Heme-Lymph Lab: Anemia

Objectives

Laboratory instructors:

1. Facilitate lab discussion and answer questions

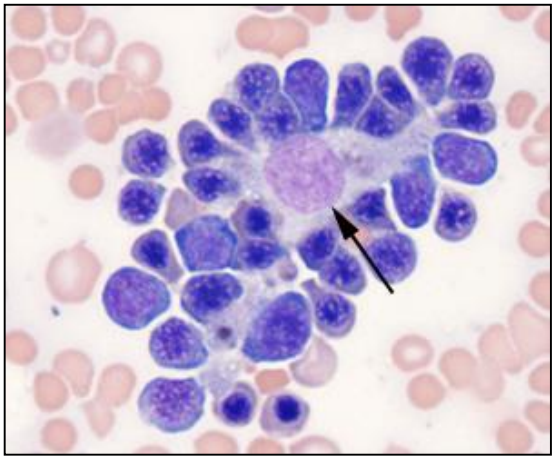
Students:

1. Review the introductory material below
2. Study and review the assigned cases and questions in small groups before the Lab. This includes the pathological material using Virtual Microscopy
3. Be prepared to present your cases, questions and answers to the rest of your Lab class during the Lab

Erythropoiesis: The process of red blood cell (RBC) production

- Characterized by:
  - Increasing hemoglobin synthesis
  - Decreasing cell size
  - Decreasing cytoplasmic basophilia (increasing pink color)
  - Progressive chromatin condensation of the nuclei
  - Extrusion of nucleus (orthochromatic stage)
  - Extruded nuclei are subsequently phagocytized
  - Loss of mitotic capability after the early stage of polychromatophilic normoblast

- Picture below: Erythroid progenitors (normoblasts) cluster around macrophages (arrows) in the bone marrow and spleen
- Macrophages store iron
- Iron is transferred from macrophages to erythroid precursor cells
- Iron is used by normoblasts for hemoglobin synthesis

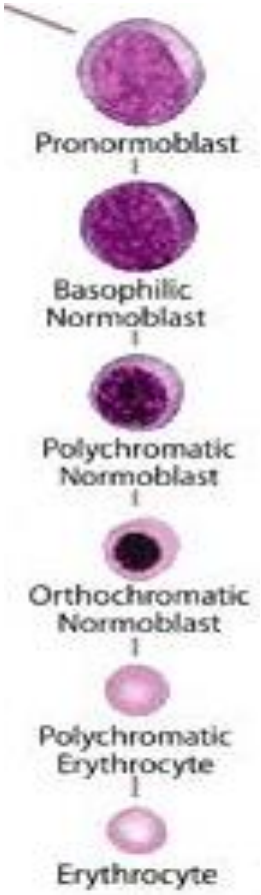


Erythroid maturation stages (Below):

- Average of 4 cell divisions during maturation

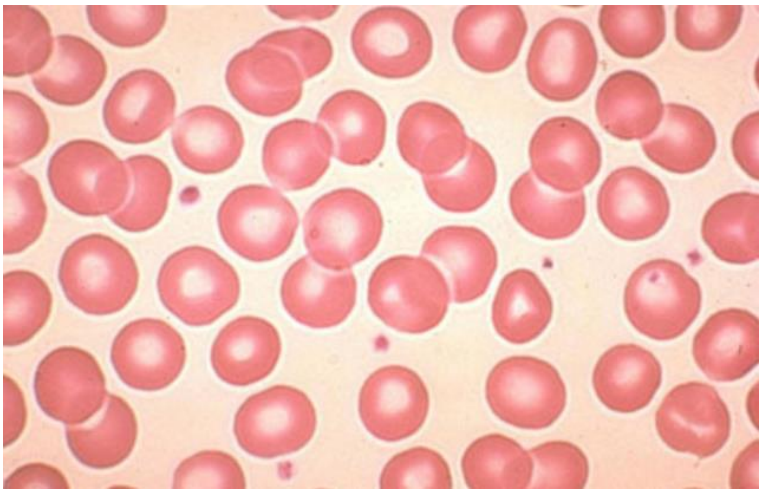
[One pronormoblast gives rise to 16 red cells]

