Procedure:  Reticulocyte Manual

I. PRINCIPLE
A reticulocyte is an immature red blood cell, which still contains remnants of nucleic acids (RNA). When these cells are stained with New Methylene Blue, a supravital stain, their RNA precipitates out in the form of blue staining granules. To be considered a reticulocyte, the cell must contain two or more granules. These granules should not near the outer margin of the cell so as to be confused with Heinz bodies.

II. SPECIMEN
A. Sample Collection
Use whole blood collected into potassium EDTA (3.7 to 5.4 mmol/ml). Na heparin and Na citrate have not been evaluated for this method in this lab and should not be used.

B. Specimen Storage
Specimens left at room temperature for up to 24 hrs may be analyzed. Specimens stored at 2 to 6 degrees centigrade are stable up to 72hrs, but there must be no hemolysis in the specimen.

III. REAGENTS
A. Reagent Preparation and Storage
1. Commercially purchased New Methylene Blue stain is used and stored at room temperature.
2. Retic-Chex control Kit (12x1ml)
   a. Retic-Chex II – Composed of stabilized human RBC in a preservative medium
   b. Retic-Chex Stain – Filtered and buffered new methylene blue

IV. EQUIPMENT
1. Pipettes
2. 12 x 75mm test tubes
3. Microscope and immersion oil
4. Gloves – Must be worn throughout the procedure.
5. Safety Shield – Work behind a shield when setting up the test.
V. PROCEDURE

A manual reticulocyte count is performed in the event that the automated reticulocyte count is beyond the reportable range of the analyzer, the sample is insufficient to perform by automated methodology or the sample is obtained from a patient with a Hemoglobinopathy. At no time are the manual and automated result reported for the same sample.

A. Staining

1. Mix equal parts of EDTA blood (2 drops) and stain (2 drops) in a 12 x 75mm tube. If a patient is anemic use 2 drops of blood 1 drop of stain.
2. Mix completely until resuspension of cells is accomplished.
3. Incubate at room temperature for at least 3 but no longer than 10 minutes. (Heinz bodies will stain if longer)
4. Make wedge film slide and allow to air dry.

B. Counting

1. Replace the microscope ocular with the Ocular that has been fitted with the Miller Disc reticle
2. Scan film at low power to verify uniform distribution of RBCs. There must be an average of six RBCs per Small Square in order to ensure an accurate count. If fewer RBCs are found make a new wedge smear that is thicker.
3. Using the Miller disc with 100x immersion oil, count all RBCs in the small square up to the minimum of 150 RBCs (approximately 20 fields). Tally the retics in seen in the whole counting area (large and small square). If a retic is found in the small square it counts as a RBC and a retic.

C. Calculation

The small square is equal to one ninth of the large square. There for the calculation is as follows:

\[
\text{Retic \%} = \frac{\text{Total \# of retics counted in large square} \times 100}{\text{Total \# of RBCs in small square} \times 9}
\]

VI. QUALITY CONTROL

There are 3 levels of controls provide by Streck Laboratories (Retic-Chex 12x1ml). The controls vials have a closed vial stability of 75 days and an open vial stability of 14 days. The Retic-Chex controls are assayed stabilized controls for evaluating the automated, semi-automated and manual reticulocyte count. In this laboratory they are used solely as a control for the manual method. Three levels of Controls are analyzed each shift.

Handling instructions
Use product immediately after removing from refrigerator
1. Mix by gentle inversion between thumb and index finger until the red blood cells are completely resuspended. Do not mix mechanically. Do not rub between palms of hands.
2. Wipe threads of vial and cap with clean tissue before replacing cap. Recap Vial.
3. Return to refrigerator immediately.

Procedure:
1. Prepare a dilution using equal drops of control and stain (1:1).
2. Incubate at room temperature for a minimum of 15 minutes and no longer than 30 minutes.
3. Mix well. Prepare a smear and air dry.
4. Follow counting procedure in the sample procedure section.
5. Enter control values into the LIS
   a. Enter function MEM
   b. At the test prompt enter RET. Do not enter controls in the worksheet mode.
   c. At the accession number prompt enter C-RETC1, C-RETC2 or C-RETC3 to enter the appropriate level.
   d. The system will alert the user if the value entered is unacceptable and if so, appropriate action should be taken. Refer to quality control procedure in hematology procedure manual (Book 1).

VII. RESULTS

A Interpretation of Results

Normal Range for Manual Results:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 – Adult</td>
<td>0.5-1.5%</td>
</tr>
<tr>
<td>7D-8Y</td>
<td>0.0-1.0%</td>
</tr>
<tr>
<td>0-7D</td>
<td>3.0-7.0%</td>
</tr>
</tbody>
</table>

B Reporting of Results

Log in-patient results on worksheet. Report the patient results using function MEM in worksheet mode. The appropriate worksheet is RET. Hide all appropriate fields that do not apply. (automated and absolute counts where RBC counts are not available).

VIII. LIMITATIONS

Results are limited by the manual nature of the test.
IX. PATHOLOGY AND BIOCHEMISTRY

Increased reticulocyte counts occur in iron deficiency anemia, megaloblastic anemia under treatment, hemolytic states and a few days after hemorrhage due to increased erythropoietic activity of bone marrow.

Persistent low reticulocyte counts in aplastic anemias, untreated pernicious anemias and sickle cell crisis indicate insufficient erythropoiesis

X. REFERENCES

Streck Laboratories Retic-Chex II stain/Control package insert

Revised 1/2013 to reflect 24 hour stability at room temperature