

Reticulocyte

RETICULOCYTE

Young red blood cell; still have small amounts of •
RNA present in them

Can be detected using supravital stain •

Tend to stain somewhat bluer than mature RBC's •
on Wright stain (polychromatophilic)

Slightly larger than mature RBC •

Effective bone marrow activity , monitoring anemia •
& response to therapy

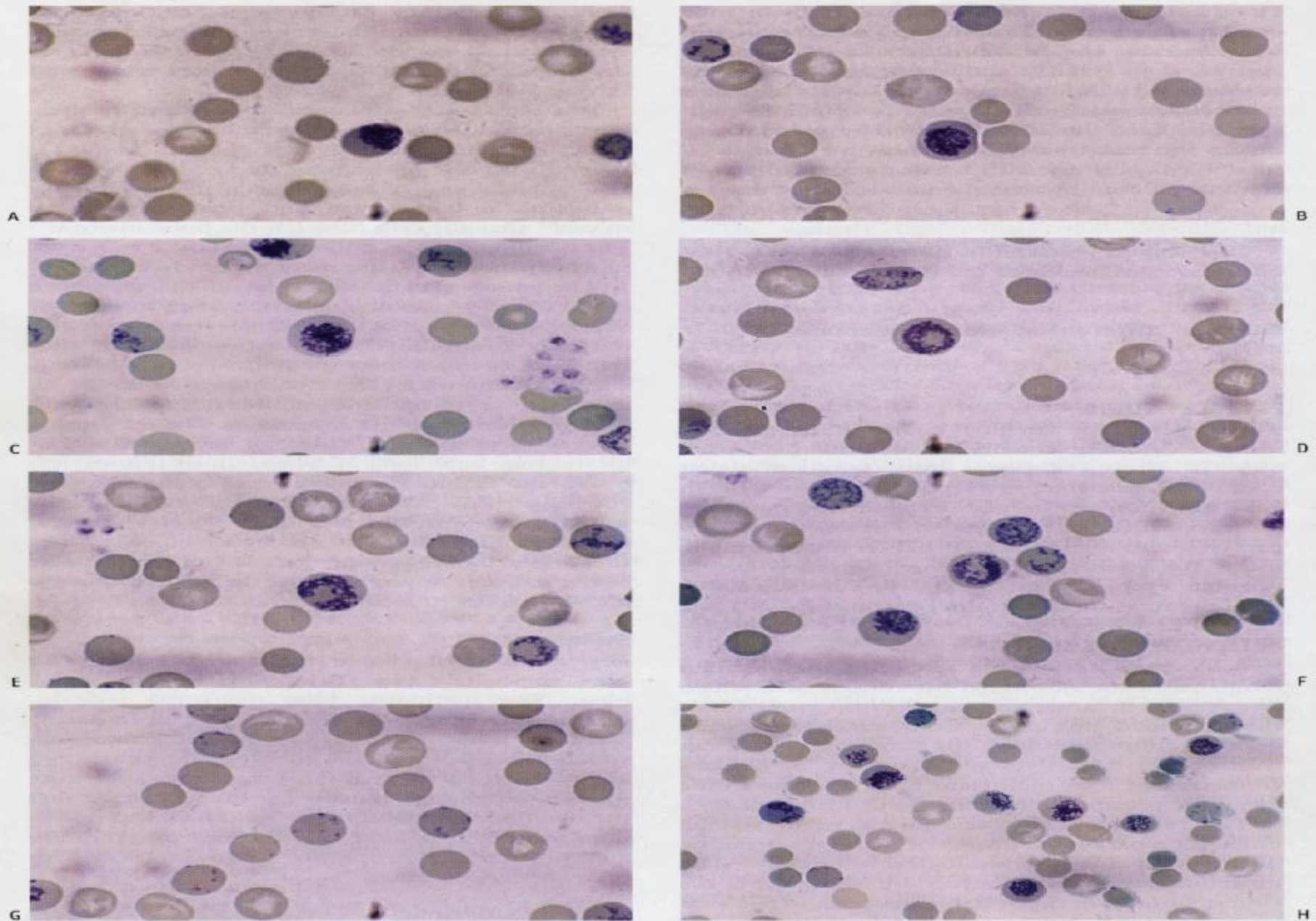


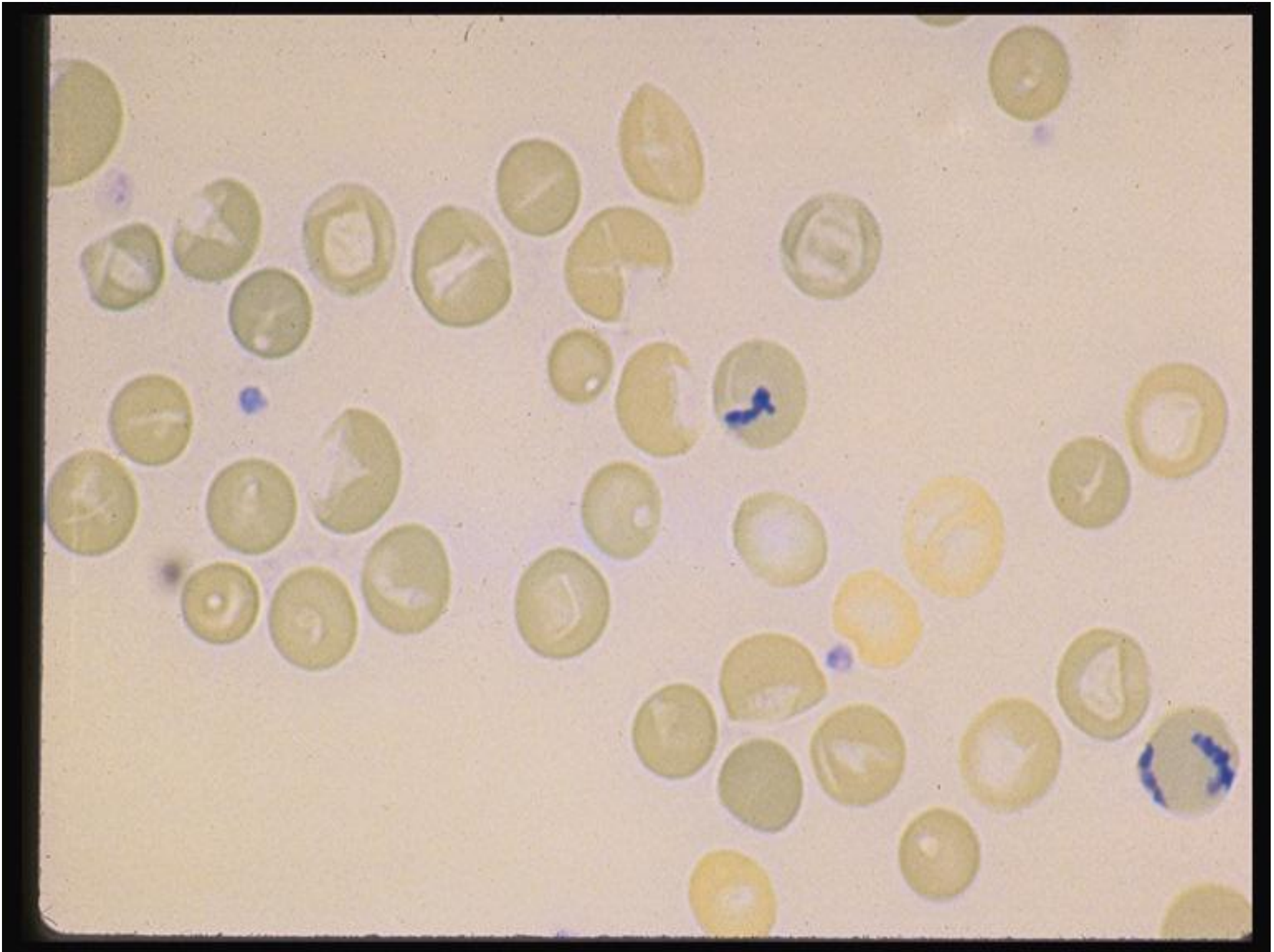
Fig. 3.4 Photomicrographs of reticulocytes showing stages of maturation. A and B, most immature (group I); C and D, intermediate (group II); E and F, later stage intermediate (group III); G, most mature (group IV); H, haemolytic anaemia, stained supravivally by new methylene blue.