- pronormoblast → reticulocyte = 7 days
- reticulocytes → mature RBC =1-2 days



aka nucleated rbc

aka reticulocyte



Mature Red Blood Cell

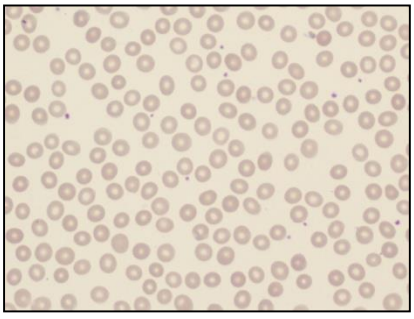
7-8 microns; round / ovoid biconcave disc with orange-red cytoplasm, no RNA, no nucleus; survives ~120 days in circulation

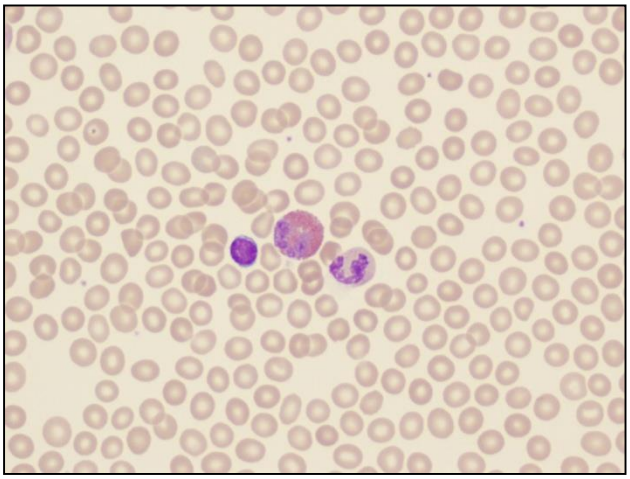
Classification of Anemia by Morphology

1. Microcytic, hypochromic anemia
   MCV < 80
   MCH < 27
   MCHC < 32
2. Normocytic, normochromic anemia
   MCV and MCH within normal range
3. Macrocytic
   MCV >100

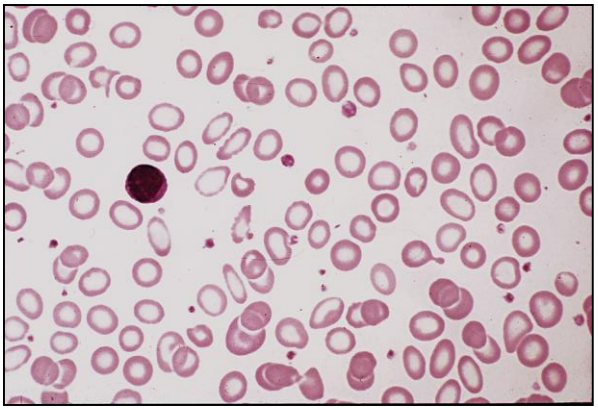
Normal Red Blood Cell Morphology

Size of normal RBC is comparable to the nucleus of a small lymphocyte
Normocytic = Normal size





<u>Normochromic (vs Hypochromic) RBCs</u>
Defined by area of central pallor:
Up to 1/3 the size of RBC= Normochromic
<1/3 of RBC size = Hypochromic



