood more challenging than those of mammals.

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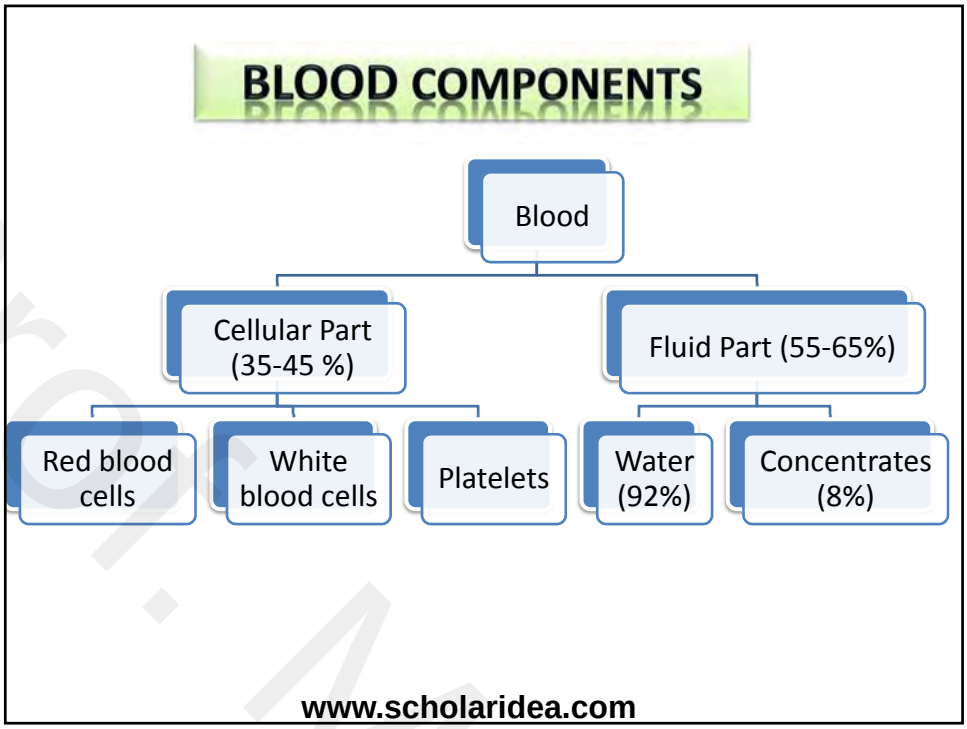

Blood Picture
Complete Blood Count (CBC)
Hemogram

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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)

Differential:

	Absolute	Normal Range Number	2 years – 6 years
Neutrophils	43 %	(3.61)	(1.50 – 8.50)
Bands	6 %	(0.50)	(0.00 – 1.00)
Lymphocytes	41 %	(3.44)	(3.00 – 9.50)
Monocytes	4 %	(0.34)	(0.00 – 0.80)
Eosinophils	3 %	(0.25)	(0.02 – 0.65)

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Evaluation of the red blood cells (RBCs)
Erythrocytes picture

Hemoglobin concentration

RBCs count

Packed cell volume

Mean corpuscular values

Erythrocytes morphology

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Erythrocytes (RBCs)

The major function of the RBCs is to transfer oxygen from the lungs to tissues, and to transfer carbon dioxide back from the tissues to the lungs.

The shape of RBCs is biconcave disc in human and most animal species

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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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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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Evaluation of the red blood cells (Erythrocyte picture)

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CBC in Dog

Test Requested	Results		Reference Range	Units
COMPLETE BLOOD COUNT				
WBC	5.9		4.0-15.5	$10^3/\mu L$
RBC	5.2		4.8-9.3	$10^6/\mu L$
HGB	11.5 (LOW)		12.1-20.3	g/dL
HCT	34 (LOW)		36-60	%
MCV	67		58-79	fL
MCH	22.4		19-28	pg
MCHC	34		30-36	%
Comment				
RBC MORPHOLOGY	NORMAL			
Differential	**Absolute**	**%**		
Neutrophils	3422	58	2060-10600	/µL
Lymphocytes	1888	32	690-4500	/µL
Monocytes	295	5	0-840	/µL
Eosinophils	295	5	0-1200	/µL
Basophils	0	0	0-150	/µL
Platelet Estimate	Adequate			
Platelet Count	327		170-400	$10^3/\mu L$

