

AVIAN LEUKOPET™ METHOD FOR PERFORMING TOTAL WHITE BLOOD CELL COUNT IN AVIAN 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 #5877 procedure which is no longer available. A recent study utilizing blood samples from birds and reptiles concluded that results obtained with Avian Leukopet™ approximated those that would be previously obtained with the BD Eosinophil Unopette®¹.

Materials provided:

0.1% stabilized Phloxine solution in prefilled screw cap tubes
25 ul Minipet pipetter
Disposable pipet tips

Additional required materials*:

Hemacytometer with Neubauer ruling
Hemacytometer cover glass
Microscope
Single or multi-place tabulator

*Available from Vetlab® Supply

Storage requirements

Keep tubes in dark, cool area. Exposure to light can cause breakdown of the Phloxine stain.

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.
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. Allow tube to incubate for 10 minutes but no longer than 1 hour.
9. Using the rinsed pipet, aspirate a sample from the tube and charge the hemacytometer.
10. Allow to stand for up to 10 minutes for cells to settle.
11. Using the 10X objective, count the heterophils and eosinophils in both chambers of the Neubauer hemacytometer.
12. Use the following formula to calculate the total leukocyte count:

Total Heterophil + Eosinophil (both chambers) X 1.1 X 16 X100

Total WBC/ul =

%Heterophils + %Eosinophils
(From differential count)

¹Kristine Trotta, Pamela M Torres, Jill Arnold, and Joan Mauer: 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

For Orders and Technical Support Contact:

Vetlab Supply

18131 SW 98th Ct.

Palmetto Bay, FL 33157

800.330.1522 • 305.232.8421 Fax • info@vetlab.com

www.vetlab.com

Rev. 11/2009