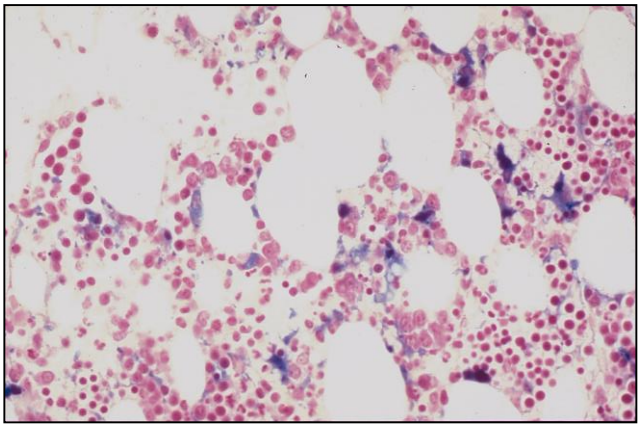
Hypochromic Microcytic Anemia

The peripheral smear shows:

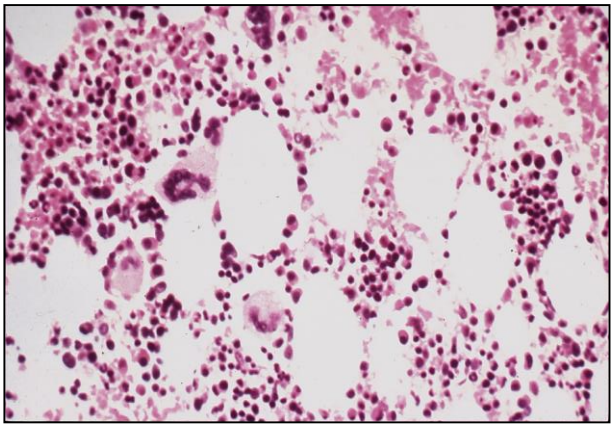
- Aniso- (variation in size) and poikilocytosis (variation in shape) of RBCs
- The red blood cells are microcytic since many are smaller than the nucleus of the lymphocyte
- The erythrocytes are hypochromic with a increased central pallor (>1/3 of cell size)
- Elliptocytic and pencil-shaped forms are present.

Below: Iron Stained Bone Marrow
Prussian Blue Stain

Normal bone marrow biopsy:
Iron (Blue stain) is present in the reticuloendothelial cells



