Leukopet<sup>tm</sup>

**Avian Leukocyte Counting System** 

For over 10 years Leukopet<sup>tm</sup> has provided veterinarians with a complete in-clinic solution for performing WBC counts on avian species.





Each Leukopet<sup>tm</sup> kit contains:

- 50 tubes prefilled with 1% Phloxine.
- 25 ul Minipet for dispensing blood samples.
- 50 disposable pipet tips.
- Economical 100 test kit also available.

The Leukopet™ system utilizes the same procedure, dilutions, and formulas as the original Unopette® method.

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# 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.

### Materials provided:

1% stabilized Phloxine solution in prefilled screw cap tubes 25 ul Minipet pipettor 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 in dark, cool area. Exposure to light can cause breakdown of the Phloxine stain.

## Summary Procedure (See complete product package insert.)

1.

- 2. Make a blood smear by routine methods and stain with Wright Giemsa or another appropriate differential stain.
- 3. Perform the differential blood count as for mammalian species substituting the heterophil for the neutrophil. Record results.
- 4. Attach a clean, unused disposable pipet tip to the 25 ul pipettor.
- 5. Unscrew the cap of one of the prefilled Phloxine tubes and place in a tube rack.
- 6. Using the pipettor, aspirate 25 ul of freshly drawn anticoagulated blood.
- 7. 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.
- 8. Cap the tube and mix well by inverting several times. Do not shake.
- 9. Allow tube to incubate for 30 seconds to 1 minute. (See notes.)
- 10. Using the rinsed pipet, aspirate a sample from the tube and charge the hemacytometer.
- 11. Allow to stand for a minimum of 5 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:

Total WBC/ul =

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

%Heterophils + %Eosinophils (from differential count)

References:

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

Thrall, Mary Anna et.al: "Hematology of Birds" in Veterinary Hematology and Clinical Chemistry; Lippincott, Williams, Wilkins 2004, pp. 240-242

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

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Legal Notice: The responsibility for achieving accurate and clinically useful results with the Leukopet™ system rests solely with the Veterinarian and/or individual performing the test. The Leukopet™ system has not been approved for use in human testing.