Exotic Hematology Lab
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Introduction

While the general set-up for manual reptile and avian Complete Blood Counts (CBC) is almost identical to that in mammals, the morphology of the cells is vastly different. Without practice or access to reference materials, evaluating an avian or reptilian blood slide can be quite challenging.

Some of the specific differences in avian and reptile blood from mammals are the presence of heterophils instead of neutrophils, the existence of azurophils, the species of haemoparasites seen, and the fact that the red blood cells are nucleated. It is due to the nucleated red blood cells found in birds and reptiles that so far has prevented automated CBCs, and provided job security for many veterinary technicians!

A Complete Blood Count consists of four parts: the Packed Cell Volume (PCV) and Total Solids (TS); a Differential Count; a Total Leukocyte Count; and an evaluation of the morphology of the cells. The focus of this lab is to perform the differential count, total leukocyte count and morphology evaluation on both an avian and reptilian blood sample.

Supplies Needed

The supplies needed to perform a CBC on a bird or reptile include:
- Microscope with a 100x objective
- Immersion oil
- Microscope slide
- Cell Stain such as Diff Quick or Wright’s Giemsa
- 5-button cell counter (not necessary but useful)
- Microhematocrit tubes and clay
- Crit centrifuge
- Crit reader card
- Refractometer
- Eopette® or Leukopette®
- Hemocytometer
- Blood sample pulled in a pre-heparinized syringe

Many of these items can be purchased through your regular vendor for lab equipment and supplies. Other retailers include Vet Lab Supply at www.vetlab.com or Exotic Animal Solutions at www.exoticanimalsolutions.com.

As with any other diagnostic test, reference materials to assist with the identification of the cells, or to aid in determining abnormal findings, are beneficial. A couple of these include Avian and
Instructions for Setting Up a Manual Avian or Reptile Complete Blood Count

1. Collect at least 0.2cc of blood in a pre-heparinized syringe using a 25g needle. A smaller needle may lyse the cells.
2. Create a blood smear using the spreader slide or cover slip method. Apply a slightly larger drop of blood on the slide when preparing a reptile smear due to the thinness of the blood. Once the smear is dry, stain the slide according to the stain manufacturer’s instructions.
3. Fill a microhematocrit tube at least half full with blood and seal one end using the clay. A second crit tube can be made for verifying results. Spin the crit tube in a centrifuge for 5 minutes and record the results of the PCV and TS.
4. Prepare the Eopette® or Leukopette® according to the manufacturer’s guidelines. The basic instructions are to add the specified amount of whole blood to the eosinophilic stain and mix well. Let sit for 5-10 minutes. Do NOT leave the sample in the stain much longer than the specified time or the red blood cells will also become stained making the total leukocyte count much more difficult and less accurate. After the specified time in the stain the sample is loaded into the hemocytometer. The amount applied to the hemocytometer should be just enough to cover the silver grid and applied equally to both sides of the counter. Let the sample sit in the hemocytometer for 5 minutes to allow the cells to settle and stop moving around the grid.
5. While the blood is in the Eopette® or Leukopette® stain, or settling on the hemocytometer, you can start the differential count. This is accomplished by recording one hundred identifiable white blood cells. The count should be performed in a monolayer area of the slide while looking through the oil objective. A 5-button cell counter can greatly aide in the differential count.
6. During, or after, the differential count the morphology of the blood cells should be evaluated for any abnormalities. This can include presence of haemoparasites, toxic changes, elevated or depressed number of immature cells, or variations in cell shape. All abnormalities should be reported.
7. After the sample has settled in the hemocytometer the total leukocyte count is done by counting the darker cells in all nine of the primary hemocytometer squares. Again, a cell counter can make the total leukocyte count much more accurate and efficient. Select one button on the counter and use this for counting all the dark cells on the grid of the hemocytometer. For cells sitting on the border between primary squares, a consistent rule should be followed. Whether it is counting them the first time they are encountered, or only counting the dark cells on the top and left border of the primary square, consistency is what is important. Both grids on the hemocytometer should be counted and compared for similarity. The count from each side of the hemocytometer are referred to as Side A and Side B in the following formula.
8. To calculate the total leukocyte count and absolute cell counts use the following formulas:

