CLS 291 Clinical Hematology 1

جامعة الملك سعود
King Saud University

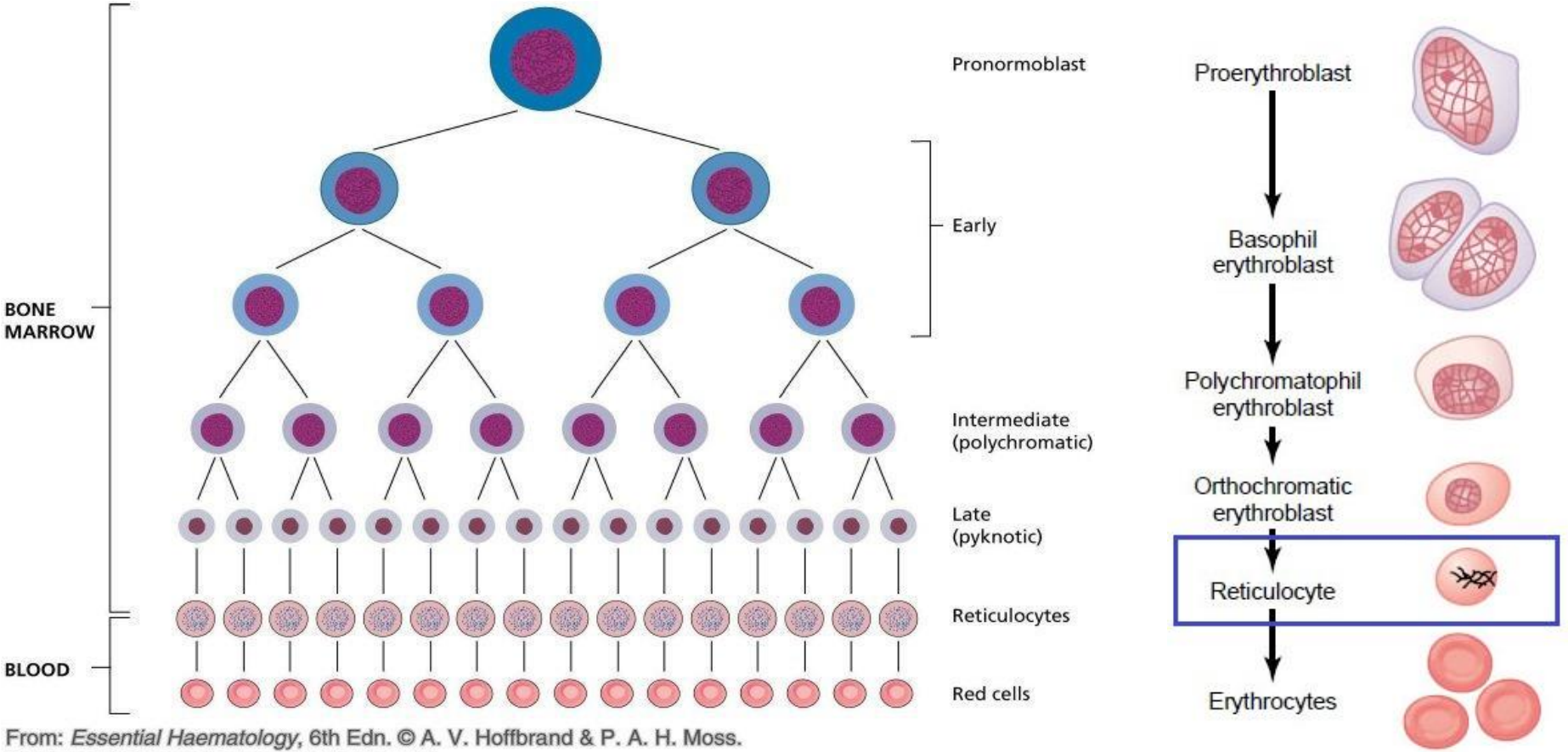
Lecture 8

Reticulocyte Count

Outlines

I. Erythropoiesis
II. Contents of reticulocytes
III. Reticulocytes Stages of Maturation
IV. Stains used for counting reticulocyte
V. Reticulocyte Stains principles
VI. The Difference between retic. and Hb H cells.
VII. Aim of counting reticulocytes.
VIII. Conditions associated with high and low reticulocyte count
IX. Counting method and calculation