Iron deficiency:
There is no iron staining



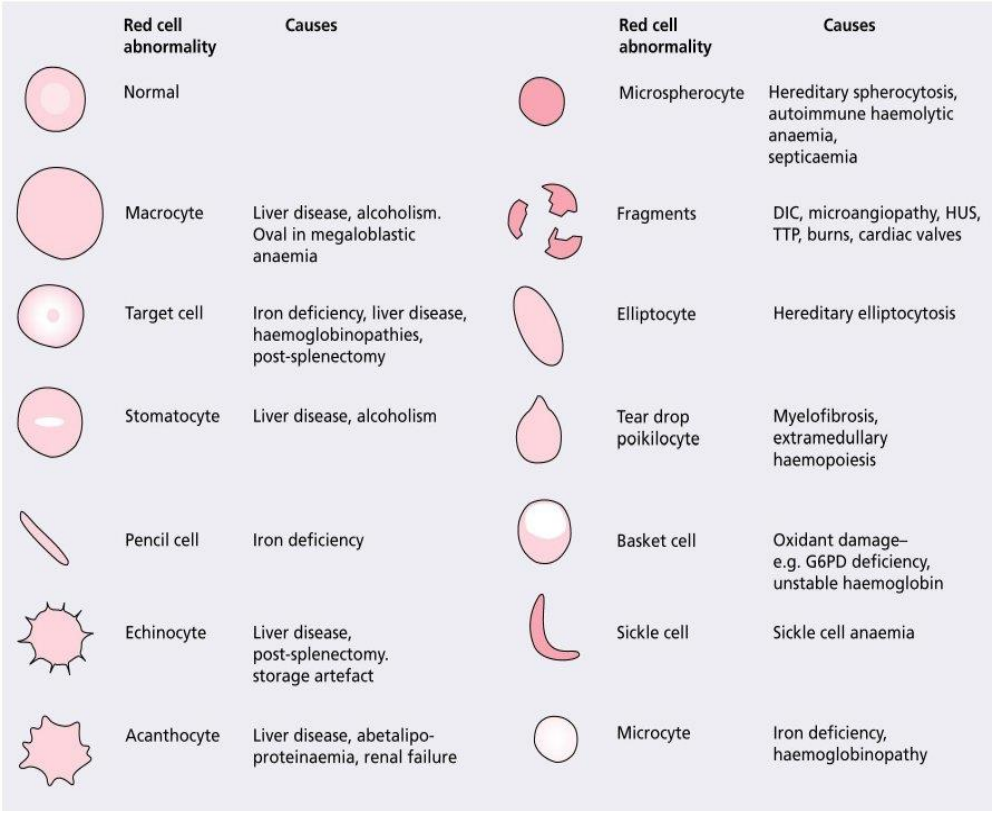
Classification of Hemolytic Anemias

- **Intracellular Causes**
  - Red cell membrane defects
  - Enzyme defects
  - Hemoglobin defects
    - Thalassemia
    - Sickle cell disease
    - Hemoglobin C
- **Extracellular causes**
  - Autoimmune
  - Microangiopathic

Laboratory Markers of Hemolysis

- Anemia: CBC shows low Hb
- Evidence of marrow activity: high reticulocyte count in the blood (and nucleated rbcs in extreme cases)
- Increased breakdown products of Hb (high indirect bilirubin, lactate dehydrogenase), hemoglobinuria
- Decreased binding protein (haptoglobin-consumed)

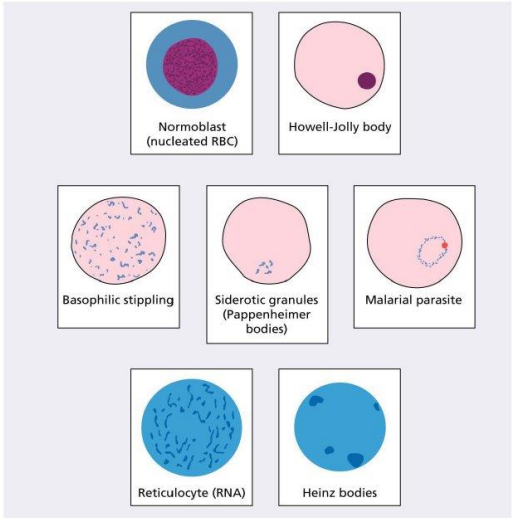
Red Blood Cell Shapes



Red cell abnormality	Causes	Red cell abnormality	Causes
Normal		Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
Echinocyte	Liver disease, post-splenectomy. storage artefact	Sickle cell	Sickle cell anaemia
Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure	Microcyte	Iron deficiency, haemoglobinopathy

Red Cell Inclusions (Right figure)

- The reticulocytes and Heinz bodies are only demonstrated by supravital staining (like new methylene blue, bottom 2 pictures).
- Heinz bodies are oxidized denatured hemoglobin
- Reticulocytes contain remnant RNA
- Pappenheimer bodies are siderotic granules (contains iron)
- The Howell-Jolly body is DNA remnant
- Basophilic stippling is denatured RNA



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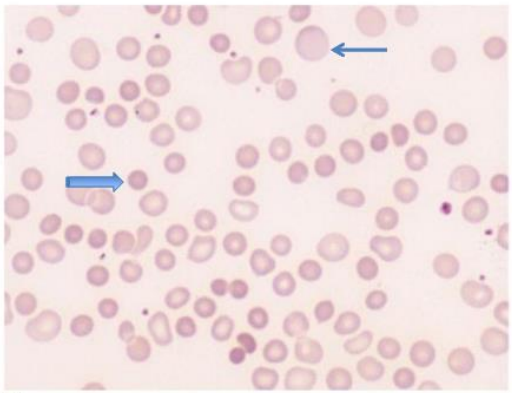
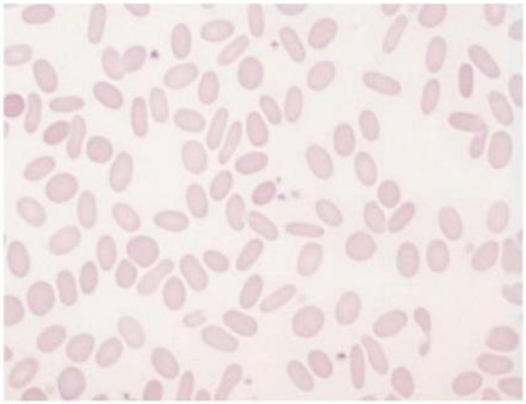
Red Cell Membrane Defects