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\text{Total Leukocyte Count} = \text{Side } A + \text{Side } B \times 1.1 \times 16 \times 100 \\
\% \text{ heterophils} + \% \text{ eosinophils} + \% \text{ basophils}
\]

\[
\text{Absolute Cell Count} = \text{Total Leukocyte Count} \times \text{each WBC Differential } \% 
\]
“Normal” Ranges by Species

The results of the CBC are interpreted by the veterinarian. Reference values are available by species through the International Species Information System (ISIS). This database collects physiologic values from mainly zoo collections. Reference ranges can also be found in the Campbell or Clark texts, however individual species variation does exist and should be considered. For reptile reference ranges, *Reptile Medicine and Surgery* by Dr. Douglas Mader, is an excellent resource.

The Wildlife Center of Virginia is working on developing “normal” healthy CBC values for wildlife species taken just prior to release. There are published values for some species (Black PA, McRuer DL, Horne L. Hematologic Parameters in Raptor Species in a Rehabilitation Setting Before Release. *Journal of Avian Medicine and Surgery*. 2011; 25(3): 192-198) and work continues on this project to expand the list of species.
Blood Cell Identification Flow Chart

Type of Cell

Red Blood Cell

White Blood Cell

Thrombocyte

Round nucleus, oval cell, clear cytoplasm, can clump, easily confused with reptile lymphocyte

Mature

Oval shaped, pale pink cytoplasm

Immature

Larger, darker cytoplasm, more circular shaped

Granules in the cytoplasm?

Yes

Heterophil

Rod/oval shaped granules in cytoplasm, segmented nucleus, purple-pink granules

Eosinophil

Round granules, segmented nucleus, “hot pink” granules, distinct granular border

Basophil

Round granules, round nucleus, dark purple-blue granules, distinct granular border

Azurophil

In reptiles only, tiny purple-blue granules, cytoplasm has “tie dye” appearance, round to oval eccentric nucleus

Lymphocyte

Round cell with round nucleus, in birds can stick to RBC and get stretched, purple nucleus with blue cytoplasm, high N:C ratio, in reptiles may have very little cytoplasm visible

No

Monocyte

Larger than lymphocyte, vacuoles in cytoplasm, purple nucleus with pale blue cytoplasm, dimpled nucleus, more cytoplasm visible then in lymphocyte
**Identification Guide**

- **Heterophil**: granulocyte, rod/oval shaped granules in cytoplasm, granules are a pink-purple color, segmented nucleus, granule borders not always distinct.

- **Eosinophil**: granulocyte, circular shaped granules in cytoplasm, “hot pink” granules, segmented nucleus, granule borders are distinct.

- **Basophil**: granulocyte, circular granules in cytoplasm, dark purple granules typically the same color as the nucleus, granule borders are distinct, more common in reptiles than birds.

- **Lymphocyte**: mononuclear, round cell with round nucleus, purple with blue cytoplasm, high nucleus:cytoplasm ratio, often sticks to red cells in birds.

- **Monocyte**: mononuclear, round cell with dimpled nucleus, larger than a lymphocyte, vacuoles in cytoplasm, more cytoplasm visible than in lymphocytes.

- **Azurophil**: found in reptiles only, fine granules in cytoplasm, granules are a purple to blue color taking on a “tie-dye” appearance, round-oval shaped nucleus eccentrically located.

- **Thrombocyte**: “platelets”, round dark nucleus with clear cytoplasm, oval shaped cell, smaller than a lymphocyte, easily confused with a lymphocyte especially in reptiles.

- **Plasmodium**: cause of avian malaria, inclusion in red blood cell, oval shaped, takes up less than half of cytoplasm of the red blood cell.

- **Haemoproteus**: inclusion in red blood cell, “smile” shaped and wraps around nucleus, can take up more than half of the cytoplasm of the red blood cell.
Leukocytozoan- inclusion in white blood cell (typically the lymphocyte), causes cell’s nucleus to move into eccentric position, inflates the size of the cell, mature parasite causes “wings” from cell.

Haemogregarine- reptile blood parasite found in reptiles, inclusion in red blood cell, “smile” shaped and wraps around the nucleus, darker band in middle of parasite.

Toxic Heterophils- 4 degrees of toxicity, basophilic inclusions in the cytoplasm, degeneration of cell. degrees of toxicity, basophilic inclusions in vacuoles in cytoplasm, karyolysis, general