Erythropoiesis



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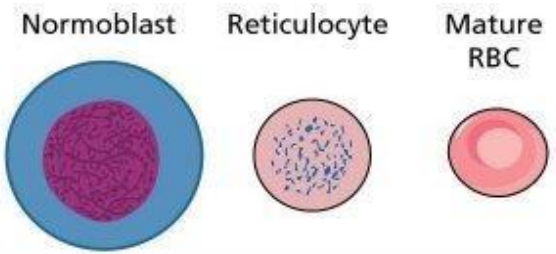
Reticulocyte Contents

- **Reticulocytes or Retic** are immature red cells.
- They contain remnants of the ribosomal ribonucleic acid (rRNA).
- Reticulocytes are derived from nucleated precursor.



Nuclear DNA	Yes	No	No
RNA in cytoplasm	Yes	Yes	No
In marrow	Yes	Yes	Yes
In blood	No	Yes	Yes

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Normal range of Reticulocyte count:

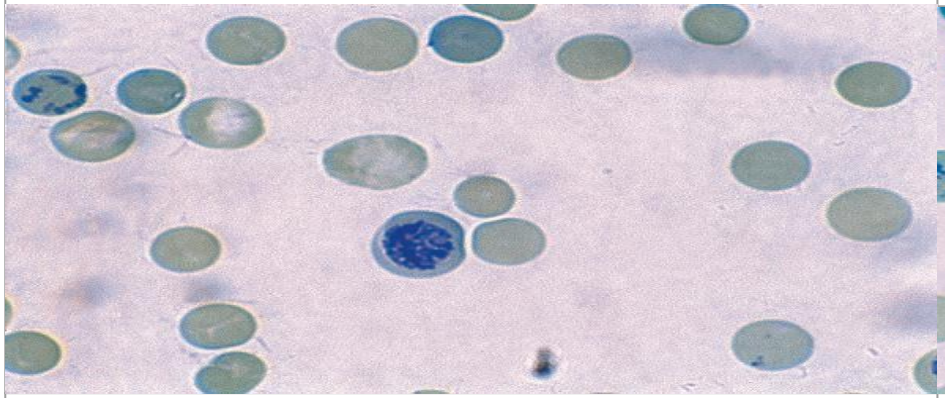
- 50–100 x10^9/l
- 0.5–2.5 %

Result interpretation:

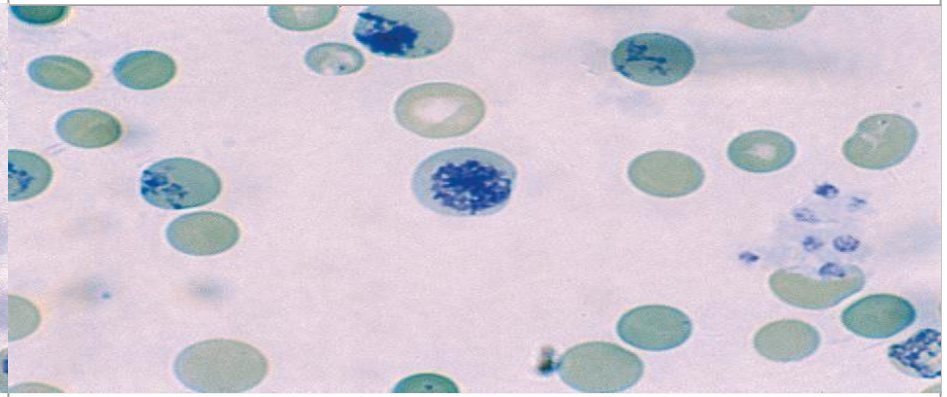
- **Above the normal range:** reticulocytosis
- **Below the normal range:** reticulocytopenia

