Red Blood Cell Morphology

Pinal Patel
Laboratory Education Specialist

Cleveland Clinic

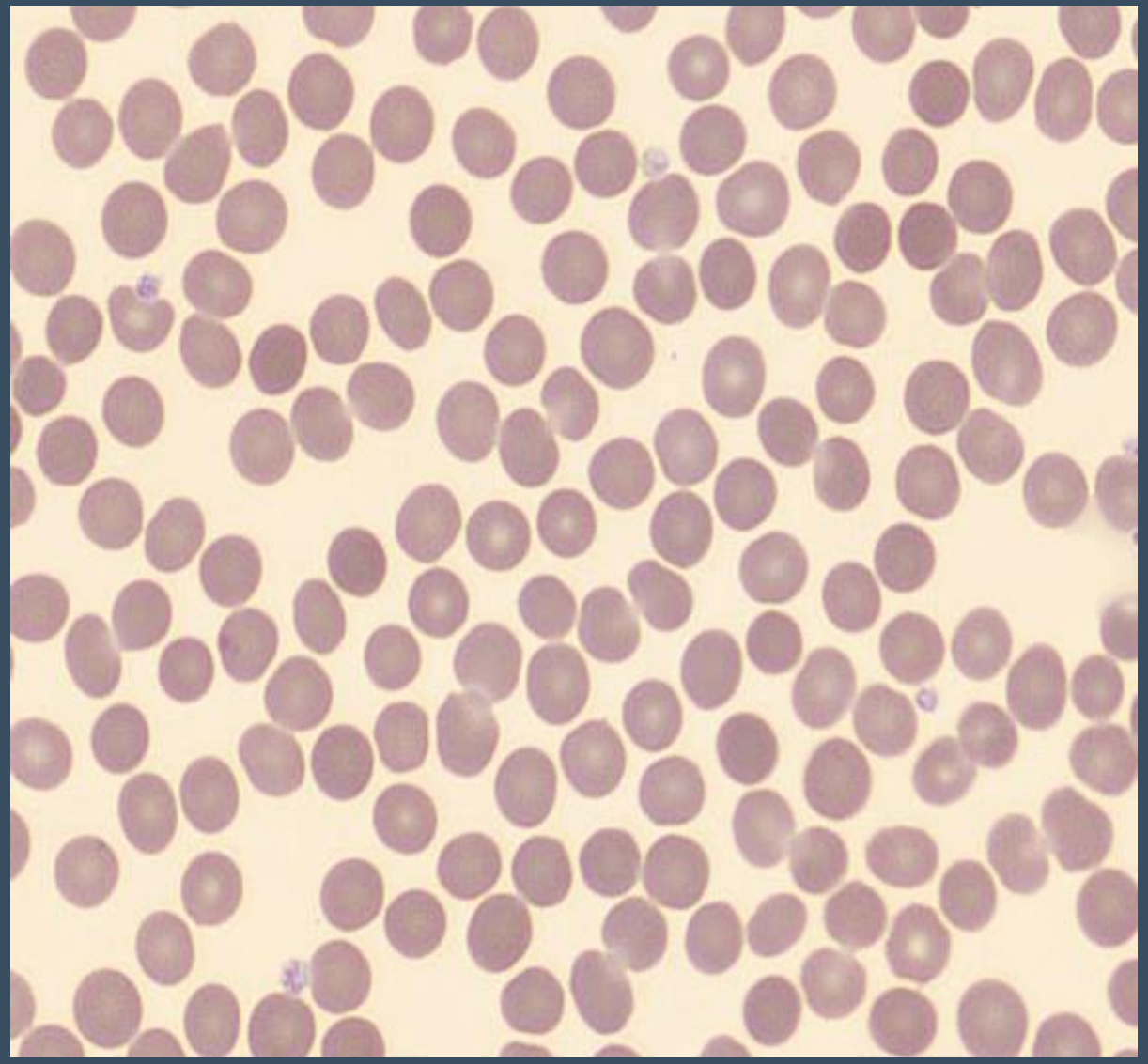
Introduction

- In contrast to WBC, circulating RBCs are anucleate and homogenous in appearance.
- Derived from bone marrow orthochromic erythroblast following extrusion of nucleus.
- While developing in marrow,erythroid cells increase in RNA content for Hgb production
- 1% of circulating RBCs get replaced every day
- Essential Nutrients for RBCs synthesis are iron, folic acid , and vit.B12.

Introduction

- The blood film should be examined in the area where the red cells are touching but not often overlapping
- Red cell morphology is assessed according to grading system
- Scan red blood cells on a slide using 100x oil immersion, observe 10 fields, red cells are observed for size, Shape, hemoglobin content and the presence or absence of inclusions.

Normal RBC

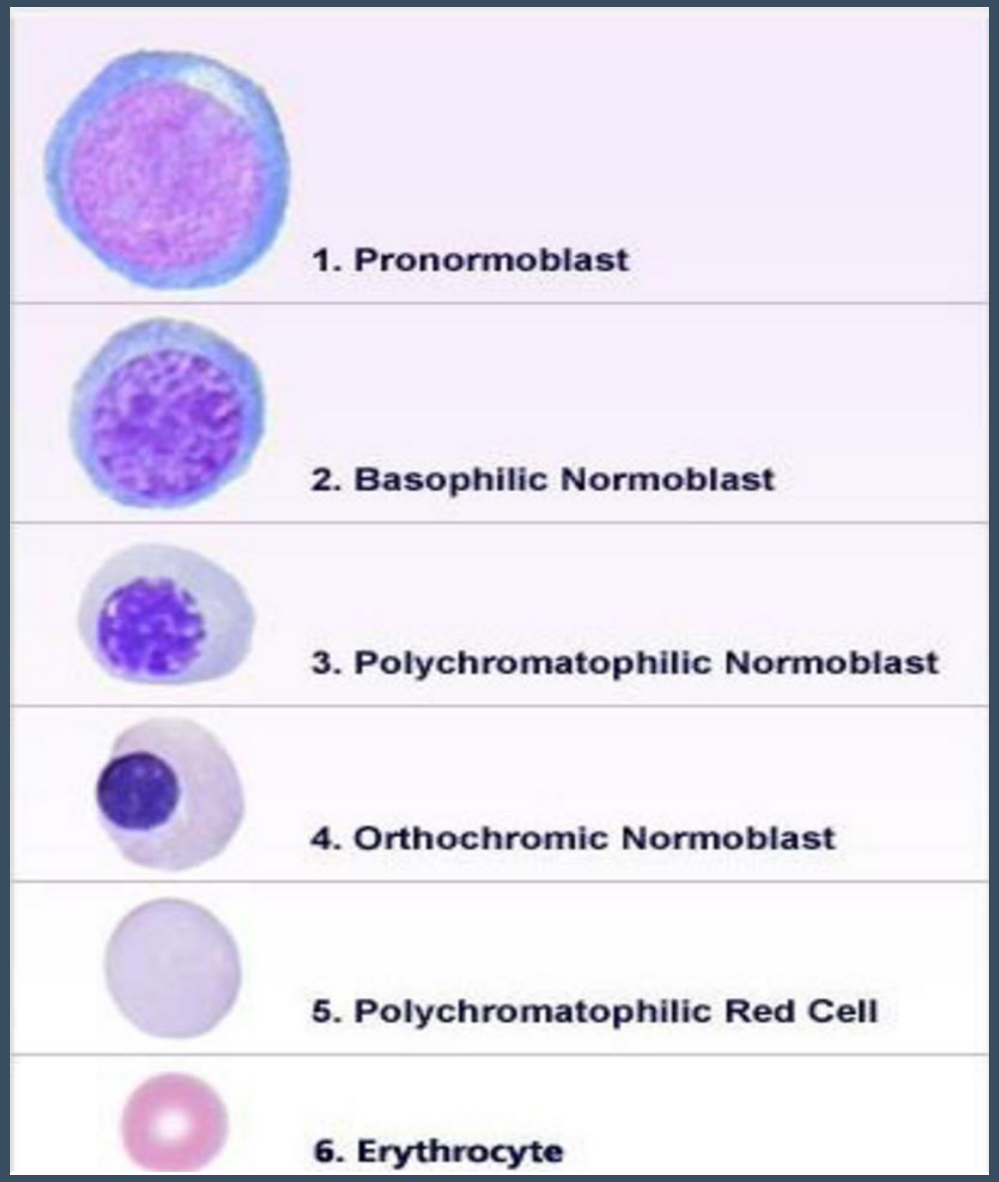


- 7 days in marrow and ~120 days in circulation
- Hemoglobin gives bright red color to RBC
- Size ~ 7 µm
- Central pallor ~1/3 of total size
- Slight variation in size and shape

Normal RBC

- Approximate size is same as nucleus of mature lymphocyte
- Circulate in blood as biconcave disk
- Biconcave disk shape allow them to stacks and flex to smooth the flow through the narrow blood vessels and entering small capillaries

Stages of RBC Maturation



- As the RBC go through the six maturation stages, overall size of the cell decreases, and hemoglobin content increases.
- The nucleus become smaller in size and more condensed until it is pushed out of the cell.
- The first three stages normally take place in bone marrow .
- The orthrochromic normoblast can be seen in peripheral but not in high numbers.
- The last two cells can be seen in circulation.

RBC Physiology

Deformability

- Surface area 140 μm^2
  - An excess surface to volume ratio allows cells to stretch undamaged
  - Deformability also depends on cytoplasmic viscosity
  - Increased Hb concentrations compromise deformability and shorten the RBC life span
    - As MCHC increased, variation in deformability

RBC Physiology

- Membrane Composition
  - 52% proteins
  - 40% lipids(equal ratio of cholesterol and phospholipids)
  - 8% carbohydrates
- Transmembranous proteins
  - Assemble in one of two complexes, ankyrin or protein 4.1
  - Provides vertical membrane structural

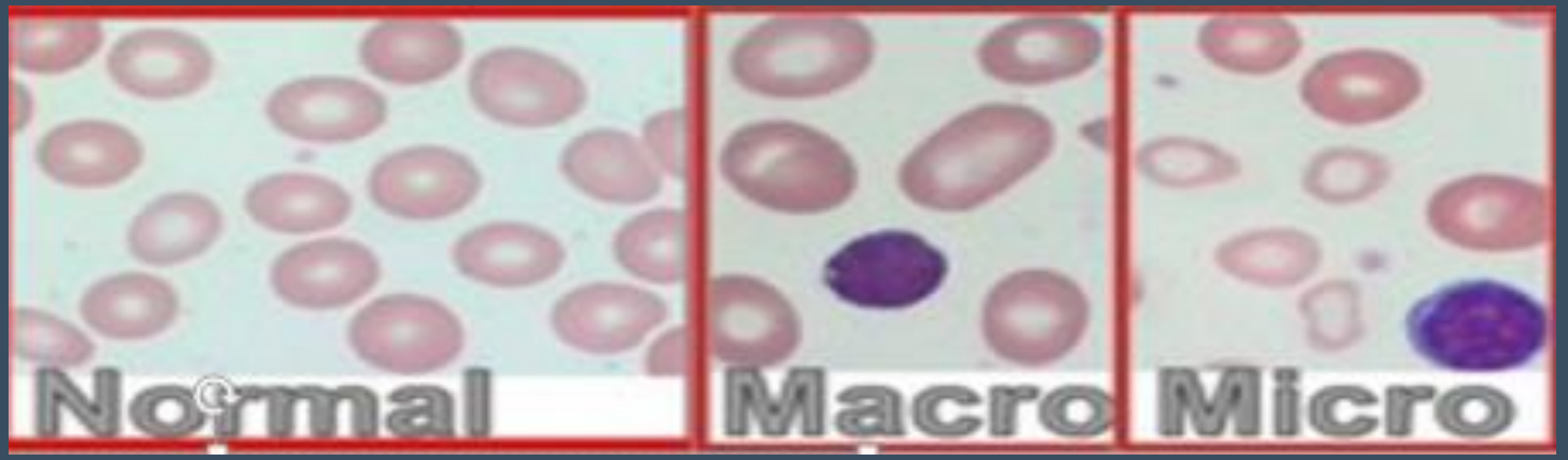
RBC Physiology

- Skeletal Proteins
  - The spectrins provide lateral membrane stability
- Mutation in ankyrin, protein 4.1, or spectrin is seen in hereditary spherocytosis
- Mutation in spectrin dimer to dimer or spectrin-ankyrin-protein 4.1 junction are seen in hereditary elliptocytosis

RBC abnormality

- Size (anisocytosis)
- Hemoglobin content -color variation
- Shape(poikilocytosis)
- Erythrocyte inclusion
- Red cell distribution

Size



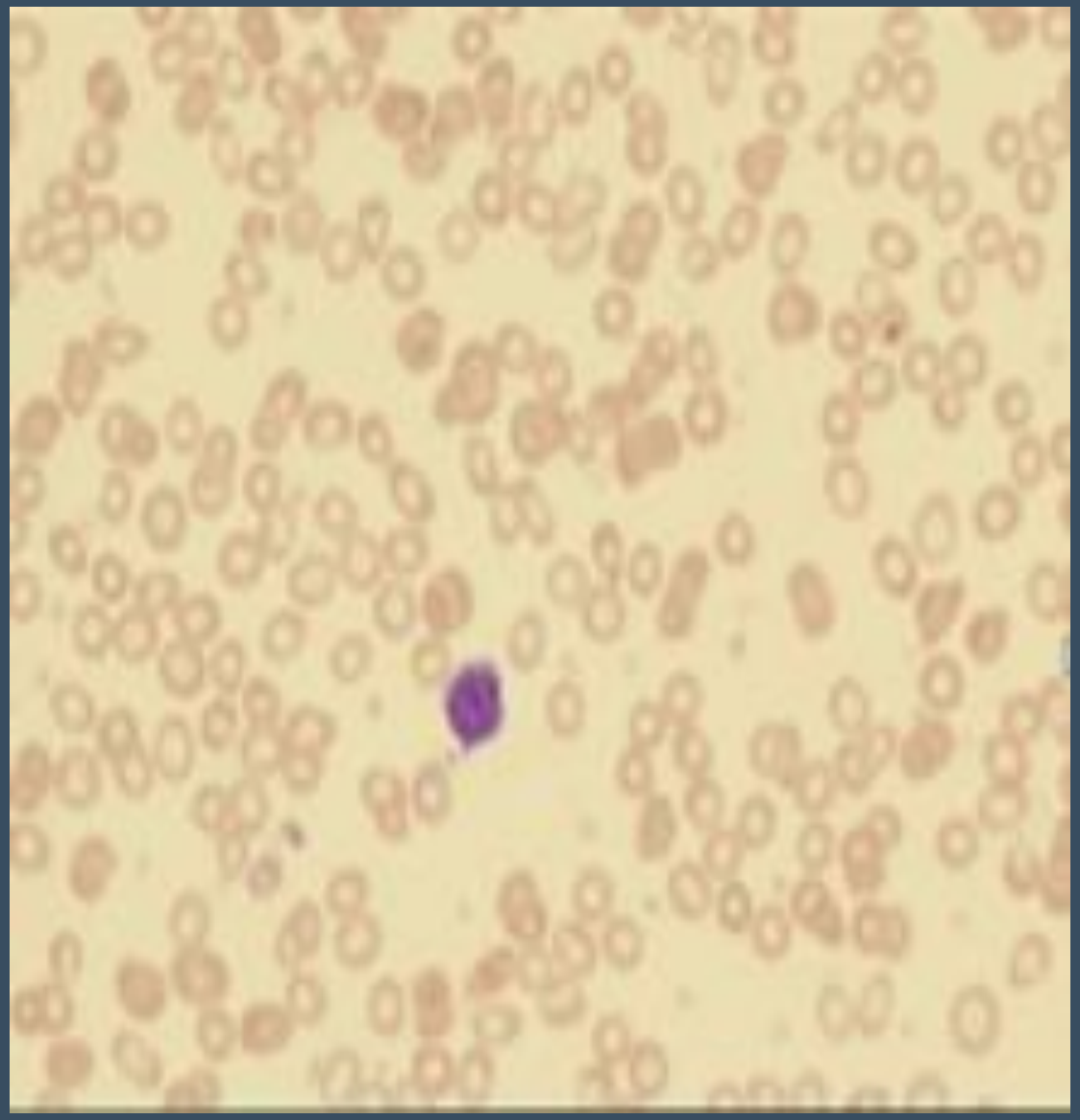
- On this basis anemia may be classified as

  normocytic /normochromic (MCV 80-100 fl)

