**CLINICAL PATHOLOGY**

I. What is clinical pathology?

A. Definition:

l. ***pathology***is the "branch of malicine that deals with the basis of disease, especially those structural and functional changes in organs and tissues caused by a disease". In general, it is the study of disease.

2*.* ***Clinical pathology***is a "subspecialty of pathology that deals with the use of laboratory methods (clinical chemistry, microbiology, hematology ,... ) for the diagnosis and treatment of disease". In general it is the study of disease in the clinical environment by use the laboratory assays.

B. ***Veterinary clinical pathologists***are specialists in the disciplines of basic pathology, hematology (study of blood), clinical chemistry (study of physiologic and biochemical reactions),cytology (study of cells), and surgical pathology (study of disease via microscopic analysis of tissue samples obtained during surgery).

C. Veterinary clinical pathologists and other laboratory professionals (medical technologists, medical laboratory technicians and veterinary technicians) often work in a clinical laboratory that limits its assay to: hematologic assays, clinical chemical assay, urinalysis, and clinical cytologic or histologic examinations. Other assays or diagnostic laboratory procedures includes specific laboratories (eg. microbiology, histopathology and toxicology) that are supervised by microbiologists, histopathologists. and toxicologists, respectively.

II. Laboratory tests should be used with other diagnostic procedures. Before laboratory tests are used, two diagnostic procedures are essential:

(1) Obtain case history.

(2) Obtain complete physical examination.

 With knowledge gained from these two basic procedures, a diagnostician can select diagnostic procedures to clarify or classify identified problems. Veterinarians frequently use the laboratory assays in conjunction with other diagnostic methods to identity or classify pathologic states that develop in domestic mammals. Some body systems (integument, nervous, skeletal and cardiovascular) are relatively evaluated via visual or imaging methods (physical examination, radiography and ultrasonography), whereas other body systems ( hemic, immune, urinary, and endocrine) are better evaluated by laboratory tests.

III. What are the major causes for analyzing patient samples via laboratory procedures?

A.To detect an unidentified pathologic state.

B.To define, classify or confirm a pathophysiologic disorders or disease state.

C.To eliminate (rule out) a possible cause of the disease.

D.To evaluate changes in a pathologic state either due to natural progression of the disease or because of medical or surgical therapy.

**SAMPLES**

**I. Blood samples and specimens:**

**A.** Most clinical laboratory assays are designed to detect or quantify substances or cells in blood samples ; the substances or cell of interest is called the **analyte**. Obtaining useful results for the analyte requires appropriate samples. Whenever there is doubt about the appropriate sample for a particular test at particular laboratory, the laboratory should be contacted prior to sample collection.

**B.** Blood sample collection

l. Blood and major components are frequently used as samples for laboratory assay. Blood must be collected and processed properly so that assay results reflect the true composition of blood rather than artifactual changes.

2. Blood is composed of blood cells (erythrocytes, and five major leukocyts types and platelets) and plasma. Blood withdrawn from a blood vessel must immediately be mixed with an anticoagulant to prevent initiation of clot formation and to maintain cells and other components in suspension.

3. Analysis or processing of whole blood must be relatively rapid because the cells die within a few hours, and thus a sample will become unacceptable for analysis. Samples must be analyzed within minutes, usually within hours, rarely within days.

**C.** Anticoagulants used for blood sample collection:

**a. Calcium-binding agents** prevent Ca2+ from participating in the formation of a blood clot.

1- Ethylene Diamine Tetra-acetic Acid (EDTA) (as Na2EDTA, K2EDTA, or K3EDTA)

a- EDTA is the preferred anticoagulant for almost all routine hematologic tests, including the complete blood count (CBC) assays.

b- EDTA chelates Ca2+ and other divalent cations (Mg2+, Cu2+ and Pb2+) but the other anticoagulants do not. EDTA attaches to Ca2+ in six places so prevent coagulation of blood. Used in either a liquid or dry form. One drop of EDTA 10 % solution sufficient to prevent coagulation of 5 ml of blood.

2- Citrate (as sodium citrate or potassium citrate).

a- Citrate is the preferred anticoagulant for most tests of the coagulation system. Citrate's anticoagulant activity is achieved by its forming an ionic bond with Ca2+ .

b- Because it has low toxicity, citrate is also preferred for collection of whole blood to be used for transfusions.

3- Oxalates (as lithium, ammonium, and potassium saIts).

a- Oxalate is used for a few laboratory tests; for example used for glucose and lactate evaluation assays. Generally, oxalates alter morphologic features of leukocytes and erythrocytes and thus are unsuitable for hematologic samples.

b- Oxalate's anticoagulant activity is achieved by its forming an ionic bond with Ca2+.

**b. Heparin** (as lithium, ammonium, potassium, or sodium salts) activates antithrombin III which then inhibits the activity of several coagulation factors (including thrombin). It is also forms an ionic bond with Ca2+,but its major action is through interfering with conversion of prothrombin to thrombin .

1- Used for several special laboratory assays (such as blood gas analysis) and can be used for many clinical chemistry assays.

2-It is used commonly in liquid or dry form.

3- Major disadvantages:

a- Alters morphologic features and staining of leukocytes.

b- Allows clotting as effects are slowly overridden by the coagulation system.

c- Allows platelet clumps to form.

**D.** Plasma

1. Plasma is the fluid component of blood that is harvested after centrifugation of an anticoagulated blood samples. Plasma will contain the anticoagulant that can interfere with some assays.

2. Plsma has two major components:

1-Water: about 92- 95 % of plasma volume; 100 ml of plasma contains 92- 95 ml of H2O.

2-Solids: about 5-8 % of plasma volume. Most solids are proteins on a weight per volume (weight/volume) basis. Other solids are glucose,lipid,hormons, urea, electrolytes, and other chemicals.

