White blood cell protocol and descriptors

Cell types to identify

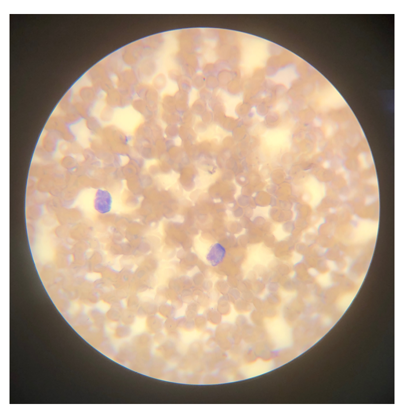
Collectively, lymphocytes and heterophils/neutrophils make up about 80% of WBCs (Davis et al., 2008); however, this is highly species dependent. Full descriptions and functions of these cells are provided in (Clark et al., 2009; Davis et al., 2008).

1. Lymphocytes: Lymphocytes are the most common WBC type in passerines (60-73% of WBCs depending on species) and are differentiated into B-cells and T-cells that are morphologically indistinguishable and that are involved in the adaptive immune response and immune memory. Lymphocytes have diverse protective roles against intra- and extracellular macro- and microparasites through various mechanisms from the production of antibodies to the coordination of other immune responses, to direct control of viral pathogens (Ruhs et al. 2020).
2. Neutrophils (mammals): Neutrophils are phagocytic cells that are the next most common WBC type (7-20% of WBCs; (Davis, 2009). These are fast-acting cells that can engulf or control microbes, typically by mechanisms involving oxidation, upon first exposure (Ruhs et al. 2020).
3. Eosinophils: Eosinophils are involved in defense against multicellular (i.e. helminth, extracellular) parasites but have diverse roles.
4. Monocytes: Monocytes are generally rare, are the largest WBC type, and are also phagocytic.
5. Basophils: Basophils are important for the inflammation response (although their precise function is unclear).

***** From the counts of neutrophil and lymphocytes, you can calculate N:L ratios as a hematological index of stress (Davis et al., 2008). This measure has been shown to scale positively with plasma levels of corticosterone, the primary vertebrate stress hormone (Davis et al., 2008). Unlike plasma corticosterone, which can increase within 2 min of acute stress associated with animal capture (Romero & Romero, 2002), prior research has shown that H(heterophil):L ratios in songbirds increase more slowly, and remain at baseline levels during routine handling for up to one hour after capture (Davis et al., 2008).

Vocabulary:

1. White blood cell count: A count measures the number of white blood cells in your blood.
2. White blood cell differential: A differential determines the percentage of each cell type present in the blood.
3. Field of view: This can be under any magnification, but for our purposes it is under 1000x. A single field of view is the circle of light you see to the right. When you are looking under the microscope, it is the whole circle highlighted on the slide.
4. Nucleated: Avian and fish red blood cells are nucleated. Other species are not

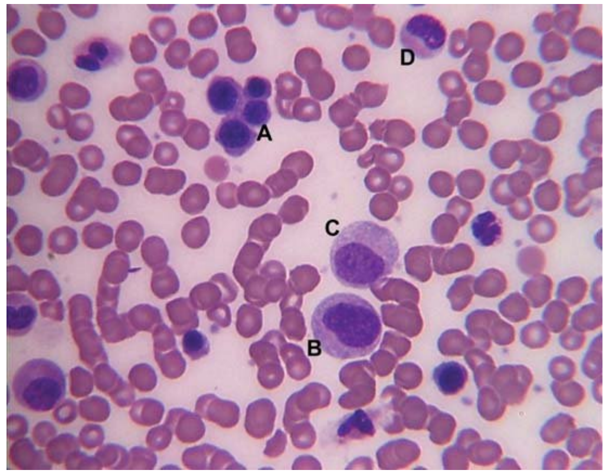


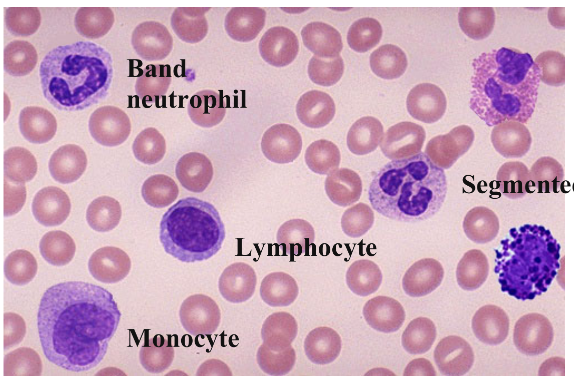
Examples of cell types

* These are partial fields of view, cropped to highlight the cell features.

Mammals:

A) Early erythrocyte – don't count
B) Neutrophil
C) Neutrophil
D) Early neutrophil





Segmented neutrophil

Eosinophil

Basophil

<< This is a perfect example. It never looks like this!