  Macrocytic (MCV >100 fl), or

  microcytic/hypochromic (MCV <80 fl)

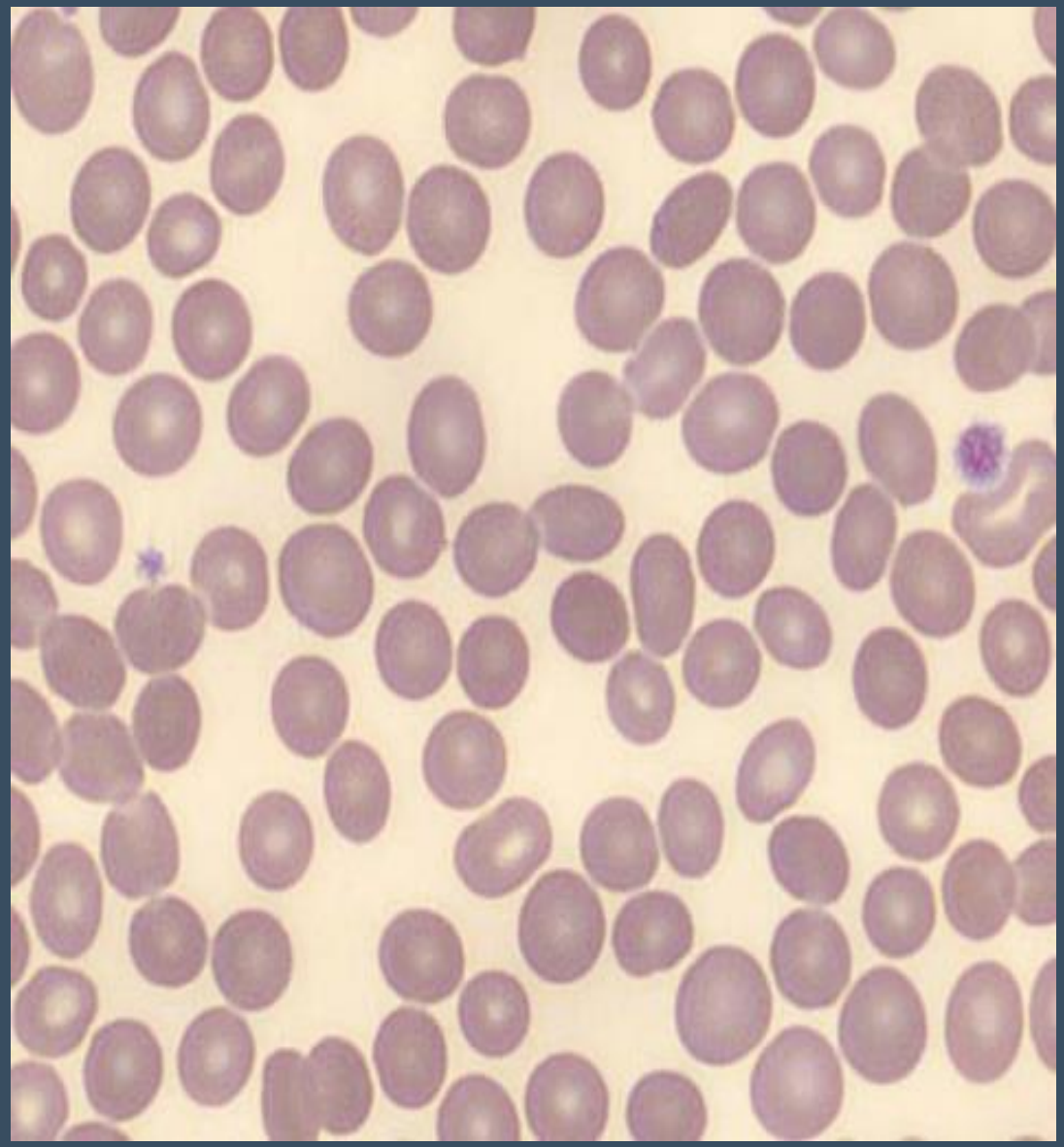
Microcytic



- Expanded central zone of pallor
- Decreased or defective globin synthesis also presents as microcytic hypochromic anemia
- Found in

  Iron deficiency anemia Thalassemia , Sideroblastic anemia , Lead poisoning and Anemia of chronic disease.

Macrocytic

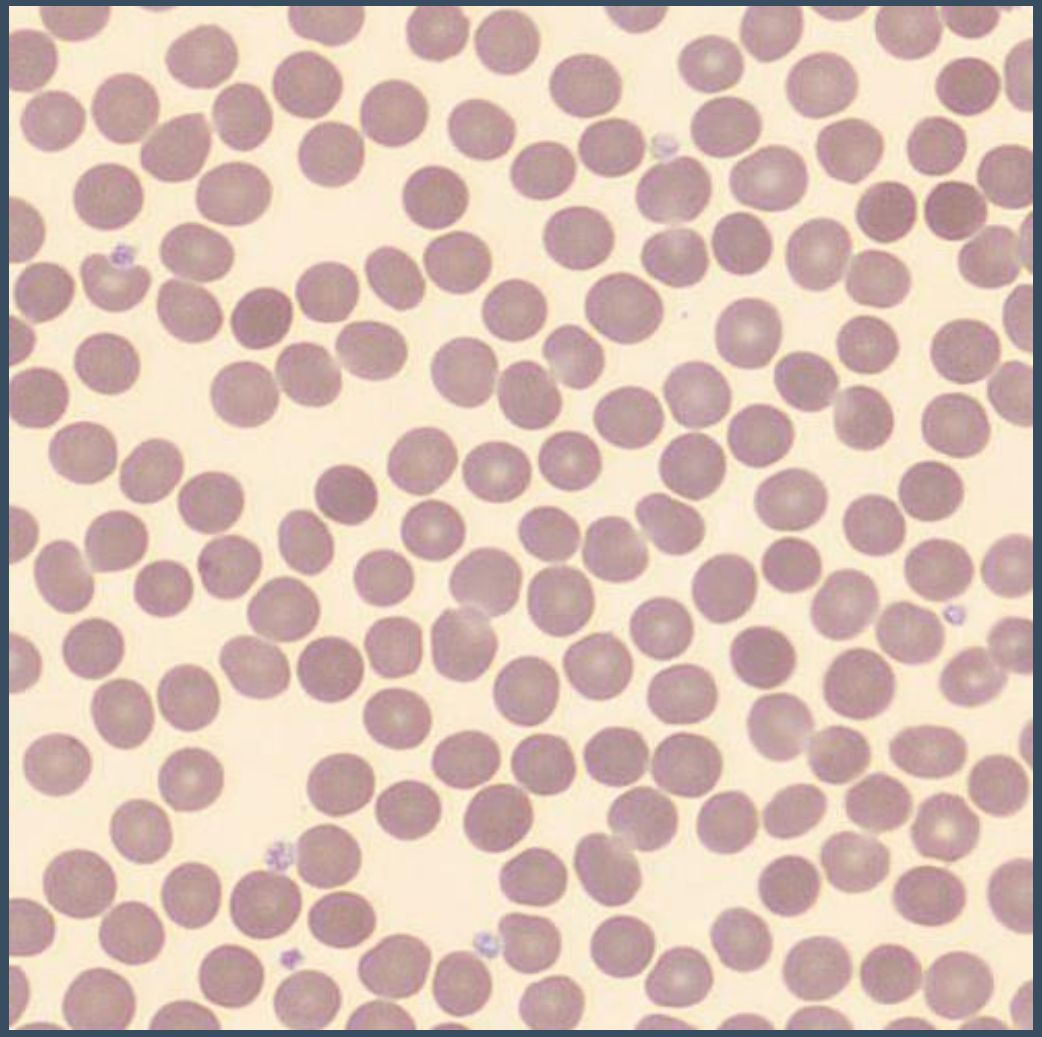


- The common cause of macrocytes is due to impaired DNA synthesis, RNA synthesis is unaffected resulting in the asynchrony between the cytoplasmic and nuclear maturation.
- Neutrophilic hyper segmentation is typically seen.
- RBC May be round or oval in shape, the diagnostics significance being different.
  - oval shape in B 12 and folate deficiency
  - round shape in liver disease and reticulocytosis
- Found in folate or B 12 deficiency, liver disease, reticulocytosis

Hemoglobin content

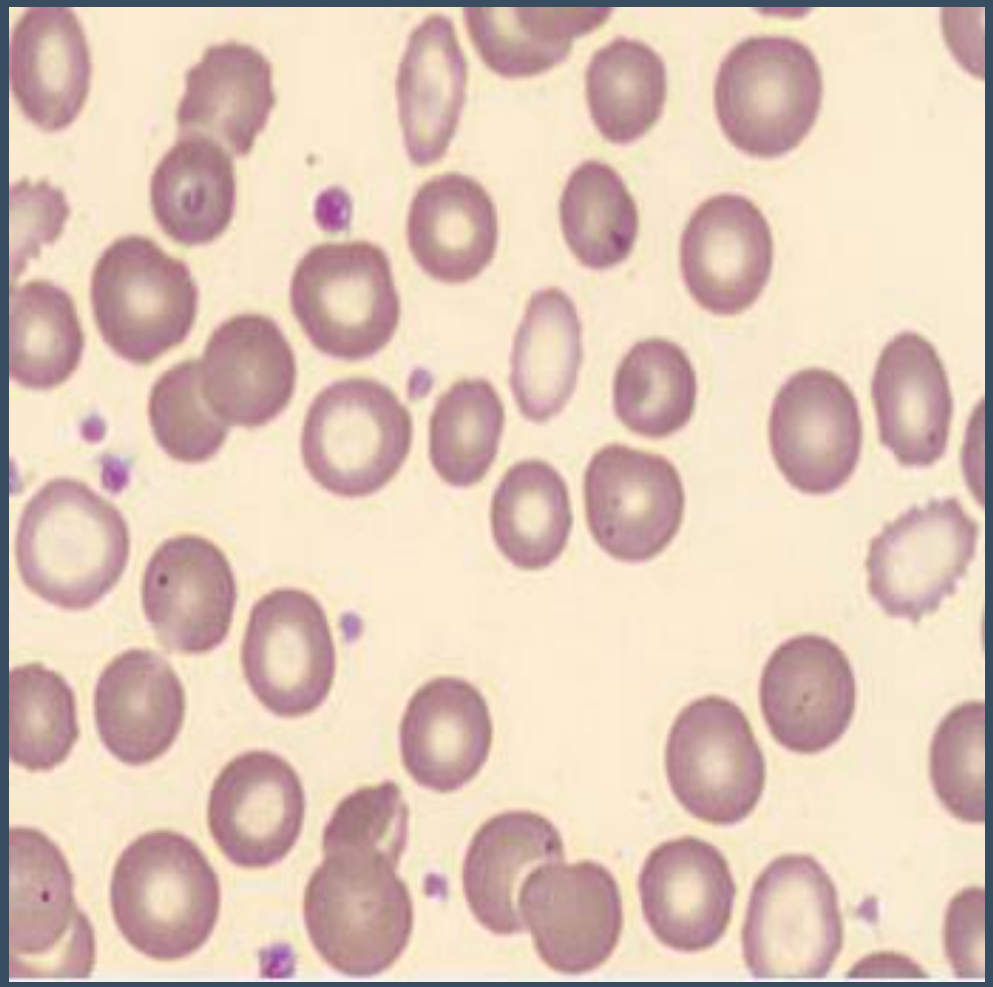
- Normochromic
- Hypochromic
- Polychromic / Polychromasia

