

Hematology and Cytology: More than pretty Colors
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Quality from the Beginning

Hematology and Clinical Chemistry data is only as good as the sample submitted

Pre-analysis factors can effect all data

Is the sample fasting?

how was the blood drawn?

how was the sample handled post-draw?

Sample Handling

Blood collection:

Label, label, label

slides and blood tubes

Use frosted end slides - label in pencil

add paper label after staining

If submitting to lab - no paper labels

Sample Handling

Use largest bore needle possible

decrease time for collection

decreases lysis of fragile RBCs

Vacutainer versus needle and syringe?

whatever gives you the best sample

Consider use of a butterfly catheter with Vacutainer connection.

Collecting the Blood Sample

Principles of good venipuncture

#21 gauge or larger facilitates rapid flow, minimizes hemolysis.

move plunger within unit to ensure syringe patency.

enter the vein keeping bevel side of needle up.

Avoid repositioning or excessive suction.

Collecting the Blood Sample

Immediately transfer blood to an EDTA collection tube.

Method 1

Remove needle and tube stopper.

Gently dispense the blood into the tube.

Method 2

Leave needle on and push it through the stopper

allow the vacuum to draw the specimen from the syringe.

Do not force the blood through the needle

Hematology

Instrumentation

Abaxis

CDC Technologies

Heska

Idexx

LaserCyte

QBC technology

Hematology

Automated hematology

Advantages

Speed

“accuracy”

Increased information

Disadvantages

Cost

Maintenance

Tend to forget the blood film

Hematology

Reference laboratories

Advantages

Large combined profiles available

CBC and Chemistry

Specialty testing

Serology

Endocrine

Experienced technologist/technicians

Pathologist available for review of abnormalities

Disadvantages

No stat testing available in most areas

Hematology

CBC

Necessary equipment

Microcentrifuge

Hemocytometer (unopette system)

Microscope

Refractometer

Hematology

Components of CBC

WBC -100 cell differential

PCV or HCT

Platelet count

Total protein

Review of the blood film

Hematology

WBC

Unopette system

Estimate from blood film

Requires good uniform film preparation
Estimate from 10X or 40x
Can be used to support manual count

Hematology

Additional information from an automated count.

RBC indices

MCV

MCHC

RBC count

Automated differential

Review of the blood Film

Allows rapid identification of significant hematologic abnormalities

Marked leukopenia or leukocytosis

Increased band neutrophils

Abnormal cell types

Allows evaluation of cell morphology not reported by automated systems

Toxic change in neutrophils

Red blood cell morphology

Macroplatelets

Review of the Blood Film

Identification of common instrument errors

Miss-sampling

Small clots in sample – decrease the volume analyzed – pancytopenia

Instruments cannot always detect when the sample flow is altered. Daily QC does not eliminate errors due to individual sample quality!

Platelet counts commonly affected by small clusters, large platelets or small red blood cells.

Hematology

Evaluation of blood film

10X

Cellularity

Estimate of WBC count –

High - > 50 / 10x field

Low - < 20 / 10x field

Feathered edge

Platelet clumps

Large cells (blasts, mast cells)

Microfilaria

Hematology

Evaluation of the blood film

40x – must use a coverslip to use this objective lens

Estimate WBC count

Average # of cells/10 fields X 2,000

Quick differential

Hematology

Evaluation of blood film

Counting area

100 cell Differential

WBC estimate

Red cell morphology

Body of film

RBCs touching or just overlapping

Platelet estimate

Hematology

WBC Methods

Estimate from blood film – provides a number to compare with automated count

Count all leukocytes in ten 40 x fields

(raw leukocyte count/# of fields counted) x 2000 = estimated leukocyte count.

Corrected count = (estimated count x (actual PCV/ normal PCV)).

Note: 40x objectives require a coverslip!!

Hematology

Example

50 cells/10 fields

$$5 * 2000 = 10,000 / \mu\text{l}$$

PCV 22

$$10,000 * 22/45 = 4,888 / \mu\text{l}$$

Platelet Estimates

Platelet estimate

Average #/oil immersion field x 20,000

Example:

7 platelets/field

$$7 * 20,000 = 140,000 / \mu\text{l}$$

>10 platelets/oil immersion field – normal platelet mass.

