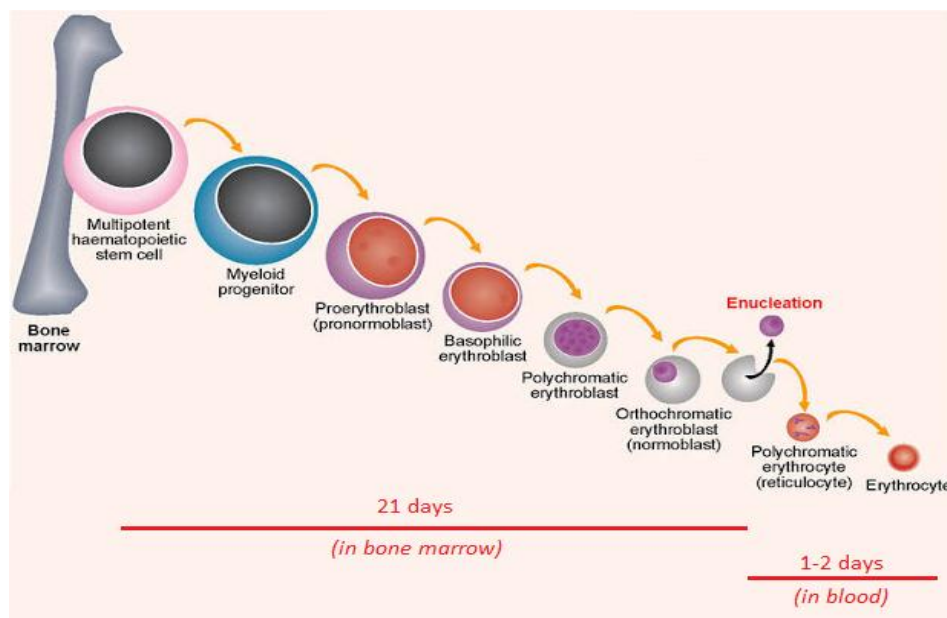


## Reticulocyte count

Reticulocytes are immature RBCs erythrocytes that contain increased amounts of ribonucleic acids (RNA) that are released in the circulation during an increased bone marrow activity aimed to compensate for blood loss or destruction. Reticulocytes are visualized by staining erythrocytes with vital stains such as new methylene blue (NMB).

### Objectives:

- recommend a reticulocyte count in order to identify bone marrow function, including production of enough red blood cells.
- They may also order a reticulocyte count to help diagnose and distinguish between different types of anemia.
- They may also use it to help monitor the progress and health after chemotherapy, radiation therapy, a bone marrow transplant, or treatment for iron deficiency anemia.



**High reticulocyte levels (reticulocytosis)** could be a sign of:

- acute bleeding
- chronic blood loss

- hemolytic anemia
- erythroblastosis fetalis, also called hemolytic disease in a newborn, a potentially fatal blood disorder that affects some fetuses and newborns
- kidney disease

**Low reticulocyte levels** (reticulocytopenia) could indicate:

- iron deficiency anemia
- aplastic anemia
- folic acid deficiency
- vitamin B-12 deficiency
- bone marrow failure caused by drug toxicity, infection, or cancer
- kidney disease
- cirrhosis
- side effects from radiation therapy

### **Procedure:**

Specimen of Whole blood collected with EDTA tube is recommended; however, any anticoagulant is acceptable.

1. Pipet five drops of new methylene blue solution into a labeled glass test tube.
2. Add an equal volume (five drops) of well-mixed blood (control or patient). Mix gently using a pasteur pipet.
3. Incubate at room temperature for 10 minutes.
4. Resuspend mixture thoroughly, and prepare 2-3 wedge smears. Air-dry the smears immediately by gently waving the slide.
5. Label the reticulocyte smears by writing appropriate information directly into the thick end of the smear.

### **Reticulocyte Counting:**

Using the oil immersion lens and an ocular fitted with a Miller disc( if present) ,select an area of the smear where the erythrocytes are evenly distributed, without

overlapping and enumerate 10 field. reticulocytes are enumerated in the following ways:

- Reticulocyte percentage
- Absolute reticulocyte count (This is reticulocytes numbers in 1 mm<sup>3</sup> of whole blood. This is not % of the RBCs).

Count a total of 1000 RBCs under the 100 x oil immersion lens.

$$\text{\% Reticulocyte count} = \frac{\text{Reticulocytes count} \times 100}{1000 \text{ RBCs}}$$

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$$\text{Absolute reticulocytes count} = \frac{\text{\%Reticulocyte count} \times (\text{RBC count}) (10^{12}/\text{L})}{100}$$

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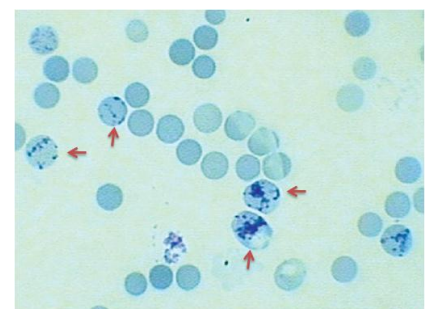
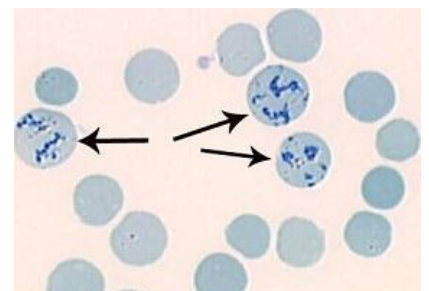
$$\text{Corrected reticulocytes count} = \frac{\text{\%Reticulocyte count} \times \text{Patient Hct}}{45(\text{average normal Hct})}$$

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Adult Males: 0.5 - 2.5%

Adult Females: 0.5 - 4.0 %

Newborn: 1.8 - 8.0%



### **Technical sources of error:**

- a. Other RBC inclusions** (Pappenheimer bodies, Howell-Jolly bodies, and Heinz bodies) will be stained with new methylene blue. Heinz bodies appear as light bluegreen inclusion located at the periphery of the erythrocyte. Howell-Jolly bodies are usually one or two round, deep purple staining inclusions and are also visible on Romanosky stains. Pappenheimer bodies are indistinguishable from reticulum of reticulocytes. If Pappenheimer bodies are suspected, a Prussian blue iron stain should be performed to verify their presence.
- b.** The whole blood-stain mixture should be resuspended prior to making the smears. Reticulocytes have a lower density than mature erythrocytes, and therefore will be located near the top during incubation.
- c.** Poor drying or moisture may result in the presence of refractive artifact on the smears. This refractive artifact may be confused with precipitated reticulum. However, precipitated reticulum is not refractive, and fine focus adjustment will reveal the difference.
- d.** Increased glucose levels may cause the reticulocytes to have a pale stain.
- e.** Counterstaining with a Romanosky-type stain is no longer recommended, as it may obscure the precipitated reticulum.

### **References:**

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