Evaluation of the red blood cells (RBCs) Erythrocytes picture

Determination of RBCs count (/mm^3 or T/l)

Determination of Hemoglobin concentration (g/dl)

Determination of Packed cell volume (%)

Determination of Mean corpuscular values (MCV, MCH, MCHC)

Determination of erythrocytes morphology

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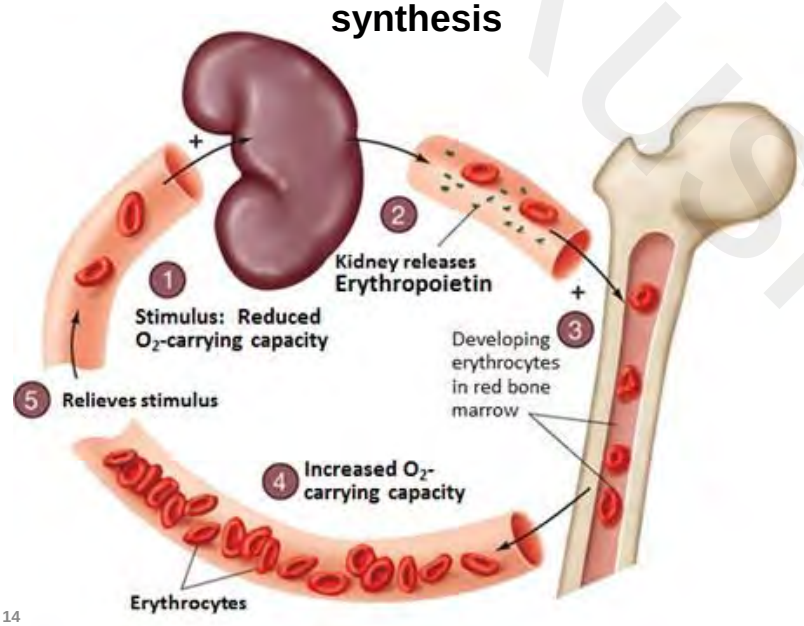
Mechanism of stimulation of Red blood cells synthesis



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Blood sample

Site of collection

Tail or Ear vein or medial canthus of Eye

Rat and Mice

Ear vein in pigs and Rabbits

Saphenous, Cephalic or Jugular vein

Dogs and Cats

Jugular vein

Ruminants and Equines

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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

Blood swab —Sample→ Swab

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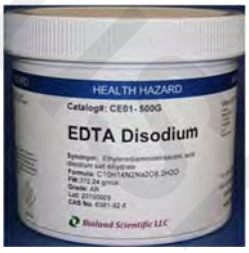
Anticoagulants

1. **Ethylene Diamine Tetra-acetic acid (EDTA).**
2. **Heparin.**
3. **Ammonium and potassium oxalate mixture.**
4. **Sodium citrate.**
5. **Sodium fluoride and potassium oxalate mixture.**

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.**

Heparin

Dose: 0.1 ml of 0.75 % solution/ 5ml blood

Mode: Inhibit thrombin

Advantages:

- ✓ **Suitable for Haematocrit determination.**
- ✓ **For measuring acid base balance.**

Disadvantage

- ❖ **Preserve the blood sample for 8 hours.**
- ❖ **More expensive.**

AMMONIUM AND POTASSIUM OXALATE MIXTURE

Amount required

Ammonium oxalate 1.2 gm.
potassium oxalate 0.8 gm.
D.W. 100 ml.

