

The ABCs of RBCs

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October 9, 2012

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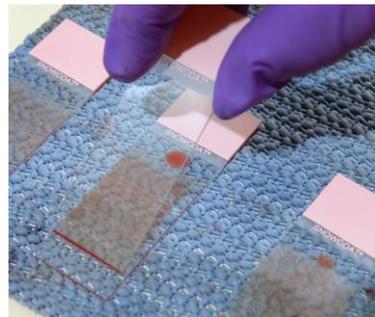
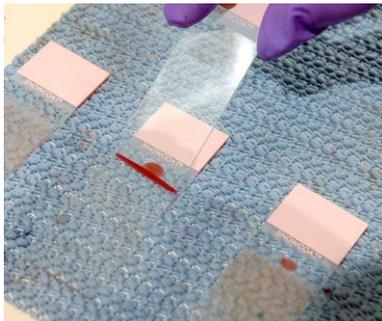
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The first step in obtaining meaningful results from complete blood counts (CBC's) is drawing the blood. The physiological state of the patient influences the CBC results. Excitement or exercise immediately preceding the blood draw can result in splenic contraction and increase in the red blood cell (RBC) count. The blood sample should be drawn by a vacutainer needle directly into an EDTA tube or by syringe and needle and placed into an EDTA tube by puncture of the stopper and vacuum draw into the tube. Upon filling the EDTA tube, the sample should be immediately mixed by inversion of the tube several times. Improper filling of the EDTA tube can result in erroneous results for CBC analysis.



Before performing cells counts, hematocrit or other analysis, the sample must be evaluated for blood clots. Blood clots invalidate the results of the CBC.

Blood smears should be made from either fresh blood (preferred) or well mixed EDTA anticoagulated blood immediately after placement in EDTA. This will prevent artifact resulting from prolonged exposure to EDTA or to the glass tube.



The CBC differential count (ie white blood cell (WBC) distribution and morphology, RBC morphology, platelet estimate and morphology) must be performed on the blood smears. WBC differential counts from automated hematology analyzers are not reliable. Microscopic assessment of the stained blood smear is necessary to determine RBC and WBC morphology, evaluate for abnormal cells, determine platelet estimate and morphology and to screen for parasites or inclusions.

RBC's are assessed for size, color and shape. There is substantial species variation in RBC size.

Species	RBC size (um)	RBC morphology
Canine	7.0	Uniform size; central pallor
Feline	5.8	Slight to no central pallor; mild aniso; crenation common; HoJo's
Bovine	5.5	Aniso; slight to no central pallor; crenation
Equine	5.7	No central pallor; rouleau
Porcine	6.0	No central pallor
Ovine	4.5	Similar to cows but smaller
Caprine	<4.0	Aniso common; no central pallor

Polychromatophilic RBC's are immature anucleated RBC's with stainable cytoplasmic RNA which appears slightly blue in a Wright's stained blood smear. All polychromatophilic RBC's are reticulocytes (ie immature RBC's) but not all reticulocytes have sufficient cytoplasmic RNA to stain polychromatophilic in Wright's stained blood smears. Basophilic stippling in RBC's is the presence of fine to coarse blue to dark purple dots of aggregated ribosomes throughout the RBC cytoplasm. In contrast, siderotic granules are usually found in clusters in the cytoplasm of the erythrocyte. Howell Jolly bodies are nuclear remnants free in the cytoplasm of the RBC. Howell Jolly bodies can be found in healthy animals, especially cats and occasionally in dogs and horses. The number of Howell Jolly bodies increase in some regenerative anemias and may increase in the peripheral blood following splenectomy. Heinz bodies are denatured hemoglobin which is often precipitate on the cell membrane. Heinz bodies appear as lightly stained areas in the RBC cytoplasm which often protrude from the membrane or may be in the extracellular space separate from the RBC's.

