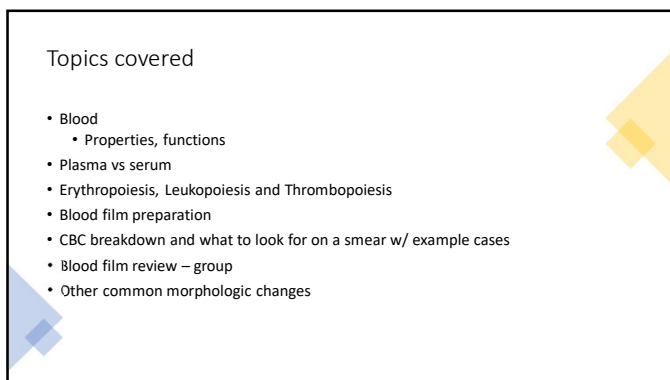
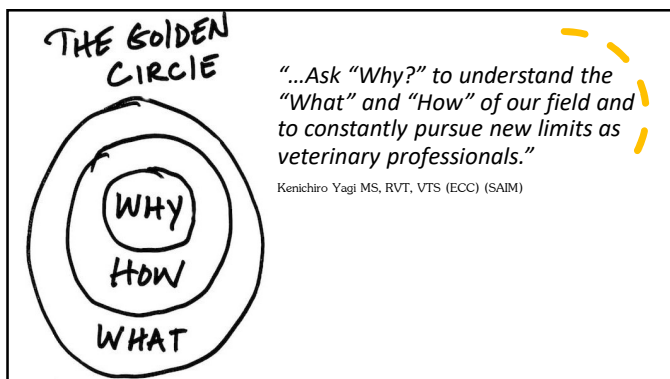




1



2



3



Blood

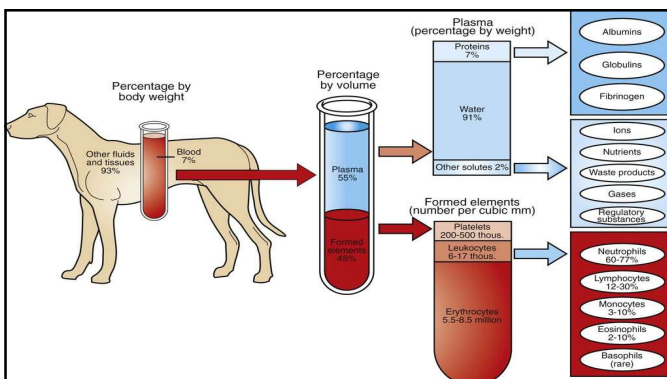
- Connective tissue
- Flows through our arteries and veins
- Most important function – transporting oxygen to cells and removing carbon dioxide

4

So what's blood made of?

STUFF AND THINGS

5



6

Plasma vs Serum

- **Plasma** = **Product of unclotted blood**, composed mostly of water, proteins, clotting factors, and other substances
- **Anticoagulant** = Substance used to tie up a clotting factor... To prevent blood from clotting.



EDTA
Inhibits calcium
(clotting factor #4)



Lithium Heparin
Inhibits thrombin and
factor x



Sodium Citrate
Inhibits Calcium

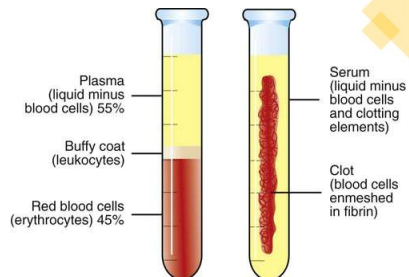


Heparin
Prevents conversion of
prothrombin to
thrombin

7

Serum

- **Serum** = **Product of clotted blood**
- Serum is plasma in which fibrinogen has been removed.
- During clotting, fibrinogen is converted to a fibrin clot matrix. Fluid left over = serum



8

ETDA



- EDTA preferred for hematology
- Excess EDTA/not enough blood = cells shrink and invalidates most cell counts
- Mix well – gentle inversion
- Refrigerate if not ran within one hour. Let warm to room temp
- Platelets start to clump after 30 minutes

9

Blood Functions



10

Transportation

- Hemoglobin transports O₂ to tissues
- Nutrients to arteries and capillaries
- Blood carries waste products from cellular metabolism to lungs/kidneys
- Transports hormones from endocrine glands to target organs
- Transports WBC and platelets



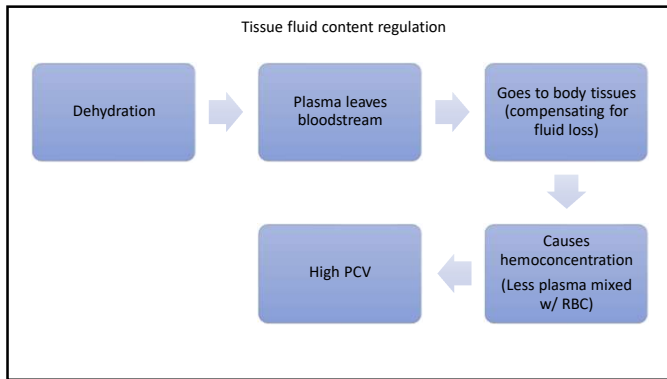
11

Regulation

- Aids in the regulation of body temperature.
- Regulates blood pH. Blood must maintain between 7.35 and 7.45 pH (slightly alkaline).
- Body tissue fluid is kept as constant as possible



12



13

Defense

- Blood carries WBC to tissues exposed to foreign invaders
- Carry platelets to vessel damage sites to aid in hemostasis.

