RBC? Neuts?? Eos??? What's the Diff!?

Eclinpath

https://eclinpath.com/

Aka: Your very best friend

Also where most of these photos are from...

Making a Blood Smear (aka Blood Film)

Tips and Tricks:

- Make many bad slides!
- Practice making slides by using old blood that is prepared to be thrown away.

<u>Monolayer</u> refers to a layer of cells in which no cell is growing on top of another, but all are growing side by side and often touching each other on the same growth surface

Zones of a blood smear



https://pressbooks.umn.edu/cvdl/chapter/module-7-1-preparin g-a-diagnostic-blood-smear/

Making Film - Angles

Approx. 45° - Goal

Too low - long slide

Too high - short slide



https://tvmdl.tamu.edu/2022/02/21/how-to-pr operly-prepare-a-blood-smear/

Making Slide - Speed

Speed will help determine the width of the blood smear when applying the spreader slide.

Ideal - Mostly, but not quite to the edge. Red Too fast - The smear is narrow. Green Too slow - The smear nearly encompasses the entire width of the slide. Blue (Better than fast)





Stain - Diff-Quik Stain (aka Romanowsky Stain)

- 1) Methanol Fixative
 - a) Fix cells in place, which is why flame fixing is not necessary.
- 2) Pink Eosin
 - a) Chemical is acidic.
 - b) Binds basic areas i.e. Cytoplasm
- 3) Purple Azure
 - a) Chemical is basic.
 - b) Binds acidic parts of cells i.e. Nucleus



https://eclinpath.com/hematology/sample-collection-h eme/diff-quick/

Check yourself- Can you see the Rainbow?

Pre Stain: Hold it up to the light: Rainbow?

Post Stain: Different shades of red to purple from Red blood cells \rightarrow Platelets \rightarrow Neutrophil cytoplasm \rightarrow Neutrophil nucleus





Befriend the Film



Initial Drop:

Where the film started. Can have weird clumpy artifact in this area.

Body: IMPORTANT to scan on 10x for platelet clumps to be seen.

Monolayer:

Diff here!

Cells not on top of each other and RBC has nice pale centers.

Feathered Edge: "C's" Hide here!

Clumps (Platelets) Critters (Microfilaria/HW) Cancer (big atypical cells)



4x

10x



40x



100x











Made the slide now what? Scan on 10x

THE WHOLE ENTIRE THING! - Look for things like platelet clumps or parasites. (Microfilaria/Heartworm)

Heavy things can get pulled to the edge - Scan this area especially for platelet clumps.



https://eclinpath.com/hematology/morphologic-featur



White Blood Cells (WBC): Count vs Differential

- Count is the actual number of cells:
 - 40x objective:(# per "n" fields / n) * 1500
- Differential is their distribution between types of cells
- Differential: count to 100 WBCs noting how many of what type
 - This gives you the percentage of each type of wbc
 - Then you multiply by the total number of wbc to get the count per cell type



White Blood Cells (WBC): <u>Neutrophils</u>

- Nuclei look like sausages, expect it to be the most common cell.
- Bands vs Segmented (seg): based on the level of maturation.
 - If the body is desperate, (i.e. significant inflammation) it will pump out neuts before having enough time to mature into seg.
- Toxic change More purple, darker spots in cytoplasm (<u>Döhle bodies</u>), "foamy" cytoplasm
 - Also means inflammation, so they are being pumped out of the marrow at a faster pace.
- Increased or decreased both mean inflammation
 - Like soldiers during battle



Fun Tidbit: Pelger-Huet anomaly

- Aussies get weird kidney bean nuclei None are segmented
 - Eosinophils will not be segmented either Can differentiate between Band vs. Pelger
- Functionally normal, just fun to see!





https://eclinpath.com/june-2016-case-mo nth/2/

WBCs: <u>Lymphocytes</u>

- Small vs. Big (Reactive vs. Blasts aka Cancer): Should be smaller than a neutrophil.
 - Blasts often have prominent nucleoli as well.
- **Decreased lymphocytes**: Due to steroids or stress. (i.e. Sick animals generally have low lymphs)
 - Absence of low lymph in a sick pet makes <u>Addison's</u> concerning
- Increased lymphocytes: Many various causes like Thymoma; Hypoadrenocorticism; Ehrlichiosis; Being under 4 months old; Lymphoproliferative disease; Inflammation; and post-vaccine.







Blasts

Lymphoma - aka Nerd Art











WBCs: <u>Monocytes</u>

- Monocytes have bigger, lacy, spread out nuclei
- Increases due to inflammation and steroids.
- Decreases are not significant.