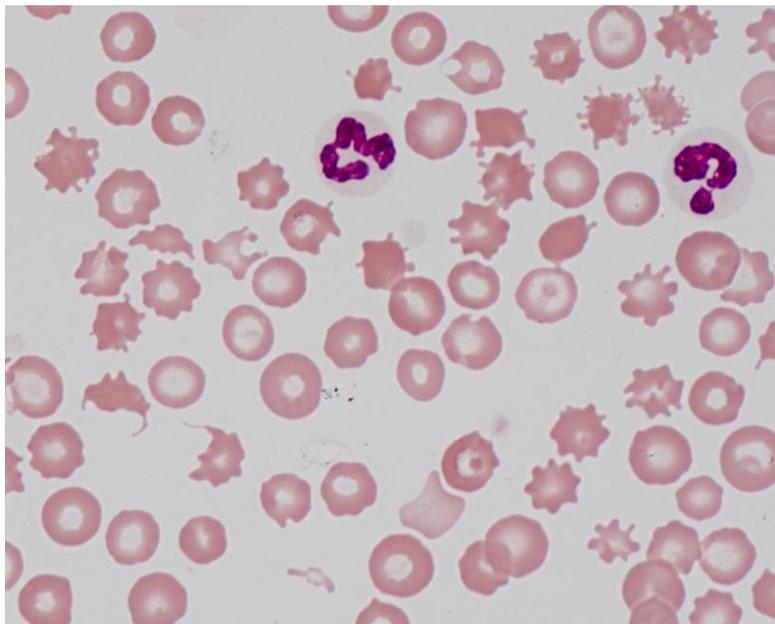
A number of erythrocyte parameters are either measured or calculated by automated hematology analyzers. The mean corpuscular volume (MCV) is directly measured and reported in femtoliters. The $MCV = \text{hematocrit (Hct)} \times 10 / \text{RBC count (millions)}$. Immature RBC's generally have increased MCV. Regenerative anemias in animals with peripheral reticulocytosis are macrocytic. A form of congenital macrocytosis occurs in poodles. Feline leukemia virus infected cats often have macrocytic RBC's. Agglutination of RBC's may cause spurious macrocytosis as the RBC aggregates are included in the calculation of

the MCV. Microcytosis may occur in iron deficient anemia and with portosystemic shunts and occurs normally in Akita dogs.

The mean corpuscular hemoglobin concentration (MCHC) is the most accurate of the indices because the calculation of this parameter does not involve the RBC count. $MCHC = \text{hemoglobin (Hb) concentration} \times 100/\text{Hct}$. Increased MCHC results from hemolysis. Decreased MCHC may occur with reticulocytosis and with iron deficient anemia. In cats, iron deficient anemia may not be manifest by hypochromia. The mean corpuscular hemoglobin (MCH) = $\text{Hb concentration} \times 10/\text{RBC (millions)}$. Factors affecting the MCH are similar to the MCHC. The red cell distribution width (RDW) is determined by some automated hematology analyzers. The RDW is the coefficient of variation of the RBC volume distribution. And is an index of the degree of anisocytosis. Anemias with significant microcytosis or macrocytosis will have increased RDW. Reticulocytosis results in increased RDW.