An illustration of three football players in orange jerseys and green helmets, in a three-point stance on a green field. The player in the center is slightly ahead of the other two.

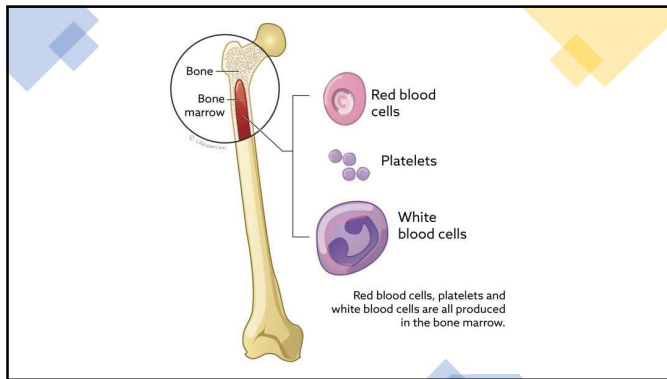
14

Where does blood come from?

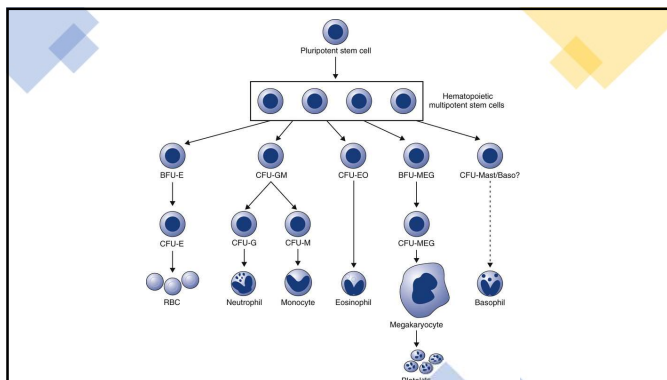
- **Hematopoiesis = the production of all blood cells (continuous process)**
- Fetus = Liver and spleen
- Newborn animal = Red bone marrow
- Mature animal = Red bone marrow, but have more yellow (more fat cells)
- Liver and spleen (times of great need)

The diagram shows four bones representing different stages of life: Infancy, Childhood, Adolescence, and Early Adulthood >25 years. A legend indicates that blue represents Cartilage, red represents Red Marrow, and yellow represents Yellow Marrow. In infancy, the bone is mostly red with blue cartilage at the ends. In childhood, the red marrow expands. In adolescence and early adulthood, the marrow becomes predominantly yellow, indicating a shift from red to yellow marrow.

15



16

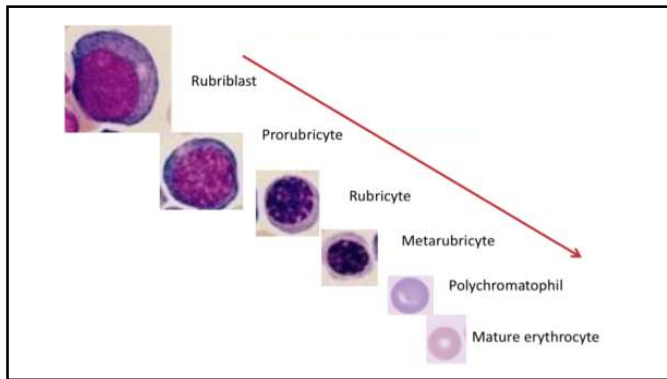


17

Erythropoiesis

- Stem cells differentiate into many other stages of RBC, lose their nuclei, start producing hemoglobin and then 3 stages later become mature
- This entire process takes 1 week in a dog
- Controlled by erythropoietin
 - Hormone produced by kidneys
 - Regulated by blood oxygen levels in kidneys
- Hypoxia stimulates erythropoietin production.

18



19

Leukopoiesis

- **Leukopoiesis** = the general term for production of WBC. Each specific type of WBC has its own stimulus for production
- **Granulopoiesis** = the production of granulocytes (neutrophils, eosinophils and basophils).
- **Lymphopoiesis** = the production of lymphocytes
- **Monopoiesis** = the production of monocytes

White blood cells

The diagram shows five types of white blood cells arranged in a row. From left to right, they are: neutrophil, eosinophil, basophil, monocyte, and lymphocyte. Each cell is represented by a small circular icon with its characteristic internal structure.