Normochromic



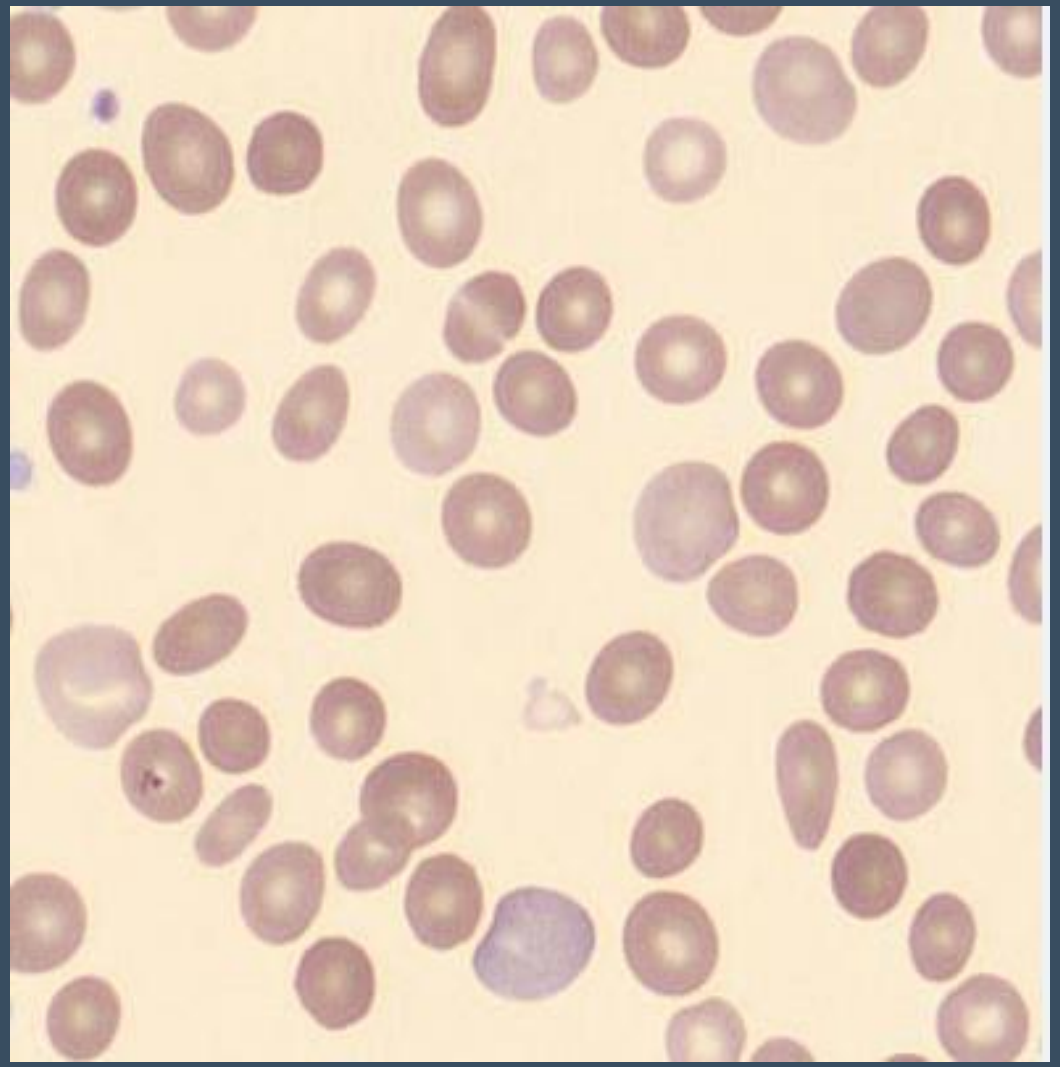
- The term normochromic indicates the red cells is essentially normal in color
- RBC has a Well hemoglobinized cytoplasm with a small distinct Zone of central pallor.
- Normochromic term is used to describe an anemia with a normal MCHC and MCH. when used in conjunction with normal MCV ,the anemia is described as a normochromic normocytic anemia.

Hypochromic



- Increased central pallor and decreased hemoglobin concentration.
- Direct relationship between the amount of hemoglobin deposited in the RBC and the appearance of red cells when stained.
- Found in
  - iron deficiency, thalassemia, any of the conditions leading to microcytosis

Polychromasia

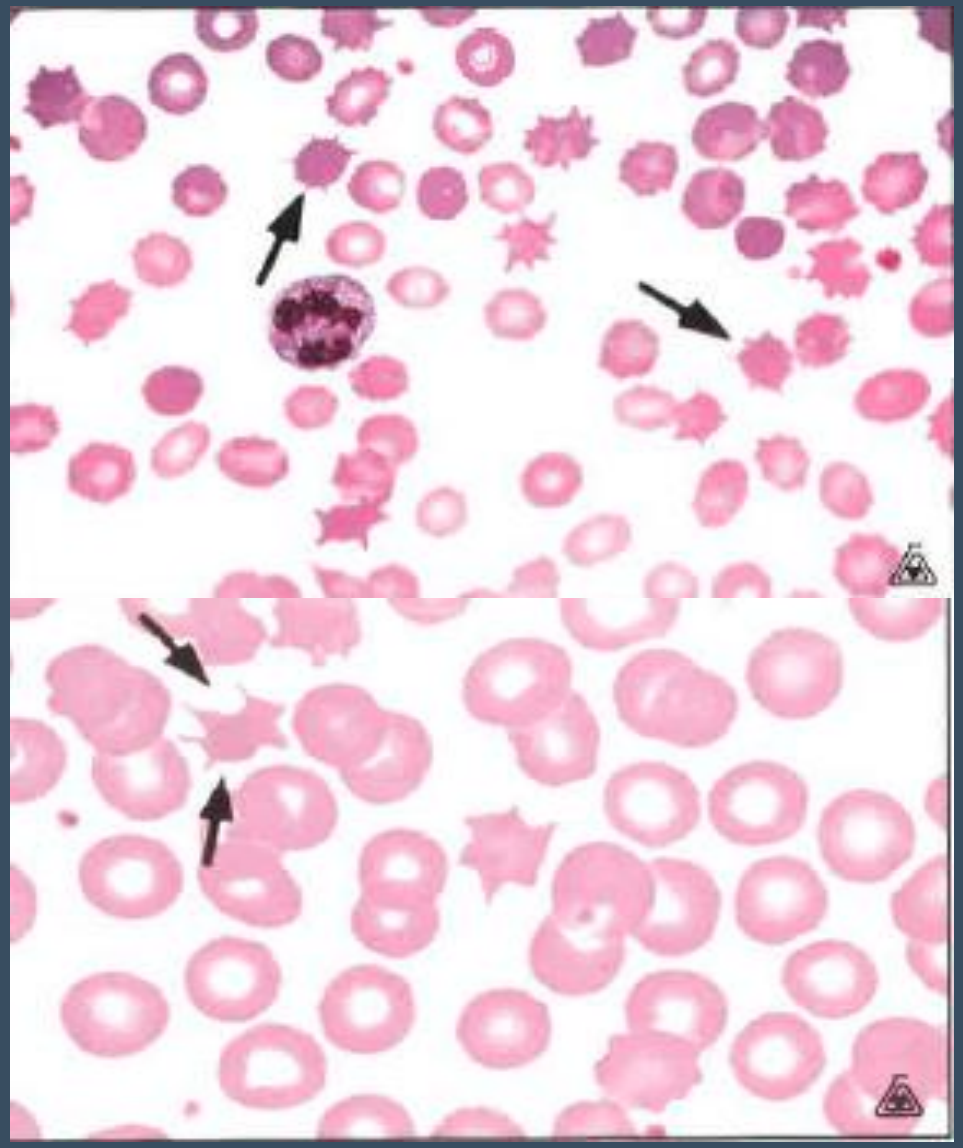


- Red cells stain shades of blue-gray as a consequence of uptake of both eosin and basic dyes by residual ribosomal RNA.
- Any clinical condition in which marrow is stimulated particularly RBC regeneration will produce a polychromatic blood picture.
- Found in
  - Erythropoiesis
  - Bleeding, hemolysis etc.

Shape abnormalities of RBCs

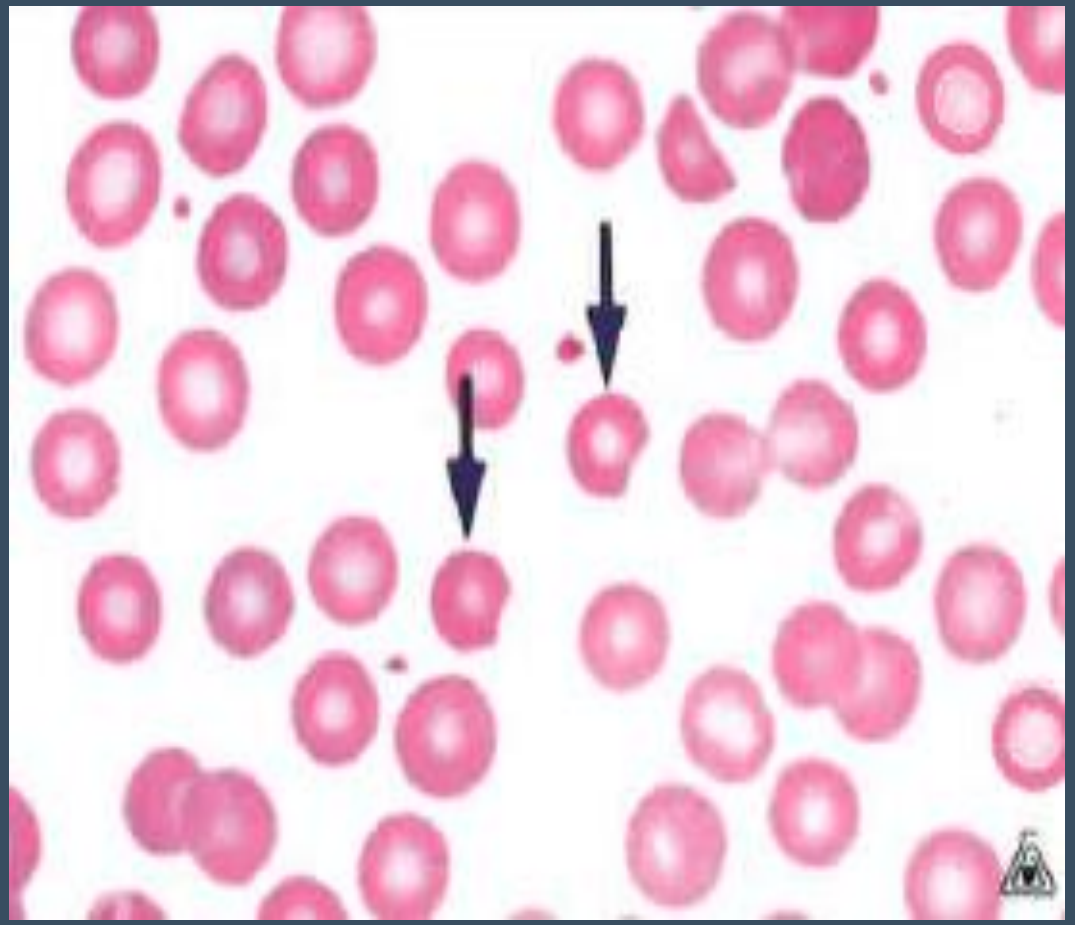
- Poikilocytosis is the general term for mature RBCs that have a shape other than the round biconcave disk
- Poikilocytes can be seen in many shapes (for example acanthocyte, spherocytes, teardrops...)

Acanthocyte



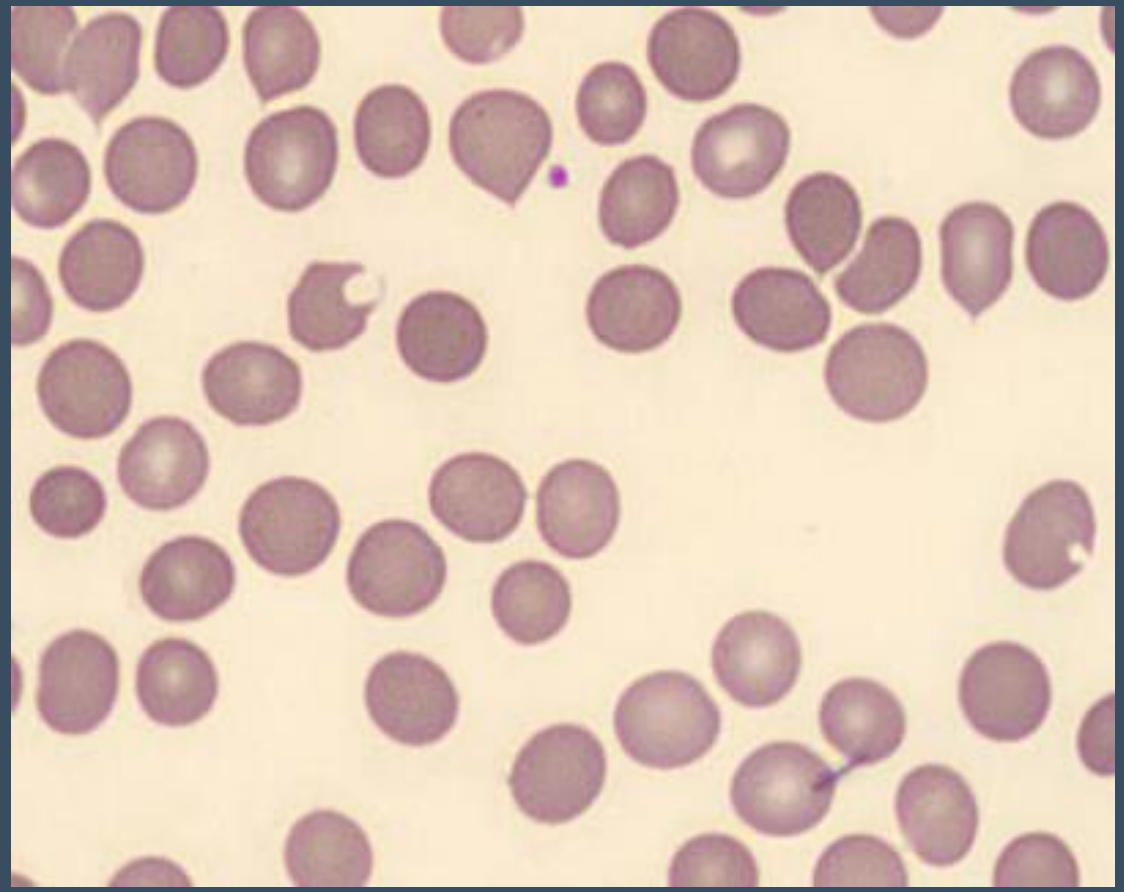
- RBCs with irregularly spaced projections or spikes
- Deficiency of LCAT
  - Important for membrane
  - Results in acanthocyte or target cells and splenic sequestration
- Found in
  - Liver disease
  - Post splenectomy
  - Abetalipoproteinemia
-