(a) **Hereditary Spherocytosis**

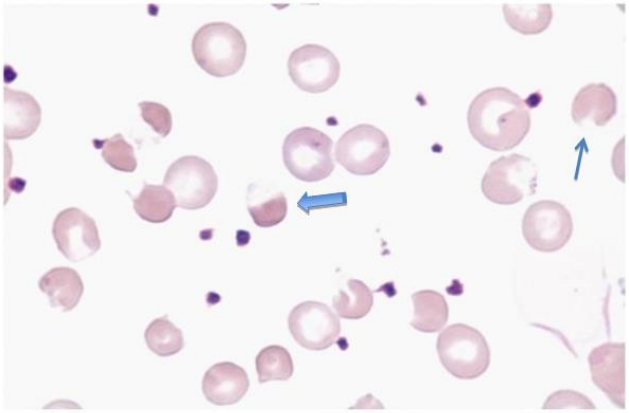
Note the small deeply staining red cells without area of central pallor (thick arrow) (spherocytes). At the top (thin arrow), note the larger polychromatic reticulocyte (confirmed by a supravital stain)



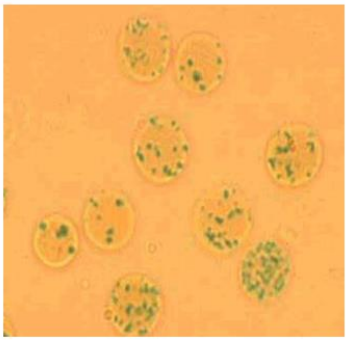
(b) **Hereditary Elliptocytosis**

Note the oval, elongated red cells with rounded ends distinguishing them from pointed ends in sickle cells

Anemia from Enzyme Defect: G6PD Deficiency

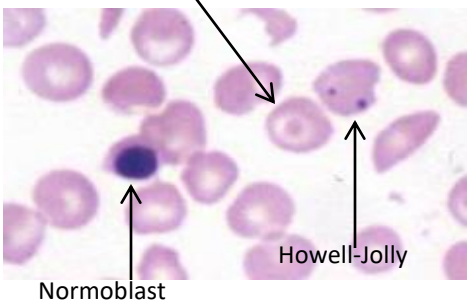


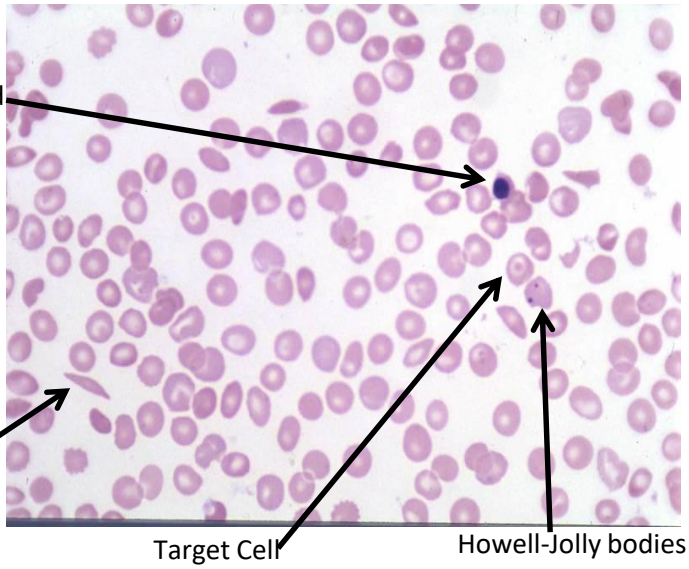
Note loss of cytoplasm in some cells due to oxidant stress (thin arrow). Also, separation of hemoglobin from cell membrane (hanging basket cells) (thick arrow)