staining

- New methylene blue
- 1.0 g of NMB in 100 ml of iso osmotic phosphat buffer pH 6.5 or citrate saline
- A= $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (150mmol/L) 23.4 g/l
- B= Na_2HPO_4 (150mmol/L) 21.3g/l
- 66 ml A + 34 ml B
- 1part citrate sodium 30g/l + 4 part NaCl 9 g/l
- Lifespan of stain is one month

Method

- 2 or 3 drops of the dye solution with 2-4 volumes of blood
- Keep the mixture at 37° for 15-20 min
- Remixing by 10 complete inversions
- Make at least 2 films on glass slide
- the exact volumes of blood to be added to the dye solution for optimal staining depends upon the red cell count



Anticoagulant must be EDTA •

24 hours in 2-6° •

6-8 hours in RT •

It is recommended retic count is done •
immediately after sample collection

•

Count & report

Distribution of cells •

Using of miller ocular micrometer disk •

At least 300 RBC are counted = 2700 RBC

retic in 20 large squares x 100 •

Retic percentage= •

erythrocytes in 20 small squares x 9 •

Edge rule : ~~the cells overlapping two of the four borders~~ •
are not counted.

The number of cell to be counted to achieve a reasonably •
precise reticulocyte count

The number of cell to be counted to achieve a reasonably precise reticulocyte count

Retic count	Approximate number of cells to be counted in small squares for CV of 10%	Equivalent to total count of
1-2%	1000	9000
3-5%	500	4500
6-10%	200	1800
20-25%	100	900

Important factors that affect the accuracy

- Retic preparation be well spread & well stained
- Visual acuity & patience of the observer
- The quality & resolving power of the microscope

RETICULOCYTE COUNT

Absolute Value

= Retic % x RBC Count•

eg 0.01 x 5,000,000 = 50,000—

Normal up to 100,000•

More accurate way to assess •
body's response to anemia

RETICULOCYTE COUNT

retic production Index

To be useful the reticulocyte count must be adjusted for the patient's hematocrit. Also when the hematocrit is lower reticulocytes are released earlier from the marrow so one can adjust for this phenomenon. Thus:

Corrected retic. = Patients retic. x (Patients Hct/45) •

$$\text{RPI} = \% \text{ Retic} \times \text{Hct}/45 \times 1/\text{CF}$$

<u>Hct</u>	<u>Correction factor (CF)</u>
45	1.0
35	1.5
25	2.0
15	2.5

Normal RPI = 1 (for non-anemic pt)

RPI < 2 : hypoproliferative

RPI ≥ 2 : hyperproliferative

Retic Production Index

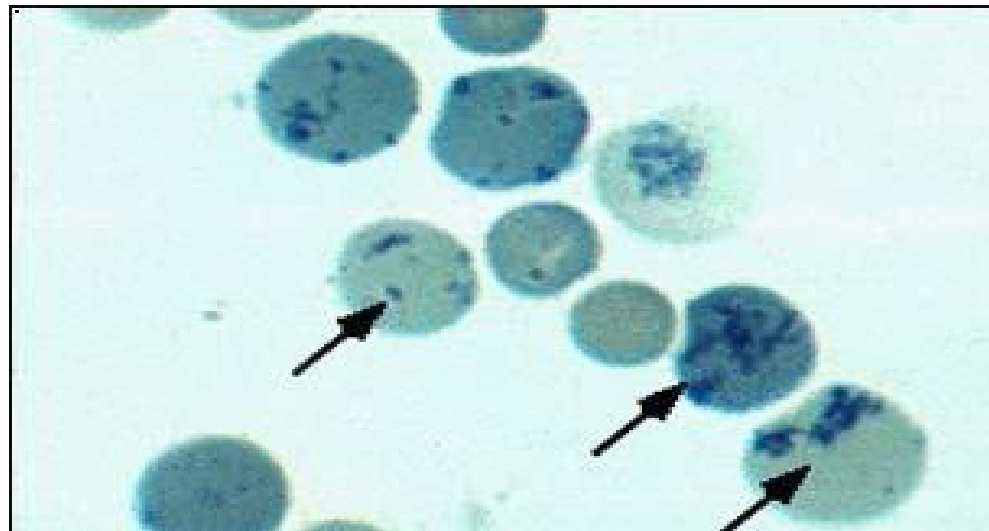
RPI < 2 hypoproliferative •
(inadequate response)