1ml of the solution in a tube, then dry at 60 ° C. This is sufficient for 10 ml blood.

Mode: Binding ionized calcium

Advantages

- **It is cheaper than EDTA.**

Disadvantages

- **It doesn't prevent clumping of platelets.**
- **It is poisonous**

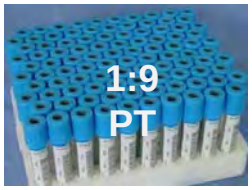
Sodium citrate

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.**

Sodium fluoride and potassium oxalate mixture

Amount: 4 parts sodium fluoride to 5 parts of potassium oxalate. 0.5 ml of 2.25% /5 ml blood.

Mode of action: Binding ionized calcium.

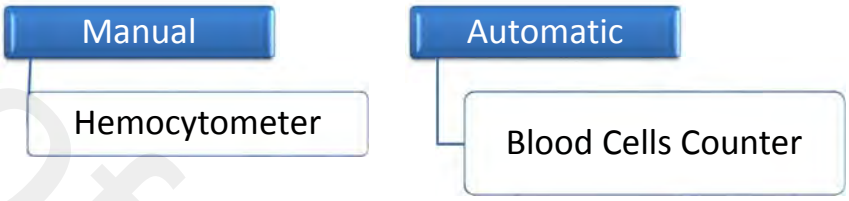
Advantages

- ✓ **Blood glucose level --- Inhibit the glycolytic enzymes**

Disadvantages

- ✓ **Poisonous.**

Total erythrocytes counts



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Physiological Factors affecting the RBCs count:

- **Life span of the erythrocytes**

  The life span of the erythrocytes varies according to the animal species (in cattle 150 days, in equine 145 days, in feline 70 days).

- **Exercise.**
- **High altitude.**
- **Emotional condition, fear or excitement.**
- **Age, sex and pregnancy.**

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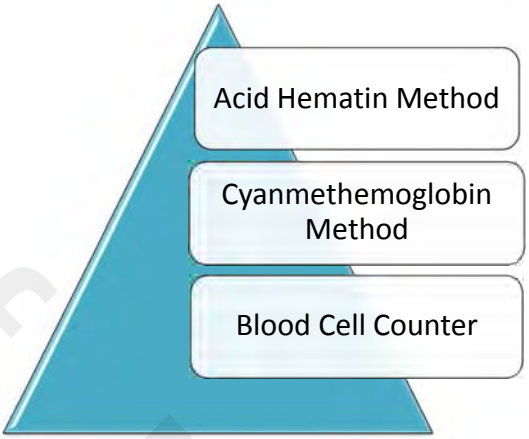
Hemoglobin concentration



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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 (HCT)
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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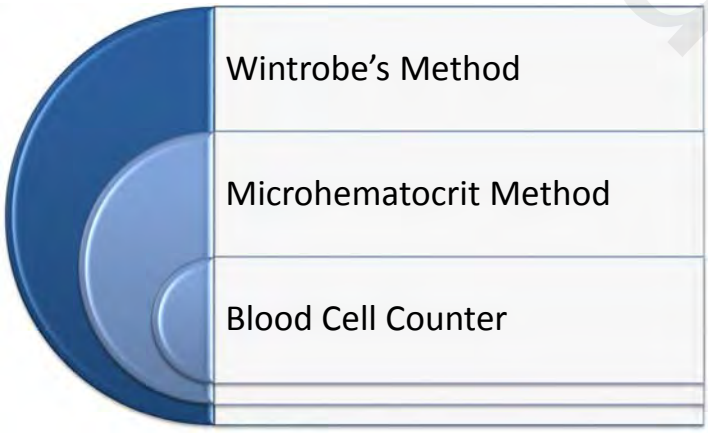
Packed cell volume (PCV)



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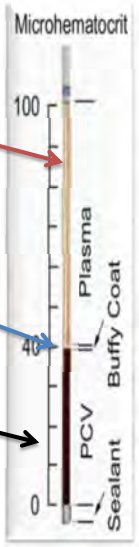
Hematocrit
Packed cell volume (PCV)



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.