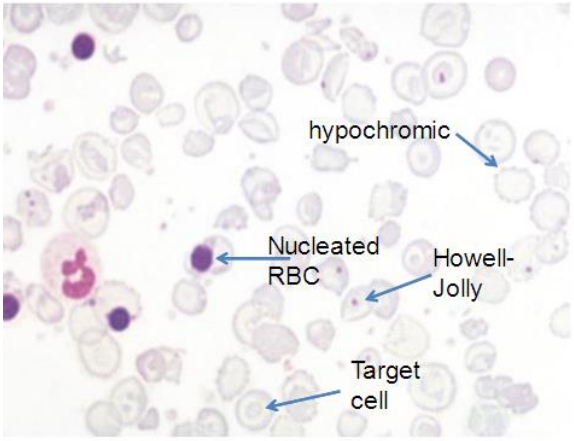
Heinz bodies representing denatured hemoglobin shown by supravital stain. Heinz bodies are also seen in Thalassemia due to excess globin chains

Hemoglobin Defects: Sickle Cell Disease





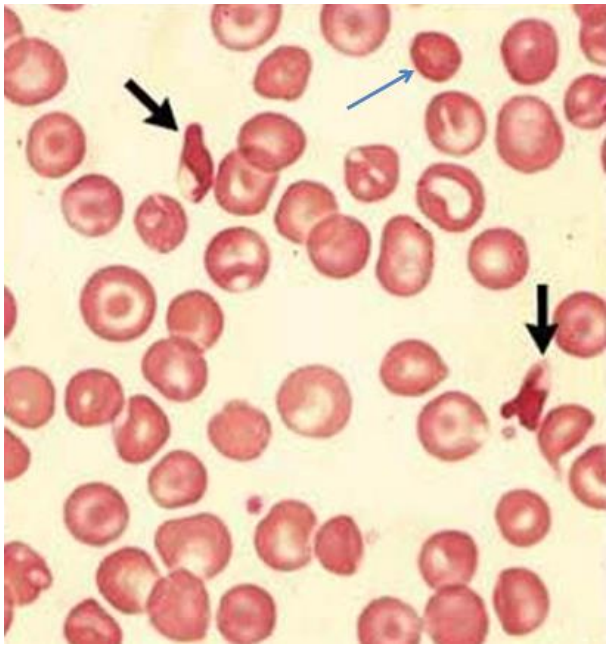
Hemoglobin Defects: β-Thalassemia Major



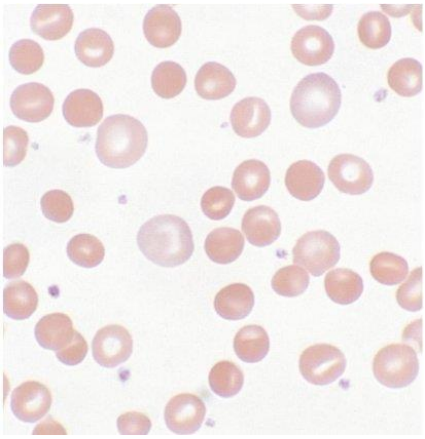
Blood film in β-thalassaemia major post-splenectomy. There are hypochromic cells, target cells and many nucleated red cells (normoblasts). Howell-Jolly bodies are seen in some red cells.

