RETI-STAIN

DETERMINATION OF RETICULOCYTES IN WHOLE BLOOD

Manual Method
Reticulocytes
Stain Solution

SUMMARY

REAGENTS

Reticulocytes Stain Solution, product number 2818, optimized new methylene blue stain solution, liquid and ready for use. Store at room temperature.

For stabilities refer to expiry date printed on the product label.

PRINCIPLE

Freshly produced erythrocytes that have just entered the peripheral circulation from the bone marrow are called reticulocytes. While these cells no longer have nucleus, they still possess some ribosomal material, RNA and other organelles. These materials will not normally stain appreciably with Romanovsky type stains but the RNA containing structures can be visualized by staining the cells with this new methylene blue stain solution.
Incubation of a blood sample (or control) with equal amounts of stain for 15 to 30 minutes will cause the RNA and associated organelles to precipitate in the form of a reticular network inside the cells. When viewed with the 100x oil immersion objective of a light microscope this reticulum can appear in the cells as a dark blue-stained filamentous network. To be considered a reticulocyte however, a cell must display at least 2 dark blue staining granules that are located at some distance from the cell periphery. Counting the number of cells containing such precipitated material on a defined portion of a peripheral smear allows the laboratory technician to estimate the percentage of reticulocytes in the sample. Expected values for normal individuals are in the 0.5% to 1.5% range.

SAMPLE MATERIAL

Patient samples should be handled according to the Laboratory's normal procedures. Control samples should be handled according to the manufacturer's instructions but otherwise should be prepared exactly as a patient sample.

EQUIPMENT

Light microscope equiped with a 10x objective for surveying smears under low power and a 100x oil immersion objective for actually counting reticulocytes and RBC's. Immersion oil, glass microscope slides, test tubes for mixing samples with stain, transfer pipettes, cell counters, Millers disc insert for microscope ocular (optional).

QUALITY CONTROL

Control blood with known concentration or commercially available control material with established values are recommended for control of precision and accuracy.

Reticulocytes Control Set	R28759G	3 x 1.5 ml	assayed

RETI-STAIN

PROCEDURE

1. Transfer 300 µl of whole blood (or control) and 300 µl Reticulocytes Stain Solution (2818) to a clean test tube using a clean transfer pipette. Mix well and allow to incubate for 15 to 30 minutes at room temperature.
2. Gently mix again and prepare blood smears in duplicate from the blood/stain mixture. Fan eventually the slides to dry each smear.
3. Examine smears initially under low power (10x) objective to determine an area for counting. In this area the RBC's should be evenly distributed and close to another, but not touching or overlapping.
4. Reticulocytes will be counted as a percentage of RBC's. The following two methods both provide the percentage of reticulocytes in a sample but neither corrects for varying hematocrits. See reference 1 and 2 for these methods.

5. Standard Method for Counting Reticulocytes: After selecting the starting area of the smear, count all the RBC's in the field of view with one cell counter.

5a. Enumerate the number of reticulocytes present using a second counter but the same area.

5b. Move the slide to consecutive fields using the cross sectional method until al reticulocytes in 1000 red blood cells are counted.

5c. A second technician should repeat the count on the duplicate slide. If the two counts do not agree within 20% of each other (3) a third smear should be counted. Alternatively, your Laboratory Director should set the limits of acceptable agreement between two counts. The reticulocyte % equals the number of reticulocytes in 1000 RBCs/10.

For example, using the Standard method - technician #1 counts 1000 RBC's in contiguous fields of one slide and also saw 15 reticulocytes among those 1000 RBC's. Technician #2 counts 1000 RBC's on the duplicate slide of that sample and counted 13 reticulocytes. The average reticulocytes count is 14 and the reticulocyte % = 14/10 or 1.4%.

6. Miller Disc Method for Counting Reticulocytes - The use of a Miller ocular micrometer disc in the microscope eyepiece will facilitate counting.

6a. Count reticulocytes in the large square including those in the smaller square.

6b. Count the total red cells in the small square only.

6c. Repeat this on 20 consecutive fields of view or until at least 120 RBC's are counted. The formula for converting these two sets of counts to a reticulocyte percentage is:

$$\frac{\text{Total retics in large squares}}{\text{Total RBC's in smaller squares} \times 9} \times 100\ \% = \ \%\ \text{Reticulocytes}$$

For example, using the Miller disc method - if the total retics counted in the largest squares was 8 and the total RBC's in the smaller squares was 120 the % retics = 0.74. Counts on a duplicate slide should agree within 20% or the limits set by your Laboratory Director.

PRODUCTS

Products	Product no.	Quantity
Reticulocytes Stain Solution	2818	1 x 100 ml

NOTES

1. For in vitro diagnostic use only.
2. For professional use only.
3. Always contact INstruchemie for the complete product insert and latest edition.
4. Printed in the Netherlands, July 2015 – version 3.0