Evaluation of the Erythrocyte

The Erythrocyte

Erythropoiesis – the formation of red cells

Erythrocyte function – oxygen transport

Red Cell Shape

The normal red cell is a biconcave disc

Perfect shape with just the right amount of surface area to exchange oxygen in the lung and tissue

Cell membrane is

~45% protein

~45% lipid

~10% carbohydrate

A protein structural “net” holds the membrane in shape

Erythropoiesis

The formation of erythrocytes

Occurs primarily in the bone marrow

Can also occur in the spleen (extra-medullary hematopoiesis EMH)

RBCs are formed in sinusoids and have to move through the walls of the sinusoids to get into the blood stream (must be flexible)

Oxygen Transport

Hemoglobin

Four molecules of Heme (each with one atom of iron)

One molecule of globin (protein)

95% of the red cell dry weight is hemoglobin

Each heme/iron unit transports one Oxygen molecule

Schistocytes

Hallmark of RBC

fragmentation

Shearing of RBC by

intravascular fibrin strands

Microangiopathy

Turbulent blood flow

Caval syndrome

Valvular stenosis

Intrinsic RBC abnormalities

Severe iron deficiency

Chronic doxorubicin toxicosis

Spherocytes

Hallmark of immune-mediated anemia (often large numbers)

Can be seen with RBC fragmentation (small numbers)

Hypophosphatemia

Toxins (zinc)

Heinz Bodies

Most common in cat

Onions

Acetaminophen

Propylene glycol

Metabolic disease

Diabetes mellitus

Renal disease

Lymphoma

Eccentrocytes

Oxidative injury

Fused inner cell membrane

More commonly

seen in dogs

Basophilic Stippling

Punctate aggregates of RNA

Stain with Wrights-Giemsa

Associated with regeneration

Lead Poisoning

Howell-Jolly Bodies

- Micronuclei or nuclear remnants
- Regenerative anemia
- Post splenectomy
- Can be indicator of marrow injury

Evaluation of the Erythrocyte

- Polycythemia – increased red cell number
 - Relative polycythemia
 - Absolute polycythemia
- Anemia – decreased red cell number
 - Regenerative
 - Non-regenerative

Evaluating the Erythrocytes

- Clinical signs seen with increased RBCs - Injected (red) mucous membranes
- Clinical signs seen with decreased RBCs
 - Pale mucous membranes
 - Icteric mucous membranes

Anemia

- Classification of anemia is based on degree of regeneration.
 - Regenerative anemia – bone marrow is functionally responding to the decrease in erythrocytes
 - Increased erythrocyte production
 - Release of young erythrocytes (reticulocytes)

Anemia

- Non-regenerative anemia – bone marrow cannot respond to the need for erythrocytes
 - Can be primary marrow failure
 - Can be due to non-marrow disease
 - Suppression of erythropoiesis
 - Lack of erythropoietin

Anemia

Changes in the RBC indices with Regeneration

Macrocytosis - increased Mean Corpuscular Volume (MCV)

Dog MCV – 60-77 fl

Some breeds of dog can have an MCV greater than the normal range (Poodles) or smaller (Akitas).

Cat MCV – 39-55 fl

Cats can have a non-regenerative macrocytic anemia associated with FELV.

Cats often have a more prominent increase in MCV during regeneration than dogs.

Anemia

Evidence of Regeneration from the blood film.

Presence of polychromasia.

Blue tinge to RBC on a Wright's Giemsa stain.

Difficult to appreciate with some quick stains.

Polychromatophilic RBCs have a “muddy” blue color.

Anemia

Evidence of regeneration from reticulocyte counts.

Why do reticulocyte counts if there is polychromasia?

Not all reticulocytes appear as polychromatophilic cells.

Is quantitative rather than qualitative.

Anemia

Reticulocytes

Dog – aggregate reticulocytes only

Cat – both aggregate and punctate reticulocytes.

Can count both

Aggregate indicates recent regeneration

Punctate indicates regeneration some time in the past.

Anemia

Reticulocyte count

Count number of reticulocytes/5 fields

in the body of the blood film

(approx #1000 RBCs)/10 = raw %

The raw % can be misleading.

Must be evaluated in light of the degree of anemia

The simple corrected reticulocyte count.