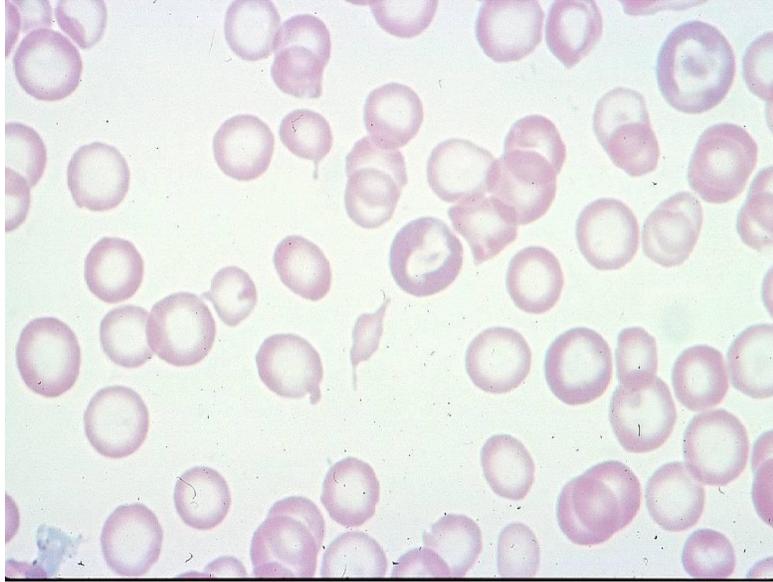
Poikilocytes are RBC's with abnormal shapes. Echinocytes or crenated cells are spiculated erythrocytes with evenly placed, uniform membrane projections. They are often found as an artifact but can be associated with uremia, pyruvate kinase deficiency, doxorubicin administration and malignant lymphoma.

Acanthocytes, also known as burr or spur cells have irregularly spaced surface membrane projections. These cells are found with hemangiosarcomas, splenic, and hepatic disorders. Acanthocytes are occasionally associated with lipid abnormalities associated with renal disease or with high cholesterol diets.



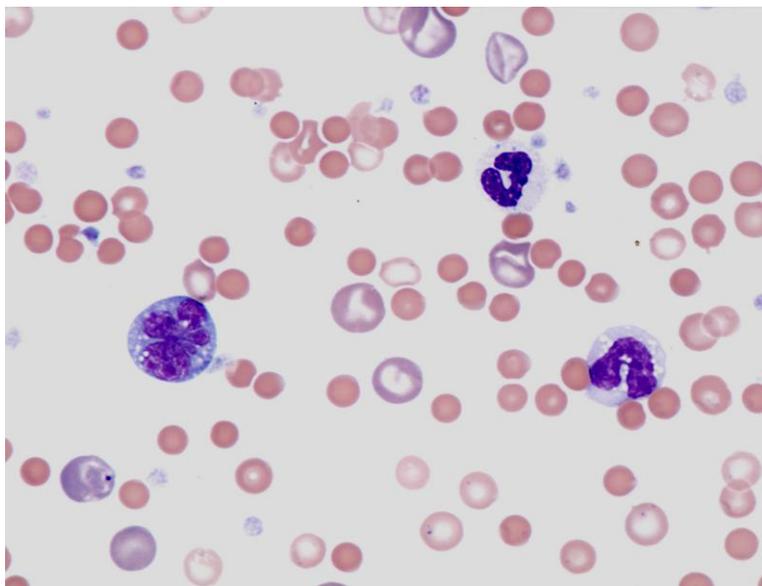
RBC acanthocytosis

Schistocytes are irregular erythrocytic fragment resulting from intravascular trauma secondary to a variety of conditions, including intravascular coagulation, vasculitis, hemangiosarcoma, caval syndrome and endocarditis.



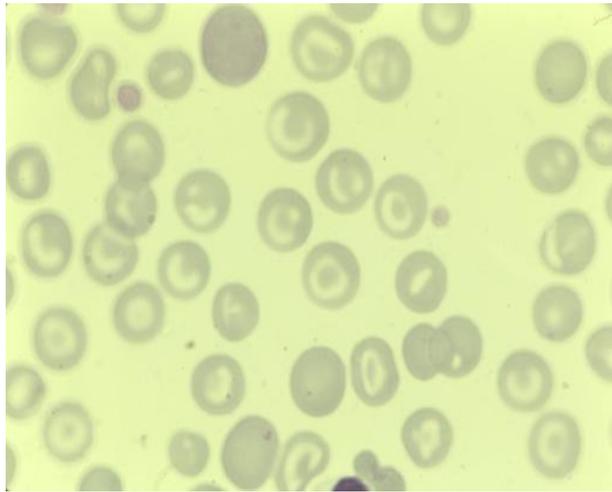
RBC schistocyte and hypochromia

Spherocytes are RBC's with decreased central pallor and increased density of staining. Spherocytes can only be readily recognized in dogs because of the normal central pallor of the canine erythrocyte. Spherocytes are observed primarily in immune mediated hemolytic anemia and have decreased lifespan in the circulation due to decreased deformability.