Reticulocytes Stages of Maturation

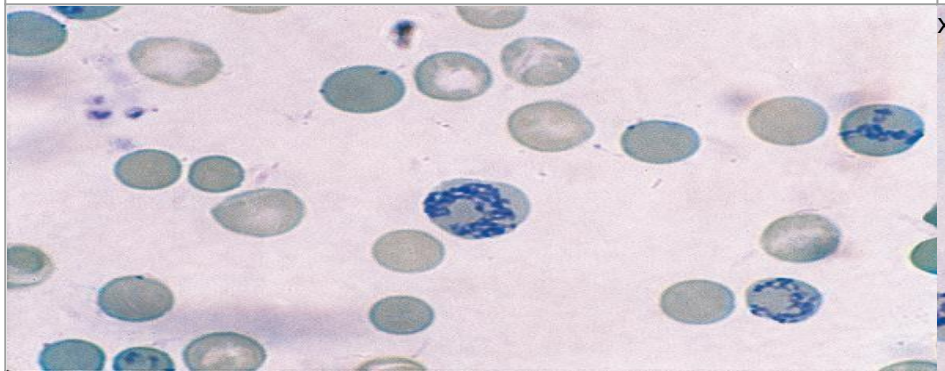
Stage I (Most immature reticulocytes)



Stage II (Intermediate maturation of reticulocytes)

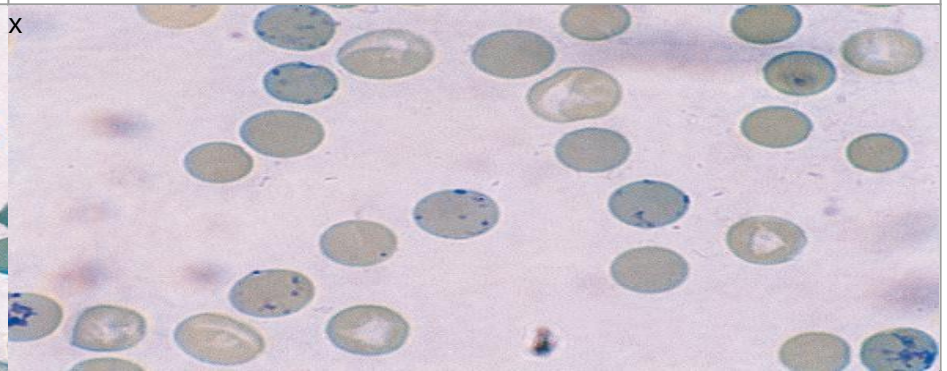


Stage III (Later stage intermediate stage)



Stage IV (Most mature reticulocytes)

x



Reticulocytes Stains

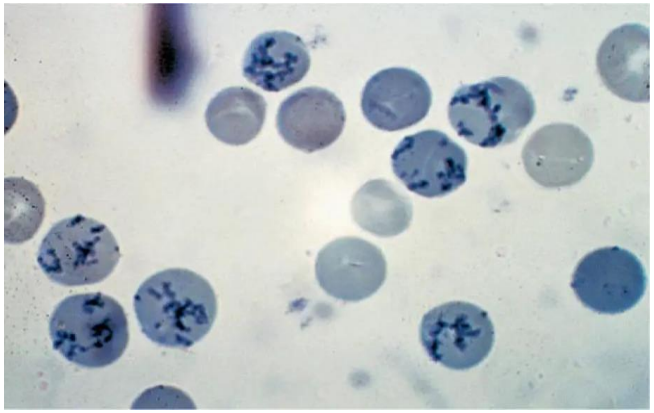
- They are called **reticulocytes** because of the **reticular** (mesh-like) network of ribosomal RNA (rRNA) that becomes visible under the microscope with certain stains.

- Reticulocytes are visualized **by one of the following stains:**

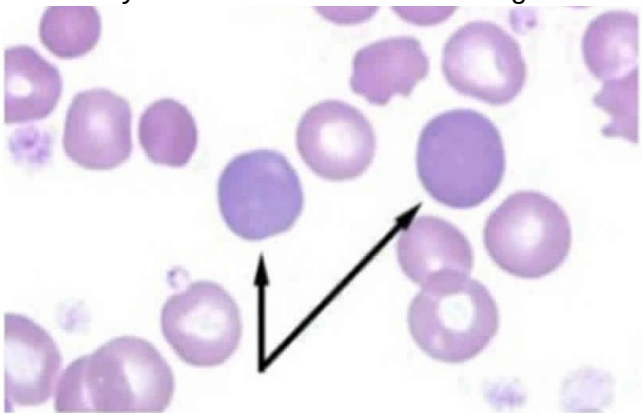
1. **Supra-vital staining** such as:
   a. **New methylene blue (NMB)**
   b. **Brilliant Cresyl Blue**

   - This stain precipitates the rRNA in an unfixed smear.

2. **Wright stain**
   - The Reticulocyte appears **polychromatophilic** red blood cell.