Protocols
* All: Dried blood smears should be stained with a Giemsa-Wright (Sigma-Aldrich) stain (Owen, 2011) and examined under a compound microscope using oil immersion at 1000x magnification.
**Oil for 1000x magnification:
https://www.amazon.com/gp/product/B007PD4IZS/ref=ppx_yo_dt_b_asin_title_o01_s00?ie=UTF8&psc=1v

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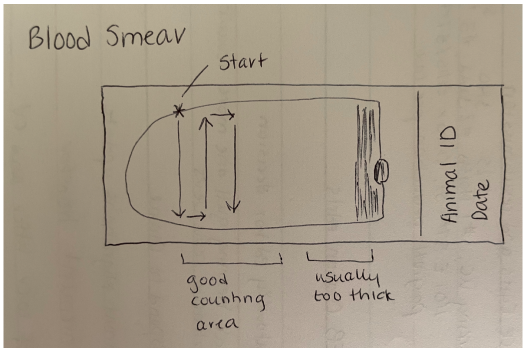
***** Watch this first:**
https://www.youtube.com/watch?v=bhgKCva4dBE



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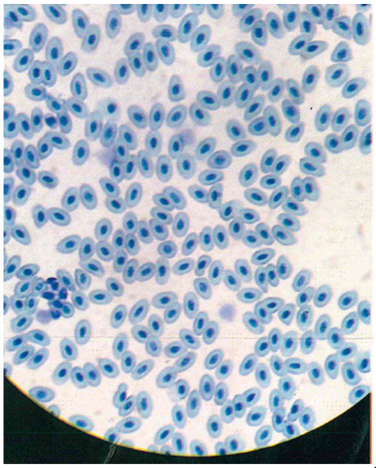
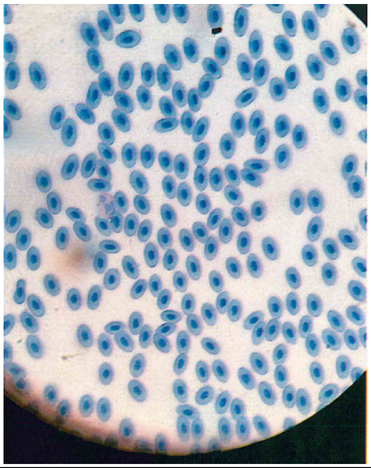
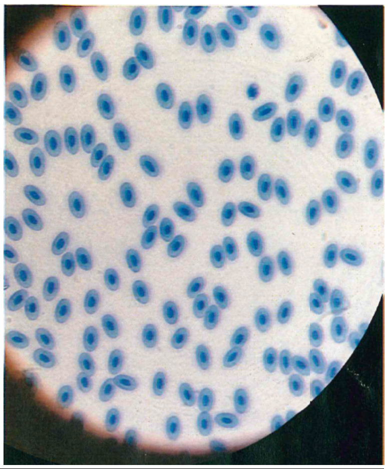
1. White blood cell count (or a "total cell count")
    a. Under 1000x magnification and oil immersion, count for 50 fields of view. Here you assume that an average FOV represents 200 red blood cells in a complete monolayer. If there isn't a complete monolayer, estimate the percentage of coverage and adjust the number of FOV accordingly. For example, 67 FOV when coverage is only 75%.
    b. Coverage is considered 100% when red blood cells are nearly touching but not overlapping (see linked video above and also the pictures below which counted the number of rbcs)
    c. Total leukocyte count should be expressed as the number of leukocytes per 10,000 red blood cells.



300 cells/FOV



250 cells/FOV



150 cells/FOV

2. White blood cell differential
    a. Under 1000x magnification and oil immersion, count until you view 100 white blood cells. Depending on the species or individual this may take a varying amount of field of views. For example (in my experience), it takes very few fields of view to count 100 white blood cells in a gorilla, but many fields of view to count 100 white blood cells in a small songbird.

3. Calculating a "stress" index
    a. This is a simple calculation. Once your count or differential is complete, divide the number of heterophils or neutrophils by the number of lymphocytes. This number can vary and can't tell you a ton by itself (i.e. for one individual), but when comparing across individuals or species, an animal with a higher number indicates greater "stress". See Davis et al. 2008 for more details.

Additional resources

1. See highlighted portions of Owen 2011 (PDF)
2. https://wildlifehematology.uga.edu
3. https://www.theowenlab.com/?page_id=264
4. https://thefishsite.com/articles/hematology-and-blood-chemistry-for-fish-species

Citations

1. Campbell. Avian and exotic animal hetatology and cytology.
2. Clark et al. 2009. Atlas of clinical avian hematology
3. Davis et al. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists.
4. Owen, J.C. 2011. Collection, processing, and storing avian blood: a review.