20

Thrombopoiesis

- **Thrombopoiesis** = the production of platelets
 - Megakaryocyte = Large multinucleated cell that never leaves the bone marrow. Pieces of the cytoplasm are released into the peripheral blood as platelets. Thrombopoiesis takes up to 7 days

21

CBC

Complete Blood Count

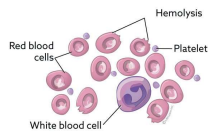


22

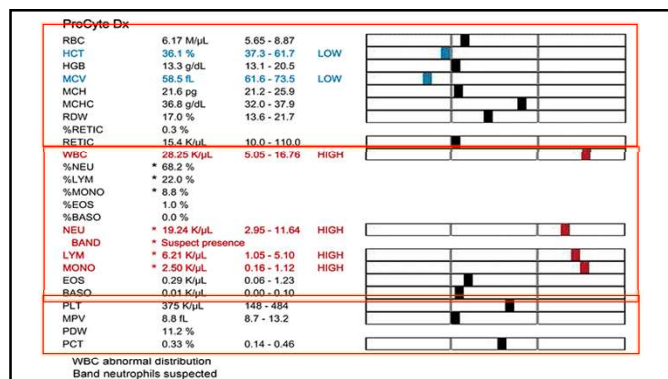
CBC – Complete Blood Cell Count

• Consists of

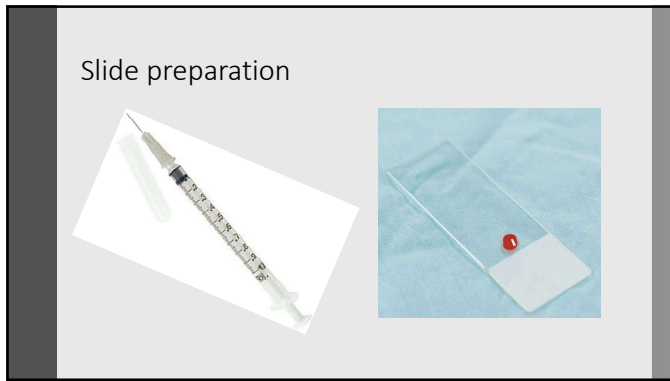
- Total red blood cell (RBC) count
- Packed cell volume (PCV)
- Plasma protein concentration
- Total white blood cell (WBC) count
- Blood film examination: differential WBC count, erythrocyte and leukocyte morphology, and platelet estimation
- Reticulocyte count when the patient is anemic
- Hemoglobin concentration
- Erythrocyte indices



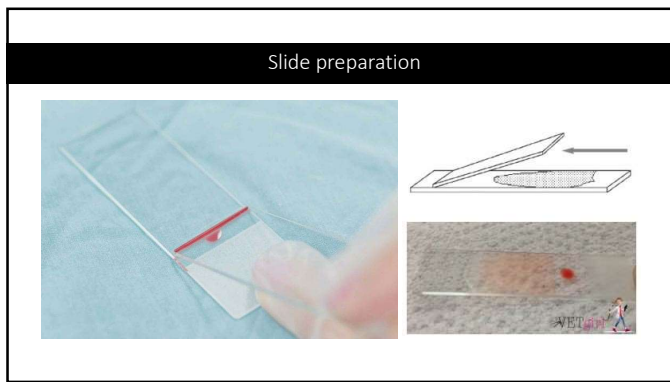
23



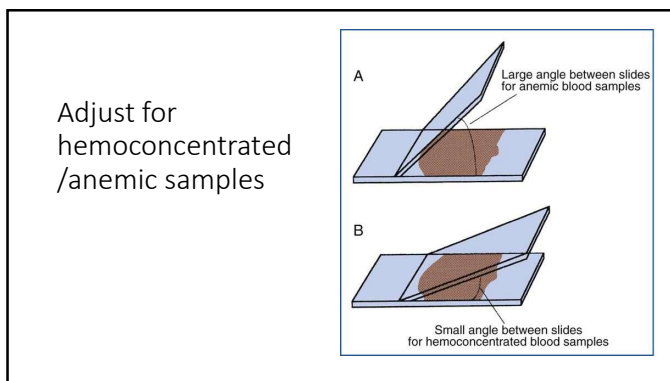
24



25



26



27

Staining Blood Films

Dip Quick Stain - Jorgensen

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DIRECTIONS FOR USE

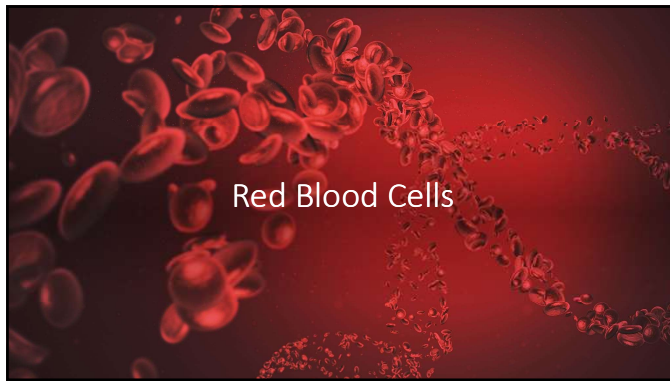
1. The slide is allowed to air dry.
2. After air drying the slide is repeatedly dipped directly into the wide mouth bottle marked #1 fixative for a total of 5 seconds. The slide is held up out of the bottle and excess fixative drained off.
3. The slide is repeatedly dipped directly in bottle with component #2 for a total of 5 seconds. It also is held up out of the bottle to drain off excess stain.
4. The slide is finally repeatedly dipped in component #3 bottle for 3-5 seconds.
5. Gently rinse with distilled water and gently blot dry with a paper towel.