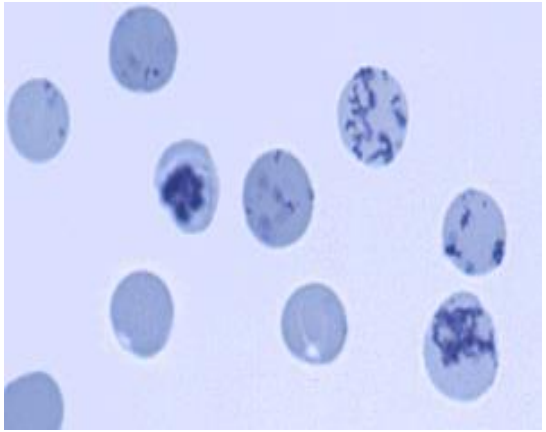
Reticulocytes stained with NMB showing rRNA.

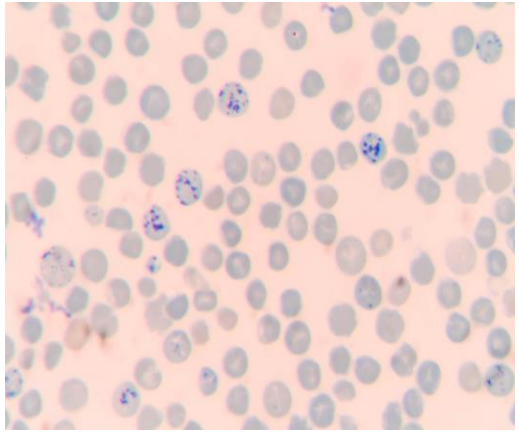


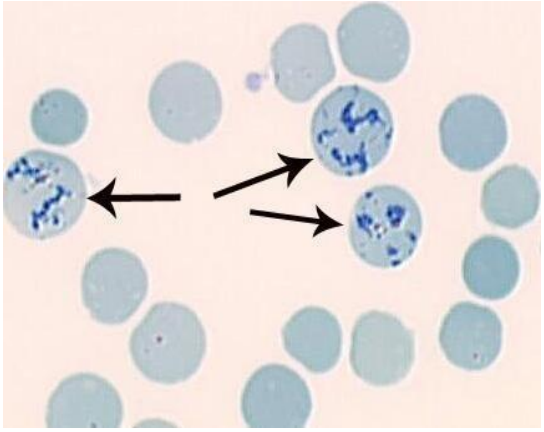
Reticulocytes stained with Wright stain.

Reticulocytes Stains

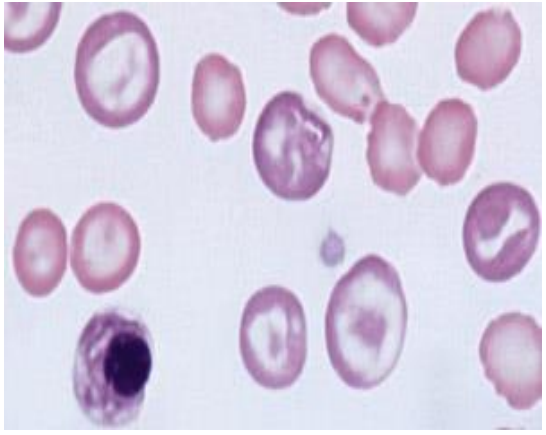
Reticulocytes stained with NMB showing rRNA.

