



Reticulocyte Count (New Methylene Blue)

Reagents Needed:

New Methylene Blue, Brecher
or New Methylene Blue "N"

RICCA CHEMICAL COMPANY Cat. No. 5280
RICCA CHEMICAL COMPANY Cat. No. 5285

Recommended Method:

1. For venous blood, Add 3 or 4 drops of New Methylene Blue "N" to an equal volume of well-mixed EDTA-anticoagulated blood in a small test tube and mix.

For peripheral (capillary) blood, draw blood up to the 0.5 mark in a leukocyte diluting pipet, then draw in an equal volume of Brecher New Methylene Blue or New Methylene Blue "N" (to the 1.0 mark). Alternatively, mix approximately equal volumes of blood and stain solution on a clean slide and draw up the mixture into a capillary pipet.

Note: proportions of blood and stain have little effect on staining of reticulum, but do determine depth of coloration of erythrocytes. A slight excess of blood gives optimal color contrast.

2. Draw the blood and stain up into the bulb of the pipet, mix well, and allow to stand for 10-15 minutes (10 minutes is usually sufficient). The staining time may be adjusted due to personal preferences for staining intensity.
3. Mix again.
4. Expel a small drop of the blood-stain mixture onto a clean slide and smear in the usual manner. Make several slides.
5. Dry rapidly by waving slides in the air.
6. Examine the dry smear under an oil immersion microscope without fixation or counterstaining.

Note: If desired, smears can be counterstained with Wright's Stain (RICCA CHEMICAL COMPANY Cat. No. 9350 or 9360).

Satisfactory Staining Results:

Normal erythrocytes stain light greenish-blue.

Reticulocytes stain deep blue and are sharply outlined.

Normal Values Found:

For normal healthy young adults, Reticulocytes comprise 0.3 - 1% (usually 0.6 - 0.8%) of the total number of Erythrocytes.

In young infants, the percentage is 2 - 4 times higher than for young adults (0.6 - 4%).

Some sources give higher figures: 0.5 - 2% for adults and 5 - 10% for infants.

Consult RICCA CHEMICAL COMPANY Technical Reference Document TRD14 for details on Reticulocyte count using Brilliant Cresyl Blue stain.

This is a typical staining procedure. These reagents may be suitable for other staining procedures. Consult staining reference books or standard operating procedures for other suitable uses of these products.