Homozygous C Disease

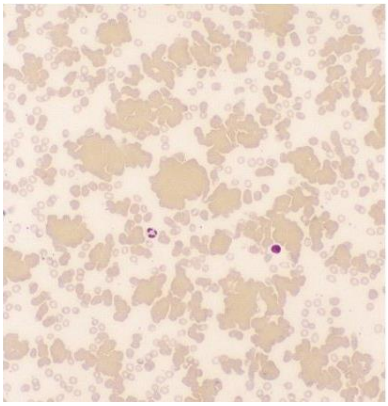
- Hemoglobin C produces an envelope shaped or rhomboidal shaped cells (arrows) as opposed to the sickled shaped cell
- Target cells and microspherocytes are common
- Hemoglobin C Trait is asymptomatic



Extracellular causes of Hemolysis: Autoimmune

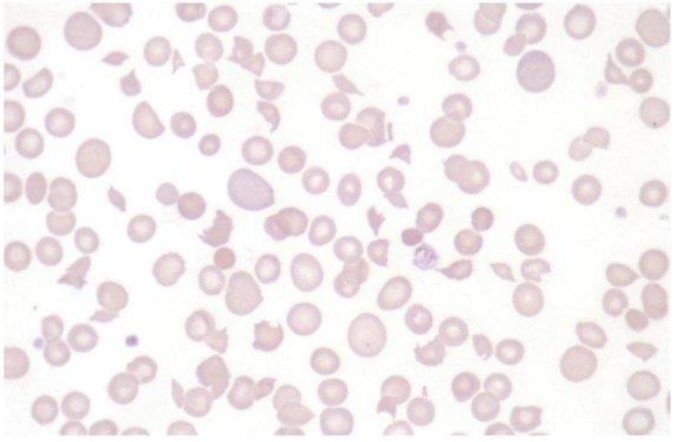


Left: Note the microspherocytes (small deeply staining cells without central pallor) and the larger polychromatic reticulocytes. This picture is usually associated with warm antibody hemolysis



Left: Note the clumping (agglutination) of red cells usually associated with cold agglutinin disease (cold antibody-mediated autoimmune hemolysis)

Extracellular Causes of Hemolysis: Microangiopathic



Blood film in microangiopathic hemolytic anemia. Note the numerous contracted and deeply staining cells (spherocytes) and broken RBCs (schistocytes)

Laboratory Testing in Hemolytic Anemias: Coombs Test

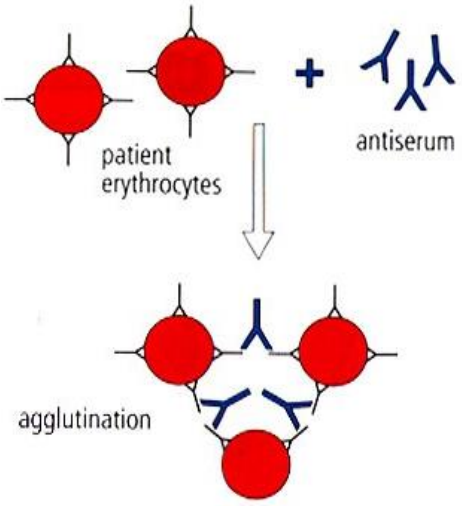
Determines the presence of immunoglobulins (Ig)/or complement on the red blood cell surface (direct) or the presence of anti red blood cell Ig in the serum (indirect)

Direct Coombs

The patient erythrocytes are incubated with Coombs reagent which contain:

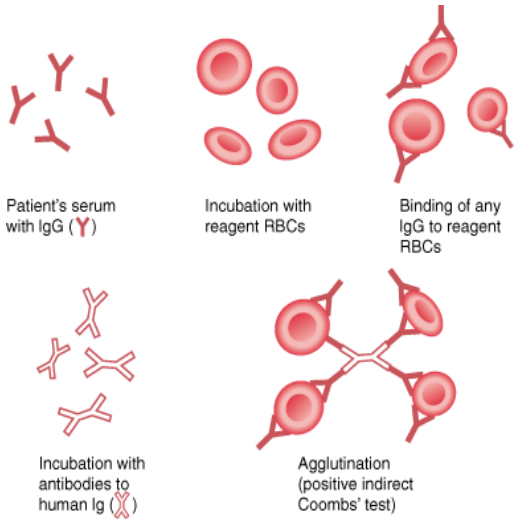
- Broad spectrum, or
- Type-specific **antibodies** (Anti-IgG, -IgM, or -Complement)