- Iron def. anemia
 - B12/folate def.
 - Chronic disease
- Sideroblastic anemia
 - Aplastic anemia
- Myeloproliferative

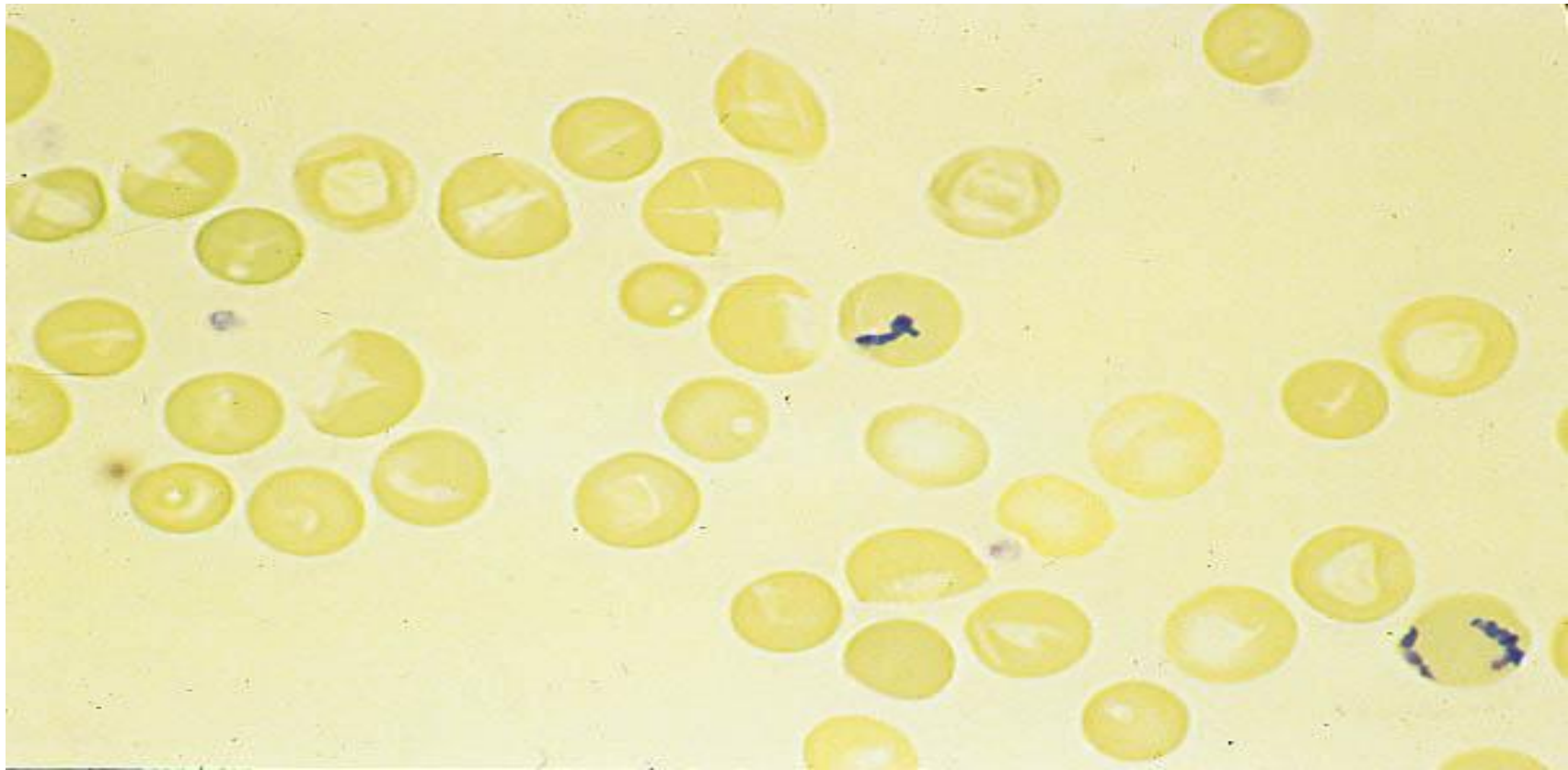
RPI \geq 2 : •
hyperproliferative
(adequate response)

