Natt and Herrick Solution
For Direct Counting of Avian and Reptilian Blood Cells

Explanation of the Procedure:
The method developed by Natt and Herrick allows for direct counting of both erythrocytes and leukocytes in avian and reptilian species. Furthermore, since the same dilution is used for both red and white cells, a total leukocyte and a total erythrocyte count can be obtained simultaneously from the same charged hemacytometer.

Materials Provided:
Natt and Herricks Solution

Additional Requirements (Not provided)*
Neubauer Hemacytometer
Hemacytometer Cover Glass
Filter Paper; #1 Whatman
Red Blood Cell Diluting Pipet or alternative method for preparing a 1:200 dilution.

Procedure:
Inspect the Natt & Herrick's stain solution and filter if any precipitate is noted. Using a clean test tube or vial and a standard red blood cell diluting pipette, dilute whole anticoagulated blood with the Natt and Herrick's solution at the rate of 1:200. Dilution may also be accomplished using standard pipettes and micro capillaries. Cap the tube, mix well, and allow to incubate for approximately 5 minutes. (See notes). Before charging the hemacytometer, mix several more times. Allow the charged hemacytometer to stand for 1-2 minutes to allow the cells to settle.

Performing the Total Erythrocyte Count
Using the high dry (40X) objective of the microscope, count the total number of red blood cells (easily recognizable by their nuclei) in the four corner and central squares of the central large square of the counting chamber. Count all cells that overlap the top and left border. Do not count any cells that overlap the bottom or right borders. Multiply the total number of cells counted by 10,000 to obtain the total erythrocyte count per/ul.

Performing the Total Leukocyte Count
As previously described, the same 1:200 dilution is used for counting white cells. White blood cells tend to stain dark blue to purple and may exhibit some granularity. The total leukocyte count is obtained by counting all leukocytes present in the nine large ruled squares of the hemacytometer. Again, count all cells that overlap the top and left border. Do not count any cells that overlap the bottom or right borders. The computation for total leukocyte count is as follows:

Total WBC/ul = (Total WBC in 9 squares + 10%) X 200

Notes
Incubation times can vary from species to species. It may be necessary to increase or decrease for optimum results. Prolonged incubation can results in pronounced RBC staining in some patients.

References:
Campbell, Terry W.: Avian Hematology and Cytology, Second Edition. Iowa State University Press 1995. pp 7-11

*Available from Vetlab Supply

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