**LEUKOCYTES : LABORATORY EVALUATION OF LEUKOCYTES**

**I. White blood cell count**

**II. Blood smear evaluation**

**III. Bone marrow examination, evaluate bone marrow aspirates**

**I. WHITE BLOOD CELL COUNT**

A. Methods of determination

1. Manual counts: use hemocytometer chamber, and microscope. Inherent error is about 20%, even with excellent technical skills.

2. Automated cell counters: Results are more acceptable than by manual counting. Inherent error is about 5%.

B. Abnormalities in the total WBC (leukocyte) count:

**1-Leukocytosis**: is an increase in total leukocyte count above the normal upper limit of animal. Causes: 1-generalized infection, 2-localized infection, 3-intoxicosis (metabolic disturbance, chemicals, drugs and venoms),4-rapidly growing neoplasms, 5-acute hemorrhage(particularly into one of the body cavities), 6-sudden hemolysis of RBC, 7-leukemia and 8-trauma.

**2-Leukopenia**: is a reduction in leukocyte count below the normal value. Causes: 1-viral infection, 2-bacterial infection, 3-endotoxins(gram-ve bacteria),4- x-rays, 5-chemical agents (eg.antibiotic) and 6-anaphylactic shock.

**II. BLOOD SMEAR EVALUATION**

A. Microscopic evaluation of the blood smear.

B. Differential leukocyte count

1. The percentage of a given leukocyte type multiplied by the total WBC count gives the absolute number of that leukocyte type/μL of blood. As a cross check, the cumulative total of all the percentages of the various leukocyte subtypes should equal 100%. The cumulative total of the absolute cell counts for the various leukocyte subtypes should equal the total WBC count.
2. Interpretation of the leukogram should be based upon the absolute cell counts/μL of blood and not upon the relative percentages.
3. The suffix “philia” or “cytosis” (e.g., neutrophilia, eosinophilia, basophilia, lymphocytosis, and monocytosis) indicates an increase in number of a particular leukocyte.
4. The suffix “penia” or “cytopenia” (e.g., neutropenia, eosinopenia, basopenia, lymphocytopenia, and monocytopenia) indicates a decrease in number of a particular leukocyte.

(3)The blood concentration of various granulocytes (neutrophils, eosinophils, basophils) and agranulocytes ( monocytes, lymphocytes) results from changes in:

1.the rate of release of these cells from the bone marrow into the blood.

2.the distribution of these cells between circulating and marginal pools within the vasculature.

3.the rate of emigration of cells from the blood into tissues.

(4)Granulocytes and monocytes within the blood are in transit from the bone marrow to the tissues where they will perform their functions. The concentration of lymphocytes in the blood, is primarily a reflection of changes in the kinetics of lymphocyte.

Physiologic factors should be considered in the interpretation of leukocyte counts:

1. Age of animal, effect on both total and differential leukocyte counts. Total leukocyte counts in dog and calf is high at birth. Young animals have differential counts varies from that normally found in adult.
2. Breed or species of animal.Ranging from predominant of lymphocytes in bovine blood, and predominant segmented neutrophils in canine blood.
3. Degree of excitement and muscular activity of the patient during blood collection.
4. Stage of pregnancy.
5. Stage of estrus.
6. Stage of digestion.

Mammalian leukocytes include the neutrophil, monocyte, eosinophil, basophil, and lymphocyte.

All leukocytes participate in body defense, but each is functionally independent.

**I. NEUTROPHILS**

**A. Morphology**

1. Mature neutrophils (segmented neutrophil) of mammals(about 15-45% in bovine):

1. Have multiple nuclear lobes (2-5 lobes) separated by constrictions(polymorphonuclear) except in equine segmented neutrophils (unclear nuclear lobes)
2. Have colorless cytoplasm except in bovine neutrophils (orange to pink cytoplasm) .
3. Cytoplasmic granules (generally do not stain) are lysosomes and contain
4. microbiocidal elements (1-myeloperoxidase, 2-lysozyme,3-defensins)
5. enzymes (1-acid hydrolases, 2-neutral proteases, 3-elastase).

2. Immature neutrophils (band neutrophils about 0-1% in bovine) are uncommon in the peripheral blood of the healthy bovine.The nucleus is U- or S-shaped and of uniform thickness.

**B. Function**

1. Phagocytosis and microbicidal action are the primary functions of neutrophils. This activity is conducted efficiently in tissue but not in blood.
2. Contribute in pathologic events by releasing inflammatory mediators into the surrounding tissues(e.g., rheumatoid arthritis).
3. Amplification of acute inflammation.