3. Generally, the chemical composition of plasma is very similar to interstitial fluid in most tissues. Plasma and interstitial fluid are the extracellular fluids , one intravascular and one extravascular. Difference differentiation

**E**. Serum

1. serum is the fluid component of blood that is harvested after centrifugation of a coagulated (clotted) blood sample. The clotting involves platelets and coagulation proteins. To get the maximal amount of serum from the clotted sample, centrifugation should not be started prior to the retraction of the clot (which typically takes at least 30 min. if a clot activator is not present in the tube). If samples are centrifuged prior to clot retraction, some serum will be trapped in a soft fibrin clot.

2. Serum has the same composition as plasma except serum does not contain most of the coagulation proteins. The major protein that is absent in serum but present in plasma is fibrinogen.

3. During the clotting process, substance. released from cells alter the analyte concentrations in serum. For example, platelets release K+, and thus serum K+ is greater than plasma K+ .

**III. Urine Samples**

A. Urine is the most common sample analyzed by laboratory assays. Urine must be collected and processed properly so that the assay results reflect the true composition of the product of the urinary system.

B. To prevent artifactual changes in urine, it should be processed soon after collection.

**IV. Milk Samples:** milk is the sample analyzed during subacute mastitis.

**V. Other Body Fluid samples**

A. Pleural fluid, peritoneal fluid, synovial fluid, and cerebrospinal fluid samples are collected to characterize body cavity effusions, joint diseases and central nervous system disorders respectively.

**Haematology**

**Haematology**is the study of the cellular elements of the blood, which can divided into three types:

1. The erythrocytes or red blood cells.
2. The leukocytes or white blood cells.
3. The thrombocytes or platelets.

**Production** of all these types of cells occurs in **bone marrow** under **hormonal control**.

**I. The ERYTHRON**

A. This mass of erythroid cells includes circulating erythrocytes (RBC) and bone marrow precursor, progenitor, and stem cells.

B. The function of RBC are **oxygen/carbon dioxide** transport between the lungs and the tissues of the body, which is mediated by **hemoglobin** as the O2/CO2 carrier, within the erythrocytes. Erythrocytes: membrane, shape, cytoskeleton, and metabolic processes indicate survival of them against the stresses of circulation and various injurious substances.

C. **Hemoglobin** is transported in erythrocytes.

D. Hemoglobin consists of heme and globin, and each hemoglobin molecule:

1. heme group contains an iron atom in the (Fe2+).

2. The complete hemoglobin molecule is a tetramer, containing four heme units and four globin chains.

3-**Iron** present in hemoglobin and myoglobin. Iron responsible for many enzyme reactions that have role in energy generation, prostaglandin synthesis, free radical detoxification, synthesis of DNA and fatty acids. Also it is very important mineral for erythropoiesis, brain and immunity. The main storage of iron in the body in liver, bone marrow and spleen (bound to **ferritin** or **hemosiderin**) while in blood bound to **transferrine** , all these about 50%. More than half of the total body iron present as **hemoglobin**.

4- Hemoglobin level decrease after the body iron store depleted.

**II. Heme synthesis**

1. Heme synthesis is controlled by the enzyme **δ-aminolevulinic acid synthase**, whose synthesis is controlled by heme concentration within the erythrocyte. Inhibition of heme synthesis caused by lead (poisoning) and chloramphenicol (treatment).

**III. Globin synthesis**

1. Each hemoglobin molecule is comprised of four globin chains, each of which binds to a heme group.

**IV. IRON METABOLISM**

a-Absorption of iron is regulated by the amount of storage iron (large iron stores decrease absorption) and rate of erythropoiesis (increase speed erythropoiesis increases absorption).

b- Iron classified into three types:

1- **Functional** iron e.g. Hemoglobin and myoglobin.

2- **Storage** iron e.g. ferritin and hemosiderin.

3- **Transport** iron e.g. transferrine and lactoferrin.

c. Iron is transported in blood bound to the δ-globulin call **transferrin**.

1. Iron bound to transferrin is measured as serum iron (SI).

d. Conditions with decreased SI:

(1) Iron deficiency

(2) Acute and chronic inflammation or disease

(3) Hypoproteinemia

(4) Hypothyroidism

(5) Renal disease

e. Conditions with increased SI:

(1) Hemolytic anemia

(2) Accidental lysis of erythrocytes during sampling (hemolysis)

(3) Glucocorticoid excess in the dog and horse. In contrast, SI is decreased in cattle with

glucocorticoid excess.

(4) Iron overload

(5) Non-regenerative anemia

D. Iron is stored as ferritin and hemosiderin.

E. **Ferritin** is a water-soluble iron-protein complex.

1. Ferritin is the more labile storage form of iron.

2. Small amounts circulate that can be measured as serum ferritin, which is an indirect

measurement of the storage iron pool. A species-specific immunoassay is required.

a. Serum ferritin concentration is decreased in iron deficiency.

b. Serum ferritin concentration is increased in the following:

(1) Hemolytic anemia

(2) Iron overload

(3) Acute and chronic inflammation

(4) Liver disease

(5) Some neoplastic disorders (e.g., lymphoma, malignant histiocytosis)

(6) Malnutrition (cattle)

F. Hemosiderin is a more stable, but less available, storage form of iron that is contain ferritin and protein. It is not water-soluble.

**V. ERYTHROCYTE METABOLISM**

Metabolism is limited after the reticulocyte stage because mature erythrocytes lack mitochondria for oxidative metabolism. Biochemical pathways found in mature erythrocytes are :

**A. Embden-Meyerhof pathway (anaerobic glycolysis)**

This pathway generates adenosine triphosphate (ATP) and NADH. ATP is essential for membrane function and integrity, whereas NADH is used to reduce methemoglobin. Enzyme deficiencies (pyruvate kinase and phosphofructokinase) in this pathway can lead to hemolytic anemia.

