The Reticulocyte Count Revisited

Reticulocytes are non-nucleated immature red cells in peripheral blood, containing residual RNA. After erythroid precursors lose their nuclei, another 4 days is required for the resulting reticulocytes to mature and lose their RNA. Normally the first 3 days are spent in the marrow, and the last day in peripheral blood. The reticulocyte count is useful as an index of effective red cell production. It is usually expressed as a percentage of total red cells and as an absolute count (# of reticulocytes per ul). The percentage value is falsely elevated in patients with anemia; this is overcome by correcting the percentage according to the patient's hemoglobin. The percentage value reported by Saint Luke's Regional Laboratories has already been corrected in this way. The absolute reticulocyte count does not require correction.

Previously, the reticulocyte count was performed by a manual microscopic method, based on supravital staining of the RNA-containing reticulocytes. Today, automated reticulocyte counting is available on most high-volume automated hematology analyzers. The automated methods have the advantage of enumerating large numbers of cells, thereby greatly improving precision, accuracy and efficiency.

Indications for a reticulocyte count include the following:

- Investigation of anemia
- Decrease in hemoglobin of greater than 1.5g/dL without a known cause
- Monitoring the effect of hematinic therapy of anemia (eg iron, vitamin B12)

The reticulocyte count is one of the major parameters used in the initial classification of anemia, allowing one to distinguish hypoproliferative from hyperproliferative causes.

<table>
<thead>
<tr>
<th>Reticulocyte Count</th>
<th>Causes</th>
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| Increased          | • Hemolytic anemia  
|                    | • Acute blood loss  
|                    | • Response to replacement therapy (eg iron, B12) |
| Decreased          | • Aplastic anemia  
|                    | • Marrow suppression by drug, toxin, or viral infection  
|                    | • Pure red cell aplasia  
|                    | • Bone marrow replacement (leukemia, lymphoma, carcinoma) |
| Normal             | • Iron deficiency anemia  
|                    | • Anemia of chronic disease  
|                    | • Chronic renal failure  
|                    | • Megaloblastic anemia (B12 or folate deficiency)  
|                    | • Myelodysplasia |

In patients with anemia a second correction is recommended, because increased erythropoietin production in this situation leads to premature release of reticulocytes into peripheral blood, resulting in an increase in reticulocyte maturation time in peripheral blood, and a false elevation of the reticulocyte count. This correction is achieved by dividing the reticulocyte percentage by a factor which depends on the degree of anemia.

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Correction factor</th>
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<tr>
<td>35</td>
<td>1.5</td>
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<tr>
<td>25</td>
<td>2.0</td>
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<tr>
<td>15</td>
<td>2.5</td>
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For example, if the hematocrit is 25 and the reticulocyte count is 6%, the corrected count would be 3% (6% divided by a factor of 2). This doubly corrected reticulocyte count is termed the reticulocyte production index, and is a rough estimate of the rate of effective erythropoiesis. For example, the above value of 3% would indicate an approximate 3-fold increased rate of erythropoiesis by the marrow. In chronic hemolytic states the marrow is capable of increasing red cell production up to 8-fold the normal rate.
Automated reticulocyte counting methods have led to the ability to measure stages of reticulocyte maturity, based on RNA content. A relatively new parameter is the "immature reticulocyte fraction" or IRF (expressed as a ratio of immature reticulocytes to total reticulocytes). This value provides a very early and sensitive index of marrow erythropoietic activity. A rise in the IRF has been shown to be one of the earliest indicators of hematopoietic recovery following bone marrow transplantation or intensive chemotherapy. The IRF can also be used as an early indicator of response to erythropoietin therapy in patients with chronic renal failure and other diseases.

In summary, the reticulocyte count should be one of the initial tests ordered in the investigation of anemia. The reference range for the reticulocyte percentage is 0.4-1.8%, and for the absolute reticulocyte count is 28-88 th/uL in males and 17-77 th/uL in females. The reference range for the IRF is 0.0-0.1. The percentage and absolute values are always reported, together with the IRF. The reticulocyte count is available 24 hours a day, 7 days a week, and one 5mL lavender top tube is required (the same tube used for the CBC).

**Cold Agglutinins**

Cold agglutinins are nonspecific IgM antibodies which agglutinate red blood cells at cold temperatures between 0 and 30°C. In the past, cold agglutinin titers were often used as a surrogate test for Mycoplasma pneumonia, since mycoplasma infections are often associated with elevated anti-I titers. Numerous conditions besides Mycoplasma pneumonia will give an elevated titer including: viral infections, hemolytic anemia, liver disease and pregnancy. More specific mycoplasma IgG and IgM immunoassays are preferred to diagnose Mycoplasma pneumonia. Cold agglutinin titers should only be ordered to diagnose cold autoimmune hemolytic anemia.

Cold agglutinins are present in 95% of healthy patients at titers < 1:16. Titers less than 1:32 are considered negative. Specimen requirement is one 10 mL red top tube (preferably without gel) of blood. The tube should be immediately placed in a 37 C water bath and allowed to clot. After 10 minutes, the tube can be centrifuged and the serum transported to the laboratory at ambient temperature. If a water bath is not available, the tube should be transported at ambient temperature without centrifugation.