% retics x patients PCV /45 (dog) or 35 (cat)

Corrected count

<1.0 = non-regenerative

1.0-4.0 = adequately regenerative

>4.0 = highly regenerative (commonly hemolytic)

Reticulocyte Count

Example

Total reticulocytes in five 100x oil immersion fields = 28

$28/10 = 2.8\%$

Corrected for degree of anemia

$2.8\% * 21/35$ (for a cat, or 45 for a dog) = 1.7%

1.7% indicates a mildly regenerative anemia

Nucleated RBCs (nRBC)

Metarubricytosis

Appropriate metarubricytosis

Part of the regenerative process

Must also see polychromasia

Metarubricytosis

Effects WBC count on Automated analysis

NRBCs are counted as leukocytes

Need to correct the WBC

$(100/(100 + \text{number of NRBCs counted on differential})) \times \text{WBC count.}$

Example: 10 NRBCs noted during Differential Count, WBC count is 11,000 / μ l

$$(100 / (100 + 10)) \times 11,000 / \mu\text{l} = 10,000 / \mu\text{l}$$

Metarubricytosis

Inappropriate metarubricytosis

Lead toxicity

Myeloproliferative diseases

Erythemic myelosis in the cat

Hypoxia and bone marrow necrosis

Extramedullary hematopoiesis (EMH)

Sepsis/ endotoxemia (marrow injury, splenic compromise)

Neoplasia (splenic hemangiosarcoma)

Hypochromasia

Visual assessment

MCHC (mean corpuscular hemoglobin concentration) measured
assessment of hypochromasia

Decreased with iron deficiency – true decrease in hemoglobin

Decreased with marked regeneration – large polychromatophilic cells have less hemoglobin/volume

Increased MCHC is always an artifact

What to look for if MCHC is increased

Hemolysis (in vivo or artifact at collection)

Heinz bodies

Lipemia

Paraproteinemia

Regenerative Anemia

Blood loss

External blood loss

Anemia

Hypoproteinemia

Internal blood loss

Anemia

Normal proteins

Usually a moderately regenerative anemia.

Must be chronic to result in iron deficiency.

Not common in adult animals.

When seen in adults often associated with bleeding gastrointestinal lesion.

Iron deficiency in young animals.

Usually nutritional or due to internal or external parasites.

Regenerative Anemia

Hemolysis - Intravascular hemolysis

Associated with hemoglobinemia and hemoglobinuria

Zn toxicity, Tylenol, copper toxicity

Some forms of immune-mediated anemia

Some hemoparasites – babesia in acute infections.

DIC – microvasculopathy

Sepsis – Leptospirosis, clostridial diseases

Anemia

Intravascular hemolysis - relatively rare.

Hemoglobinemia results in artifactually increase in MCHC.

May see “ghost cells”

May be difficult to differentiate from increased fragility – lyse in vitro

No hemoglobinuria if the hemolysis occurs during collection of the sample.

Anemia

Extravascular hemolysis

Most common

May be associated with jaundice.

Most forms of immune-mediated anemia

Hypersplenism

Some hemoparasites – M. hemophilia, babesia

No hemoglobinemia, or hemoglobinuria.

Removal of altered RBCs by the

Macrophage phagocyte system.

Spleen, liver, bone marrow

Can be associated with RBC metabolic abnormalities (Pyruvate kinase deficiency).

Immune-mediated Anemia

Can be associated with agglutination.

Must differentiate true immune agglutination from non-specific agglutination.

Non-specific due to abnormal “sticky” proteins.

Saline test.

Add 5 drops of blood to

2 mls of saline – mix, centrifuge, pour off supernatant.

Repeat once more.

Evaluate wet mount on 10X.

Polycythemia

Polycythemia – increased red cell count

Most often associated with dehydration

Dehydration = “relative polycythemia”

High PCV/HCT, High Total Protein

Will return to normal with fluids

Can be a primary disease

Polycythemia Vera

High PCV / HCT, Normal Total Protein

Can be secondary to hypoxia (lack of oxygen)

Pneumonia, large thoracic masses

Leukocytes

Evaluation of leukocytes

Differential count

100 cells categorized by type

200 cells should be counted if the WBC count is greater than
30,000 / μl

Unclassified cells should be included in differential

Note abnormal morphology

Toxic change

Reactive lymphocytes

Leukocytes

Normal leukocytes and their function

Granulocytes

Neutrophils

Eosinophils / Basophils

Lymphocytes

Monocytes

Leukocyte dynamics

Normal resting state

Inflammation

Stress

Excitement

Abnormal Leukocytes: leukemia

Granulocytes

Neutrophil:

Most common granulocyte in circulation in dog and cat.

“Neutral” staining granules.

Granules contain enzymes and antibacterial substances to kill and
degrade bacteria

Primary function.

