National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual-
Haematology and Immunohaematology Department

Fourth Year, Semester (7)
Anemia's and Haemoglobin Disorders Investigations (MLS-HAEM-411)

Student Name: ……...

ID: ……**Batch**…….....…...

Instructions

- Wear lab coat
- Wear Gloves
- Avoid swallow any chemical
- Follow the procedures provided
- Write your results in this manual

Content

Preparation of blood smear:

Blood film should be made on clean glass slides. Films made on cover glass have negligible advances and are unsuitable or modern laboratory practice. Films may be separate by hand or by means of separated slide spreader.

Manual method:

Principle of smear preparation:

A small drop of blood is placed near the frosted end of a clean glass slide, a second slide used as a spreader. The blood is streaked a thin film over the slide. The slide is allowed to air dry and then stained.

Sample:

Ethylene di amietetra-acetic acid (EDTA) anticoagulant blood is preferred. Blood smear can also made from finger stick blood directly onto a slide.

Heparinized blood should not generally be used because it's staining blue background.

Equipment and supplies:

1. Glass slides 75 * 25 mm and approximately 1 mm thick, ideally they should be forested at one end to facilitate labeling.
2. Spreader glass slide

Procedure:

1. Place a small drop of blood in the center line of slide about 1 cm from the frosted end.
2. Then place spreader in front of the drop at an angle of about 30 degree to the slide and move it back to make contact with the drop.
3. The drop should spread out quickly along the line of contact with a steady movement of the hand, spread the drop of blood log the slide.
4. The spreader must not be lifted off until the .last trace of blood has been spread out. With correctly sized drop, the film should be about 3 cm in length. It is important that the film of blood finished at least 1 cm before the slide end.
5. The film should be allowed to dry in the air. In humid condition the films may be exposed to current warm air (e.g., from a hairdryer), but this should be in a microbiological safety hood.

Note:

The thickness of the blood film can be regulated by varying the pressure and speed of spreading and by changing the angle at which the spreader is held. With anaemic blood, the correct thickness is achieved by using a wider angle, while with polycythaemia blood, the angle should be narrower.

Labeling blood films:

1. The film should be labeled immediately after spreading
2. Write either a laboratory number or the name of the patient and the data in pencil on the frosted end or on the film itself (write on the thickest part, which is least suitable for microscopic examination).

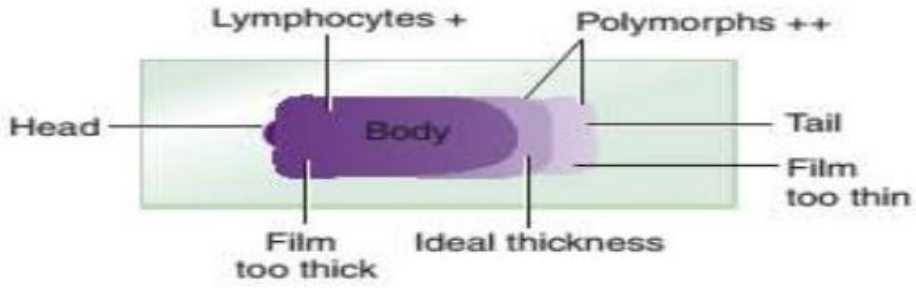
Characteristics of a good smear:

1. The smear preparation will be thick at drop end end thin at the opposite end.
2. Should occupy 2\3 of the total slide area
3. The blood smear should not touch the edges. Except for point of application.
4. Should be margin free.

Common causes of poor blood film:

1. Drop of blood too large or too small
2. Spreader slide pushed across the slide in a jerky manner
3. Failure to keep the spreader side to a 30 degree angle with the slide
4. Failure to push the spreader slide completely across the slide
5. Holes in film (slide contaminated with fat)