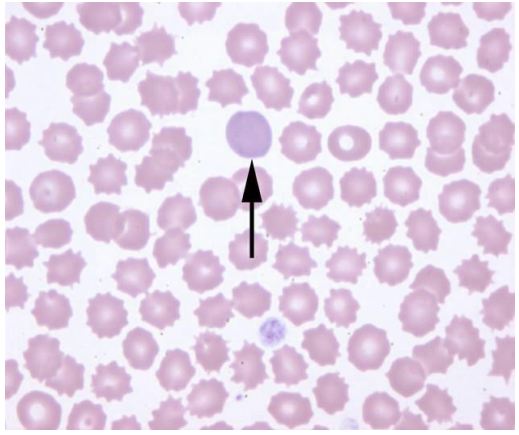


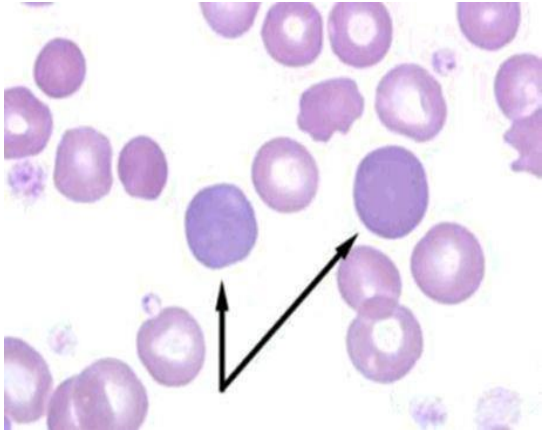




Reticulocytes stained with Wright stain.





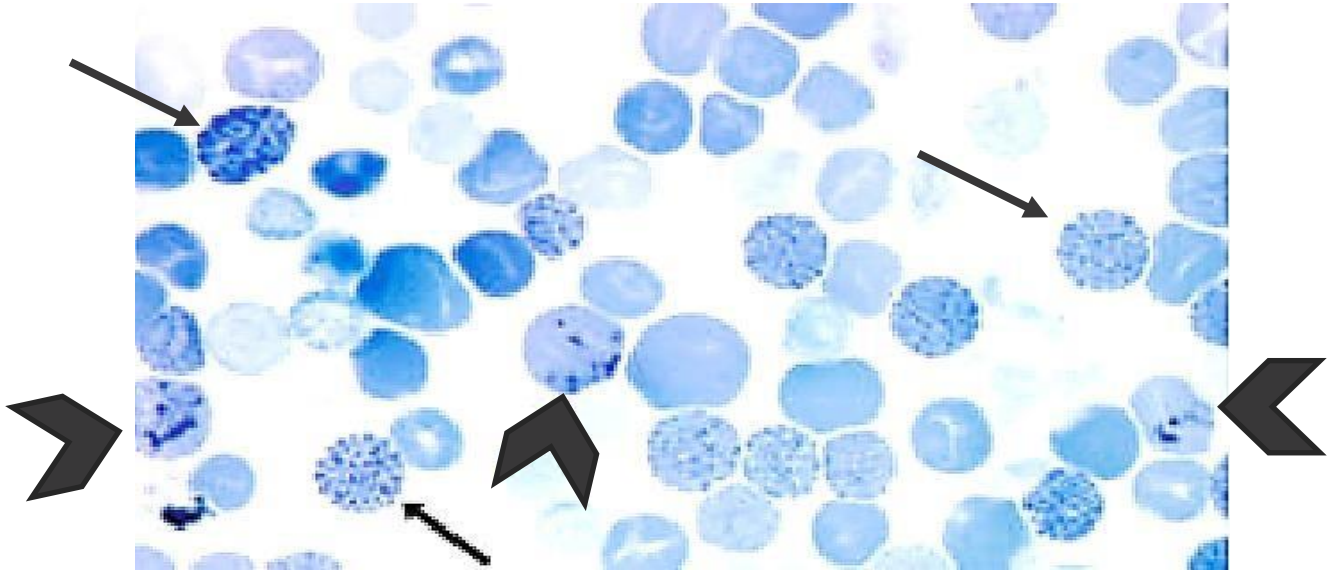


Reticulocytes Stains Principles

- **The Supra-Vital stain:**
  - It precipitates the residual ribosomal RNA within the reticulocytes.

- **The Wright stain:**
  - The reticulocyte visualized by the Wright stain is stained by the two components of the dye (acidic and basic parts of the stain) because of the presence of the Hb and the remaining RNA thus giving a polychromatic grayish-blue color .

The difference between retic. and Hb H cells



Reticulocytes (arrow head)	**Hb H cell Golf ball (arrow)**
Shows a mesh-like network of ribosomal RNA (rRNA).	Multiple fine, rounded, deeply stained deposits (golf ball cells).

The Purposes of Counting Reticulocytes

I. As a follow-up to abnormal results on a complete blood count (CBC), RBC count, hemoglobin, or hematocrit to help determine the cause.

II. To determine if the bone marrow is functioning properly and responding adequately to the body's need for red blood cells.