**B. Pentose phosphate pathway (Hexose-monophosphate pathway)**

This anaerobic pathway produces NADPH, which is a major reducing agent in the erythrocyte. NADPH serves as a co-factor for the reduction of oxidized glutathione. Reduced glutathione neutralizes oxidants that can denature hemoglobin. Enzyme deficiency in glucose-6-phosphate dehydrogenase results in hemolytic anemia.

**C. Methemoglobin reductase pathway**

Hemoglobin is maintained in the reduced state (i.e., oxyhemoglobin; Fe2+) necessary for transport of oxygen by this pathway. Enzyme deficiency (Methemoglobin reductase) results in methemoglobin accumulation (methemoglobinemia). Methemoglobin (Fe3+) cannot transport oxygen, and cyanosis in blood and mucous membranes and exercise intolerance.

**D. Rapoport-Luebering pathway**

This pathway allows formation of 2,3 diphosphoglycerate (2,3 DPG), which has a regulatory role in oxygen transport. Increased 2,3 DPG help oxygen release to tissues by lowering the oxygen affinity of hemoglobin. Usually anemic animals have increased 2,3 DPG concentrations and deliver more oxygen to tissues with a lesser amount of hemoglobin (a compensatory mechanism).

**VI. ERYTHROKINETICS**

1. In mammals, erythropoiesis occurs extravascularly in bone marrow parenchyma.

2. Characteristic morphologic changes take place during maturation from the rubriblast to the mature erythrocyte .

a. Cells become smaller.

b. Nuclei become smaller and their chromatin is more aggregated:

(1) Cell division stops in the late rubricyte stage when a critical intracellular concentration of hemoglobin is reached.

(2) The nucleus is **extrude** at the metarubricyte state, and a reticulocyte is formed in

mammals.

c. Cytoplasmic color changes from blue to orange as hemoglobin is formed and RNA is lost.

3. In mammals, reticulocytes and erythrocytes migrate into the venous sinus of the bone marrow through transient openings in endothelial cell cytoplasm.

a. Reticulocytes of most species remain in the bone marrow for two to three days before release and finally mature in the peripheral blood or spleen.

b. In health, the reticulocytes of cattle and horses mature in the bone marrow; mature

erythrocytes are released.

4. The time from stimulation of the erythropoietic progenitor cell until reticulocytes are released is approximately five days.

5. Starting with the rubriblast, three to five divisions produce eight to 32 differentiated cells.

6. The bone marrow has the capacity to increase erythropoiesis.

a. Erythrocyte production can be increased up to seven times the normal rate in humans,

providing the necessary stimulation and nutrients are present. This capacity to increase

production varies with the animal species. It is greatest in birds and dogs and least in cattle and horses.

7. Regulation of erythropoiesis

a. **Erythropoietin (Epo)**

(1) The majority of Epo is produced by the kidney in response to hypoxia, but the liver may account for 10% to 15% of Epo production.

(2) Androgens increase Epo release. In contrast, estrogens and corticosteroids decrease Epo release.

(3) Actions of Epo

*(a)* Inhibition of apoptosis (cell death) of newly formed progenitor cells and prorubricytes, allowing them to differentiate into mature erythrocytes.

*(b)* Stimulation of hemoglobin synthesis in dividing erythroid cells.

*(c)* Switching of hemoglobin synthesis in sheep from type to another.

**b. Interleukin-3 (IL-3)** and **colony-stimulating factors** (GM-CSF and G-CSF).

(1) IL-3 is produced by activated T-lymphocytes; GM-CSF by activated T-lymphocytes,

macrophages, endothelial cells, and fibroblasts; and G-CSF by macrophages, monocytes, neutrophils, endothelial cells, and fibroblasts.

d. Thyroid and pituitary hormones alter the tissue demands for oxygen, so stimulate erythropoiesis.

**VII. ERYTHROCYTE DESTRUCTION**

A. The average erythrocyte lifespan in circulation varies with the species: cow, 160 days; sheep, 150 days; horse, 145 days; dog, 110 days; pig, 86 days; cat, 70 days; bird, about 35 days. Thus, ruminant blood smears have infrequent reticulocytosis in health, while avian blood smears may have 4% to 5% reticulocytes in health. In certain disease states, anemia may develop more quickly in birds and cats than in large animals because of the normally short erythrocyte lifespan.

B. Aging of erythrocytes is accompanied by changes in enzyme content and cell membrane structure that make the cells less capable of survival and subject to removal by the spleen.

C. In health, erythrocytes are removed from circulation by two routes:

1. **Phagocytosis** by macrophages is the major route of erythrocyte removal.

a. Within the phagosome, the erythrocyte releases its hemoglobin, which is divide into heme and globin.

b. Globin is broken down to its constituent amino acids, which are reutilized.

c. After releasing the iron, heme is cleaved by heme oxygenase, forming: iron, carbon monoxide and biliverdin.

d. Biliverdin is reduced by biliverdin reductase to bilirubin, which is excreted into the blood, where it binds with **albumin** for transport to the liver(excreted with bile).

2. **Intravascular lysis** with release of hemoglobin into plasma is a minor route of erythrocyte removal .

a. Free hemoglobin in the plasma binds to the **haptoglobin** forming the **hemoglobin-haptoglobin complex**(HH **complex**), that is cleared from plasma by the liver, preventing loss of hemoglobin in the urine.

b. **If intravascular lysis is excessive**, the serum haptoglobin may become saturated. The free hemoglobin then separate into dimers, which can pass via the glomerular filter. This does not occur in health.