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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, as a result of decreased secretion of erythropoietin.
- Malignant tumors.
- Leukemia.

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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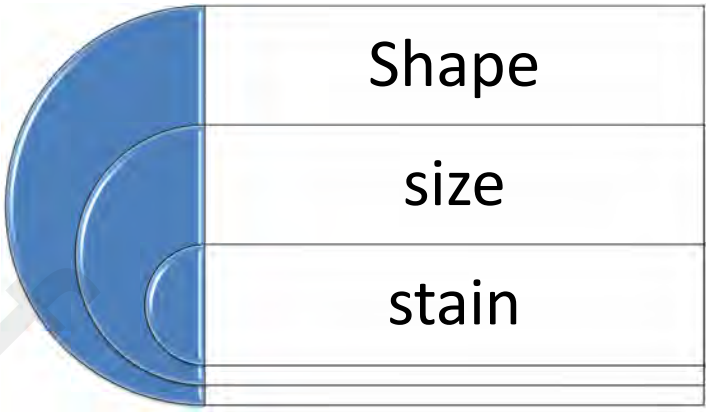
Determination of erythrocytes morphology



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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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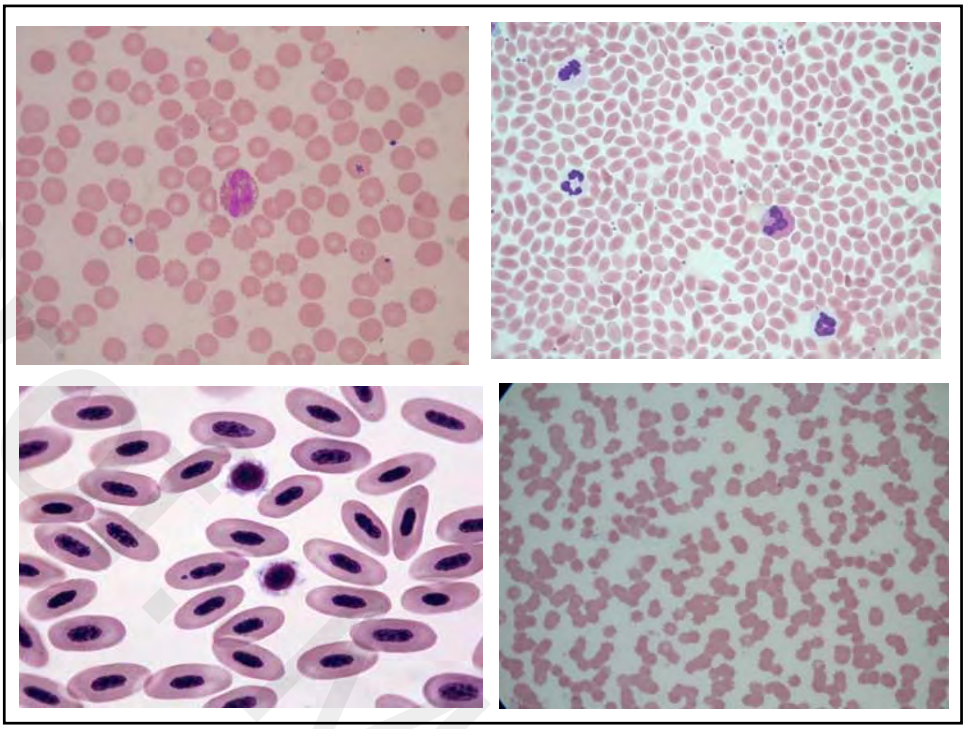
Morphological examination of erythrocytes

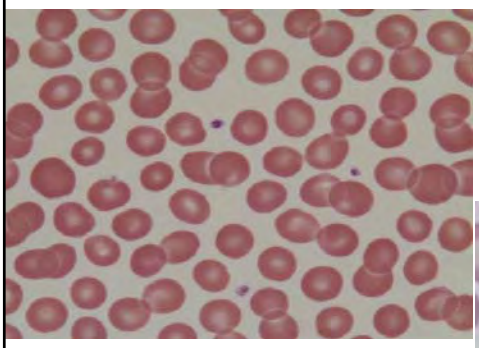
Morphological examination of erythrocytes is carried out by:

- 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).

