RETICULOCYTES COUNT

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Reticulocyte

**Definition:**

- Immature RBC that remains 24h in the circulation before they transform into mature RBC.
- They contain remnants of ribosome and RNA which were present in large amount in the cytoplasm in the precursors from which they were derived.
Reticulocytes = polychromatohilic
Reticulocytes count

**Purpose of testing:**
- Reticulocyte count help in monitoring anemic patients under treatments
- Determine the state of increased erythropoietic activity
- Assess bone marrow activity
Principle of test

- The peripheral blood sample is stained with supravital stain (brilliant cresyl blue or New methylene blue).

- These are basic dyes that have the ability to react with ribosome and nucleic acids of reticulocytes while it still alive.

- The nucleic acid-dye reaction forms a blue precipitate of granules or filaments.
Staining method

- Deliver 2-3 drops of the dye solution into a 75x10 mm glass or plastic tube using Pasture pipet.

- Add twice the amount of the patients EDTA blood to the dye solution and mix.

- Keep the mixture for 37°C for 15-20 min.

- Resuspend the Red cells by gentle mixing.

- Make film on the slide in the usual way.
Counting reticulocytes

1. Look into the slide area using 10x objective
2. Add a drop of oil
3. Move to 100x
4. Count all red cells (reticulocytes + RBC) using a manual counter
5. Make sure not to count WBC (large nucleated cell)
6. The number of reticulocytes is counted also on outside paper to be able to calculate.
Calculations

- % retics = \frac{\text{no. Of retics}}{\text{Total no. Of red cells}} \times 100

- Total no. Of red cells = retics and RBC in 10 fields.
- If the count of RBC is 200-25-/ filed
- Meaning that in 5 filed we will have total of 1000RBC and retics
- Thus calculation will be the following:

- % retics = \frac{\text{no. Retics}}{1000} \times 100 \Rightarrow \%\text{retics} = \frac{N}{10}

N.R in adults = 0.5% - 1.5%  
N.R in neonates = 2.5% - 6%
Source of errors:

- Improper mixing of the specimen with the stain
- Improper time of incubation of the stain with the dye.
- Improper count.
- Count Heinz body as reticulocytes.
Condition of high and low count of reticulocytes

- Increased reticulocytes:
  - Effective erythropoiesis
  - Anaemic patient under treatment
  - B.M assemant after bleeding

- Decreased reticulocytes:
  - Ineffective erythropoiesis
    - Ex: BM disease or chemotherapy associated
    - Hypoproliffrative conditions