Bite cells



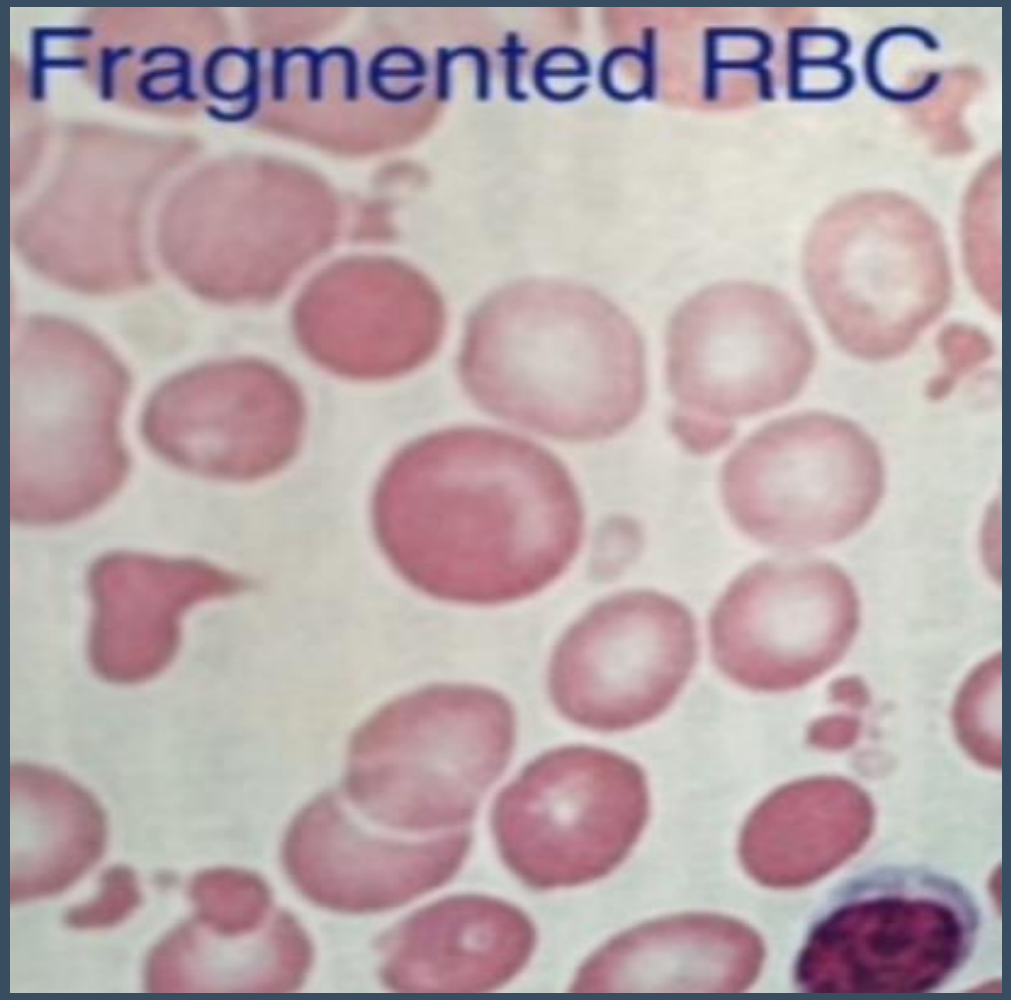
- An abnormally shaped red blood cell with one or more semi circular portions removed from the cell margin
- These bite cells results from the removal of denatured hemoglobin by macrophages in the spleen.
- Found in
  - G6PD deficiency , in which uncontrolled oxidative stress causes hemoglobin to denature and form Heinz bodies, is a common disorder that leads to the formation of bites cells.

Blister cells



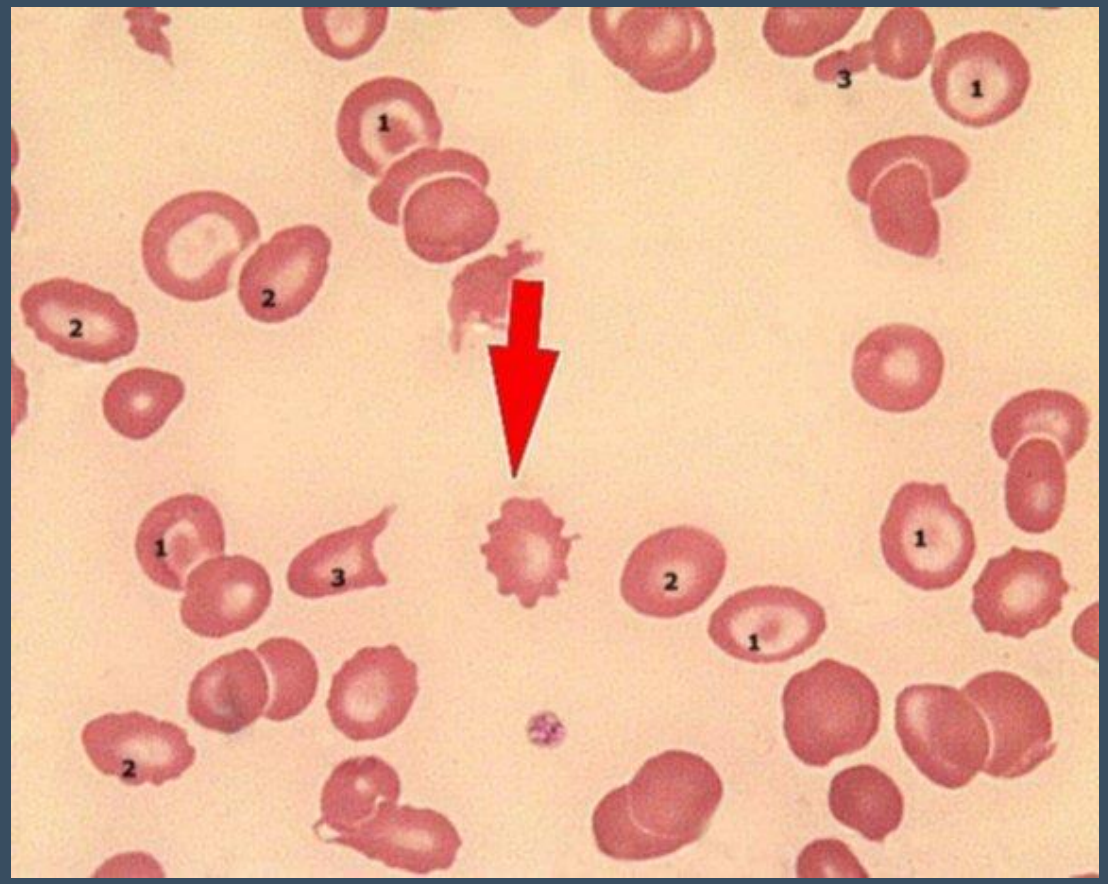
- Have a centric hello area resemble a purse or handbag
- Found in
  - Micro angiopathic hemolytic anemia

Schistocytes



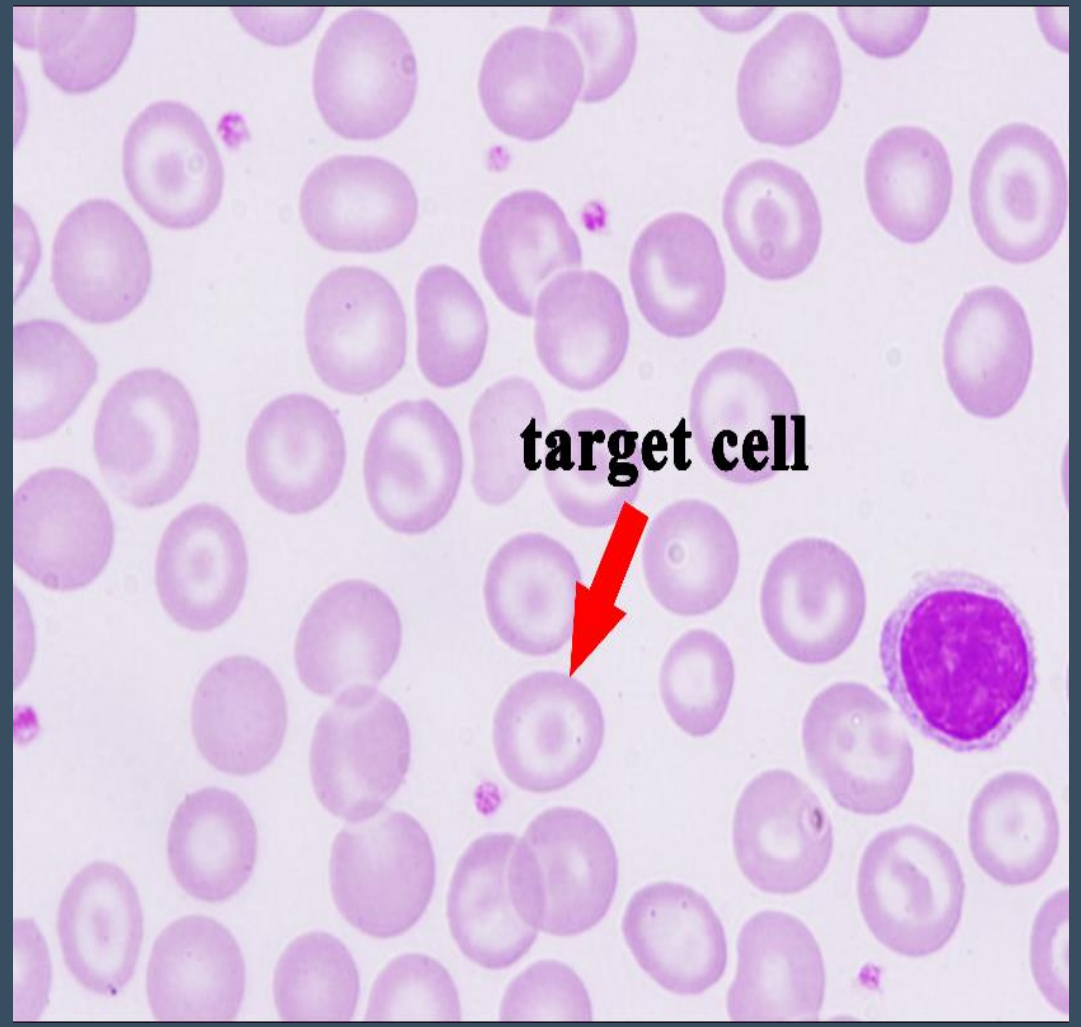
- Read cell fragments that are irregular in shape and size.
- Mechanical damage of formerly normal RBCs
- Found in
  - DIC, TTP
  - micro angiopathic hemolytic anemia
  - Burns

Echinocyte (Burr cells)



- Red cell with many short blunt projections which are regularly distributed on their surface
- Found in
  - Usually artifactual- the result of slow drying smear under humid condition
  - Sometimes are non-artifactual indicating uremia and chronic renal disease, pyruvate kinase deficiency, liver disease, megaloblastic anemia

Target cells (Codocyte)

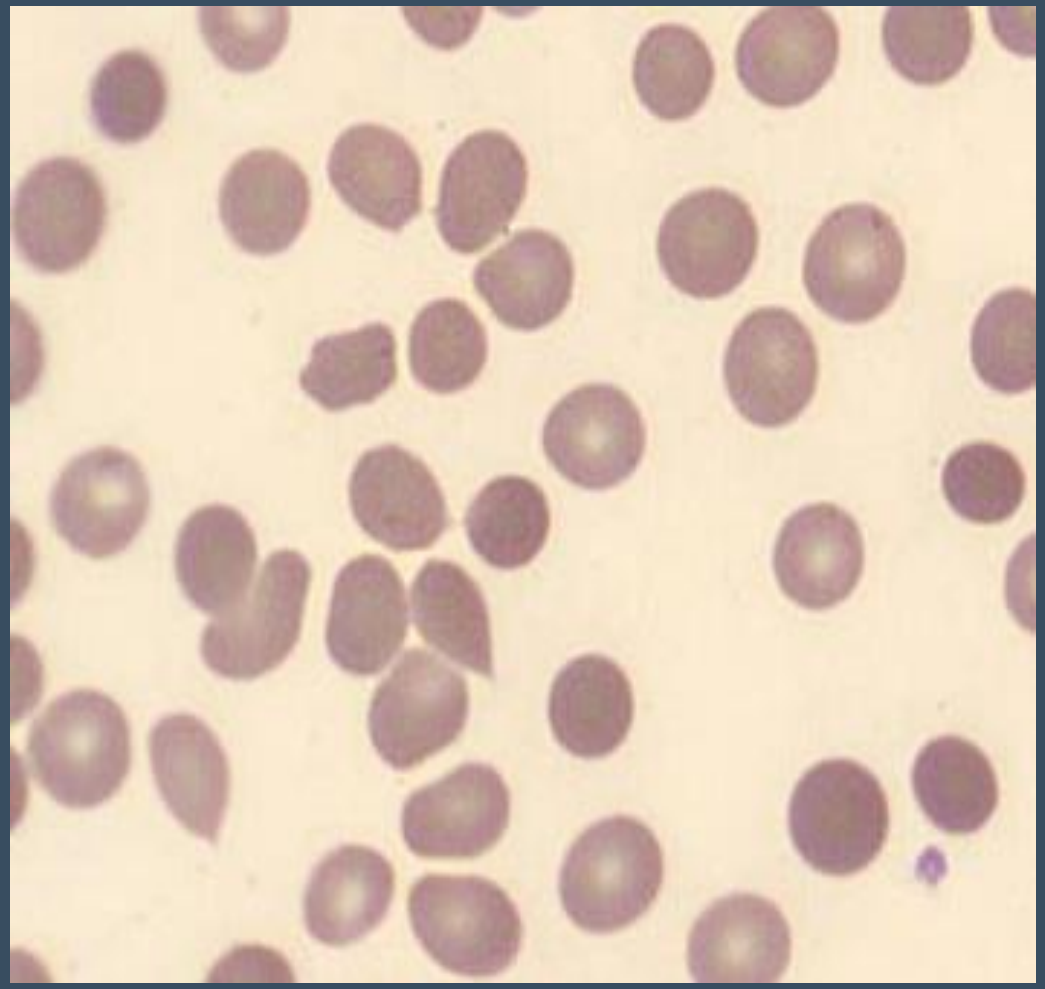


- Cellular membrane is thinner than normal
  - May be due to an excessive ratio of membrane lipid to cell volume.
  - Certain enzyme defects cholesterol is increased and becomes incorporated in the membrane.