The shape of blood film



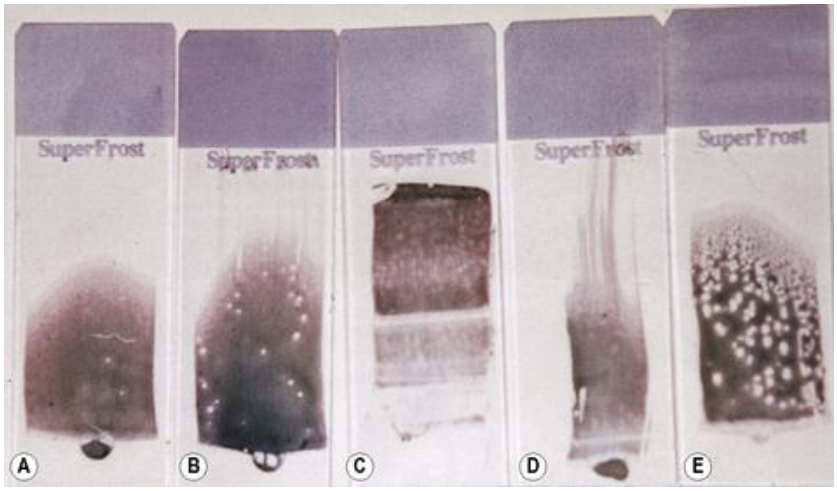


Figure 1.1 blood films made on slide. A: A well-made film. B: An irregular patchy film on a dusty slide. C: A film that is too thick. D: A film that has been spread with inconstant pressure and using irregular edge spreader. E: A film made on a heavy greasy slide.

Lab Question:

You are provided with blood sample labeled s ( ) do thin blood film.

Study questions:

1. **What is the characteristics of a good thin blood film?**

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2. **Common causes of poor thin blood film.**

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Staining of thin blood film:

Blood films are staining so that the morphology of blood cells become more easily viewed, identified, and evaluated.

Principle:

Blood films are stained by Romanowisky dyes which re compound dyes consisting of mixture of Methylene blue ad Eosin. Methylene blue stains acidic cell components such as nuclei and cytoplasmic RNA. Eosin is red and stains more basic components such as a haemoglobin.

Romanowisky stains include:

1. Giemsa stain
2. Wrights stain
3. Leishman stain
4. May – Grunwald stain

The widely used Romaowisky stains are:

1. Leishman stain
2. Write stain
3. Giemsa stain

Fixation:

Good fixation is essential for good staining ad presentation of cellular details. Contamination of methyl alcohol with water leads to poor fixation and loss of cell details.

Staining:

Leishman method:

Requirements:

1. Leishman stain
2. Buffered distilled water PH 6.8
3. Staining rack

Preparation of solution:

Leishman stain:

1. Leishman powder 1.5 g
2. Methanol 500 ml
3. Add glass peads to the mixture
4. Shake well, leave on a rotary shaker during the day, then incubate at 37 C over night

Phosphate buffer:

a) $Na\ H_2\ po_4\ 2\ H_2O$ 23.4 g\L
b) $Na_2\ H\ po_4$ 21.3 g\L

Mix 46 ml of solution (A) with 54 ml of solution (B)

Method:

1. Make a thin blood film and air dry rapidly
2. Place the film on a staining rack, flood with Leishman stain and leave for 3 min to fix.
3. Add twice as much as buffered distilled water
4. Leave to stain for 10 min
5. Wash off stain with tap water
6. Clean the back of the slide and stand upright to dry

Color response of blood cells to Romanowisky staining:

Cellular component	Color
Nuclei	
Chromatin	purple
Nucleoli	Light blue
Cytoplasm	
Erythroblast	Dark ble
Erythrocyte	Dark pink
Reticulocyte	Grey - blue
Lymphocytes	Blue
Metamyelocyte	Pink
Monocyte	Grey - blue
Myelocyte	Pink
Neutrophil	Pink
Promyelocyte	Blue
Basophil	Blue
Eosinophil	Orange
Platelet	Purple

Quality control:

Too acid stain: (red color)

1. Insufficient staining time
2. Prolonged buffering or washing
3. Old stain

Too alkaline stain: (blue color)

1. Thick blood smear
2. Prolonged staining
3. Insufficient washing

Factors giving rise to faulty staining:

1. Old staining solution and incorrect preparation o stain give pale staining blood smear
2. Stain solution not filtered form stain deposit on film
3. Inadequate fixation and blood collected into heparin as anticoagulant give raise a blue background of blood smear.

Lab Question:

You are provided with blood sample labeled as ( ) Make thin blood film and stain it

Study Questions:

1. Causes of too acidic stain of blood films:

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2. Causes of too alkaline stain and how to correct it:

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Haemoglobin Estimation by Cynomethaemoglobin Method:

This method is the most accurate and most commonly used method. A standardized method is recommended by ICSH (International Committee or Standardization in Haematology).

Principle:

Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. The latter converts Hb to methaemoglobin which is converted to cyanomethaemoglobln (HiCN) by potassium cyanide. The absorbance of the solution is then measured in spectrophotometer t wavelength 540 nm.

Preparation of solution:

Drabkins solution (PH: 7.00 – 7.4) consist of:

1. Potassium ferricyanide 200 mg
2. Potassium cyanide 50 mg
3. Potassium dehydrogen phosphate 140 mg
4. Nonionic detergent 1 ml
5. Distilled water to 1 L

The solution should be clear and pale yellow in color. When measured against water as a blank in a spectrophotometer at wavelength of 540 nm, the absorbance must be zero. The solution is unstable if exposed to light can be stored at room temperature in brown bottle for several months.