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Lab Time

MAKE A SMEAR! 😊

30



31

RBC

- **RBC** = measures the RBC in a specified volume of blood

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HCT/PCV

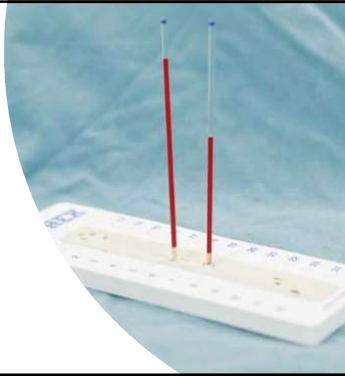
- **Packed Cell Volume or Hematocrit** (PCV or HCT) is the volume of packed erythrocytes expressed as a percentage of total blood volume.
- **PCV** generally refers to a manual count done with a microhematocrit tube
- **HCT** generally refers to an automated count done by a CBC machine.
- Useful in screening patients for anemia or dehydration

$HCT = (MCV \times RBC) \div 10$

33

Packed cell volume (PCV)

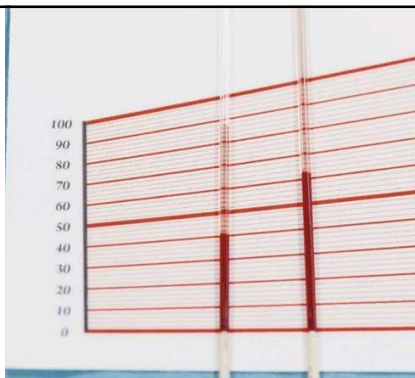
- Procedure:
 - Place whole blood into a heparinized tube or anti-coagulated blood into plain blue HCT tube.
 - Fill tubes $\frac{3}{4}$ full, fill at least 2 tubes, plug with clay
 - Centrifuge 2-5 minutes



34

PCV continued

- Line up bottom of RBC layer at the zero line and top of the plasma on the top line
- Use reader card to obtain percentage
- If using several tubes, average the PCV obtained.



35

- RBC have the highest specific gravity = bottom of tube
- Buffy coat (whitish-gray layer) = middle layer, contains WBC and platelets.
- Plasma =(clear to pale yellow) = top layer. Record any abnormalities
- Cloudy/white = lipemic
- Reddish tint = hemolyzed
- Yellow = icteric



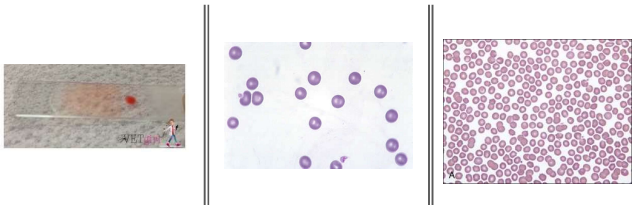
36

Normal PCV Levels

- Less than indicated range = anemia
- Greater than indicated range = polycythemia or dehydration

Canine	37-55
Feline	30-45

37



Confirming hemoconcentration with a blood smear

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Anemia

- Anemia = low PCV/HCT, lower than normal RBC count
- This results in a decreased oxygen-carrying capacity of the blood
- Reasons:
 - Loss
 - Destruction
 - Production problem

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Polycythemia

- **Polycythemia** – PCV/HCT higher than normal
- Three types
 - Relative
 - Compensatory – Result of hypoxia.
 - Polycythemia rubra vera – Rare bone marrow disorder

Why is there a greyhound on this slide??



40

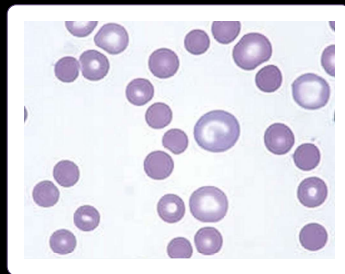
Mean Corpuscular Volume

- MCV is the measure of the average size of the erythrocytes.
 - High = Cells are macrocytic
 - Low = Cells are microcytic
 - Normal = Could be normal, or a mixture of sizes!
- Changes could be
 - Artifact:
 - Clumping, agglutination
 - Storage related changes could cause “macrocytic” artifact.
 - Pathologic:
 - Macrocytic = may be large amount of immature RBC
 - Microcytic = may be anemic, iron deficiency

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Anisocytosis

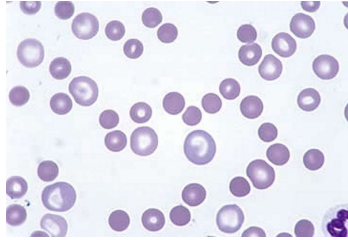
- Variation in RBC size
- Large RBC are immature RBC
- Normal in bovine blood



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RDW (Red Blood Cell Distribution Width)

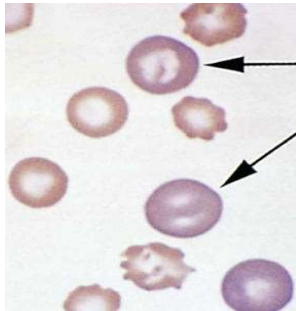
- Objective measure of cell size variation.
- Elevated level also indicates anisocytosis.
 - Whether small/large, small/normal, large/normal... etc
- Decreased level not clinically significant



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Polychromasia

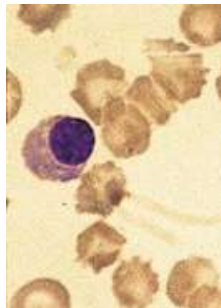
- Erythrocytes that stain a bluish tint.
- Blue tint is because of the result of organelles that are in the cytoplasm.
- Called reticulocytes when stained with new methylene blue
- Sign of bone marrow response!