Target cells

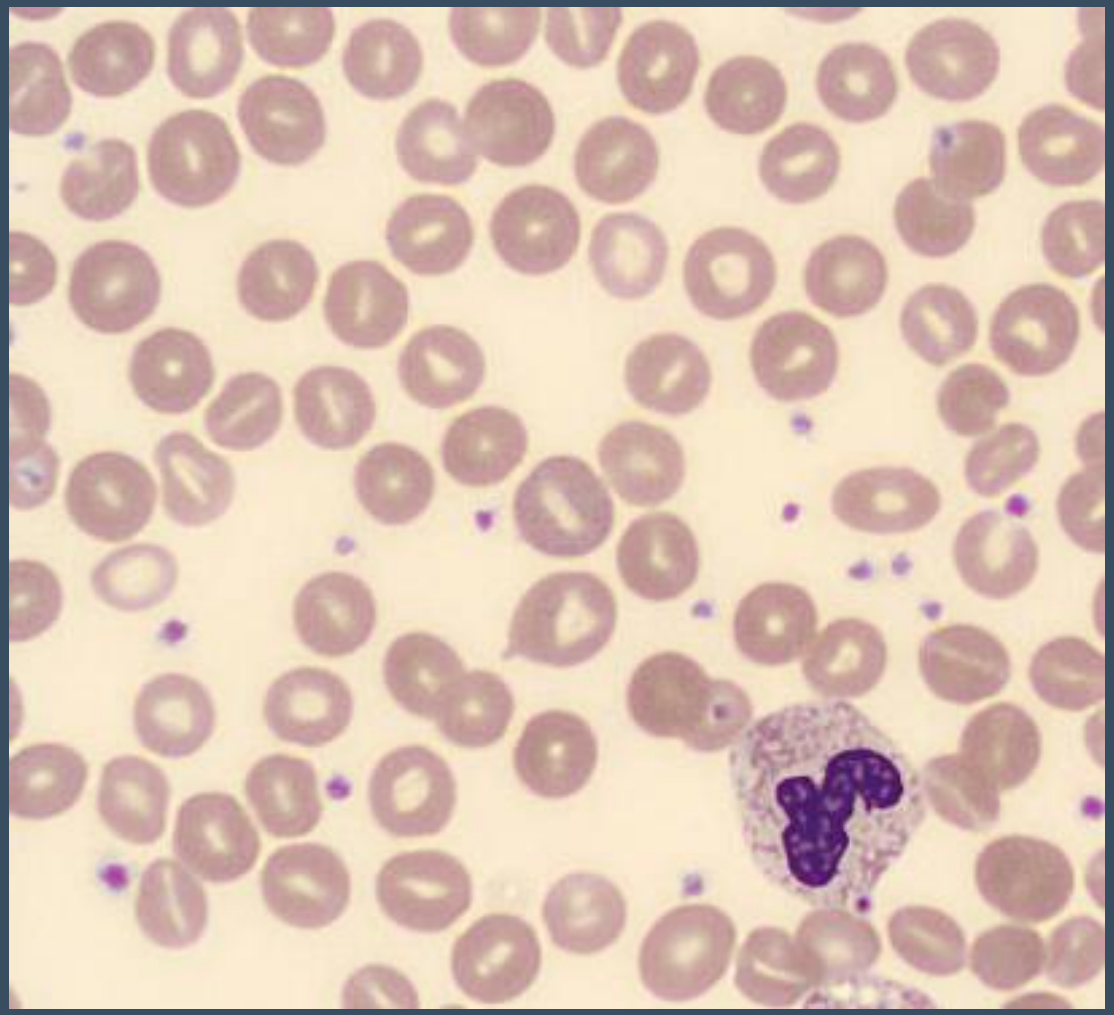
- maldistribution of an abnormal hemoglobin
- Found in
  - obstructive liver disease ,
  - severe Iron deficiency,
  - thalassemia,
  - post splenectomy,
  - lipid disorders ,
  - hemoglobinopathies

Spherocytes



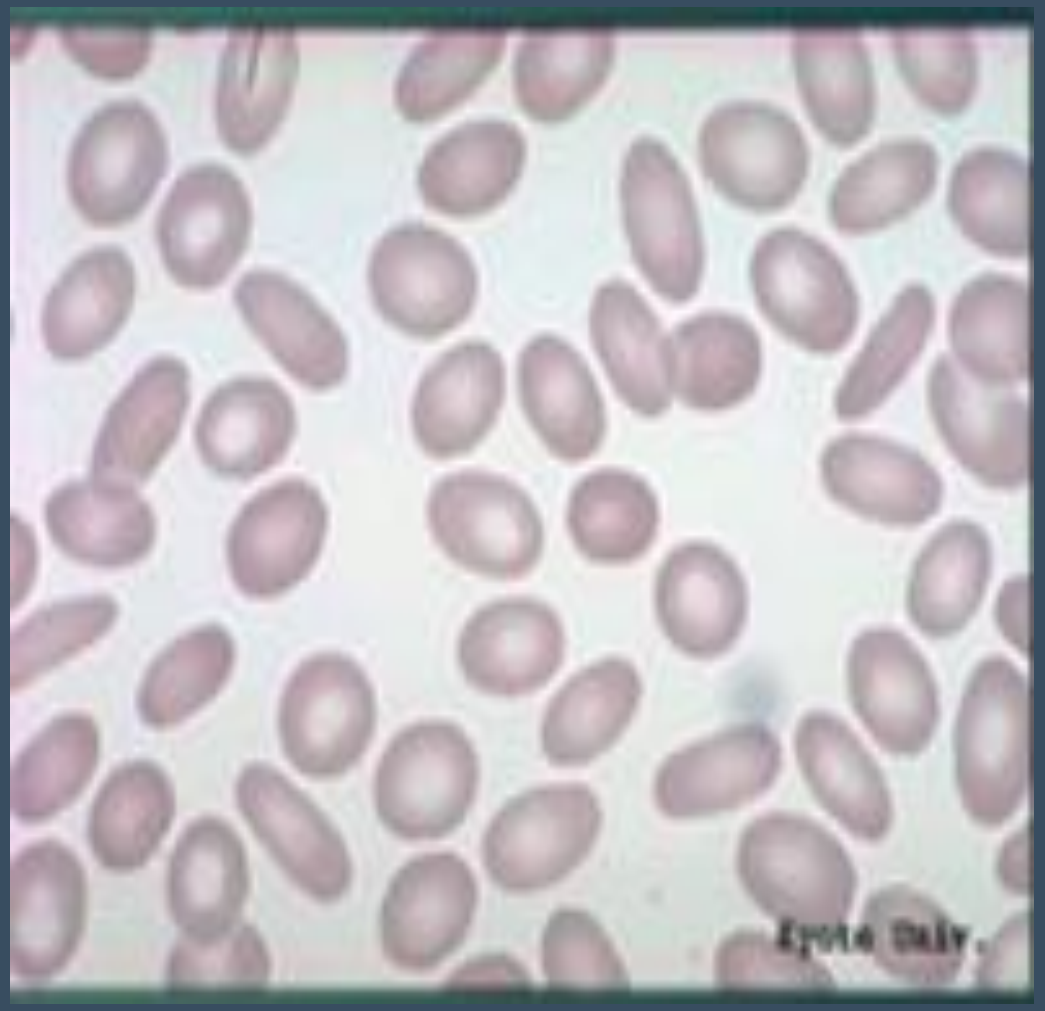
- Red cells are more spherical with absence of central pallor
- This abnormality is due to the abnormality of red cell membrane.
- The detailed mechanism for sphering is the congenital condition known as hereditary spherocytosis.
  - deficiency of the membrane proteins, spectrin and ankyrin.
- Acquired causes are
  - ABO incompatibility
  - immune hemolytic anemia
  - Microangiopathic hemolytic

Stomatocytes



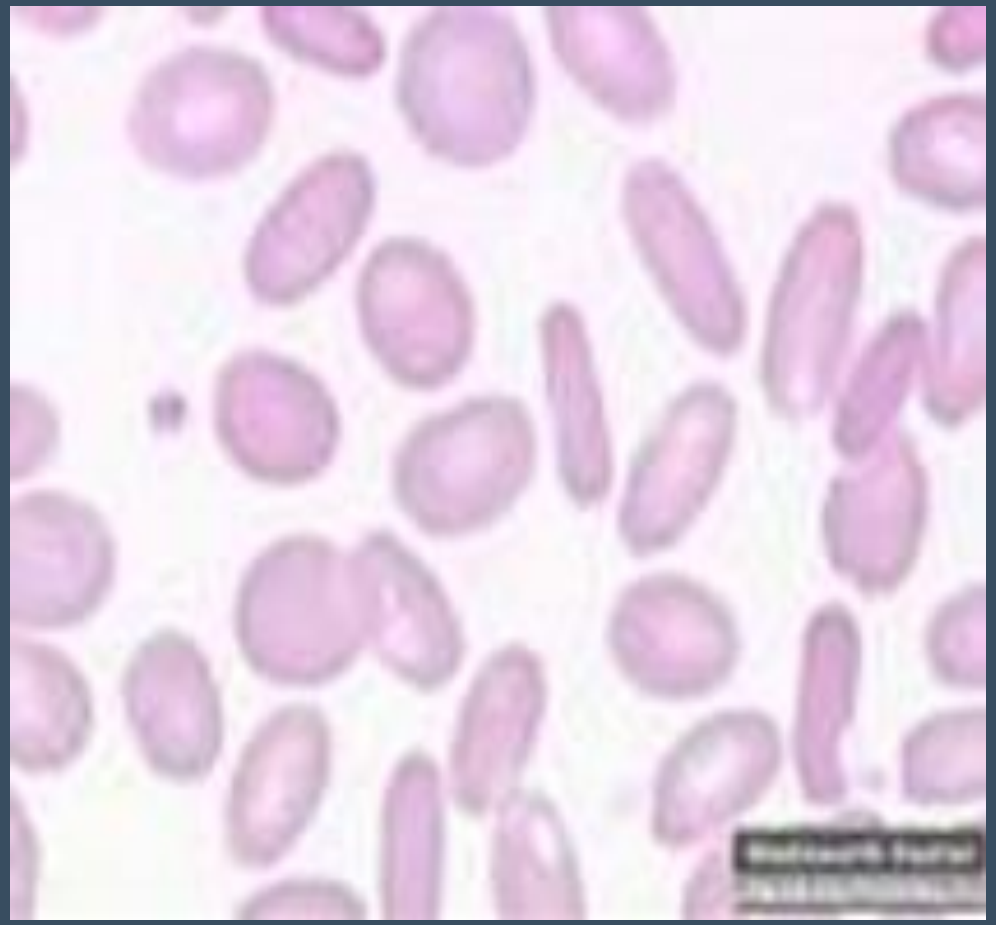
- Red cells with the central area of pallor, which is slit-like instead of round
- Caused by several different membrane defects , deficiency of membrane protein leads to increased permeability of Na and K+ cations
- Found in
  - Alcoholic liver disease, hereditary stomatocytosis

Ovalocytes



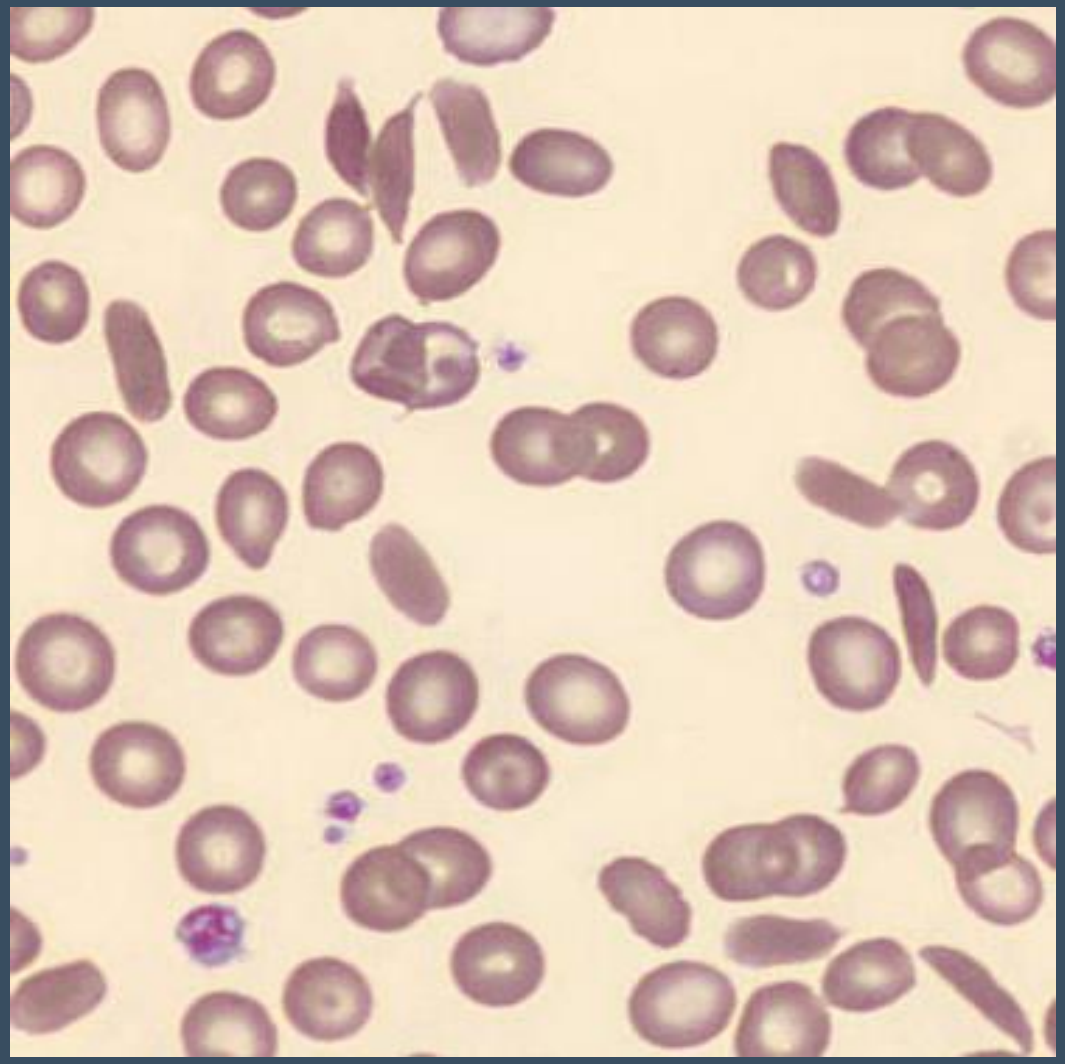
- An elongated cells
- Represent a membrane defect where RBCs suffers a loss of integrity
- Found in
  - thalassemia major
  - hereditary ovalocytosis
  - megaloblastic anemia
  - iron deficiency
-

Elliptocytes



- Elliptocytes and ovalocytes often used interchangeably
- Elliptocytes are longer and narrower
- Found in
  - Hereditary elliptocytosis

Sickle cells(Drepanocyte)