Test sample:

EDTA anticoagulated venous blood. Alternatively freely flowing capillary blood.

Method:

1. Label 3 test tubes as Test, STD Blank
2. Added 5 ml drabkins to each tube if Df 1\250 or 4 ml if Df 1\200
3. Add 0.02 ml blood to test tube
4. Add 0.02 ml of Hb STD to STD tube

5. Mix well for 2 min, incubate for 10 min at RT
6. Read the absorbance of sample on photometric colorimeter
7. Calculation:
   Hb conc. = Abs of test sample\ Abs of STD * STD conc, g\dl * Df Of TEST\Df of STD

Precautions:

1. Before the sample is read the solution must be clear
2. In case of high WBCs count centrifuge the specimen and use the sedimentation

Reference range:

Age group	Haemoglobin (g\dl)
Adult male	14.0 – 17.0
Adult female	12.0 – 14.0
Children	11.0 – 14.0
New born	14.0 – 22.0

Interpretation:

Decreased levels of haemoglobin are found in:

1. Anaemia
2. After sever hemorrhage
3. Varity of systemic diseases e.g. leukemia, lymphoma, uremia, cirrhosis, hyperthyroidism
4. Haemolysis due to transfusion of incompatible blood, bacteremia, and artificial heart valves.

Increased levels of haemoglobin are founded in:

1. Haemoconcentrtion state of blood e.g. severe burns
2. Polycythaemia
3. Congestive heart failure
4. High altitudes

Source of errors:

1. Insufficient mixing of blood specimen
2. Inadequate pipetting and use of badly calibrated pipettes
3. Inadequate mixing of blood with drabkins solution
4. Exposure of the preparation (Cyanomrthaemoglobin solution) to the direct light for long time.

Lab Question:

You are provided with blood sample labeled as ( ) you are requested to estimate haemoglobin level.

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Study Question:

1. The principle of cyanomethaemoglobin method:
2. Give 3 causes of increased and decreased haemoglobin concentration:
3. Write the source of errors in Hb estimation:
4. Student's findings (measurements or observations):
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5. comments and interpretation:
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6. Evaluation (carried out by the instructor):
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7. Name and signature of the instructor:
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8. Date: ……..\.....\.....

Hb STD Calibration Curve:

In a laboratory which tests several samples in day it is more convents to prepare a standard curve for Hb.

Purpose:

1. Detect the power stability
2. Accuracy and precision
3. Efficiency of standard materials and reagent

Reagents:

1. HiCN reagent
2. Drabkins solution

Method:

1. Prepare serial dilution of HiCN using the following formula: upper limit of STD concentration = d (n-1)
   D: different between each two points
   N: number of points
   Example:
   Concentration of HiCN standard = 15 g\dl
   15 = d 6-1)
   d= 15\5 = 3
   the concentration of serial dilution will be 0, 3, 6, 9, 12, 15 g\dl
   Require volume 5 ml
   Using R V\O
   Tube (1) 5 ml from STD HiCN + 0 ml solution
   Tube (2) ……. ml from STD HCN + …….. ml solution
   Tube (3)…….. ml from STD HiCN+ ……. ml solution
   Tube (4) ……. Ml from STD HiCN + ……. ml solution
   Tube (5) …….. ml from STD HiCN + …… ml solution
   Tube (6) …….. ml from STD HiCN + …… ml solution

2. Read the absorbance of each tube
3. Finally construct a curve with concentration on X axis and STD absorbance on Y axis.

Lab Question:

You are provided with HiCN standard conc. 15 \dl request too Hb STD curve of 6 points.

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Stud question:

Mention the purpose of Hb STD curve:

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Iron profile:

Serum ferritin:

Reflect body iron store

Serum ferritin has been widely used as a test for iron deficiency and iron over load.

measure by an immunological method:

1. Immunoturbadiometric assay (RIA): it is first reliable method to be introduce, in which excess radiolabelled antibody was react with ferritin.
2. Enzyme Lined Immunosorbent assay (ELISA): in which enzyme labelled antibody was react with ferritin
3. Latex labelled (immune turbidometery)

Reagents:

1. Ferritin: prepared from iron loaded human liver or spleen or postmortem, ferritin purify should be assessed by polyacrylamide gel electrophoresis or gel filtration chromatography.
2. Antibody to human ferritin: poly clonal antibody may be raised in rabbits or sheep, an immunoglobulin G (IgG) is extracted and labeling with enzyme. Monoclonal antibody specific or L subunit rich ferritin also may be obtained.