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nRBC

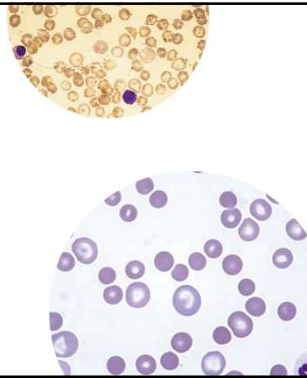
- Immature RBC
- Usually observed in anemic animals if in moderate to large quantities,
- Sign of bone marrow response – regenerative anemia!



45

Polychromasia and Nucleated RBC

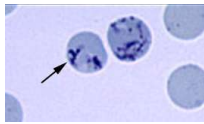
- Under normal conditions, 1% of all RBC are immature.
- The bone marrow will first send out polychromatophils in anemic conditions and then if it's really needed, nucleated RBC (nRBC)



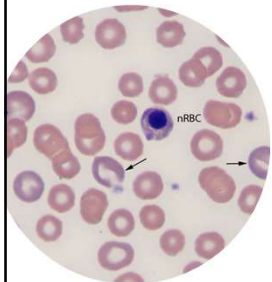
46

Reticulocytes

- **Retic** = counts # of immature RBC. Helpful in characterizing anemia as regenerative or non-regenerative



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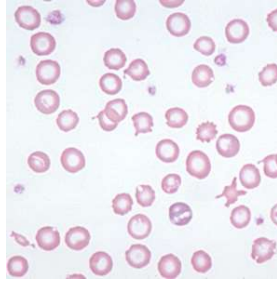


- Is this anemia regenerative or non-regenerative? Why?

48

Hemoglobin

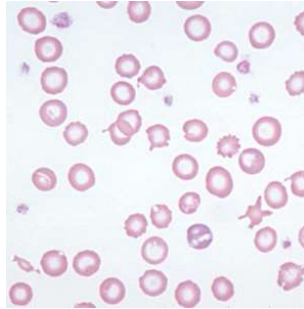
- **HGB** = measures the concentration of HGB in the RBC in a specific volume of blood
- **MCH (Mean Corpuscular Hemoglobin)** = mean weight of HGB contained in the average RBC
- **MCHC (Mean Corpuscular Hemoglobin Concentration)** = concentration of hemoglobin in the average RBC in regards to the size of the cell



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Hypochromic

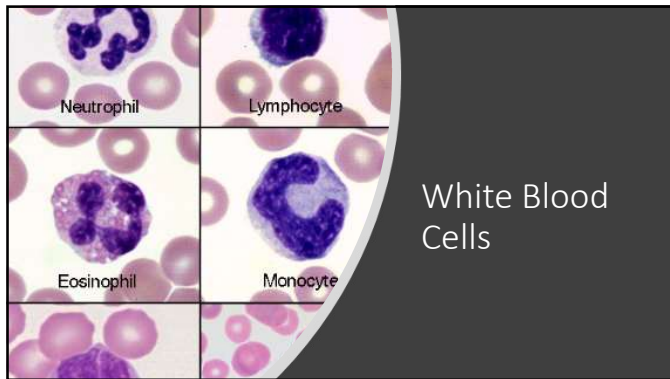
- Decreased staining intensity, caused by insufficient amount of HGB within the cell.
- Iron deficiency is often the cause
- Improper smear technique can result in similar looking artifacts.
- True hypochromic cells are also microcytic.



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CBC – RBC example

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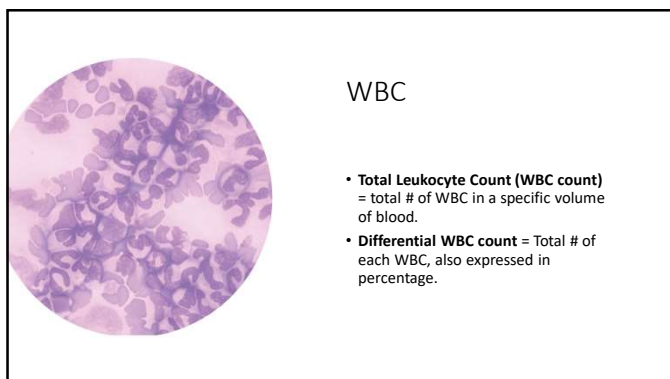


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Terms to know!

- Leukocytosis = increased WBC – non specific
- Leukopenia = decreased WBC – non specific
- **Neutrophilia = increased neutrophil count.**
- **Neutropenia = decreased neutrophils.**
- Eosinophilia = increased eosinophils.
- Eosinopenia = decreased eosinophils.
- **Basophilia = increased basophils.**
- **Basopenia = decreased basophils.**
- Monocytosis = increased monocytes.
- Monocytopenia = decreased monocytes
- **Lymphocytosis = increased lymphocytes.**
- **Lymphopenia = decreased lymphocytes.**

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WBC

- **White Blood Cells (leukocytes).** Larger than RBC
- 2 Main types, broken down into 5 specific WBC
 - Granulocytes: Prominent cytoplasmic granules
 - Neutrophils
 - Eosinophils
 - Basophils
 - Agranulocytes: No granules
 - Lymphocytes
 - Monocytes