- Hemolytic disease
- Hemoglobinopathy
(including thalassemia)
- Treated B12/folate def.

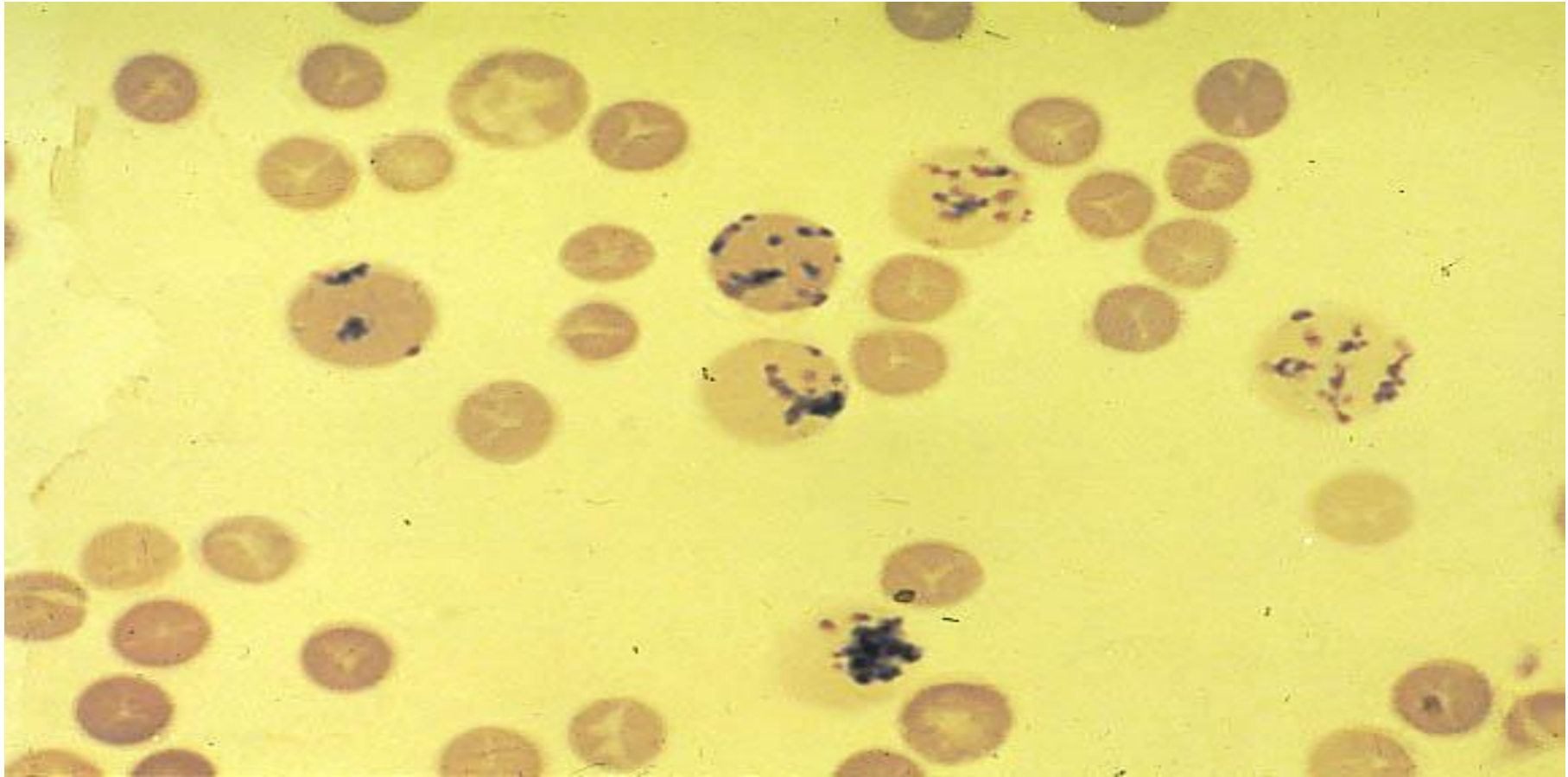
Retic count = % immature RBC
Normal 0.5-1.5% (for non-anemic)
In newborn 2.5-6.5%