1. Hemoglobin that passes into the glomerular filtrate is absorbed by the proximal tubules and catabolized to iron, bilirubin, and globin.

2. Unabsorbed hemoglobin passes into the urine, causing **hemoglobinuria**.

3. In **hemolytic anemia** similar routes of destruction occur (extravascular or intravascular hemolysis).

**METHOD OF EVALUATING ERYTHROCYTES**

**I. Packed-cell volume** (**PCV**), **Hemoglobin (HB) Concentration , and Red Blood Cell (RBC) COUNT are indicators of circulation RBC mass.**

A. **Packed-cell volume** (**PCV**) or **Hemmatocrit (**Hct) is the percent (%) of **erythrocytes** to whole blood volume.

1. Centrifugal methods give a packed-cell volume (PCV), a very accurate measurement with small natural error (±1%). PCV value increase during dehydration, vomition, diarrhea and burns ,while commonly decrease during anemia.

a. **Plasma** obtained by this method can be used for:

(1) Plasma protein concentration using refractometer.

(2) Plasma fibrinogen concentration using heat precipitation and refractometer.

(3) Plasma color and transparency:

*(a)* Normal plasma is clear and colorless (dog and cat) to light yellow (horse and cow).

*(b)* Icteric plasma is yellow.

*(c)* Hemoglobinemic plasma is pink to red and clear.

*(d)* Lipemic plasma is whitish to pink and opaque.

b. The **buffy** **coat** zone, a white layer between the RBCs and plasma, is include leukocytes and platelets. (Measurement of its width has been used to estimate white blood cell counts).

c. Microfilaria may be detected by microscopic examination of the plasma just above the buffy coat layer.

2. Calculate the PCV , determining the RBC count and the mean corpuscular volume (MCV) of the erythrocyte are important in **morphological classification anemia**.

B. **Hb concentration**

1.Colorimetric determination by the cyanomethemoglobin technique is used most frequently.

a. Heinz bodies, hemolysis, lipemia, and treatment with Oxyglobin may cause **high values.**

2. Hb concentration provides the most direct indication of oxygen transport capacity of the blood and should be about one-third the PCV if erythrocytes are of normal size.

3. Determination of Hb concentration give the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

**C. RBC count**

1. RBC counts, performed with a hemocytometer, have a large degree of error.

2. Automatic counters, if standardized for mammalian blood, allow for more accurate RBC counts.

3. The value of the RBC count is that it allows determination of MCV and MCH.

**D. Factors affecting PCV, Hb concentration, and RBC count**

1. Change in circulating RBC mass affects all three parameters:

a. **Low values** occur in **anemia**(decreased RBC mass)**.**

b. High values occur in **absolute polycythemia** (increased RBC mass)less common.

c. High values occur in **relative polycythemia** with dehydration, vomition, diarrhea, burns and excitement-induced splenic contraction (increase in PCV without increase in actual size of the erythron as a whole).

2. Change in plasma volume affects all three parameters:

a. Dehydration or fluid shifts to visceral organs cause increased values.

b. Overhydration with parenteral fluids causes a reduction in values, anemia.

**II. USES OF PCV, Hb and RBCs COUNT IN THE MORPHOLOGICAL CLASSIFICATION OF ANEMIA.**

A. **Mean corpuscular volume** (MCV): represents the red cell volume in femtoliters (fl)

1. MCV (fl) = (PCV ×10)÷ RBC count (in millions)

The results:1-more than the normal range(macrocytic)2-less than the normal range (microcytic) 3- within the normal range(normocytic)

2. The MCV is determined directly by automatic cell counters.

**3. Factors affecting MCV values**

a. Reticulocytosis is the most common cause of macrocytosis (increased MCV).

b. Immature animals have small erythrocytes cause microcytosis (low MCV).

c. Iron deficiency causes microcytosis .

d. Healthy Asian breeds of dogs often have microcytic erythrocytes.

e. Feline LukemiaVirus-infected cats often have macrocytic erythrocytes.

B. **Mean corpuscular hemoglobin** (MCH) represents how much Hb is present within an average erythrocyte in picograms (pg).

1. MCH(pg)= (Hb concentration ×10)÷ RBC count (in millions)

2. Factors affect the MCH and MCHC in a similar way; therefore, the MCH offers little

additional hematologic information about the patient, so it not used in the classification of anemia.

5. MCH is not generally used in the classification of anemia. If the MCHC and MCH differ, interpretation of the hemoglobin concentration should be based upon the MCHC, because the MCHC value more **accurate**.

C. **Mean corpuscular hemoglobin concentration** (MCHC) represents the average Hb concentration per average erythrocyte in grams of Hb/dL (100 mL) of erythrocytes.

1. MCHC(g/dL) = (Hb concentration (pg)×100)÷ Hct (%)

2. The MCHC is the most accurate of the RBC indicator because its calculation does not essentially require the RBC count.

3. The MCHC is used in the classification of anemia.

**4. Factors affecting the MCHC**

a. **Hemolysis** or treatment with Oxyglobin increased MCHC.

b. A true increase in MCHC does not normally occur; increased concentrations of hemoglobin cannot be produced within the cell.

c. In **reticulocytosis** the MCHC may be decreased,because Reticulocytes do not have their full component of hemoglobin.

d. Iron deficiency cases have hypochromia (i.e., low MCHC) .

D. **Red cell distribution width** (RDW)

It is an index of the degree of anisocytosis or variation in size of the erythrocytes.

1. This erythrocyte parameter can be determined by some automated cell counters.

2. The RDW is the coefficient of variation of the red cell volume distribution

3. Anemia with significant microcytosis or macrocytosis have an increased RDW. Reticulocytosis may result in an increased RDW.