WBCs: <u>Eosinophils</u>

- PINK
- Increase with parasites and allergies; can also be secondary to cancer
 - Also included with <u>Addison's</u> so perfect Addisonian is extremely sick, normal lymphocytes, and elevated eosinophils.



https://www.merckvetmanual.com/endocrine-system/ the-adrenal-glands/addison-disease

WBCs: <u>Basophils</u>

- Horseshoe shaped nuclei and purple inclusions in the cytoplasm.
- Uncommon Finding zero counts of Basophils
- Similar to eosinophil in reasons for change.





WBCs: Inclusions

• Is it the same or different color to the nucleus?



Red Blood Cells (RBC)

• **Dogs**: Have a pale center if you are scanning in the right area. Cats and others will not.

Relationship to each other:

- Rouleaux "stack of coins"
 - More organized
 - Not a ton of multi-direction
 - Normal for cats (and Horses)
- Agglutination cloud shaped clumps
 - Chaotic
 - Sign of <u>IMHA</u> extremely worrying

https://eclinpath.com/hematology/morpholo gic-features/red-blood-cells/patterns/



Red Blood Cells: Morphology

• Spherocytes:

- Disc made into a sphere Picture a frisbee \rightarrow A ball and then looking at it in 2D.
- Smaller around
- Darker color (no more pale area in dogs)
- IMHA

• Schistocytes - aka Sad broken cells

- Possibly due to slide making or blood draw technique double check with a new slide.
- Means something is physically breaking cells. (turbulent blood flow)
 - DIC, neoplasia of vessels (i.e. hemangiosarcoma), iron deficiency increasing risk of cell breakage.

• Echinocytes (+/- acanthocytes) - Spike balls

- Cells that dry slowly can have spikes on the outside of them. (related to liver disease in humans)
- If you picture this in 3D space \rightarrow 2D image: it can also lead to dark spots in the cells.





For all Morphology - Picture these things in 3D form.



RBCs: <u>Morphology</u>-Part 2

• Keratocyte + Eccentrocytes

- Start with damage to cell membrane by taking a small bite out of cell. (Keratocyte; aka Horn cells)
- Gets more extreme with blister forming on the side of RBC (Eccentrocyte)
- Oxidative damage causes An example, Acetaminophen

Ghost cells - A shell of what was

- $\circ \quad \mbox{Cell membrane gets } \underline{lysed} \rightarrow \mbox{insides fall out} \rightarrow \\ \mbox{leaves a pale outline of membrane.} \\$
- Could be due to IMHA, oxidation, or hemolysis.

• Codocytes - "Target cells"

• We will acknowledge them, but they have minimal diagnostic relevance.







Lysis refers to the breakdown of a cell caused by damage to its plasma (outer) membrane.

<u>RBC Inclusions</u> - aka The RBC Circle of Life

Note the happy



<u>RBCs: Inclusions</u> (Expanded)

• Nucleated RBC (nRBCs)

- \circ When anemic, the bone marrow works faster to pump cells out \rightarrow does not have the time to get rid of nucleus.
- Round nuclei vs lymph. Gives a more weird shape
- Can be misread as WBC, if more than 20 per 100 WBC- do the math to correct.

<u>Corrected WBC</u> = obtained nucleated cell count x [100 ÷ (nRBC + 100)]

Howell-Jolly Body

- Smaller remnant of nuclei extremely dark and round.
- Not clinically significant.

• Heinz Body

- Signal for oxidative damage (can happen from things like acetaminophen, zinc, or garlic)
 - Common in cats they do not cope with oxidation well.
- Pale structure clumped on the surface.
- Apply new methylene blue for visibility.

https://eclinpath.com/hematology/tests/wbc-count/



<u>RBCs: Inclusions</u> - more FYI

- **Basophilic stippling** Why do some cells look a bit purple or patchy?
 - Uncommon in both dogs and cats.
 - Immature red blood cells; could mean lead poisoning.





Platelets - What's that?!



<u>Platelets</u>

- Platelet count
- Adjust the light
- Clump vs. No clumps is key!!
 - Look everywhere for clumps



- If there are Clumps then you cannot have an accurate manual platelet count!
 - Can generally say "adequate" vs. "not adequate"
- <u>Platelet Count</u>: on 100x objective through a microscope
- Estimated platelet count/ μ L = average count in 10 fields x 15,000



Fun TidBit: Cavaliers get "<u>Macrothrombocytopenia</u>"

- Lower numbers of larger platelets are normal for them.
 - Can be 50-100k for platelet count.
 - Check PCT better correlates with bleeding risk in these cases.





End.