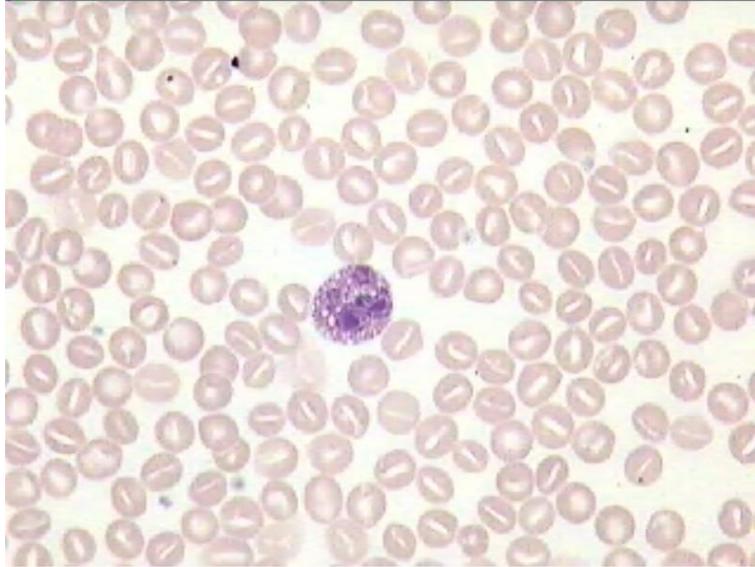
Spherocytes in canine IMHA

Codocytes or target cells are RBC's with disproportional increase in surface membrane area to volume. This is caused by either increased membrane volume or decreased hb. Reticulocytes are often codocytes. Other than in regenerative anemias, codocytes may be seen with biliary obstructive liver diseases. The mechanism of codocyte formation in these conditions is the decrease of lecithin cholesterol acyltransferase which results in increased cholesterol to phospholipase ratio increasing the surface area of the RBC membrane.



RBC codocytes (target cells) in obstructive liver disease in a dog.

Stomatocytes are RBC's with excessive membrane which folds to form an elongated area of pallor in the center. Young RBC's are often stomatocytes. Hereditary stomatocytosis is caused by a defect in the RBC membrane and has been reported in Alaskan malamutes, Drentse partijshonds, miniature Schnauzers, standard Schnauzers and a Pomeranian. In the Alaskan malamutes, this defect was associated with chondrodysplasia and in the Drentse patrijshonds concurrent hypertrophic gastritis occurred with the stomatocytosis. In the other breeds, no associated disease conditions have been reported.



Stomatocytes in a dog.

Anemia is a condition of absolute decreased RBC, Hct and Hb. Relative anemia occurs with plasma volume expansion. Anemias are classified by several parameters.

Anemia

Anemia classification by size (MCV) and Hb content:

- Normocytic
- Macrocytic
- Microcytic
- Normochromic
- Hypochromic

Anemia classification by bone marrow response:

- Regenerative
- Nonregenerative

Hallmarks of regenerative anemias:

- Polychormasia
- Reticulocytosis
- Macrocytosis and hypochromasia (reticulocytosis)
- Hypercellular bone marrow

Species which normally mount the greatest maximal reticulocyte response are the species which have the fastest rate of recovery. In decreasing order of maximal reticulocyte response:

- Dog
- Cat
- Cow
- Horse

Reticulocytosis does not become apparent for 48 – 72 hours after onset of the anemia and reaches a maximum 7 days after anemia onset (dogs). In cats, the maximum reticulocytosis may occur earlier than in dogs with a peak of aggregate reticulocytes 4 days after anemia onset. Dogs have greater reticulocyte responses than cats. In cattle, significant reticulocytosis does not develop in acute responding anemias until the hematocrit is very low. In horses reticulocytosis does not occur.