III. To help detect and distinguish between different types of anemia.

IV. To monitor response to treatment, such as that for iron-deficiency anemia.

Reticulocyte Count Result Interpretation

- Conditions associated with **reticulocytosis** (Increased reticulocyte count):

  1. Bleeding (hemorrhage).
  2. Increase RBC destruction.
  3. Polycythemia.
  4. Patients using mechanical valves.

- Conditions associated with **reticulocytopenia** (Decreased reticulocyte count):

  1. Bone marrow is not functioning normally (aplastic anemia, chemotherapy).
  2. Low level of the hormone erythropoietin.
  3. Deficiencies in certain nutrients such as iron, vitamin B12.

Method of Reticulocyte Counting

A. **Automated method:**

- It is performed using dyes or fluorochromes that combine with the RNA of reticulocytes.
- Following the binding of the dye, fluorescent cells can be counted using a **flow cytometer instrument**.
- Automated reticulocyte counting is currently incorporated in all automated blood counters (CBC instruments).

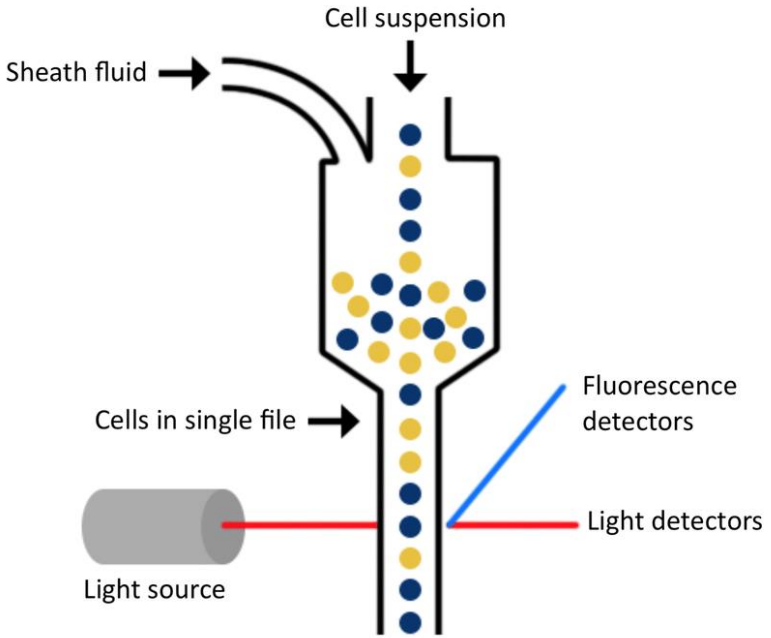


Figure. Diagram of flow cytometer instrument.

Method of Reticulocyte Counting

B. Manual method:

1. Add 2 drops of stain+2 drops of EDTA blood sample (must be fresh <48 h).
2. Incubate for 10 min at 37˚C in an incubator.
3. Mix, then use the stained blood to do a blood film.
4. Place the first slide on the microscope stage and, using the low power objective (10X), find an area where the red blood cells are evenly distributed and are not touching each other (ideal area of thickness).
5. Carefully change to the oil immersion objective (100x).
6. Counting the NO. of retic in (30 fields) and NO of RBC in 4 fields.
7. Do the calculation to find the percentage of retic.

https://www.youtube.com/watch?v=0ODP6-JR3vE

Calculation

- % of reticulocytes $= \frac{\textit{Total counts of retic in (30) fields}}{\textit{No.of fields (30) x Average No. of RBC in the 4 fields}}\ x\ 100$

- % of reticulocytes $= \frac{X}{N\ x\ Y}\ x\ 100$

- X= Total counts of retic in 30 fields
- N= No of fields =30
- Y= Average no of RBC in the 4 fields

Normal ranges:

- 50–100 x10^9/l
- 0.5–2.5%

Example:

X=Total counts of retic in 30 fields

- = (it was 35 retic cells)

N=Number of fields

- = (30)

Y=average no of RBC in the 4 fields

- (we counted 4 fields, and the NO. Of RBC were 115, 109, 98, and 89) the average is (115+109+98+89 / 4) = 102

Calculation:

% of reticulocytes $= \frac{X}{N\ x\ Y}\ x\ 100\ = \frac{35}{30\ x\ 120}\ x\ 100$

= 1.14%

Interpretation:

The result is within the normal range

Lab Evaluation Check List

Lab evaluation will be based on the following:

1. Do Counting of Reticulocytes with the calculation, and interpret your result.
2. Differentiate between Retics and Hb H cells (golf ball).