**C. Abnormal neutrophils morphology**

1. Toxic change of neutrophils

Toxic change can be associated with severe inflammatory disease. Manifestations of toxic change may occur in mammalian neutrophil:

1. Cytoplasmic basophilia.
2. Cytoplasmic vacuolation.
3. Döhle bodies.
4. Toxic granulation.

2. Neutrophil nuclear hypersegmentation

Five or more distinct nuclear lobes are observed. Causes of nuclear hypersegmentation of neutrophils:

* 1. Corticosteroid therapy
  2. Late stages of chronic inflammatory disease.
  3. Hyperadrenocorticism.

3. Neutrophil nuclear hyposegmentation

Nuclear shape is bands, Observed in some congenital defects in dogs.

**D. Abnormal Neutrophil count:**

1- Neutrophilia

1. systemic infection (Salmonellosis, Pasteurellosis and Leptospirosis) with immature neutrophils.
2. localized infection produced by pyogenic microorganism such as Staphylococcus, Streptococcus and Corynebacteria
3. Corticosteroids

d-Hemorrhage and hemolysis

e-Chemical and drug poisoning and toxemia

2- Neutropenia

a-increased tissue demand:bacterial infection, indotoxemia

b-reduced production:

1-chemotherapy or radiation

2-drug reaction:antibiotic, nonsteroidal anti-inflammatory drug

3-infectious agents:virus, mycosis

c-genetic disorder and metastatic neoplasia.

**II. EOSINOPHILS**

A. Morphology

1. Mature eosinophils (2-15% in bovine) have 2-3 nuclear lobes separated by constrictions and cytoplasmic granules that stain with acidophilic dye (red).
2. Distinct, eosinophilic granules characterize the cell .
3. These granules are lysosomes that contain major basic protein, acid hydrolases, and an eosinophil-specific peroxidase, which play a significant role in eosinophil function.

**B. Function**

1. Eosinophils attach to and kill helminths in a process mediated by antibody, complement, and T lymphocyte.
2. Eosinophils suppress hypersensitivity reactions. They are attracted by and inhibit chemical mediators liberated by mast cells during allergic and anaphylactic reactions.
3. Eosinophils promote inflammation, especially in asthma and allergic disease. They bind to IgE and are activated by antigen-IgE complexes, releasing their granule contents which contribute to tissue damage in allergic reactions.

**C.Abnormal morphology**

Degranulated eosinophils are activated during disease and have cytoplasmic degranulation and vacuolation .

**D.Abnormal eosinophils** **count:**

1. eosinophilia

a-parasitism:Ectoparasite(Arthropodes)Endoparasite(Nematodes, Protozoa and Trematodes).

b-Delayed hypersensitivity:Asthma,Dermatitis

c-Neoplasia:Eosinophilic leukemia,fibrosarcoma

d-drug reaction:tetracycline

2. eosinopenia

1. Corticosteroids adminstration
2. Catecholamine (epinephrine) release promotes eosinopenia
3. Acute infection
4. Any stress condition
5. Hyperactivity of the adrenal gland

**III.BASOPHILS**

1. **Morphology**

Mammalian basophils (0-2% in bovine) contain 2-3 nuclear lobes separated by constrictions or non-lobulated and cytoplasmic granules that stain with basophilic dye (blue) are round and stain purple in most species. Basophil granules contain histamine, heparin, and sulfated mucopolysaccharides.

**B. Function**

Specific functions of basophils include the following:

* 1. Participation in immediate and delayed hypersensitivity reactions through mediator release (e.g., histamine release in allergic reactions).
  2. Rejection of parasites (e.g., ticks).
  3. Possible tumor cell cytotoxicity.

**C.Abnormal morphology**

Degranulated basophils,affected cells appear vacuolated and lack purple granules.

**D.Abnormal basophils count:**

1. basophilia

a-parasitism:Dirofilaria immitis,Hepatozoonosis,Ancylostomiasis,Ticks

b-Allergic diseases:dermatitis,pneumonitis,gastroentritis

c-Drug reactions:heparin,penicillin

d-mast cell tumor and basophilic leukemia.

**E.MAST CELLS**

Mast cells share similar function with basophils but constitute a distinct cell line.

**A. Morphology**

a.Mature mast cells have a round nucleus and a moderate volume of cytoplasm filled with dark purple granules that often obscure the nucleus.

b.Mast cells are tissue leukocytes and are not found in the blood of normal healthy mammals.

**B. Function**

1.Mast cells mostly exist in subepithelial locations (dermis, submucosa of gastrointestinal tract), but can be found associated with near any tissue (lymph nodes, liver, spleen)

2. Mast cells promote inflammatory reactions, especially hypersensitivity.

**C. Mastocytemia**

1. Reactive conditions causing mastocytemia (increased mast cells in the blood) include :

1.Enteritis (e.g., parvoviral enteritis).