The presence of regenerative anemia suggests an extramarrow cause (ie hemolysis or blood loss). Bone marrow examination is not necessary in animals with absolute peripheral reticulocytosis to classify anemia as regenerative in most species. However, peripheral reticulocytosis does not occur commonly in horses. Increase in MCV and RDW may suggest regeneration in horses, but bone marrow examination may be necessary to classify anemia in this species.

Nonregenerative anemias indicate inadequate marrow response (ie deficient or defective erythropoiesis). In the first few days after hemolysis or blood loss, the marrow has not had time to produce a reticulocyte response, giving the appearance of lack of regeneration. Diseases directly or indirectly resulting in defective or reduced erythrocyte production are manifest as nonregenerative anemias. Nonregenerative anemias are typically normocytic, normochromic anemias with no poikilocytosis.

Regenerative anemia suggests blood loss or hemolysis.

Causes of blood loss

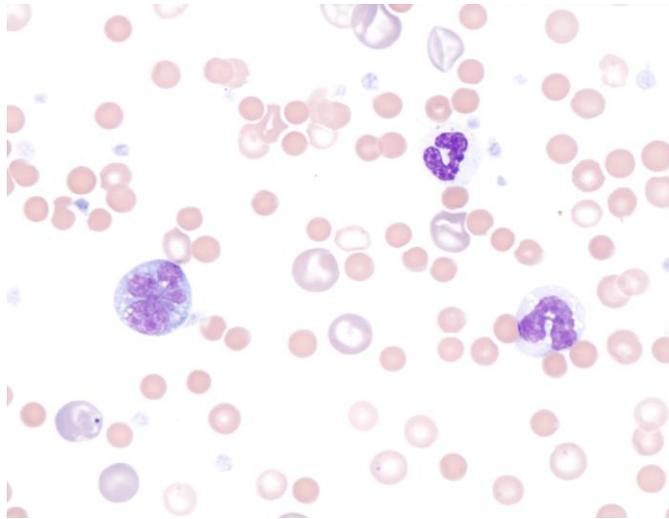
- Acute
 - Trauma
 - Surgery
 - Hemostasis abnormalities
- Chronic
 - Gastrointestinal (GI) parasitism
 - GI ulcers
 - Hematuria
 - Thrombocytopenia