Serum iron:

Principle:

Transferrin iron is separated in acid solution then converted to chromogenic solution, then the absorbance of the developing color is measured calorimetrically.

TIBC:

Principle:

Excess iron as ferric chloride is added to serum. Any iron that does not bind to transferrin is removed by excess magnesium carbonate. The iron concentration of the iron saturated serum is then measured.

Serum transferrin:

Transferrin can be measure directly by immunological method like immunonephelometric method.

TIBC = transferrin * 25

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Methaemoglobin reduction test:

Aim:

Is used as screening test for G6PD enzyme deficiency

Principle:

Sodium nitrate converts Hb to methaermoglobin (Hi). When no methylene blue is added methaemoglobin persists but incubation with of the sample with methylene blue allows stimulation of the pentose phosphate pathway in subject with normal G6PD level. The Hi is reduced during the incubation period. In the GPD deficient subject, the block in the pathway prevents this reduction.

Reagents:

1. Sodium nitrate (180 mmol\l)
2. Dextrose
3. Methylene blue

Method:

1. Prepare working reagent by mixing equal volume of methylene blue and $NaNO_2$
2. Add 2 ml patient blood (EDTA blood), and 2ml control +ve and control –ve in test tubes labeled as Test, C +ve, and C-ve.
3. Add 0.2 ml of the working reagent to each test tube, then mix genteelly
4. Incubate the test and controls at 37 C for 90 min
5. Pipette 0.1 ml volume from each tube into another clean tube
6. Add 10 ml of distilled water
7. Record the result

Interpretation of the result:

1. Brown color indicate present of methaemoglobin interpreted as G6PD deficient
2. Red color indicate reducing of Hi to Hb interpreted as normal G6PD level

3. Student's findings (measurements or observations):
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Sickling test:

Value:

To confirm diagnosis o sickle cell disease when the sickle cell shape in in peripheral blood picture.

Principle:

Red cells with abnormal Hb S convert to sickle shape when incubate with deoxygenated Na – metabisulphate 2 %, other Hb remain with normal shape.

Sample:

Fresh blood in any anticoagulant

Requirements:

1. 2 % fresh Na – metabisulphate solution
2. Red cells suspension of patient
3. Slides
4. Meting paraffin wax
5. Wet petri dish

Procedure:

1. Small portion of patient or control red cells suspension mixed with one drop of Na- metabisulphate, then placed under clean cover glass n the center of slide
2. Four side of the cover glass sealed with melting wax then placed in t petri dish for 30 min, then examined microscopic
3. Negative result require feather incubation for 12 hrs. Then examine and gives the final result.

Quality control:

1. Use only fresh reducing reagent
2. Adequate sealing
3. Void air bubble
4. +ve and –ve control run with the test

Interpretation:

Sickling formation indicate positive result

Normal cell indicate negative test

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: …….\.....\.....

Solubility test:

Value:

Use as screening test for detection of Hb S of the given blood to confirm sickle cell anaemia n adult for above 6 months.

Principle:

Red cells contain abnormal Hb S give turbid untransparent solution in phosphate deoxygenated buffer. Other Hb give clear transparent solution in t same buffer.

Reagents:

1. Dipotassium hydrogen phosphate = 215 g
2. Potassium hydrogen phosphate = 169 g
3. Na – dithionate = 5 g
4. White saponin = 1 g

Procedure:

1. Place 2 ml o working buffer in small test tub then add 0.02 ml o whole blood or paced cell 0.01 ml
2. Mix gently then incubate t RT to 3 – 5 min and observed turbidity or transparent.

Cause of false positive result

1. Leukocytosis
2. Hyperprotenemia
3. Abnormal protein
4. Unstable Hb
5. Hyperlipidemia

Causes of false negative:

1. Use un fresh buffer
2. O date reagent
3. Infant less than 6mounths

4. Very anaemic blood
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Reticulocyte count:

Background:

Reticulocyte is immature red cells contain remaining of cytoplasmic RNA result from nucleus extrude of the late normoblast. Remain two days in the bone marrow and I 24 hrs. In peripheral blood.

Principle:

Ribosomal RNA of retic stain with supravital stain (stain f living cell) sample must be fresh retic is recognized microscopically non nucleated erythrocyte contain at least two granulofilamentous material that resemble to Hb H.