2.Pleuritis

3.Peritonitis

4.Hypersensitivity conditions.

5. Visceral or cutaneous mast cell tumor .

**IV. MONOCYTES**

**A. Morphology**

1. In Romanowsky-stained blood films, monocytes are generally the largest leukocytes circulating in health(about 2-7% in bovine).
2. The nucleus is commonly oval or bean shaped or a clover leaf shape.
3. The cytoplasm is blue-gray (darker than that of immature neutrophils such as myelocytes, metamyelocytes, and bands).

**B. Function**

1. Monocytes phagocytize and digest foreign particulate material and dead or aged cells in tissues.
2. Monocytes play an important role in inflammation ,because they contain or secrete: proteolytic enzymes, interferon, IL-1, complement components, prostaglandins
3. Monocytes are a major source of CSFs and cytokines (e.g., G-CSF, M-CSF, IL-1, IL-3, TNF) involved in hematopoiesis.

**C.Abnormal Monocytes** **count:**

1.Monocytosis can occur at any time that neutrophilia occurs.

2. corticosteroids administration , except in the dog .

3.Monocytosis signs the recovery from neutropenia.

4.Bacterial endocarditis and bacteremia.

5.Disorders characterized by suppuration, necrosis, malignancy, hemolysis , hemorrhage , immune-mediated injury, and certain pyogranulomatous diseases may be associated with monocytosis.

**D. Macrophage**

* 1. The monocytes migrate into tissues and become macrophages.
  2. These macrophages are rarely in blood, but may be observed in capillary blood smears in disorders such as ehrlichiosis, histoplasmosis, leishmaniasis, and immune-mediated hemolytic anemia.
  3. Monocyte-derived macrophages include the following:
     1. Macrophages or histiocytes of exudates.
     2. Pleural and peritoneal macrophages.
     3. Pulmonary alveolar macrophages.
     4. Connective tissue histiocytes.
     5. Macrophages of the spleen, lymph nodes, and bone marrow.
     6. Kupffer cells of the liver.

**Function**

1. Macrophages secrete a variety of substances that modulate the inflammatory response (e.g., chemotactic factors, plasminogen activator, collagenase, elastase, complement components, plasmin inhibitors).
2. Macrophages function in immune recognition by phagocytosis and pro
3. cessing foreign substances for antigen presentation to T lymphocytes.

**V. LYMPHOCYTES**

**A. Morphology**

1. Mature lymphocytes

* 1. Mature lymphocytes are small cells with scant amounts of blue cytoplasm. The nucleus is round.
  2. Lymphocytes are the predominant circulating leukocyte in adult ruminants.
  3. Mature lymphocytes are not end cells; on appropriate stimulation they may transform into reactive lymphocytes. Transformation usually occurs in lymphoid tissues but occasionally may be observed in blood .

2. Reactive lymphocytes (immunocytes or transformed lymphocytes) are occasionally encountered in low numbers in blood during periods of antigenic stimulation.

* 1. These are probably T lymphocytes, but could be B lymphocytes.

3. Plasma cells represent the ultimate development of B lymphocytes in response to antigenic stimulation. They occur most frequently in lymph nodes, bone marrow, and other tissues.

**B**. **Function**

1. Antibodies production (immunoglobulins)

Antigenically stimulated B lymphocytes form plasma cells, which secrete Antibodies. This process regulated by macrophages and T lymphocytes.

2. Regulatory activity

Functions of lymphocyte- derived interleukins:

1. Mediation of humoral immunity
2. Mediation of cellular immunity
3. Activation of inflammatory cells
4. Regulation of lymphocyte production, activation, and differentiation
5. Stimulation and regulation of hematopoiesis

3. Cytotoxicity .

1. cytotoxic T lymphocyte subsets (CD8+) are cytotoxic cell for virus-infected and transformed cells.
2. Natural killer cells are cytotoxic cell but do not require previous sensitization to perform this function.

**C.Abnormal morphology**

1. Reactive lymphocytes (immunocytes). These cells are often observed following antigenic stimulation (e.g., infection, vaccination).
2. Lymphoblasts (immature lymphocytes). When observed in stained blood smears, these cells usually indicate malignant lymphoma or acute lymphoblastic leukemia.

**D.Abnormal lymphocyte** **count:**

1- lymphocytosis

a-physiologic response in young animals

b-chronic antigenic stimulation:bacterial,viral, protozoal infections and post-vaccination.

c-hypoadrenocortisim

d-lymphoma and lymphoid leukemia.

2- lymphopenia

a-drugs administration:corticosteroids,interleukin-2

b-Acute systemic infection:septicemia ,indotoxemia

c-Chemotherapeutic drugs radiation

d-Generalized granulomatous