55

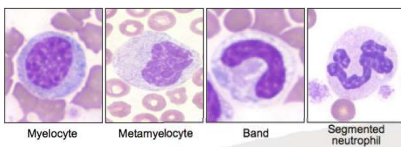
WBC functions

- **Function:** Provide defense for the body against foreign invaders.
- Two types of defense:
 - Phagocytosis
 - Immunity
- Each WBC has it's own unique role
- WBC travel from site of production → peripheral blood → their site of activity in the tissues

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Neutrophils

- Account for 40-75% of circulating leukocytes. **Most abundant WBC type of dogs, cats and horses**
- Larger than RBC, smaller than monocytes
- Band neutrophil – horseshoe shaped neutrophil. Immature neutrophil
- Segmented neutrophil – 2-5 nuclear segments



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Band neutrophils

- 14 year olds going to war
- Bone marrow releases them early to fight infection



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Neutrophil cont

- Neutrophils spend approx. 10 hours in circulation before entering tissue.
- Replaced 2.5 times per day!
- Reserve of mature neutrophils in bone marrow
- **Function: Involved in the early stages of the inflammatory response**
 - Neutrophils phagocytize microorganisms.



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Neutrophilia Types

- Neutrophilia = Increased # of neutrophils
- Immature neutrophilia = Increased # of band neutrophils, inflammatory/infectious condition. Also called a *left shift*
- Mature neutrophilia = Increased # of mature neutrophils, stress response

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Neutropenia and Leukocytopenia

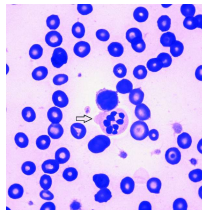
- Neutropenia = low neutrophils
- Neutrophils being "spent" faster than bone marrow can replace them
- Leads to leukocytopenia (unless there is a sharp increase in other types of WBC)
- Prognosis = Poor.
- **Example of a viral disease that causes leukocytopenia/neutropenia?**



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Hypersegmented neutrophils

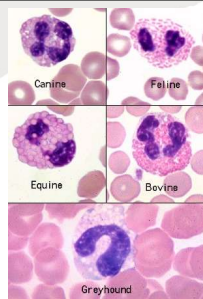
- Hypersegmented neutrophil = more than 5 segments. **Old** neutrophils **Or** indicative of a pathological condition
- Can also be an artifact from making a blood smear from old blood.



62

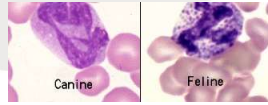
Eosinophils

- Appearance: Bilobed nucleus with pink/red granules in the cytoplasm. Slightly larger than neutrophils.
- Make up 1% to 6% of the total circulating WBC
- Stay in circulation for 3-8 hours before they migrate into tissues.
- Function:
 - Granules contain anti-inflammatory substances
 - Granules are toxic to parasites and protozoa



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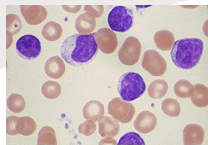
Basophils



- Least common leukocyte. Less than 1% of circulating WBC
- **Appearance:** Multilobed nuclei, similar to size of neutrophils. Large intensely staining blue cytoplasmic granules. Granules contain histamine and heparin.
- **Function:** Histamine in their granules helps initiate inflammation or allergic reactions. Heparin in their granules act as a localized anticoagulant to keep blood flowing to injured/damaged areas

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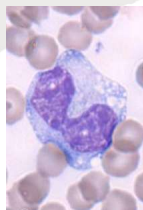
Lymphocytes



- **Appearance:**
 - Round or oval nucleus, minimal cytoplasm. Smaller than neutrophils
- Four types
 - T cells: Processed in thymus. Responsible for cell-mediated immunity. Most lymphocytes in peripheral blood are T cells
 - B cells: Rarely in blood. Involved in antibody production
 - Plasma cells: Derived from B cells in response to antigenic stimulus. Rarely in blood
 - Natural Killer Cells: Found in blood and lymph. Granular lymphocytes identify and kill virus-infected cells, stressed cells and tumor cells.

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Monocytes



- **Appearance:** Largest WBC in circulation, nucleus can be many shapes, from round to pseudolobulated. Abundant cytoplasm that stains gray, contain vacuoles
- 5% to 6% of circulating WBC
- **Function:** Participate in inflammatory responses. They don't circulate in peripheral blood for long before going to the tissues where they can live for up to 10 days. When they enter tissue they become macrophages.
- Macrophages and monocytes clean up cellular debris that remains after an infection.