Requirement

1. Well mixed fresh EDTA blood
2. Retic stain New methylene blue or brilliant cresyl blue
3. Test tube
4. Slide
5. Water path

Procedure:

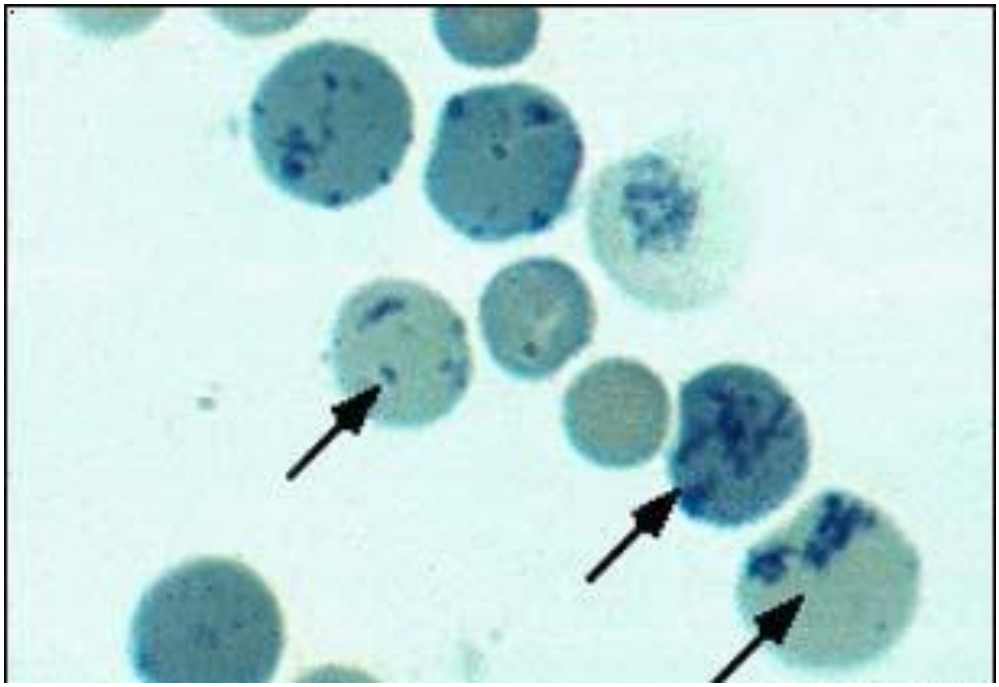
1. Mix equal volume of EDTA blood and stain
2. Incubate at water path 10 – 20 min
3. Then remix and make thin film from the mixture then count at least one thousand red cells with retic using oil immersion, then the percentage of reticulocyte using define formula:
4. Retic count = number of retic\ 1000 red cells * 100

Reference value:

0.2 – 2.0 %

Interpretation:

1. Increased reticulocyte count in the circulation indicate active erythropoiesis (e.g. a s a response to increased red cells destruction hemolysis) or hemorrhage)
2. Reticulocyte count also can be used as indicator for successful treatment of nutritional anaemia (iron deficiency anaemia and megaloblastic anemia)
3. Decreased reticulocyte count may indicate ineffective erythropoiesis or one marrow failure.



Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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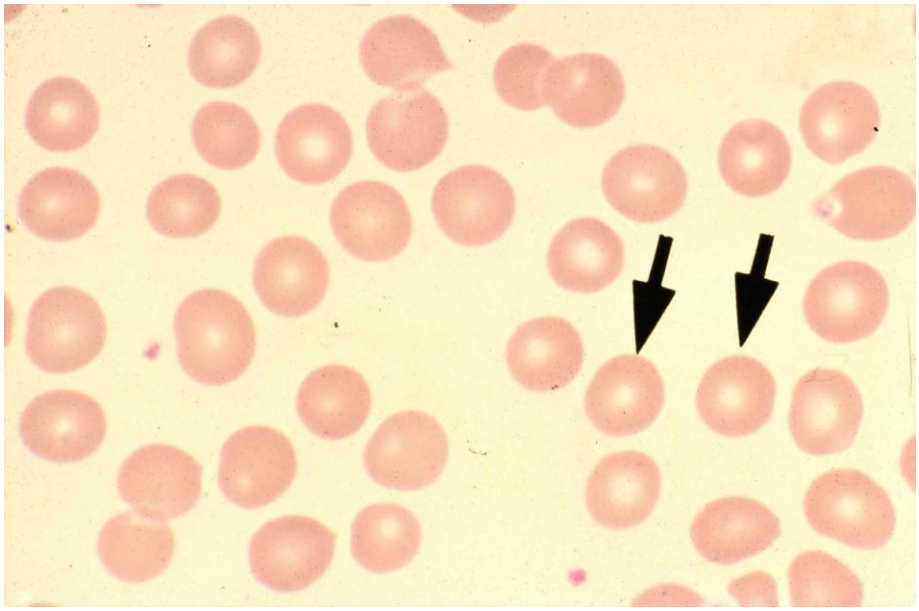
Date: ……..**.....****.....**