Reticulocyte Manual Count by Supravital Stain: Normal Count



Reticulocytes: Elevated Count



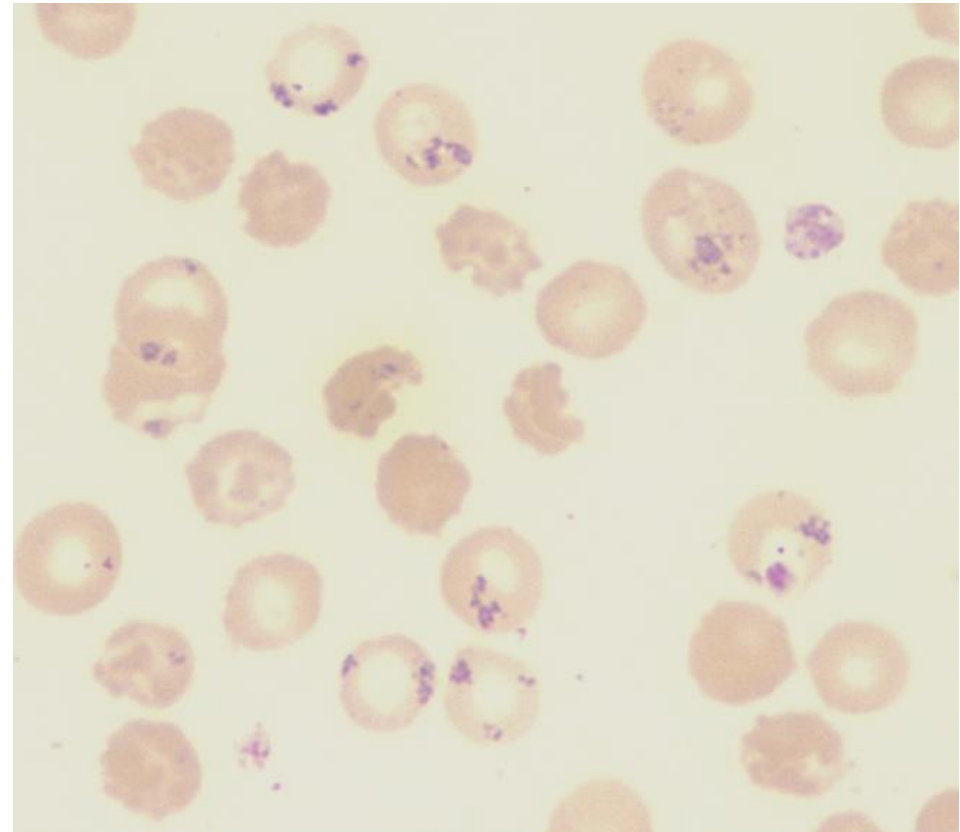
***Differentiating between
reticulocytes and other
red cell inclusions***

RBC inclusions

- Pappenheimer bodies •
- Heinz Bodies •
- Howell Jolly body •
- Hemoglobin H inclusion •