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CBC – WBC example

WBC	28.25 K/ μ L	5.05 - 16.76	HIGH	
%NEU	* 68.2 %			
%LYM	* 22.0 %			
%MONO	* 8.8 %			
%EOS	1.0 %			
%BASO	0.0 %			
NEU	* 19.24 K/ μ L	2.95 - 11.64	HIGH	
BAND	* Suspect presence			
LYM	* 6.21 K/ μ L	1.05 - 5.10	HIGH	
MONO	* 2.50 K/ μ L	0.16 - 1.12	HIGH	
EOS	0.29 K/ μ L	0.06 - 1.23		
BASO	0.01 K/ μ L	0.00 - 0.10		
PLT	375 K/ μ L	148 - 484		
MPV	8.8 fL	8.7 - 13.2		
PDW	11.2 %			
PCT	0.33 %	0.14 - 0.46		

WBC abnormal distribution
Band neutrophils suspected

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Platelets

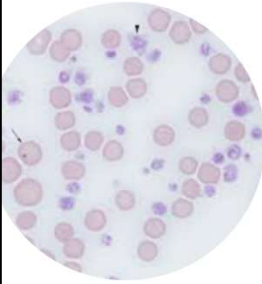


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Terms

- PLT= Number of platelets in a specific volume of blood
- MPV = Mean platelet volume. Similar to MPV for RBC
- PDW = Platelet distribution width. Similar to RDW for RBC.
 - Immature platelets (megathrombocytes) is similar to seeing immature RBC or WBC

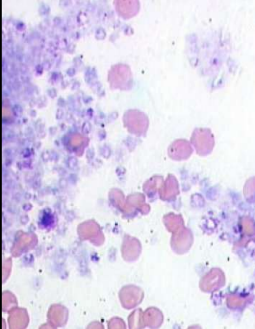
69



Platelets

- **Function: Hemostasis primarily**
- They form a platelet plug and stabilize the plug to make it irreversible
- **Platelets (Thrombocytes):**
Incomplete cells, pieces of cytoplasm megakaryocytes in the bone marrow
- Smaller than RBC, except for megathrombocyte which are seen occasionally.

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Platelets

- **Thrombocytosis** = Increased platelet count
 - Unusual. May be disease process (too much thrombopoietin) or platelets not being removed from circulation adequately
- **Thrombocytopenia** = Decreased platelet count

False thrombocytopenia common in cats – Why?
Platelets start to clump within 30 minutes of drawing your blood

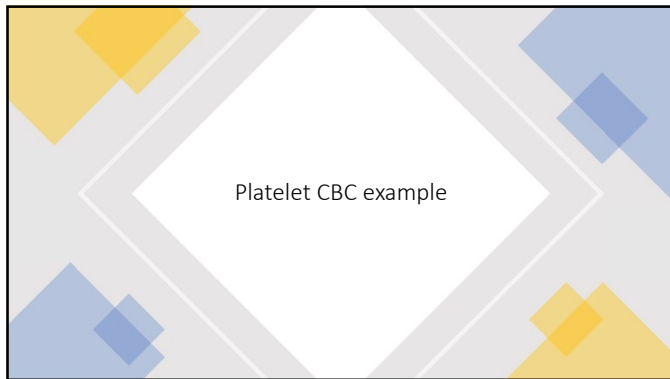
71



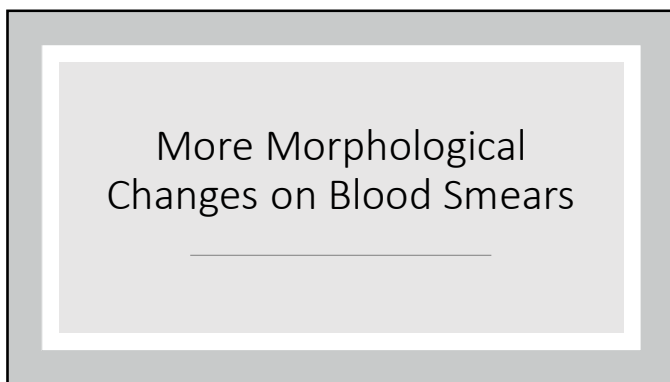
Platelets continued..

- The absence of platelet adhesion can result in bleeding disorders.
 - Low platelets – RBC may migrate through endothelial wall and may result in petechia

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Toxic Neutrophils

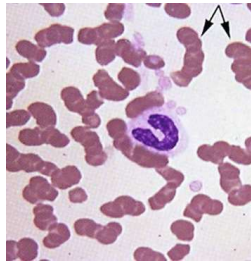
- Disease induced: Inflammation, infection, drug toxicity.
- Types of changes:
 - Dohle bodies
 - Vacuoles
 - Intensely staining granules
 - Much larger than normal neutrophils.

A microscopic image of a neutrophil with a large, multi-lobed nucleus and prominent, dark purple granules. The cell is surrounded by other smaller cells.

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Rouleaux

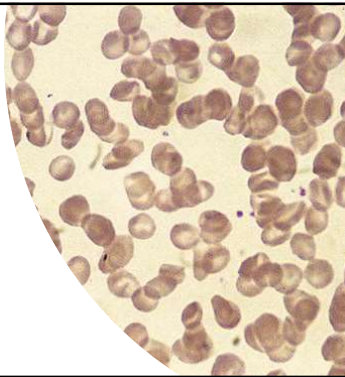
- Marked rouleaux very common in horses, maybe present in normal cats
- Pathologic: Increased rouleaux is seen with increased fibrinogen or globulin concentrations
- Artifact: Blood that was held too long before blood smear was made, blood that was refrigerated
- Differentiate from auto-agglutination



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Autoagglutination

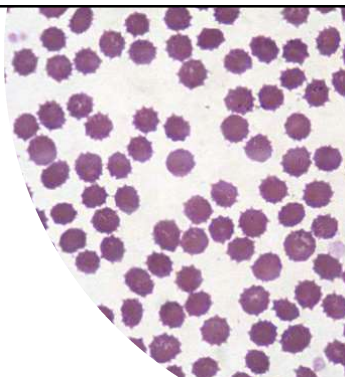
- Bridging and clumping of RBC, can be seen on a slide macroscopically and microscopically
- Pathology: Immune mediated disorders in which antibodies coat the erythrocyte
- Saline agglutination test.