**BLOOD SMEAR**

A. Staining and examination of the smear

1. **New methylene blue** (NMB).

2. **Romanowsky** **stains** (e.g., Giemsa stain, Wright’s stain, Diff-Quik®, Hemacolor®, etc.). These polychromic preparations stain certain acidic groups blue (RNA) to purple (mast cell and basophil granules and nuclear DNA), whereas basic groups stain red to orange (proteins, eosinophil granules).

**Erythrocyte morphology**

1. Normal morphology

a. **Canine** erythrocytes average **7 μm** in diameter, are uniform in size, and have central pallor (biconcave disk).

b. **Feline** erythrocytes average **5.8 μm** in diameter, have mild **anisocytosis** (i.e., variation in size), and exhibit **very slight central pallor**. **Crenation** is commonly observed. **Howell-Jolly bodies** (nuclear remnants) occur in up to 1% of the erythrocytes. **Rouleaux** formation also may be present.

c. Bovine erythrocytes average **5.5 μm** in diameter. **Anisocytosis** is common, and **central pallor** is usually slight. **Crenation** is common.

d. **Equine** erythrocytes average **5.7 μm** in diameter and **lack central pallor**. **Rouleaux** formation is common.

e. **Ovine** erythrocytes are similar to those of the cow but smaller. The average diameter is **4.5 μm.**

g. Camelidae (camel, llama, alpaca, etc.) erythrocytes are thin (about **1.1 μm**) and ellipsoidal in shape with an average diameter of **6.5** μm, and usually have high erythrocyte counts.

2. **Rouleaux formations** are groups of erythrocytes resembling stacks of coins. The degree of rouleaux tends to **positively** link with the erythrocyte sedimentation rate (ESR), and is usually associated with an altered **surface membrane charge** (zeta potential). The intensity of this charge can be a **species** characteristic or the result of **disease**. Rouleaux is common in **horses** that have a decreased membrane charge in health. In certain diseases, normal membrane surface charge may be partially covered by excess protein (hyperfibrinogenemia, hyperglobulinemia) that decreases the repel negative surface charges of the erythrocytes. Rouleaux and an increased ESR will be observed. Microscopically, rouleaux can be distinguished from autoagglutination by its diffusion in wet mounts when blood is diluted with physiologic saline solution.

a. Marked **rouleaux** formation is common in equine blood in health, but may be **absent** in the blood of severely **anemic** or cachectic(chronic disease) horses.

b. Moderate and mild rouleaux may be present in feline and canine blood in health, respectively. Marked rouleaux may be observed during inflammatory and neoplastic diseases.

c. **Rouleaux** formation is rare in ruminant blood in health and disease.

3. **Agglutination** is a grape-like aggregation of erythrocytes occurring in some blood specimens of animals with immune- (antibody-) mediated anemia.

4. **Anisocytosis** is variation in the size of erythrocytes because of the presence of macrocytes and/ or microcytes among normocytes .

5. **Macrocytes** are large erythrocytes. **Reticulocytes** are usually macrocytic and polychromatophilic (light blue-gray color when using Wright’s stain). Normochromic macrocytes may occur in certain conditions (e.g., FeLV infections, preleukemia of cats and dogs, erythroid aplasia of cats, and vitamin B12 deficiency).

6. **Microcytes** are small erythrocytes. They may be observed in **iron** and **pyridoxine** **deficiency** anemia in association with a low **MCV**. Microcytes also are associated with **hyponatremia**.

7. **Spherocytes**, associated with immune-mediated anemias, have a decreased **MCV** as a result of a **decreased membrane surface area**. Spherocytes are globoid because the remaining **smaller cell membrane** must enclose a normal amount of hemoglobin. Because spherocytes do not flatten well on the blood smear, they appear smaller than normochromic, biconcave disk erythrocytes.

8. **Polychromasia** refers to the blue-gray erythrocytes with residual RNA that are generally large (macrocytic) and seen on routinely stained blood smears. Polychromatophilic erythrocytes are synonymous with reticulocytes .

9. **Hypochromia** is decreased cytoplasmic staining intensity and increased **central pallor** of the erythrocyte caused by insufficient Hb within the red cell. The most common cause of hypochromia is **iron deficiency**, but it also occur with molybdenium poisoning and lead poisoning via inhibition of hemoglobin synthesis.

10. **Poikilocytosis** is a general term for an **abnormally shaped erythrocyte**. Blood smears should be present with CBC specimens to prevent artifactual alterations in cellular shape if there is time between blood collection and analysis. Specific types of poikilocytes include the following:

a. **Echinocytes** are spiculated erythrocytes with many evenly spaced, uniform projections on the periphery of the erythrocyte.

b. **Keratocytes** are erythrocytes with one or two projections that form a ruptured vesicle. These abnormalities often result from oxidative damage to the erythrocyte membrane, as listed for Heinz body formation.

c. **Schistocytes** (schizocytes) are irregular erythrocyte fragments that result from shearing by intravascular fibrin or by turbulent blood flow within the vasculature.

d. **Acanthocytes** are spiculated erythrocytes with two or more irregular, often blunted, projections. These cells are thought to form as a result of altered lipid:cholesterol ratios in the erythrocyte membrane. In animals, acanthocytes are associated with hemangiosarcoma (especially involving the liver), glomerulonephritis, lymphoma, and liver diseases.

e. **Fusocytes** are elongated erythrocytes that are seen in healthy Angora goats.

f. **Elliptocytes** are oval cells that are seen in healthy camelids. Occasionally, elliptocytes may be observed in iron deficiency.