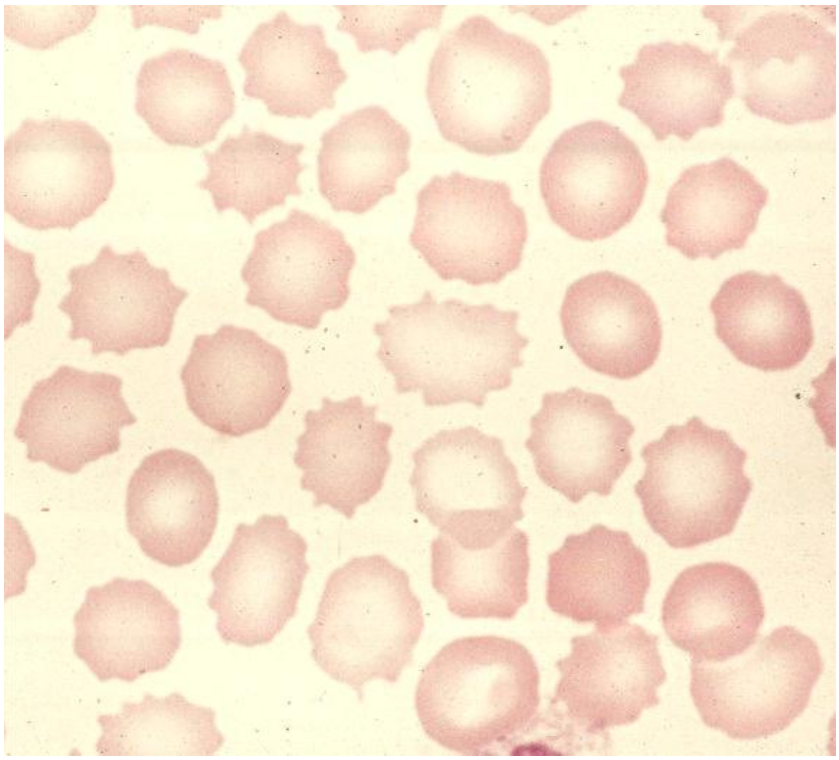
Red blood cells morphology

- **7-9 mm with 1/3 central palor**
- **Lifespan of 110-120 days**
- **About the size of nucleus of normal lymphocyte**
- **Poikilocytosis & Anisocytosis**



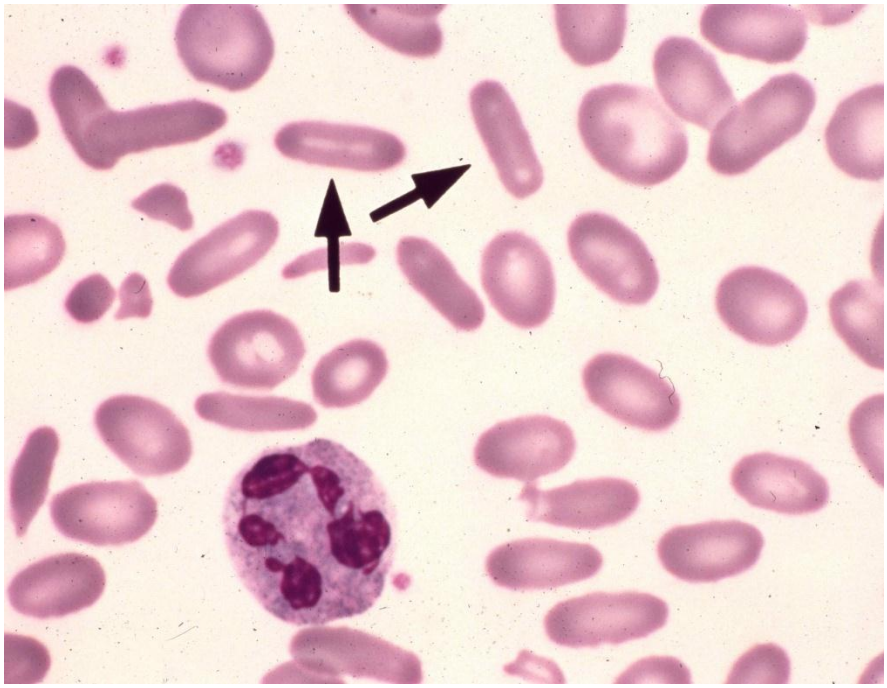
Burr cells:

- **Altered lipid in cell membrane**
- **artifact**
- **Uremia**
- **Renal failure**
- **gastric CA**
- **transfused old blood**