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Echinocytes

- Echinocytes have evenly spaced surface projections of uniform size and shape
- Often artifactual (prolonged storage of sample, slow drying of blood films, underfilled EDTA tube). This is called crenated RBC
- Pathological causes: Renal disease and lymphosarcoma in dogs, and **RATTLESNAKE BITES!**



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Spherocytes

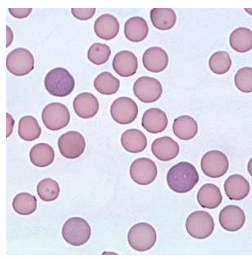
- Darkly staining RBC, reduced or no central pallor.
- Usually only found in dogs
- Suggest immune mediated destruction of RBC, Volume of cell is actually normal despite small appearance, so MCV will not be decreased



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Mycoplasma Haemofelis

- *Mycoplasma haemofelis* (formerly *Haemobartonella felis*)
- RBC parasite in felines
- Causes hemolytic anemia



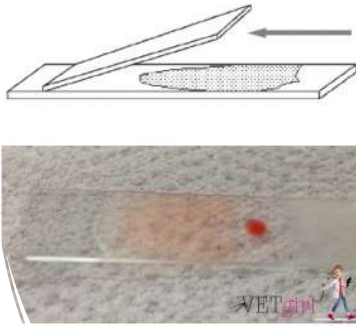
80

Reviewing a blood smear -
steps

81

Step 1

- Look at slide grossly – try to guess the hemoconcentration!
- Apply oil, then coverslip (optional) – helps w/ clarity



82

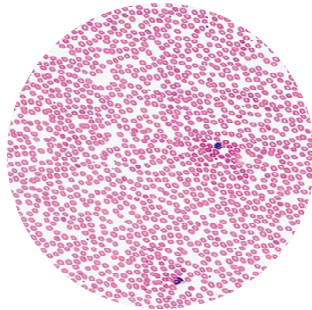
Step 2 - Start on 10x

Step 2
WBC estimate

1/5 of the "pie" x 1000

Check for platelet clumping (feathered edge)

Scan entire slide



83

WBC
estimate
on 40x

Count 10 fields

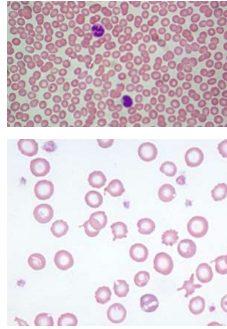
Average

Multiply by 2,000

84

Step 3 – 40x estimate hemoconcentration

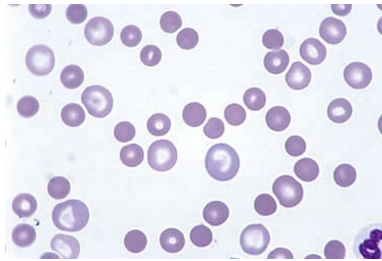
- Focus on feathered edge/monolayer
- Can evaluate an approximate hemoconcentration with practice.
- “40%, 55%, 25%”
- Or at the very least, be able to tell if it's hemoconcentrated, normal, or anemic!



85

Step 4 – 40x

- Look at RBC morphology
- Color
- Shape
- Size
- Other: parasites, agglutination, rouleaux, abnormal cells, inclusions etc



86

Quantitating morphological changes

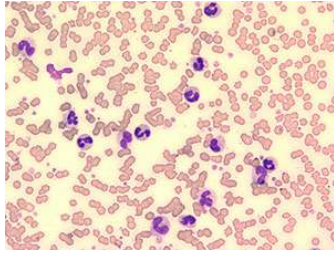
- Scale of 1+, 2+, 3+, 4+ = indicates the relative percentage of cells with the morphological change.
 - 1+ = 5-10% of cells affected
 - 2+ = 10-25% of cells affected
 - 3+ = 50% of cells affected
 - 4+ = 75% of cells affected
- “Slight” “Moderate” “Marked”
 - Slight= 10%
 - Moderate = 25%
 - Marked = 50% +

87

Step 5 – 40x

- WBC differential
- Count to 100

White blood cells



88

Performing a differential WBC cell count

- Be systematic!
- Differential is done by counting 100 WBC in the monolayer on oil immersion (or I use 40x), use a counting device!
- App for your phone



89

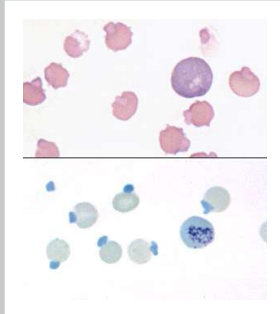
Step 7 Platelet Estimate- 100x

- First – ALWAYS look for platelet clumps.
 - Unusually large platelets (megathrombocytes) may suggest early release of platelets from the bone marrow
- Using oil immersion, count 10 fields of platelets, average and multiple by 15000 (7 platelets avg found = 140,000 platelets)

90

Heinz Bodies

- Inclusion on RBC
- Often from disease or toxin
- Example: cats who ingest tylenol



91

Blood Smear Review



92