Primary defense against bacterial disease

Can cause considerable “innocent by-stander” damage to tissues.

Eosinophils:

Function still a bit of a mystery.

Granules contain proteins that bind to parasites (Major basic Protein)

Very “caustic” – causes tissue necrosis

Associated with complex parasites.

Nematodes

Fungi

Some protozoa

Regulate allergic reactions.

Associated with immune-complex disease.

Granulocytes

Basophils:

Function not understood.

Granules are similar to mast cell granules in content.

Uncommon in circulation.

Tend to increase in number in association with eosinophils.

Lymphocytes

Lymphocytes:

Second most common circulating leukocyte in dogs and cats

Primary function: immunity

Two basic types:

B-cells (produce antibody)

T-cells (regulate immune responses)

Types not morphologically different

Can change morphology when immune system is stimulated

Monocytes

Monocytes:

Phagocytosis

Bacteria, complex organisms (fungal elements, protozoa)

Cellular debris associated with tissue necrosis

Regulate repair of tissues

Regulate immune responses

Major cell involved in red blood cell turn-over and iron recycling

Very busy cell!!

The Leukogram

Definition: Numerical and morphologic characterization of circulating leukocytes.

WBC count

Differential cell count

Morphological description of cells

Must understand normal to recognize abnormal.

Neutrophil Dynamics

Describes the normal flow of leukocytes from the bone marrow to peripheral blood.

Leukocyte compartments

Proliferating pool

Maturation/storage pool

Circulating/ marginated pool

Leukocytes

Inflammatory leukogram

Band neutrophils are the hallmark of inflammation.

Neutrophil toxicity is the hallmark of sepsis.

Dog – basophilic foamy cytoplasm and Dohle bodies.

Cat – basophilic foamy cytoplasm.

Dohle bodies are commonly seen in cat neutrophils with no evidence of toxicity.

Leukocytes

The leukocytes

Variation in band neutrophil morphology.

Dog and Cat neutrophils are not as segmented as horse or human. Bands are often over estimated on differential.

Horse bands are more consistent with human morphology.

Human laboratories often over call band neutrophils in the dog and cat.

Leukocytes

Compensated versus Non-compensated left shift.

Compensated – leukocytosis with greater number of mature neutrophils compared to bands.

Non-compensated –

Greater number of bands than mature neutrophils regardless of total count.

Normal or low count with significant numbers of bands.

Neutrophilia

Causes

Inflammation

May or may not have a left shift

May or may not have toxic change

Stress (steroid leukogram)

Does not have a left shift

Associated with lymphopenia

Excitement (epinephrine)

Associated with increases in lymphocytes as well.

Stress

Mature neutrophilia

Increased release of neutrophils from storage pool.

Neutrophils are less “sticky” and move from the marginated pool to the circulating pool.

Increased retention of neutrophils in the circulation.

Steroids make neutrophils less flexible, they can't squeeze between the cells lining blood vessels.

Lymphopenia

Lymphocytes can't get into blood vessels.

Monocytosis

Mechanism unknown – seen in the dog.

Eosinopenia

Mechanism unknown.

Excitement

Increase in blood pressure “washes” marginated neutrophils off the vessel wall into circulating pool.

Increase in lymphocytes (lymphocytosis) that may exceed the neutrophilia.

More prominent in cats (have a larger marginated pool).

No change in the other cell compartments.

Neutropenia

Causes

Severe inflammation

Usually associated with left shift

Toxic change

Bone marrow injury

Can be reversible

Infectious disease (viral, Ehrlichia)

Drugs

Chemotherapeutics

Estrogen

Antibiotics

Or irreversible

Often idiopathic

Neoplasia

also see anemia and thrombocytopenia

Endotoxemia

(Gram-Negative Sepsis)

Makes neutrophils very “sticky”.

Increases marginated pool.

Increases exit from the blood vessel to tissue.

Decreases recruitment from the storage pool.

Decreases proliferation and maturation.

Often associated with a remarkable “rebound” neutrophilia with a left shift.

These changes happen FAST!!

Lymphocytosis

Excitement

All small normal appearing lymphocytes

Usually $<20,000/\mu\text{l}$

Transient

Post vaccination (young dogs/cats)

Small normal appearing lymphocytes

Occasional large immunoblasts

Leukemia

Chronic lymphocytic

Acute lymphoblastic

Chronic canine ehrlichiosis (tick fever)

Lymphopenia

- Stress

- Loss of lymphocytes

 - Chylothorax

 - Protein-losing enteropathy- Due to dilated lymph vessels in the intestine

 - Viral disease

 - Genetic immune deficiency diseases

Monocytosis

- Stress

- Chronic inflammation

- Tissue necrosis

- Monocytopenia is not a problem.