g. **Dacryocytes** are teardrop-shaped erythrocytes that may result from the inability of the

erythrocyte to return to its pre-existing shape after deforming in the blood vessels (decreased deformability).

h. **Leptocytes** are thin cells with an increased membrane:volume ratio; they may appear folded due to the excess membrane. Leptocytes have been associated with portosystemic shunts. Polychromatophilic erythrocytes (reticulocytes) may appear as leptocytes due to increased cell membrane.

i. Target cells (**codocytes**) are a type of leptocyte that are bell-shaped, but resemble a target on smears due to the distribution of Hb centrally and peripherally in the cell. Target cells may be associated with liver disease, iron deficiency anemia, and reticulocytosis.

j. **Stomatocytes** are a type of leptocyte that are bowl-shaped with oval areas of central pallor on blood smears. This change in shape results from expansion of the inner layer of the cell membrane.

k. **Spherocytes** are small dark microcytes that lack central pallor and have a reduced amount of membrane per unit volume.

**11.Erythrocyte inclusions**

1-**Basophilic stippling** represents punctate aggregation of residual RNA in Romanowsky-stained (Wright- or Diff-Quik®-stained) cells. This often occurs in anemic sheep and cattle, and occasionally in feline anemia.

2-**Howell-Jolly** bodies are basophilic nuclear remnants within the cytoplasm of erythrocytes.These structures are observed more frequently in accelerated erythropoiesis or postsplenectomy.

3- The **Heinz body** is a round structure that protrudes from the membrane of the erythrocyte or appears as a small refractile spot in the cytoplasm. Heinz bodies are comprised of denatured, precipitated Hb caused by oxidation. They are often attached to the inner cell membrane. Because Heinz bodies are derived from hemoglobin, they are the same color as the remainder of the cytoplasm and can be indistinct with Romanowsky staining.

4- **Parasites** can occur:

1-Within the erythrocyte (**intracellular**) e.g. *Cytauxzoon felis, Babesia cati, B. felis* (cats); *Anaplasma marginale, A. centrale* (cattle); *Babesia bovis, B. bigemina* (cattle); *Theileria mutans, T. annulata* (cattle); *Babesia canis, B. gibsoni* (dogs); *Babesia equi, B. caballi* (horses); and *Babesia ovis, B. motasi* (sheep).

2-Within depressions on the membrane surface (**epicellular**) e.g.  *Mycoplasma haemofelis* (cats), *M. haemocanis* (dogs) and *Eperythrozoon wenyoni* (cattle),

3-Within the plasma (**extracellular**) e.g. (1) Microfilariae: *Dipetalonema reconditum* (dogs), *Dirofilaria immitis* (dogs), and *Setaria* sp. (horse).

(2) Trypanosomes: *Trypanosoma theileri, T. congolense, T. vivax* (cattle); *Trypanosoma cruzi,* (dogs); and *Trypanosoma brucei, T. evansi* (horses).

4- **Punctate reticulocytes** (containing small, blue-stained dots) are derived from aged aggregate reticulocytes and persist for at least two weeks. Like aggregate reticulocytes, they are increased with increased erythropoiesis. Reticulocyte counts increase in regenerative anemias.

**ANEMIA: DIAGNOSIS AND CLASSIFICATION**

Anemia is an absolute decrease in the Hct, Hb concentration, and/or RBC count. Relative anemia may occur when the plasma volume is increased (e.g., excessive parenteral fluid administration, pregnancy, neonates) or when blood specimens are improperly obtained from intravenous (pseudoanemia).

**I. DETERMINATION OF THE CAUSE OF ANEMIA.**

 **AS WITH ANY DISEASE, THE DIAGNOSIS OF ANEMIA IS MADE FROM HISTORICAL INFORMATION, PHYSICAL FINDINGS, AND LABORATORY FINDINGS.**

A. Historical information. The following findings may be important:

1. Drug administration or vaccination

2. Exposure to toxic chemicals or plants

3. Occurrence of disease

4. Diet or colostral ingestion

5. Age at onset of clinical signs

6. History of prior blood disorder

7. History of prior pregnancy of dam

B. **Physical findings**

1. Clinical signs suggesting the presence of anemia are include the following:

a. Pale mucous membranes.

b. Weakness, loss of energy, exercise intolerance.

c. Tachycardia and polypnea, particularly after exercise.

d. Syncope, depression.

e. Increased sensitivity to cold.

f. Heart murmur caused by reduced viscosity and increased instability of blood flow.

g. Weak pulse.

h. Shock, if one-third of the blood volume is lost rapidly.

2. Icterus, hemoglobinuria, hemorrhage, melena, petechiae, or fever may be observed, depending on the pathophysiologic mechanism involved.

3. Clinical signs are less obvious if the onset of anemia is gradual and the animal has adapted to the decreased erythrocyte mass and lowered oxygen transport capability (Hct of less than 10%).

C. **Laboratory findings**. Laboratory confirmation is necessary because anemias are not always accompanied by typical clinical signs. Mild anemia often is diagnosed from the laboratory data of a sick animal when its presence was not previously suspected.

1. The **PCV** is the easiest, most accurate method to detect anemia. The PCV value should be interpreted with knowledge of the patient’s hydration status and with any possible influence of splenic contraction (exercise).

2. **Hb** concentration and **RBC** count used to more classify the anemia but usually are not needed to confirm its presence.

3. This three parameters are very important in the morphological classification system of anemia.

**II.CLASSIFICATION OF ANEMIA:**

**A. Morphological Classification of Anemia:**

 The cause of the anemia should be identified during anemia because the term “anemia” does not represent a definitive diagnosis, so the classification system of anemia are used for definitive diagnosis of the cause. This morphological classification give idea about the size (through **MCV** classification) and Hb concentration (through **MCHC** classification) of the erythrocyte,so this type of classification have 6 type.