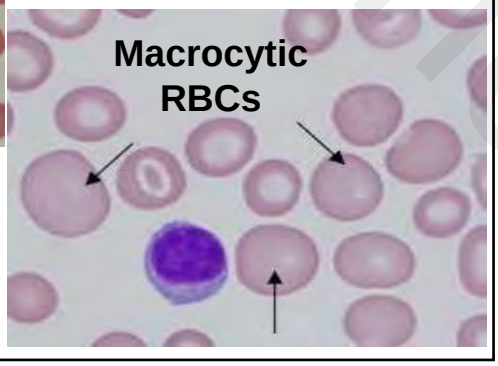
Evaluating the shape of the RBCs



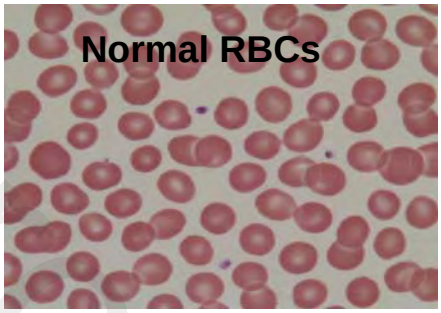
Evaluation of the Size of the RBCS

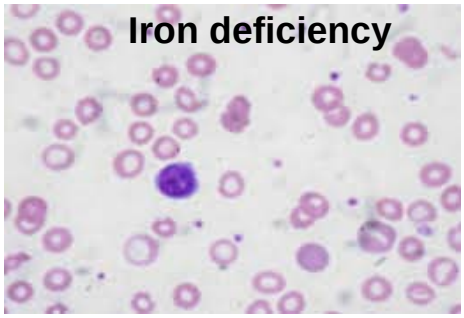


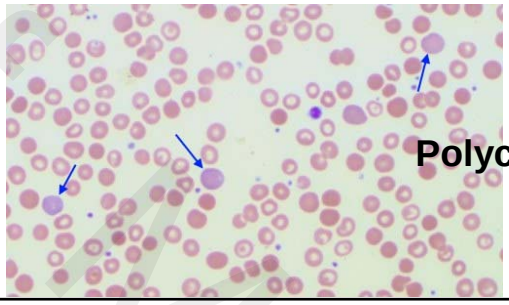
Normal RBCs



Evaluation of the stain of the RBCS







Polychromacia

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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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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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)
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PLT	192 x 10^9 / L	(150 – 450)

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ERYTHROCYTE SEDIMENTATION RATE (ESR)

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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:

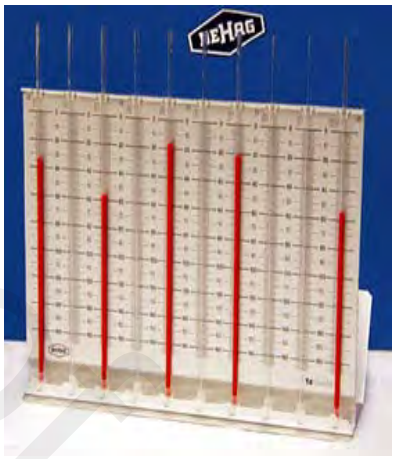
- **Rouleaux formation theory:**
  The red cells of healthy horses has a natural tendency to form chain like arrangement and thus results in rapid settling of red cells.
- **Electrical theory.**
- **Plasma protein theory.**

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.



Westergreen method

Wintrobe method

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.

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.

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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