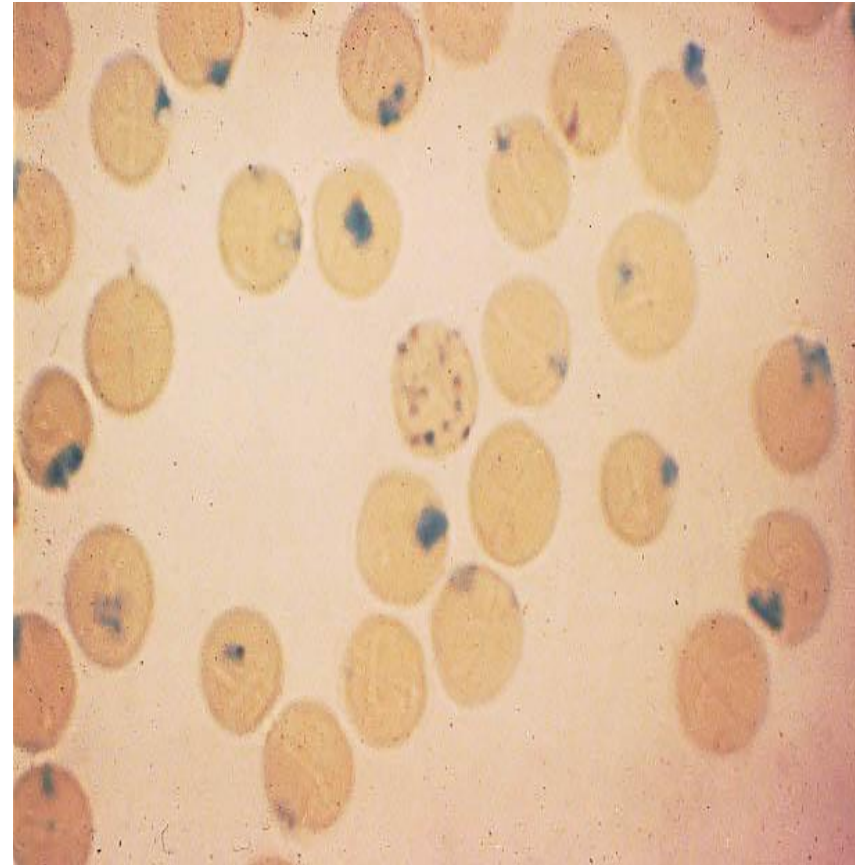
Pappenheimer bodies

- Iron-containing inclusions
- One or more granules towards the periphery of the cell
- May stain a deeper blue than reticulum
- It can be identified by over staining for iron by Perls reaction
- Hyposplenism & Fe overload



Heinz Bodies

- Denatured Hb
- Larger than Pappenheimer bodies
- Irregular in shape
- Usually attached to the cell membrane and may protrude through it
- They can be stained by a number of vital dyes
- Pale blue
- Splenectomized subjects & G6PD deficient
- Unstable Hb