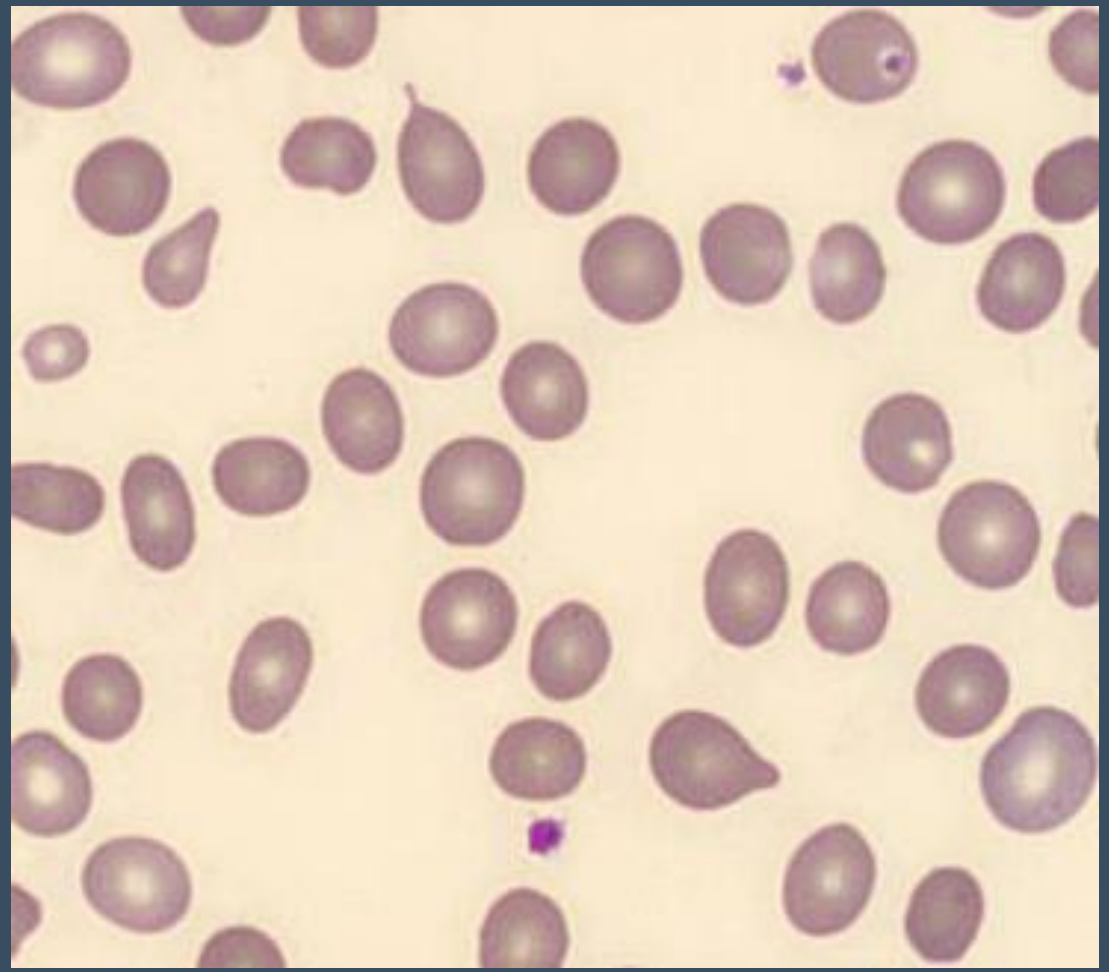


- Sickle shaped RBCs
- Single base pair mutation results in a single amino acid change
- Under low oxygen hemoglobin becomes insoluble forming long polymers this lead to membrane changes (sickling) and vasoocclusion
- Found in
  - hemoglobin S disease and trait

HGB C crystals

- The classic ‘Washington monument” appearance of a crystal distorts the cell shape and leave most of the rest of the cell clear or colorless.
- Rhomboid, hexagon or rod
- Found in
  - Hemoglobin C disease

Teardrop cells(Dacryocytes)

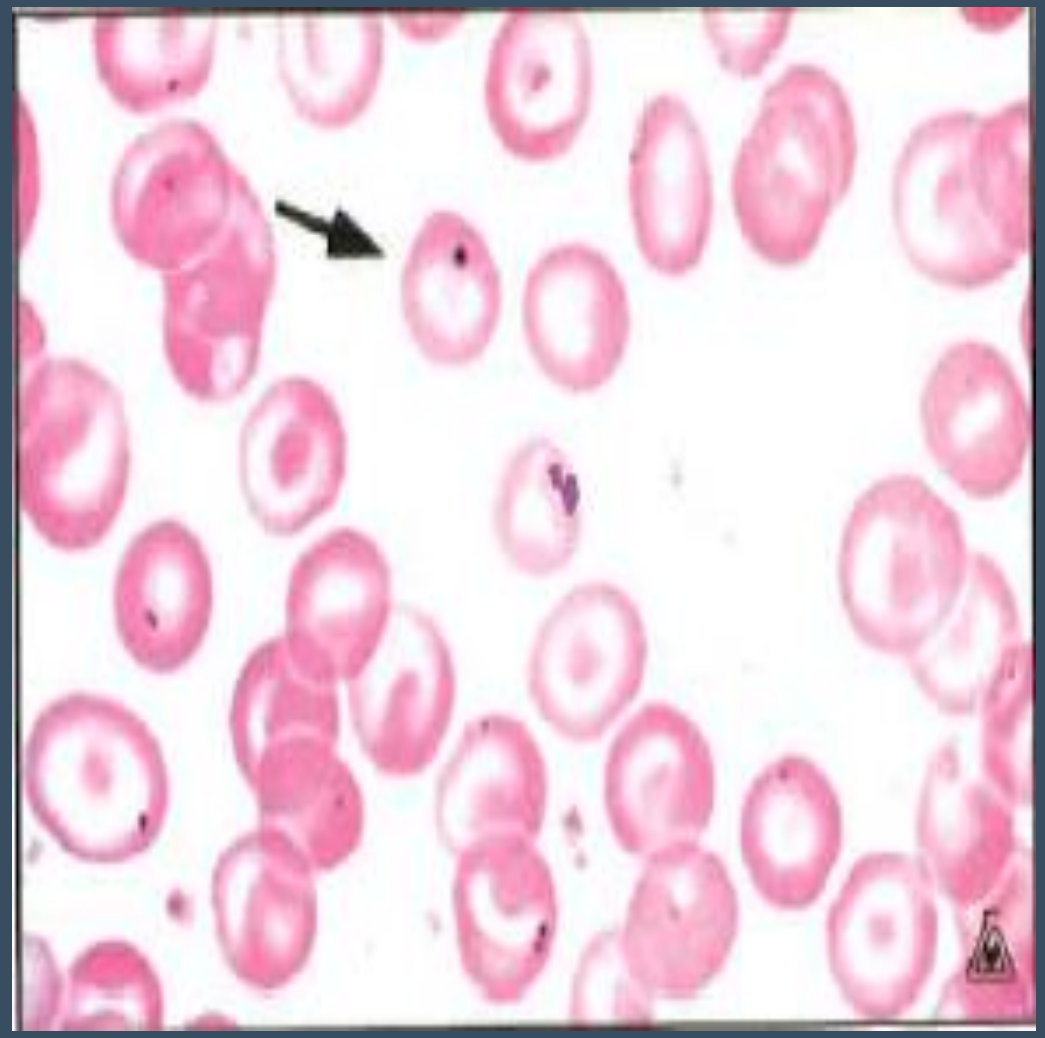


- As the term applies, these resemble tears
- Found in
  - bone marrow fibrosis
  - megaloblastic anemia
  - iron deficiency
  - thalassemia

Red Cell Inclusions

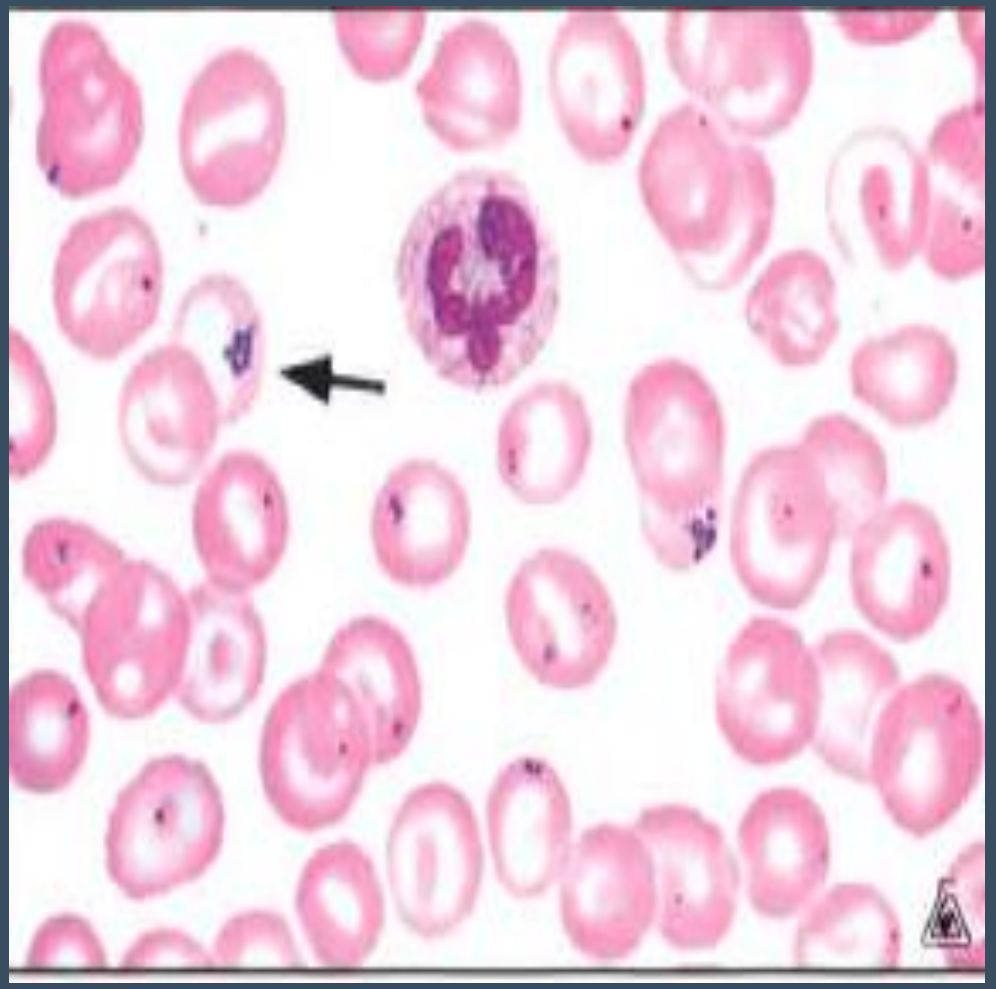
- RBC normally free from inclusions
- Result of distinctive conditions
- Presence of inclusion bodies in red blood cells is indicative of some sort of pathology

Howell-Jolly Bodies



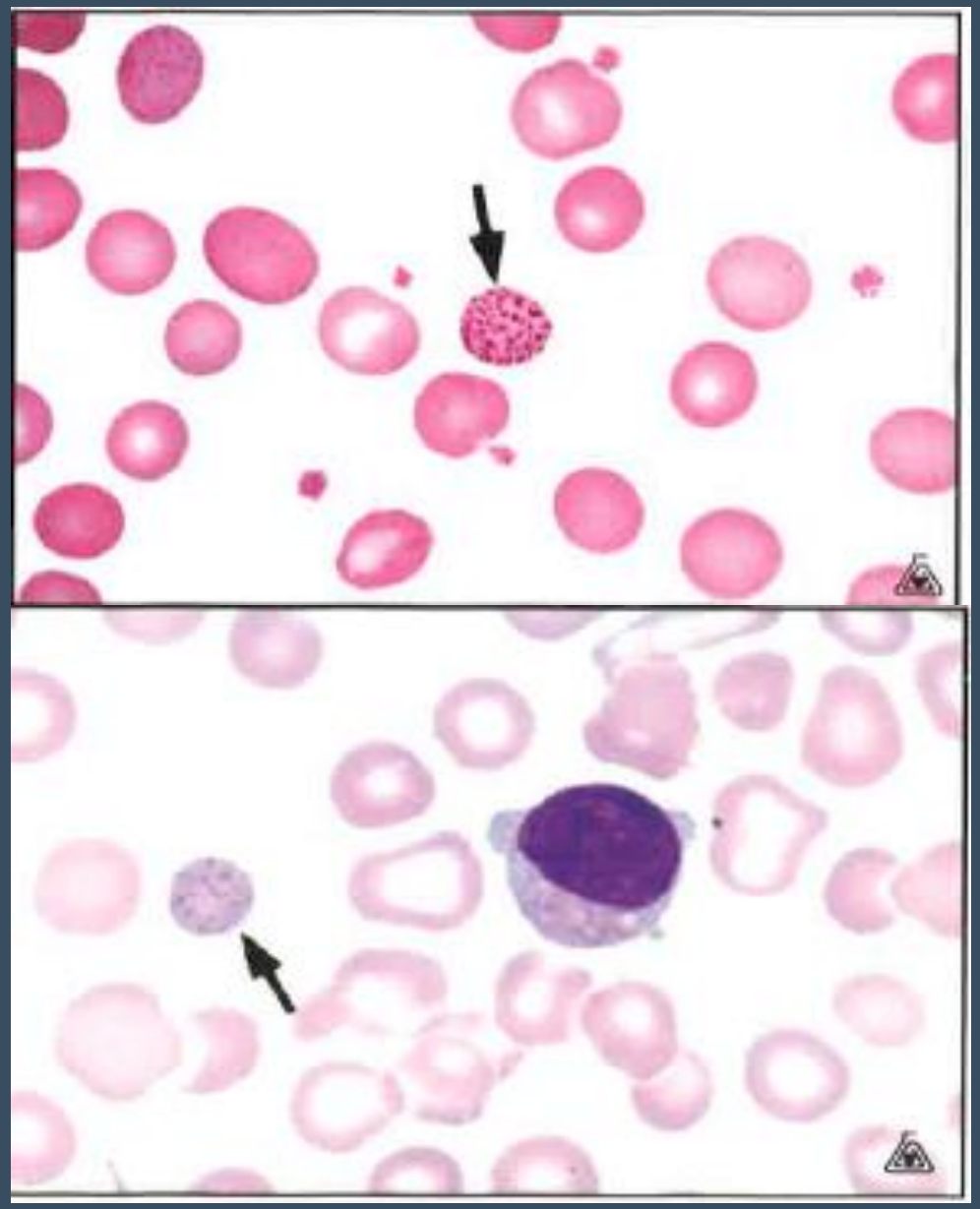
- Round, dark blue to purple inclusion, usually single and in the periphery of a red cell
- The spleen is responsible for the removal of nuclear material in the red cells, so in absence of a functional spleen, nuclear material is removed ineffectively.
- Predominantly composed of DNA
- Found in
  - Post splenectomy, megaloblastic anemia and hemolytic anemia

Pappenheimer Bodies



- Siderotic granules
- Small irregular, dark staining particles of iron visible with iron stain(Prussian blue)
- Aggregates of mitochondria, ribosomes, and iron particles
- Associated with iron loading anemias and hemolytic anemias.