If the corresponding **antigens** (broad spectrum, complement or IgG, IgM) are present on the red cell surface, there will be red cell agglutination



Indirect Coombs

- Patient serum is incubated with a variety of type 0 Rh negative reagent RBCs of known antigenic types
- The RBCs are then washed and incubated with anti-human IgG antibodies
- If the patient's serum has antibodies, which react with the reagent RBCs, the anti-IgG antibodies will cause the reagent red cells to agglutinate

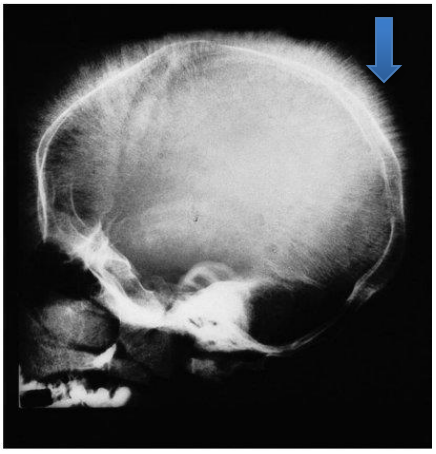


Laboratory Testing in Hemolytic Anemias:

Hemoglobin Solubility Test

- Demonstrates the presence of a sickling hemoglobin
- The clear tube contains a non-sickling hemoglobin. The hemoglobin is soluble in the buffer as demonstrated by the visible lines, which can be seen through the tube (right side tube).
- In the turbid tube (left), the lines on the grid cannot be seen. This indicates the presence of hemoglobin S which is insoluble in this reagent
- The sickle solubility test is positive.

Radiologic feature in β Thalassemia major
The expanded marrow shows a "Hair on End" appearance in the cortical bone (arrow)



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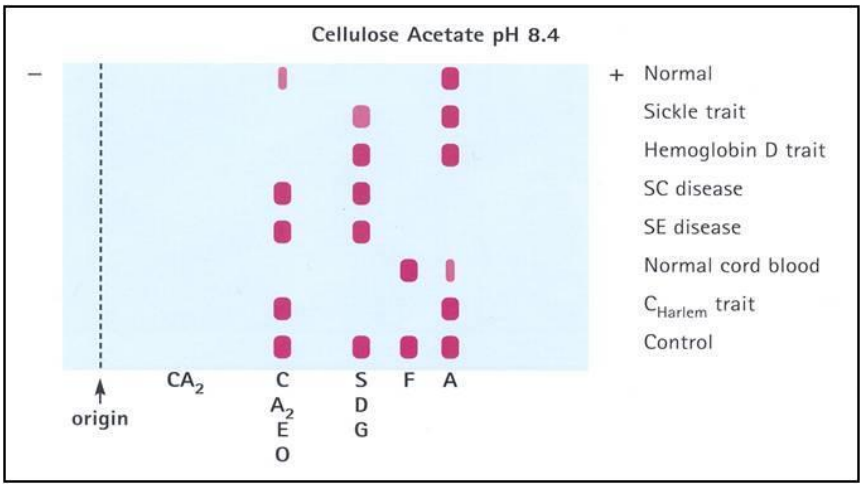
Hemoglobin Electrophoresis



- The electrophoresis runs left to right; samples loaded at the origin (arrow)
- Hemoglobins are separated by their net electric charge
- Hb C (crawls), A^2, E and O co-migrate near the origin
- Hb S (slow), D and G are next
- Hb F (fast) runs between S and A
- Hb A (accelerated)
- Note that in 'trait', the A is more concentrated than the abnormal Hb