A- normocytic normochromic anemia

B- normocytic hypochromic anemia

C- macrocytic normochromic anemia

D- macrocytic hypochromic anemia

E- microcytic normochromic anemia

F- microcytic hypochromic anemia

1. The main corpuscular volume (MCV) classify the anemia as **normocytic**(the average erythrocyte volume is within the reference interval), **macrocytic**(increased volume), or **microcytic** (decreased volume).

2. The main corpuscular hemoglobin concentration (MCHC) classify the anemia as **normochromic** (Hb concentration is within the reference interval), or **hypochromic** (Hb concentration is decreased).

3. Hyperchromasia (hyperchromic) is rarly occur because erythrocytes do not overproduce hemoglobin. Erythrocyte hemolysis or Oxyglobin administration lead to Hyperchromasia. Also in spherocytosis (with decreased pcv) may be associated with hyperchromasia because of decreased erythrocyte volume (rare conditions).

**B. Classification according to bone marrow response:**

**1. Regenerative anemia**

a. The bone marrow actively responds to the anemia by increasing production of erythrocytes.

b. Signs that refer to regenerative anemia from erythrocytes (blood smear):

(1) **Polychromasia**

(2) **Reticulocytosis** with **anisocytosis** and increased RDW.

(3) **Macrocytosis** (increased MCV) and **hypochromasia** (decreased MCHC) associated with reticulocytosis.

(4) **Basophilic stippling** of erythrocytes in ruminants.

(5) **Hypercellular** bone marrow with a decreased M:E ratio due to erythroid hyperplasia

d. The presence of regeneration suggests an extramarrow etiology: **blood loss** (e.g., hemorrhage) or **lysis** (e.g., Heinz bodies, immune-mediated) of erythrocytes of sufficient duration (two to three days) for a regenerative response to be evident in the blood.

e. Examples of regenerative anemias include: **hemolysis**, **hemorrhage**, or regeneration after the cause of a nonregenerative anemia has been resolved.

**2. Non-regenerative anemia**

a. Nonregenerative anemia suggest the lack of an erythroid response in the bone marrow. Lack of erythropoiesis response could be the result of **inadequate time** for erythropoiesis to occur or **inadequate iron, protein or hormones** .

b. Examples of **non-regenerative** anemia include: **anemia of inflammatory disease** (AID), **renal failure**, **iron deficiency anemia**, **aplastic anemia**, **pure red cell aplasia**, and **endocrine disorders**.

c. Also during the first two to three days after the onset of peracute or acute **hemorrhage** or **hemolysis**, the anemia may appear **non-regenerative**.

d**. Polychromasia, reticulocytosis,** and **basophilic stippling** (ruminants) are absent.

e. **Bone marrow examination** occasionally may reveal cases of nonregenerative anemia.

f. Horses do not release **reticulocytes** into blood; therefore, all anemias appear **non-regenerative** in **this** species.

**C. Etiological classification: according to pathophysiologic mechanisms**

**1-Hemorrhagic anemia(**blood loss anemia**) :** occur due to hemorrhage that may be **acute** or **chronic** and **external** or **internal** hemorrhage.

**A. In acute hemorrhage**

**1- Clinical signs** depend upon the **amount** of blood lost, period of **time** during which bleeding occurred, and **site** of hemorrhage. Hemorrhage from multiple sites or delayed onset of clotting at sites of vascular damage suggest **clotting test abnormalities** .

**2- Laboratory findings**

 **Thrombocytopenia** and **clotting test abnormalities** indicate for hemorrhage.

1. A **regenerative** response occurs .

2. The **Hct** initially is within the reference interval because all blood components (i.e., cells and plasma) are lost in similar size. The animal may be in hypovolemic shock if more than 33% of the blood volume is lost rapidly.

3.**Hypoproteinemia** (decreased plasma protein concentration) also observed.

4.**Thrombocytosis** during the first few hours after hemorrhage. Persistent thrombocytosis may suggest continued blood loss.

5. Neutrophilic leukocytosis commonly occurs.

**3-Acute hemorrhage causes:**

1.Gastrointestinal ulcers.

2.Hemostasis defect: bracken fern toxicosis, sweet clover or warfarin toxicosis, hemophilia and thrombocytopenia.

3.Neoplasia: Gastrointestinal.

4.Trauma,surgery.

**B. In chronic hemorrhage**

**1- Clinical findings**

1. Anemia develops slowly and hypovolemia does not occur.

2. The Hct can reach low values before clinical signs of anemia become clear because the slow onset of anemia allows for physiologic adaptations.

**2- Laboratory findings**

1. A regenerative response occurs

2. Hypoproteinemia .

3. Persistent thrombocytosis.

4. Anemia usually microcytic hypochromic due to Iron deficiency may develop because body iron stores are depleted.

**3-Chronic hemorrhage causes:**

1.Gastrointestinal ulcers.

2.Neoplasia: splenic hemangioma, splenic hemangiosarcoma.

3.Hematuria.

4.Hemophilia.

5.Vitamin K deficiency.

6.Parasitism: ancylostoma,coccidiosis, hemonchus,strongylosis,fleas,ticks and lice.

**2- Hemolytic anemia**:occur due to excessive erythrocyte destruction (decreased erythrocytic life span) by extra- or intravascular hemolysis. Extravascular hemolysis is much more common than intravascular hemolysis.

**A-Clinical and laboratory characteristics of extravascular hemolysis:**

1.Hemoglobinemia(red plasma) and hemoglobinuria are **absent.**

2**.**Regenerative response (**reticulocytosis**)

3.Normal or increased plasma protein concentration.

