Leukopet™ Method for Performing Total WBC 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 species. This will help to differentiate them from that individual's eosinophils. For in depth information regarding avian/reptilian cell morphology, there are a number of excellent publications available. (See references.)

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 #5877 procedure. 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® [1].

Materials provided:
0.1% stabilized Phloxine solution in screw cap tubes prefilled to 775 ul.
25 ul Minipet pipettor
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 deteriorate Phloxine that can affect staining quality. 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 pipettor.
4. Unscrew the cap of one of the prefilled Phloxine tubes and place in a tube rack.
5. Using the pipettor, 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.
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 30 seconds to one minute. Prolonged incubation can result in staining of the red cells and some species may require immediate charging of the hemacytometer to prevent RBC uptake. *See important technical notes
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. Place the charged hemacytometer in a humid chamber for a minimum of 5 minutes to allow the cells to settle. (A petri dish with a moistened gauze sponge in the bottom works well.)
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:

$$\text{Total WBC/ul} = \frac{\text{Total Heterophil + Eosinophil (both chambers) X 1.1 X 16 X100}}{\text{\%Heterophils + \%Eosinophils (From differential count)}}$$

TECHNICAL NOTES

1. Samples to be tested should generally be drawn in EDTA. It has been reported that some species such as ducks, jays, ravens, crows, magpies, and ratites, as well as most fish, sharks, and reptiles may yield better results with heparinized samples.
2. Always use the specific hemacytometer cover glass. Standard cover glass may bend slightly altering the volume of the chamber and ultimately the cell count.
3. We recommend using the Hausser "Brightline" hemacytometer. Due to the refractive properties of the Phloxine stain, the rulings on some lesser hemacytometers may be difficult to see.
4. Note the incubation time required for the cells to settle into one plane following charging of the hemacytometer.
5. RBC's are not lysed by this method. Their presence is indicative of stain uptake that can result from prolonged incubation. If this occurs, shorten the incubation time of the blood/phloxine mixture.
6. It has been reported that some species (particularly passerines) may require a longer incubation time for complete staining of the leukocytes. Adjust accordingly.
7. Visible darkening of the Phloxine may occur when processing samples from animals with elevated hematocrit.[3]
8. 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.
9. Phloxine is very light-sensitive. Prolonged exposure to light or heat can affect staining quality.

Footnotes:

[1]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:

Campbell, T.W. Avian Hematology and Cytology, 2nd edition. Iowa State University Press, Ames, IA, 1995, pp 3-5

Campbell, T.W.: (2012) Hematology of Birds in Thrall, M.A., Hausser, G, Allison, R, Campbell T (eds.) *Veterinary Hematology and Clinical Chemistry*; 2nd edition Ames, IA: Wiley-Blackwell pp. 256-258

Reorder Information:

HEM-AVLPF50	Leukopet[tm] 50 test kit
HEM-AVLPF100	Leukopet[tm] 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

Orders and Technical Support:

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