Causes of hemolysis

- Immune mediated

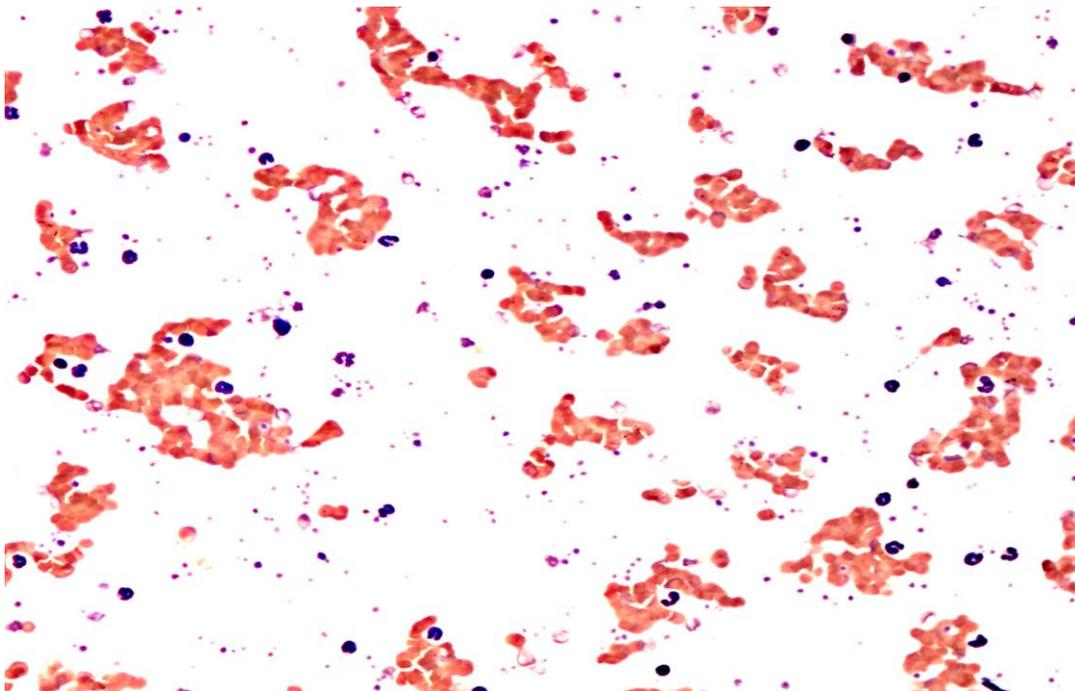
- Autoimmune
- Neonatal isoerythrolysis
- Transfusion reactions
- Secondary to infectious disease agents
 - Erythrocytic parasites
 - Leptospira
 - Clostridium sp.
- Fragmentation
 - Thrombosis
 - Hemangiosarcoma
 - Heartworms
 - Hemolytic uremic syndrome
 - Vena caval syndrome
- Toxins
 - Onions
 - Red maple leaves
 - Rye grass
 - Benzocaine
 - Acetaminophen
 - Copper deficiency
 - Propylene glycol
 - Ricin
 - Snake venom
- Inherent RBC metabolic defect
 - Glucose 6 phosphatase deficiency
 - Phosphofructokinase deficiency
 - Pyruvate kinase deficiency
 - Erythrocytic porphyria
 - Hereditary stomatocytosis
- Selenium deficiency (cattle)

Immune mediated hemolytic anemia (IMHA) is the most common form of hemolytic anemia seen in small animals. There are two common presentations. A regenerative anemia with abundant spherocytes and marked polychromasia is the most common manifestation. .



IMHA in a dog – numerous spherocytes and polychromatophilic RBCs.

Some cases of IMHA in dogs are associated with cold agglutinins causing gross and microscopic agglutination of RBC's.



Autoagglutination in dog blood with IMHA.

Nonregenerative anemias are caused by reduced or defective erythropoiesis. The bone marrow associated with nonregenerative anemias cannot maintain

effective erythropoiesis. The clinical course is long and onset is insidious. The bone marrow failure may be primary or secondary.

- Primary bone marrow failure
 - Inadequate stem or progenitor cells
- Secondary bone marrow failure
 - Nutrient lack
 - Erythropoietin lack
- Selective bone marrow failure
 - Only erythropoietic line affected.
 - Variable severity – complete aplasia to suboptimal response
- Diffuse marrow failure
 - All cell lines affected
 - Variable severity – complete aplasia to suboptimal response

Differential diagnosis of nonregenerative anemia

- Normocytic, normochromic anemia with normal or increased neutrophil and platelet numbers; usually increased M/E ratio due to hypocellular erythroid series
 - Erythropoietin lack anemia
 - Chronic renal disease
 - Endocrinopathies
 - Anemia of chronic disease
 - FeLV associated nonregenerative anemia
 - Pure red cell aplasia
 - Selective loss of erythroid precursors in marrow
 - Mechanism believed to be immune mediated
 - Bone marrow suppression due to chronic inflammatory disease
 - Inadequate marrow nutrients for erythropoiesis
 - Parvoviral infection
 - Unknown mechanisms
 - Trichostrongyle infection
 - Liver disease
 - Vitamin E deficiency
 - May produce nonregenerative anemia in swine
 - Marrow hyperplastic with dyserythropoiesis
- Normocytic, normochromic anemia with neutropenia (except myeloproliferative disorders) and/or thrombocytopenia. M/E ratio variable
 - Aplastic anemia
 - Acellular, fatty bone marrow
 - Disease of multipotential stem cell or marrow microenvironment leading to pancytopenia. Leukopenia and thrombocytopenia usually precede anemia due to shorter lifespan

