

Natt & Herrick's Stain

For Direct Counting of Avian and Reptilian Blood Cells

Explanation of the Procedure:

The method developed by Natt & 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 & Herrick's Stain Solution

Additional Requirements (Not provided)*

Neubauer Hemacytometer

Filter paper; #1 Whatman

Red Blood Cell Diluting Pipette or alternative method for obtaining a 1:200 dilution.

Procedure:

Inspect the Natt & Herrick's stain solution and filter if any precipitate is noted. Using a standard red blood cell diluting pipette, dilute whole anticoagulated blood with the Natt & Herrick's solution at the rate of 1:200. Dilution may also be made with standard pipettes and micro capillaries. Allow the diluted blood to mix for a minute or two before discharging into the hemacytometer counting chamber. After charging the hemacytometer, allow the contents to settle for approximately 3 minutes.

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 noted, 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 leukocytes in 9 squares + 10% of total WBC's) X 200

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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