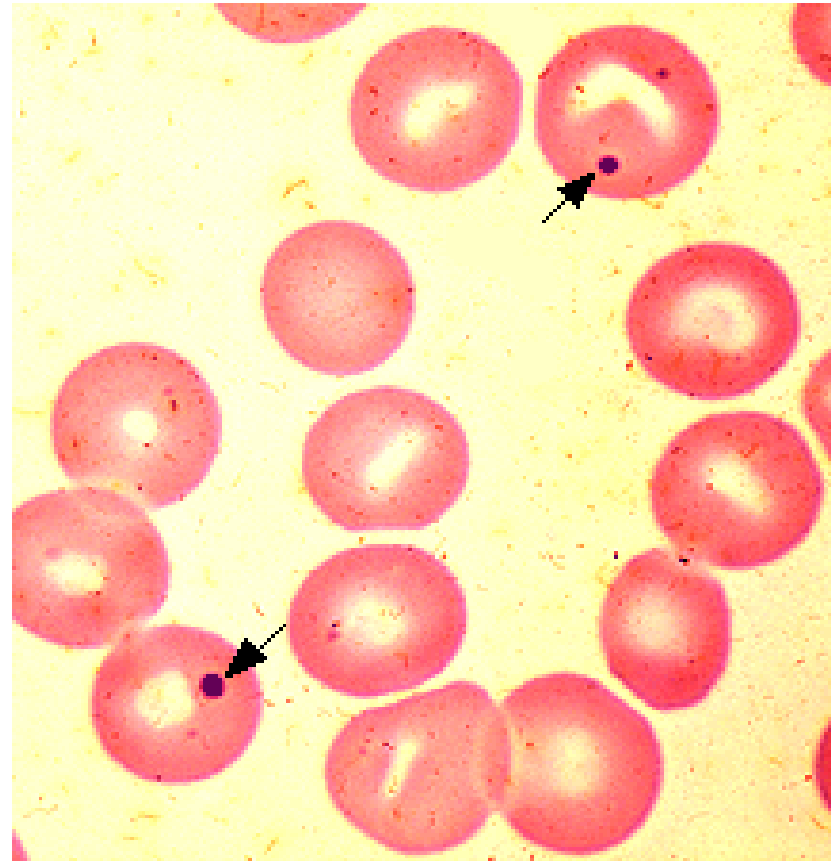


Specific dye for Heinz bodies

- brilliant green
- Whole blood is mixed with 0.5% neutral red
- Mixture is counterstained with 0.5% brilliant green
- Several thick smears are prepared from the final mixture
- Heinz bodies stain green, while reticulocytes & Howell-Jolly bodies stain a deep red

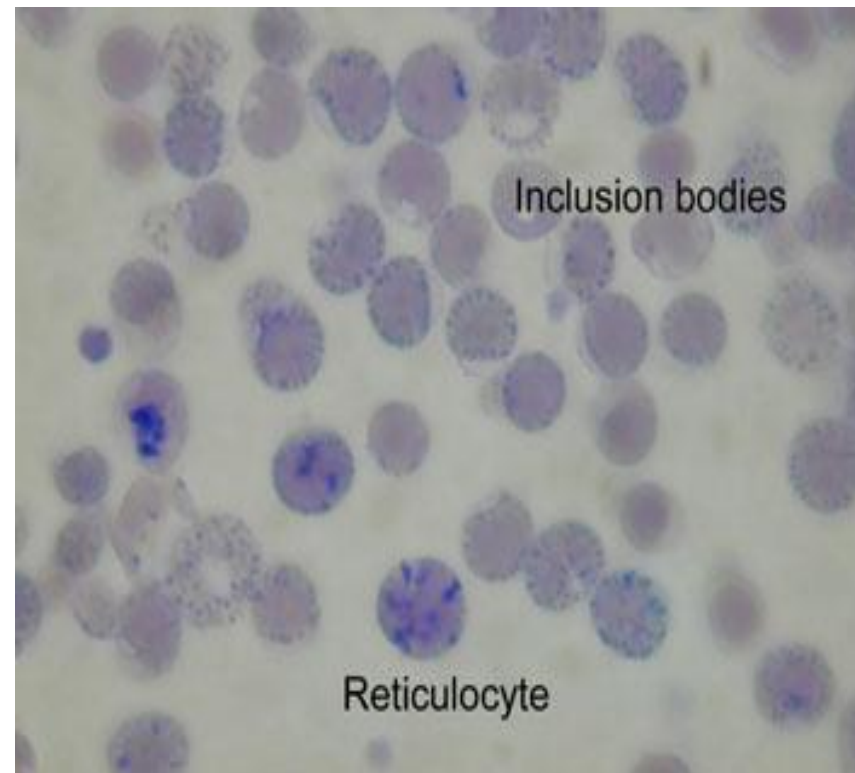
Howell Jolly body

- Nuclear remnant – DNA
- Larger than Pappenheimer bodies
- regular in shape
- Distant from the cell membrane
- Pale blue
- hemolytic anemia
- absent or hypo function spleen



Hemoglobin H inclusion

- Denatured Hemoglobin H (β_4) in α thalassemia
- Usually do not form with short incubation periods(2 hours)
- If present they are multiple and spherical, giving a “golf-ball” appearance
- Pale greenish-blue
- in α thalassemia trait 0.01-1%
- Enrichment



Automated reticulocyte count

- A peripheral blood specimen is stained with a fluorescent dye that binds to RNA
- Dyes: aramin o ,thiazol orange, acridine orange ,thioflavin T
- Large number of cells counted increased precision & accuracy
- Presence of Howell-Jolly bodies, NRBC, giant platelet, malaria parasite false positive
- Immature reticulocyte fraction (IRF)
- IRF indicates BM response to Fe, folic acid, erythropoietin

Questions?