- Drugs, chemicals, plants, irradiation, cytotoxic T cells, infectious agents (FeLV)
 - Myelophthisic anemia
 - Marrow replaced by abnormal proliferation of cells
 - Myeloproliferative disorders
 - Leukemia, hematopoietic malignancies
 - Myelofibrosis
 - Osteosclerosis
 - Diffuse granulomatous osteomyelitis
 - Metastatic cancer
 - Leukoerythroblastosis may occur
 - Anemia associated with infectious agents
 - Ehrlichiosis
 - FeLV
 - May have anemia with leucopenia and pancytopenia
 - Parvoviral infectious
- Microcytic, hypochromic anemia; variable neutrophil and platelet numbers; M/E ratio variable
 - Iron deficiency
 - Usually due to chronic hemorrhage
 - Transient dietary iron deficiency in young, rapidly growing animals on all milk diet
 - Early anemia associated with ineffective erythropoiesis; later marrow becomes hypoplastic
 - Laboratory findings:
 - Low serum iron
 - Variable TIBC
 - Low saturation of transferrin
 - Low serum ferritin
 - Increased free erythrocytic protoporphyrin
 - Microcytosis
 - Hypochromasia
 - Poikilocytosis
 - Hypercellular marrow with disproportionate number of late rubricytes and metarubricytes
 - Pyridoxine deficiency
 - Copper deficiency
 - Dyserythropoiesis in English springer spaniels
 - Polymyopathy
 - Cardiac disease
 - Microcytic nonregenerative anemia with metarubricytosis and dysplastic erythroid changes
 - Microcytosis with mild anemia in animals with portosystemic shunts
- Macrocytic normochromic anemia; variable neutrophils and platelet number. M/E ratio usually low because of hypercellular erythroid marrow.

- Ruminants on cobalt deficient or molybdenum pastures
- Vitamin B and folic acid responsive anemias
- Erythemic myelosis
- Congenital dyserythropoiesis and progressive alopecia of polled Hereford calves
- FeLV infection

Polycythemia

The term polycythemia is often used to describe a condition more precisely termed “erythrocytosis”. Erythrocytosis refers to increased number of RBC’s in peripheral blood manifest by increased Hct, increased Hb and increased number of RBC’s/uL. Spurious or relative erythrocytosis occurs when RBC mass is normal but there is decreased plasma volume resulting in a relative increase in these parameters (ie hemoconcentration). This may also occur with transient redistribution of erythrocytes due to epinephrine release and splenic contraction with delivery of high Hct blood into general circulation (primarily in horse or cat).

Absolute erythrocytosis is a result of increased erythrocytic mass in peripheral blood. Causes include:

- Secondary, appropriate erythrocytosis
 - Right to left vascular shunts
 - Chronic pulmonary disease
 - High altitude
 - Hyperthyroidism
 - Erythropoietin levels increased
- Secondary, inappropriate erythrocytosis
 - Renal neoplasia, cyst or disease
 - Other neoplasms
 - Erythropoietin levels increased
- Primary erythrocytosis
 - Primary erythrocytosis
 - Polycythemia vera
 - Clonal myeloproliferative disorder with neoplastic proliferation of all marrow cell precursors
 - Erythrocytosis, leukocytosis and thrombocytosis
 - Erythropoietin levels normal or decreased
- Idiopathic erythrocytosis