4.Hyperbilirubinemia(**icterus**) with unconjugated bilirubin in early disease but at late time became conjugated bilirubin accompanied by(**bilirubinuria**).

5.**Neutrophilia**, **monocytosis** and **thrombocytosis** occur commonly.

6.Splenomegaly result from increased macrophage activity and extramedullary hematopoiesis.

7.Abnormal erythrocyte morphology occur in variety anemia e.g. RBC parasite, spherocytes,shistocyte and keratocyte indicate for excessive phagocytosis of erythrocytes.

**B-Intravascular hemolysis**

1.Destruction of the RBC within the circulation lead to hemoglobin release into plasma and this removed by **liver** or **kidney**.

**2.Mechanisms of Intravascular hemolysis 1-Complement-mediated lysis:** lysis by complement and with or without IgM. **2-Physical injury**: Trumatic disruption and disseminated intravascular coagulation

 **3-Oxidative injury**: Heinz bodies or eccentrocytes indicate for oxidative damage by the oxidants(free radicals).

 **4-Osmotic lysis**: a. Hypophosphatemia:post-parturent hemoglobinurea,diabetes

 c. Hypotonic intravenous fluids lead to osmotic lysis.Cold hemoglobinurea in cattle when drink cold water.

 **5-Membrane lysis**:caused by:castor bean,snake venom, bacterial toxin

 **6-Hemolysis by Intravascular phagocytosis:** recent transfusion of incompatible blood and recent ingestion of colostrums.

**3. Clinical and laboratory characteristics of intravascular hemolytic anemia**

 Usually presents as a peracute or acute disease.

 1- A regenerative response (**reticulocytosis**) occurs in 2-3 days.

 2- **Hemoglobinemia** is the principal feature lead to **hyperchromasia** (increased MCH and MCHC) and **hyperproteinemia**. Hemoglobinemia is usually detected by the following:

 (1) Red discoloration of plasma

 (2) Increased MCHC and MCH

 (3) Decreased serum haptoglobin and hemopexin concentrations.

 3- Hemoglobinuria occur after 12 to 24 hours following hemolysis.

 4- Hemosiderinuria

 5- Hyperbilirubinemia(**Icterus**) occur after 8 to 10 hours of the hemolysis.It is unconjugated bilirubin and then conjugated bilirubin becomes more prominent with time become the major form and this accompanied by bilirubinuria .

 6- Abnormal erythrocyte morphology include:schistocytes, keratocytes, Heinz bodies, eccentrocytes and erythrocytic parasites.

**C.Causes of hemolytic anemia**

A- Extravascular (phagocytic)hemolysis

**1.RBC parasites:** *Anaplasma spp., Eperythrozoon spp., Hemobartonella spp., Theileria spp.,Trypanosoma spp., Sarcocystis spp., Ehrlichia spp.*

2.Viral infection: equine infectious anemia virus, feline leukemia virus

**3. Immune-mediated hemolysis**:autoimmune hemolytic anemia, lupus erythematosus

**4. Intrinsic erythrocytic defects**: erythrocytic porphyria, pyrovate kinase deficiency

**5.Fragmentation**: disseminated intravascular coagulation

B- Intravascular hemolysis

**1. Bacterial infection**: *Clostridium hemolyticum* and *leptospira* spp.(bacterial toxin)

**2. RBC parasites**: *Babesia* spp.

**3. Dietary deficiencies**: copper , phosphorus, selemium and vitamin K

**4.Chemicals and plants:** snake venoms, onions ,phenothiazin, zink, molybdenium and lead.

**5. Immune-mediated hemolysis**:Autoimmune hemolytic anemia, neonatal isoerythrolysis and incompatible blood transfusions.

**6. Hypo-osmolality hemolysis**: cold water hemoglobinuria, hypotonic fluids and water intoxication.

**3. Reduced or defective erythropoiesis (bon marrow depression anemia).**

a-Anemia caused by reduced or defective erythropoiesis are **nonregenerative**. They are characterized by an **abnormal bone marrow** that cannot maintain effective erythropoiesis. The clinical course is **long** and **dangerous**.

b-Nonregenerative anemias (bon marrow depression anemia), such as the anemia of inflammatory disease(**AID**), are observed commonly in veterinary medicine.

c-Ability of the bone marrow to maintain erythrocytic mass requires the following items to be adequate:

a. Precursor cells (i.e., multi- and unipotential stem cells)

b. Nutrients (e.g., iron and B vitamins)

c. Stimulation (e.g., Epo, IL-3, G-CSF, GM-CSF)

d. Microenvironment

**4. Nutritional deficiency anemia.**

a-Causes of this type of anemia include the following:

**1. Mineral deficiencies** (Iron deficiency , Copper deficiency, Cobalt deficiency)

**2.Vitamin deficiencies** (Vitamin B12 deficiency, Folic acid deficiency, Niacin deficiency ,Pyridoxine(B6) deficiency, Riboflavin(B2) deficiency)

**3.Protein deficiencies (hypoproteinemia)**

1-Relative hypoproteinemia(hp):occur during :

1.excessive administration of intravenous fluids.

2.Intestinal water shift into the plasma after acute blood or plasma loss.

3.Pregnancy.

2-Hypoalbominemia occur with pregnancy, lactation, intestinal malabsorption, malneutrition, exocrine pancreatic insufficiency and chronic liver disease.

3-Accelerated loss of albomin occur with hemorrhage, proteinurea due to renal disease, severe exudative disease, burns, intestinal parasite.

4-hypoglobulinemia occur due to :

1.Failure of colostrum transfer to neonate.

2.Severe combined immunodeficiency disease of Arabian foals.

5-Hypofibrinogenemia due to disseminated intravenous coagulation.