Basophilic Stippling

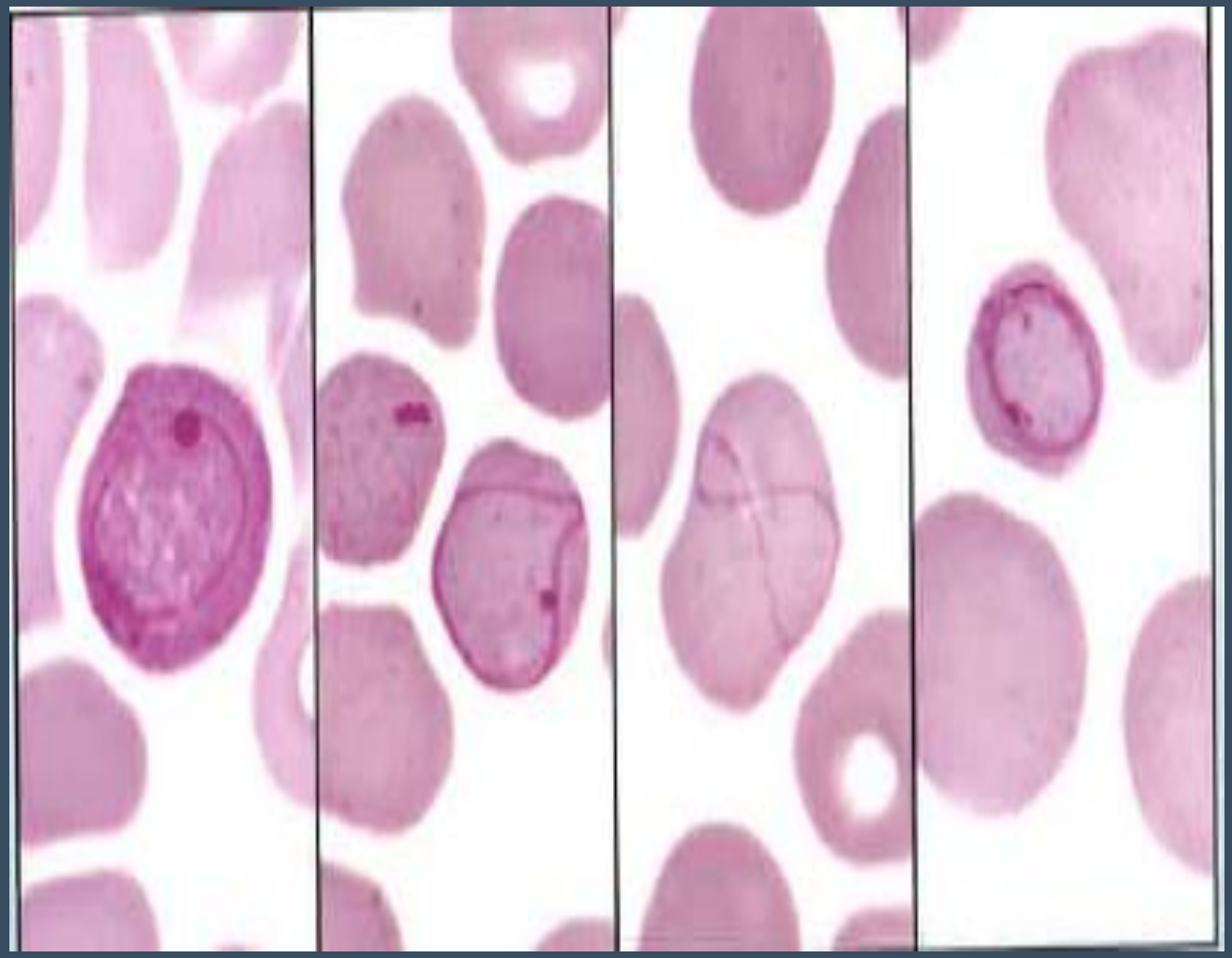


- Tiny, round, solid-staining dark blue granules
- Composed of ribosomes and RNA
- Usually evenly distributed
- Course basophilic stippling (Punctate stippling) are larger and considered more serious
- Associated with lead poisoning, disturbed erythropoiesis

Heinz Bodies

- Seen with stain such as crystal violet
- Represent precipitates of denatured hemoglobin and are clinically associated with the hemolytic anemia, G6PD deficiency,oxident drugs etc.

Cabot rings

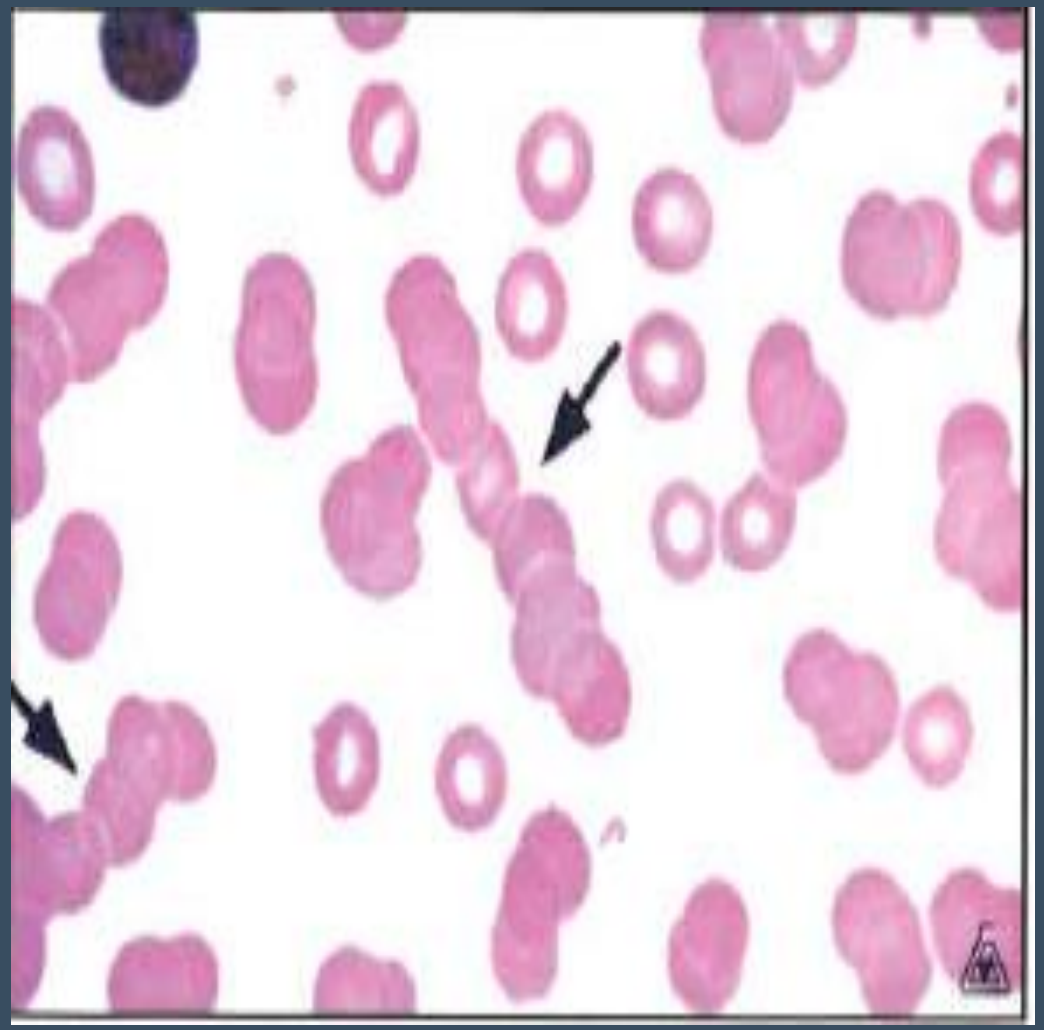


- These are ring shaped figure of eight or loop shaped Red or reddish purple with right stain and have no internal structure
- Observed rarely in
  - pernicious anemia
  - lead poisoning

Abnormal Distribution of RBCs

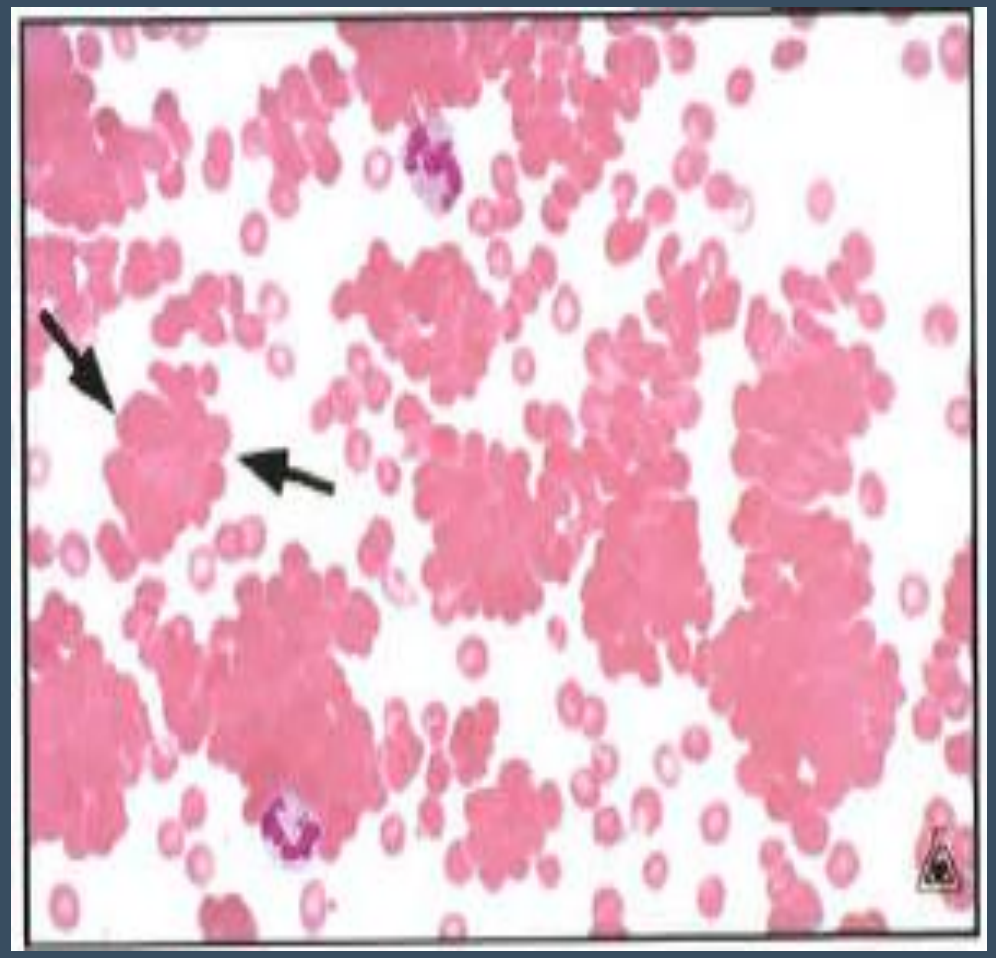
- Rouleaux formation
- Agglutination

Rouleaux formation



- Staking of RBCs due to increased plasma proteins coating RBCs(resembling a stack of coins)
- Found in
  - Hyperfibrinogenaemia
  - Multiple myeloma
  - Waldenstrom’s macroglobulinemia
-

Agglutination



- Red cells cluster and clumps together in irregular mass in the thin area of smear
- Cold agglutinin- IgM antibody
- Factitiously lowered RBC in automated analyzer

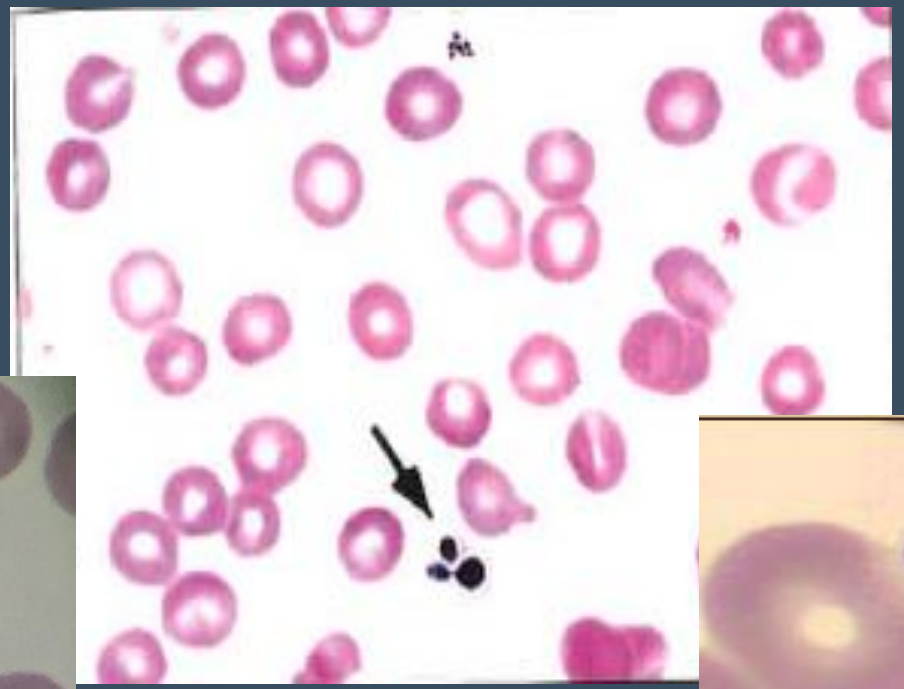
Protozoan Inclusions

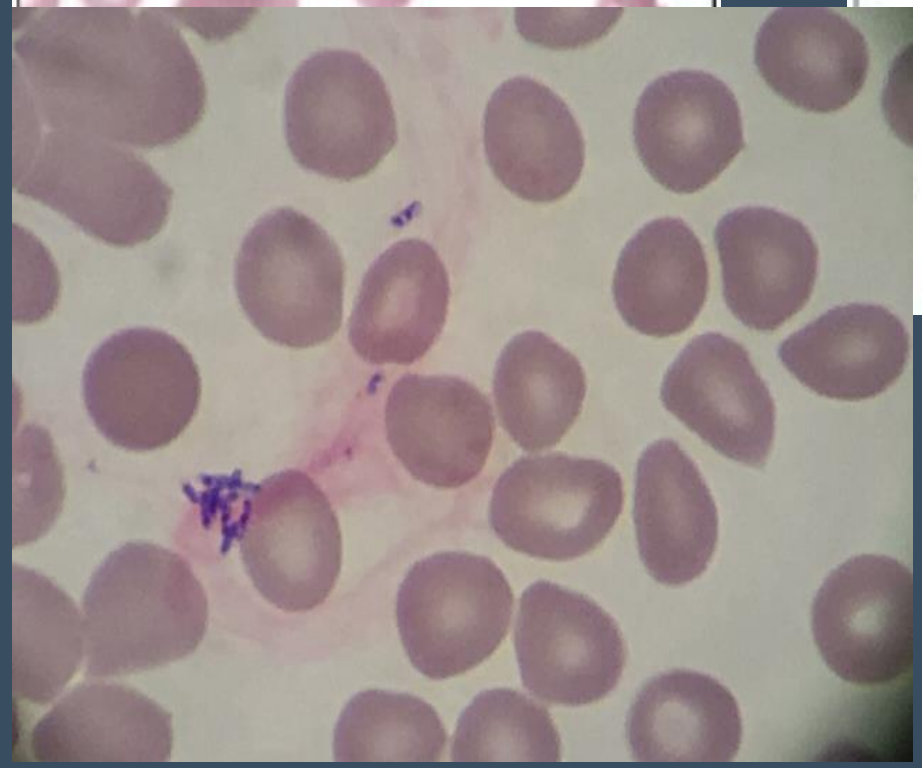
- All four species of malaria parasite will invade RBCs. (plasmodium vivax, plasmodium malaria, plasmodium falciparum, and Plasmodium ovale)
- They are transmitted by mosquito.
- It is important to recognize this abnormality as a parasite and not confused with normal morphology such as platelet laying over red cells .

