LEUKOPET™ METHOD FOR PERFORMING TOTAL
WHITE BLOOD CELL COUNT IN AVIAN AND REPTILIAN SPECIES

Avian white blood cell morphology differs from mammalian in that the predominant polymorphonuclear leukocyte is the heterophil vs. the neutrophil in mammalian species. The remaining avian leukocytes, i.e., lymphocyte, monocyte, eosinophil, are morphologically similar to their mammalian counterparts.

Avian heterophils more closely resemble eosinophils than neutrophils. Because heterophils tend to be more abundant in the vast majority of cases, it is helpful to scan the slide prior to beginning the differential count in order to determine the specific heterophil characteristics for that particular bird. This will help to differentiate them from that bird’s eosinophils. For in depth information regarding avian cell morphology, there are a number of excellent references available.

In order to determine avian total white blood cell counts, a methodology must be utilized that selectively stains heterophils and eosinophils. Avian heterophils and eosinophils both contain well-defined granules that stain orange to red. This methodology utilizes a 0.1% Phloxine solution and a standard Neubauer hemacytometer with cover glass. It is a modification of the previously published Unopette® procedure which is no longer available. A recent study utilizing blood samples from birds and reptiles concluded that results obtained with Leukopet™ approximated those that would be previously obtained with the BD Eosinophil Unopette®.

Materials provided:
- 0.1% stabilized Phloxine solution in screw cap tubes prefilled to 775 ul.
- 25 ul Minipet pipetter
- Disposable pipet tips

Additional required materials*:
- Hemacytometer with Neubauer ruling
- Hemacytometer cover glass
- Microscope
- Single place tally counter (Multi-place will also work)
- Multi-place tabulator
*Available from Vetlab® Supply

Storage requirements
Keep tubes in opaque bag in dark area when not in use. Exposure to light can cause breakdown of the Phloxine stain. Store kit at room temperature.

PROCEDURE

1. Make a blood smear by routine methods and stain with Wright Giemsa or another appropriate differential stain.
2. Perform the differential blood count as for mammalian species substituting the heterophil for the neutrophil. Record results.
3. Attach a clean, unused disposable pipet tip to the 25 ul pipetter.
4. Unscrew the cap of one of the prefilled Phloxine tubes and place in a tube rack.
5. Using the pipetter, aspirate 25 ul of freshly drawn anticoagulated blood. Wipe the outside of the tip with a lint-free wipe being careful not to draw the wipe over the opening of the tip.
6. Dispense the blood sample into the tube of phloxine and rinse the pipet tip thoroughly by aspirating and dispensing the phloxine/blood solution at least 6 times. IMPORTANT: It is critical that all of the blood be rinsed from the pipet tip to insure a proper dilution. Depending on the viscosity of the sample, this may require additional rinsing of the tip.
7. Cap the tube and mix well by inverting several times. Do not shake.
8. Place the tube in an upright position and allow the tube to incubate for 10-15 minutes but no longer than 1 hour.
9. Prior to aspirating the sample, gently rotate the incubated tube 3 to 4 times to insure complete mixing.
10. Using the rinsed pipet, aspirate a sample from the tube and charge both chambers of the hemacytometer.
11. Allow to stand for up to 10 minutes for cells to settle.
12. Using the 10X objective, count the heterophils and eosinophils in both chambers of the Neubauer hemacytometer.
13. Use the following formula to calculate the total leukocyte count:

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\text{Total WBC/ul} = \frac{\text{Total Heterophil + Eosinophil (both chambers)} \times 1.1 \times 16 \times 100}{\% \text{Heterophils} + \% \text{Eosinophils}} \\
(\text{From differential count})
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TECHNICAL NOTES

- Always use the specific hemacytometer cover glass. Standard cover glass may bend slightly altering the volume of the chamber and ultimately the cell count.
- We recommend using the “Brightline” or “Lumicyt e” brand hemacytometers. Due to the refraction properties of the Phloxine stain, the rulings on some lesser hemacytometers may be difficult to see.
- Note the incubation times needed for the cells to stain completely and to settle into one plane following charging of the hemacytometer.
- Some species (particularly passerines) may require a longer incubation time for complete staining.
- After dispensing the blood sample into the stain vial and rinsing several times, a small amount of liquid may remain in the pipet tip. This minute amount should have little bearing on the final cell count however prior to charging the hemacytometer, aspirating and dispensing one time following incubation will replace it with a like suspension of cells.

Reorder Information:
HEM-AVLPF50        Leukopet 50 test kit
HEM-AVLPF100       Leukopet 100 test kit
HEM-HCY3180000     Brightline Hemacytometer with Neubauer Ruling
HEM-HCYT001200     Hemacytometer Cover Glass Pkg. 12
HEM-EQB4117100     Single Place Tally Counter
HEM-EQBC6         6 Place Tabulator (9 place and Electronic models also available.)
STN-MDL5316     Quik III 3-step Wright’s Stain

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Kristine Trotta, Pamela M Torres, Jill Arnold, and Joan Maurer: Validation of Vetlab Supply’s Avian Leukopet. Poster Presentation at the Annual Conference of Zoo veterinary Technicians. October 1-6, 2009.; Jackson, WY

References:

Rev. 7/2015