Eosinophilia

- Allergy

- Parasitism

- Fungal disease

- Neoplasia (mast cell tumor)

- Hypereosinophilic syndrome

Eosinophilia

- Most common diseases

 - Allergic dermatitis

 - Asthma - Idiopathic or parasitic

 - Eosinophilic enteritis - Idiopathic or parasitic

 - Heartworm disease

Atypical Circulating Cells

- Reactive lymphocytes

 - Antigenic stimulation

- Neoplastic cells

 - Myeloid – neutrophil origin

 - Lymphoid – lymphocyte origin

 - Myelomonocytic – both neutrophils and monocytes

Monocytoid – monocyte origin

Erythroleukemia – red cell origin

Reactive Lymphocytes

Present in small numbers.

Associated with strong immune response.

Commonly seen in infectious disease.

Canine distemper

Atypical Circulating Cells

Genetic Abnormalities

Pelger-Huët

Decreased lobulation of granulocyte nuclei

Not associated with disease

Chediak-higashi Syndrome

Lysosomal storage diseases

An introduction to Cytology

Cytology

A useful tool in veterinary medicine.

Ease of collection

Inexpensive

Quick

Relatively low-risk to patient with possibility for return of valuable information.

Cytology

Disadvantages

No architecture to evaluate.

Histology usually required as a follow-up in neoplastic diseases.

Requires patience to obtain good samples.

Cytology: the formula

Assess cellularity.

Assess degree of hemodilution.

Note number of intact cells.

Characterize the primary cell populations.

Evaluate background.

Search for etiologic agents if indicated.

Types of Cytological Preparations

Imprints

Of surface lesions.

Of excised tissue.

Scrapings

Fine needle aspirates of mass lesions

Aspirates of body cavity fluids

Sample Preparation

Handling of air-dried slides.

Slides should not be exposed to formalin.

Causes poor staining and loss of cellular detail.

Slides should not be placed in a refrigerator.

Causes water condensation and cell lysis.

Sample Preparation

Body cavity fluids.

Pleural fluid, abdominal fluid, synovial fluid, pericardial fluid.

Always submit in an EDTA tube (LTT).

Only exception is CSF (RTT).

If culture is needed – split the sample and submit portion for culture in clot tube (RTT).

Sample Submissions

Fine needle aspirates

direct preparations.

Air-dried slides – do not fix.

To prestain or not to prestain??

“Squash”prep, blood film prep, pull film.

The primary questions???

Is the lesion inflammatory or neoplastic?

If the lesion is inflammatory:

Is it septic.

If the lesion is neoplastic:

What is its most likely origin?

Mesenchymal? Epithelial? Etc.

Is its benign or malignant.

Inflammatory Lesions

Classification is based on cell type.

Suppurative -> 85% neutrophils.

Granulomatous/pyogranulomatous

Macrophages +/- giant cells, plasma cells and lymphocytes.

Pyogranulomatous – if significant numbers of neutrophils

Eosinophilic – “significant” #'s of eosinophils

Neoplastic lesions

Classification based on cell of origin.

Mesenchymal – sarcoma.

Epithelial – adenoma/adenocarcinoma.

Round cell – histiocytoma,, mastocytoma, lymphoma

Neoplasia –cellular characteristics

General cell shape.

Cellularity of sample.

Tendency to form clusters.

Pattern of cell clusters.

Cytoplasmic borders.

Benign vs. Malignant

Cytoplasmic characteristics.

Nuclear characteristics.

Presence of inflammation.

Reactive hyperplasia can mimic neoplasia.

Characteristics of Malignancy

Nuclear molding/

Multiple, variably-sized nucleoli.

Bizarre mitotic figures.

Anisocytosis and anisokaryosis

Epithelial cells

Polygonal, caudate, or round.

Distinct cell borders.

“Organized” clusters.

Moderated to high cellularity.

Mesenchymal Cells

Spindle to stellate.

Indistinct cytoplasmic borders.

Low to moderate cellularity.

Disorganized clusters.

Mesenchymal Neoplasia – Examples

Round Cell Neoplasia

Individualized cells.

Round nuclei.

Distinct cell borders.

High cellularity.