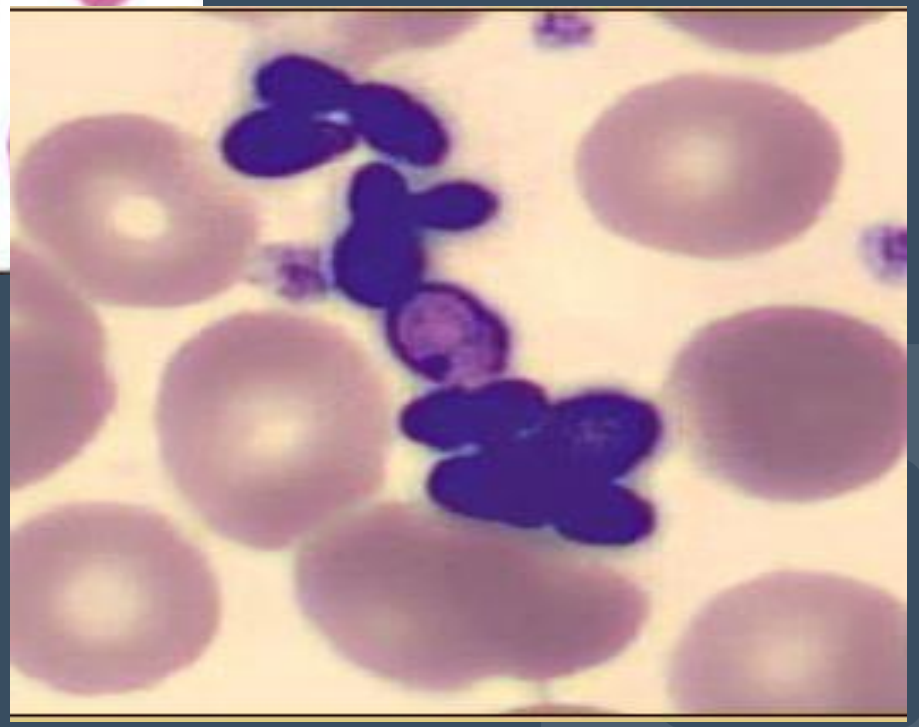
Protozoan Inclusions

- Babesia microti is also an organism that Invade red cells
- it is transmitted by tick bites and may appear as a ring forms resembling some forms of malaria
- They may appear in groups outside the erythrocyte on the blood smears

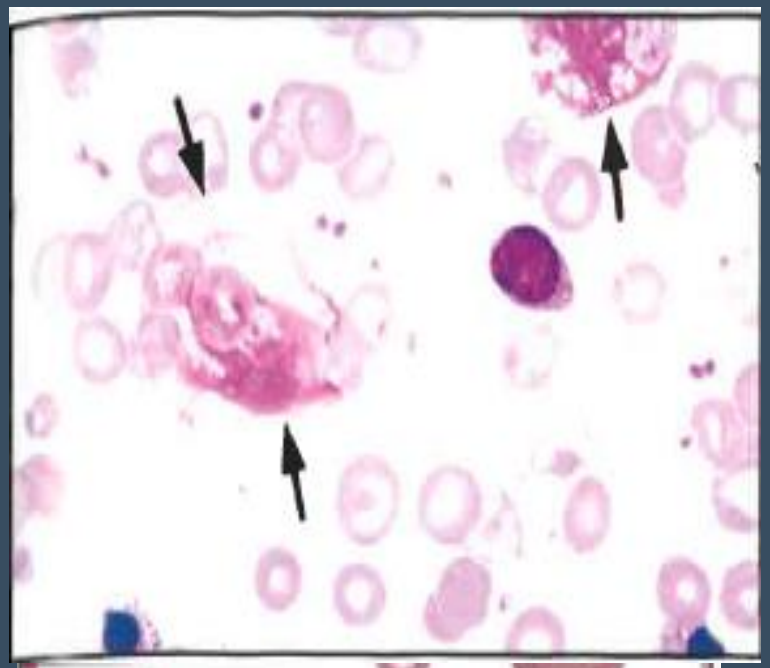
Microorganisms

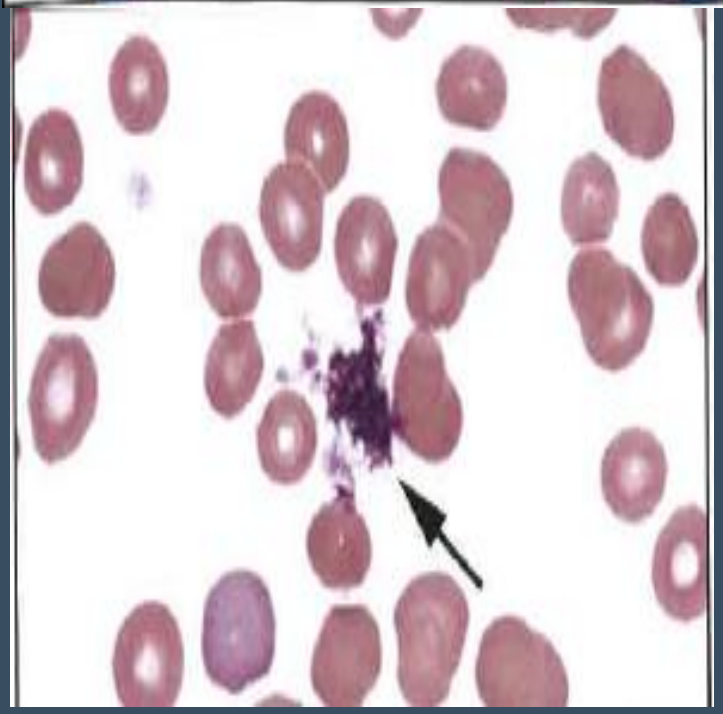


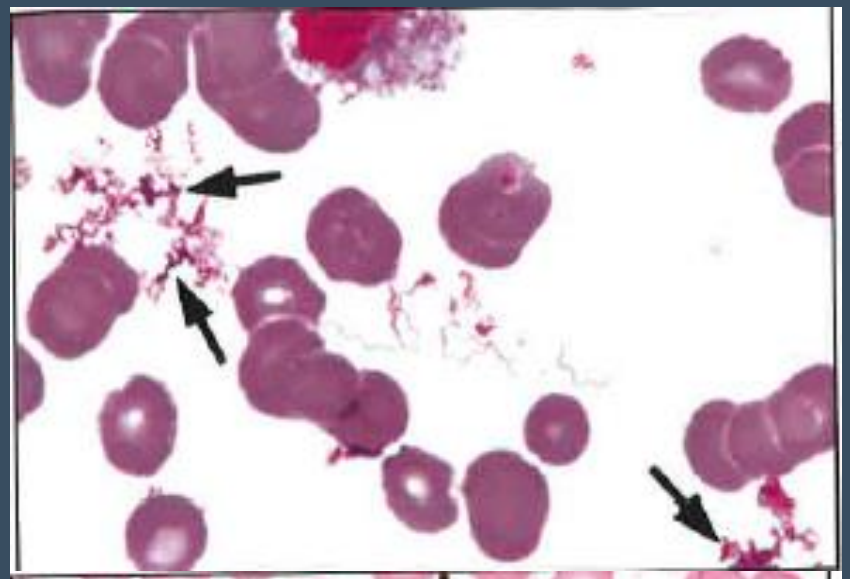


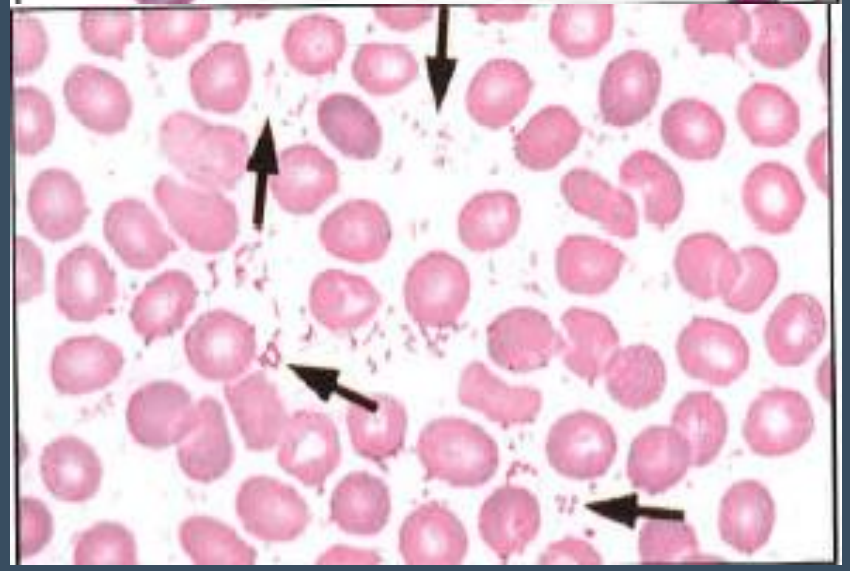


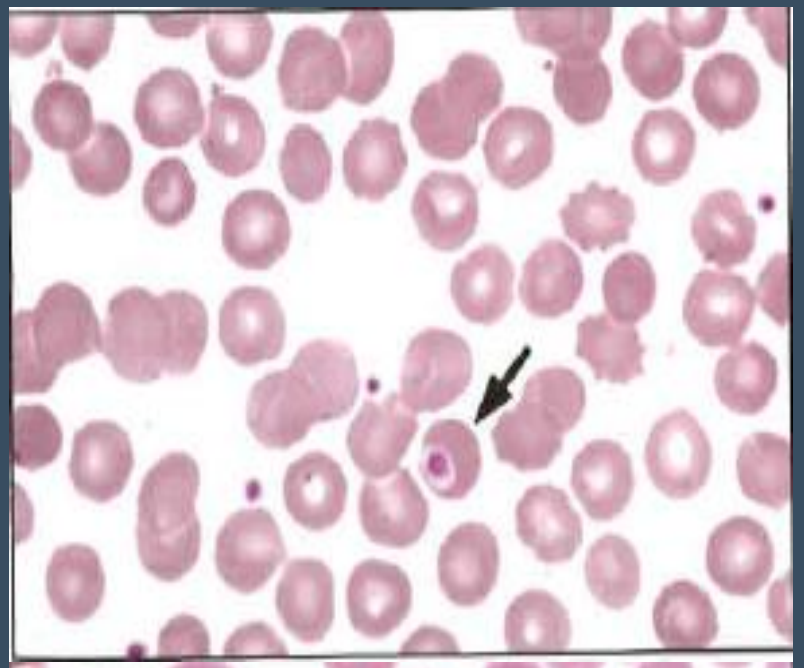
Artifacts

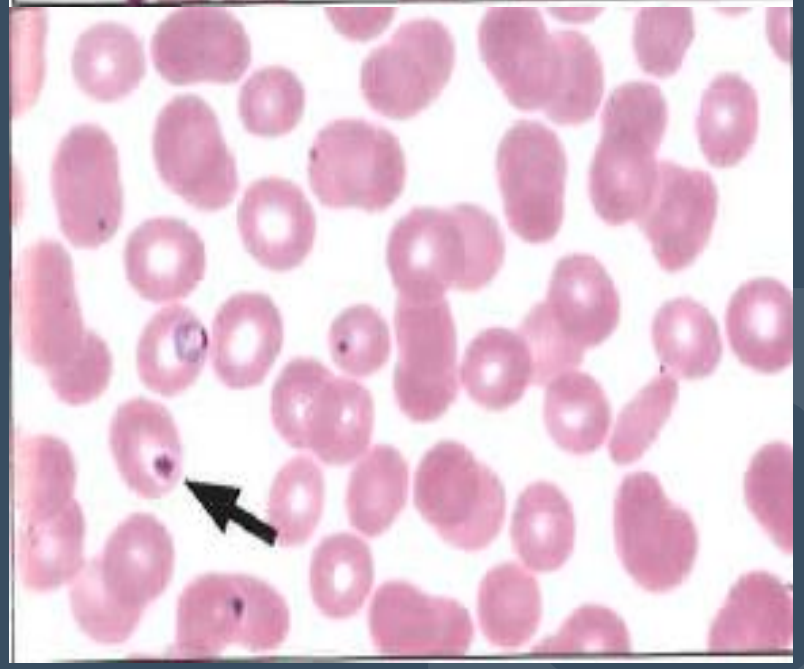












Grading of RBC Morphology

High Frequency RBC's	1+ (Few)	2+ (Moderate)	3+ and 4+ (Many)
	# of Cells/Field	# of Cells/Field	# of Cells/Field
Tear Drops	1 - 6	7 - 12	> 12
RBC Fragments	1 - 6	7 - 12	> 12
Spherocytes	1 - 6	7 - 12	> 12
Targets	1 - 20	21 - 60	> 60
Sickle Cells	1 - 20	21 - 60	> 60
Acanthocytes	1 - 20	21 - 60	> 60
Stomatocytes	1 - 20	21 - 60	> 60
Ovalocytes	1 - 20	21 - 60	> 60

	Slight	Moderate	Marked
	# of Cells/Field	# of Cells/Field	# of Cells/Field
Polychromasia	1 - 10	11 - 20	> 20
Low Frequency RBC's	Occasional	Present	
	# of Cells/Field	# of Cells/Field	
Baso Stippling	< 2	≥ 2	
Pappenheimer	< 2	≥ 2	
Howell Jolly Bodies	< 2	≥ 2	
Bite Cells	< 2	≥ 2	

Anisocytosis, RBC cold agglutinin, dimorphic population or rouleaux are documented as present if noted in blood smear.

Thank you!