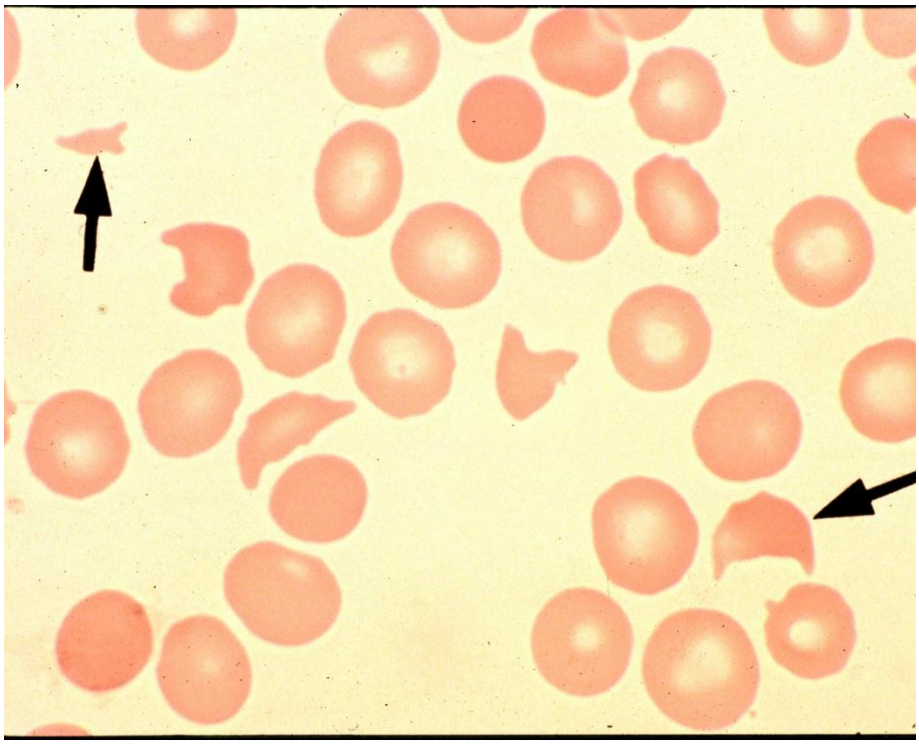
Elliptocytes/ovalocytes:

- **Aproteins**
- **Hereditary bnormal cytoskeletal elliptocytosis**



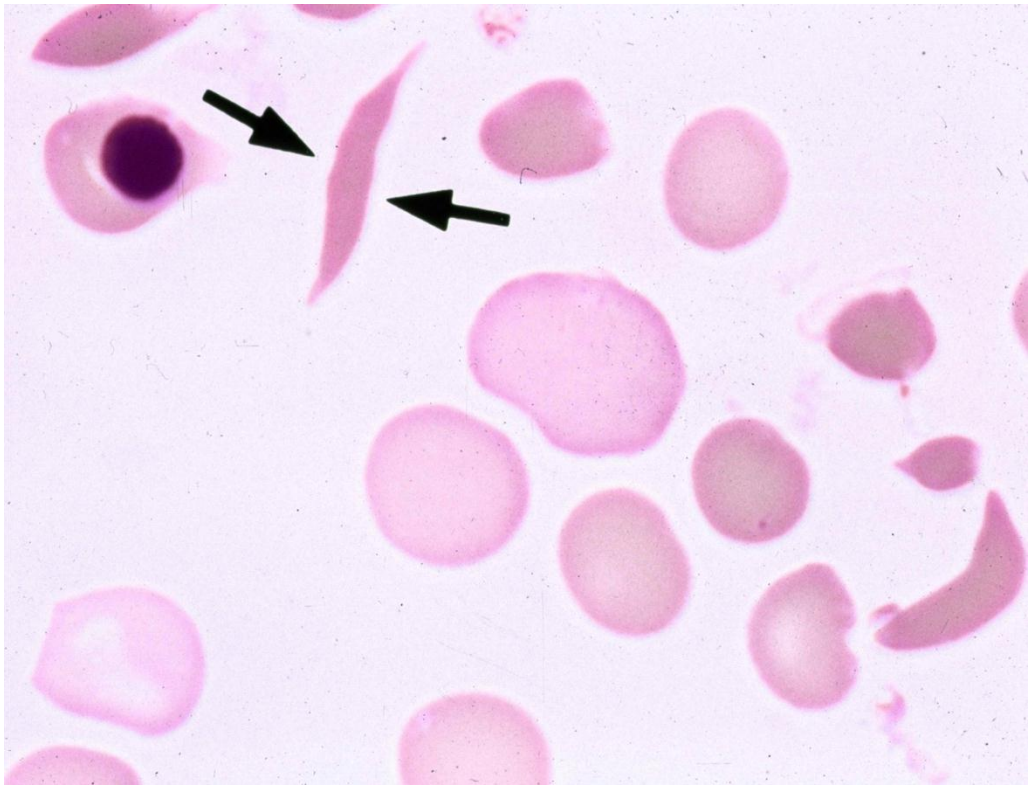
Schistocyte/helmet cells:

- **Fragmented (mechanical or phagocytosis)**
- **DIC**
- **TTP**
- **HUS**
- **Vasculitis**
- **prosthetic heart valve**
- **severe burns**



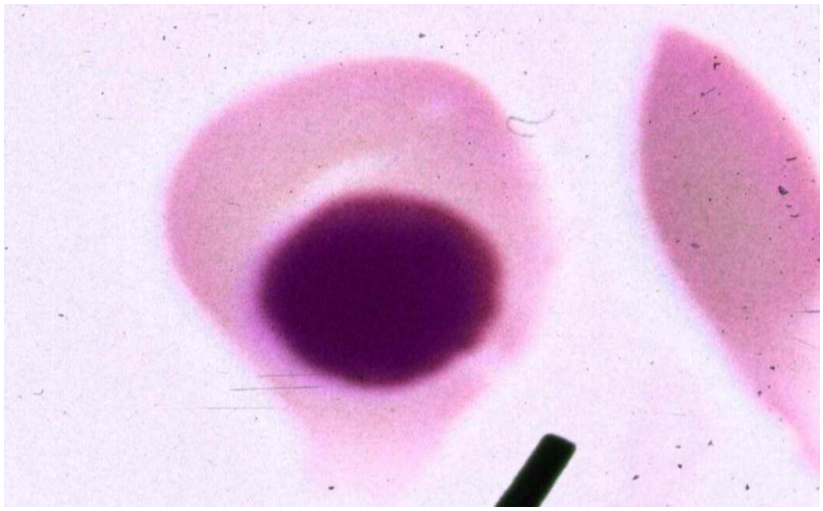
Sickle cells:

- **Molecular aggregation of Hgb-S**
- **SS, SC, S-thal**
- **rarely S-trait**



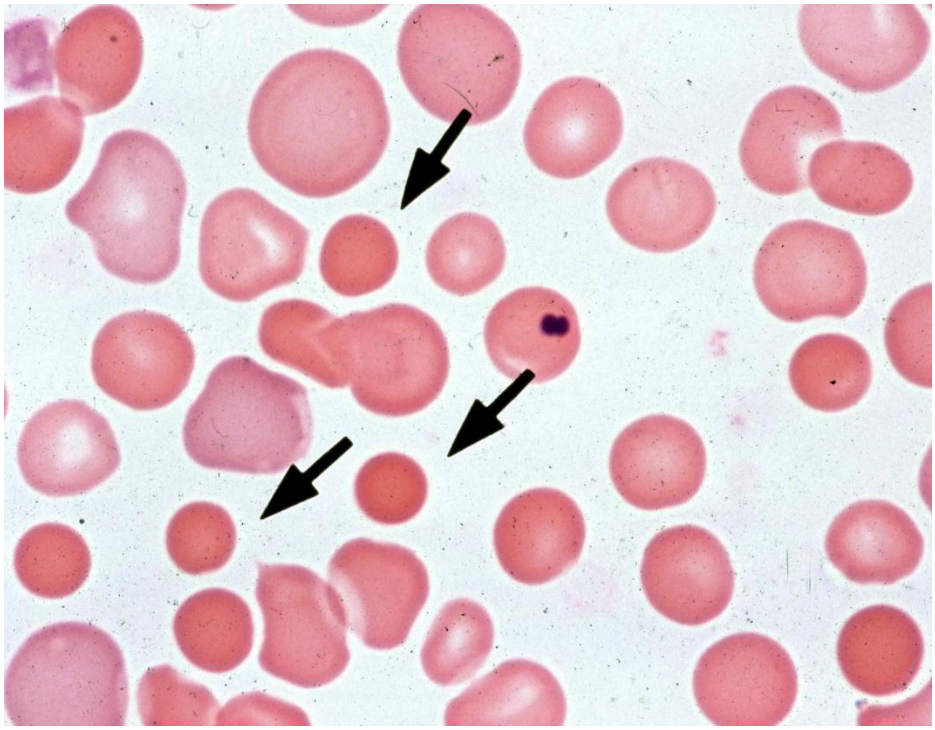
NRBC:

- **Common in newborn**
- **severe degree of hemolysis**



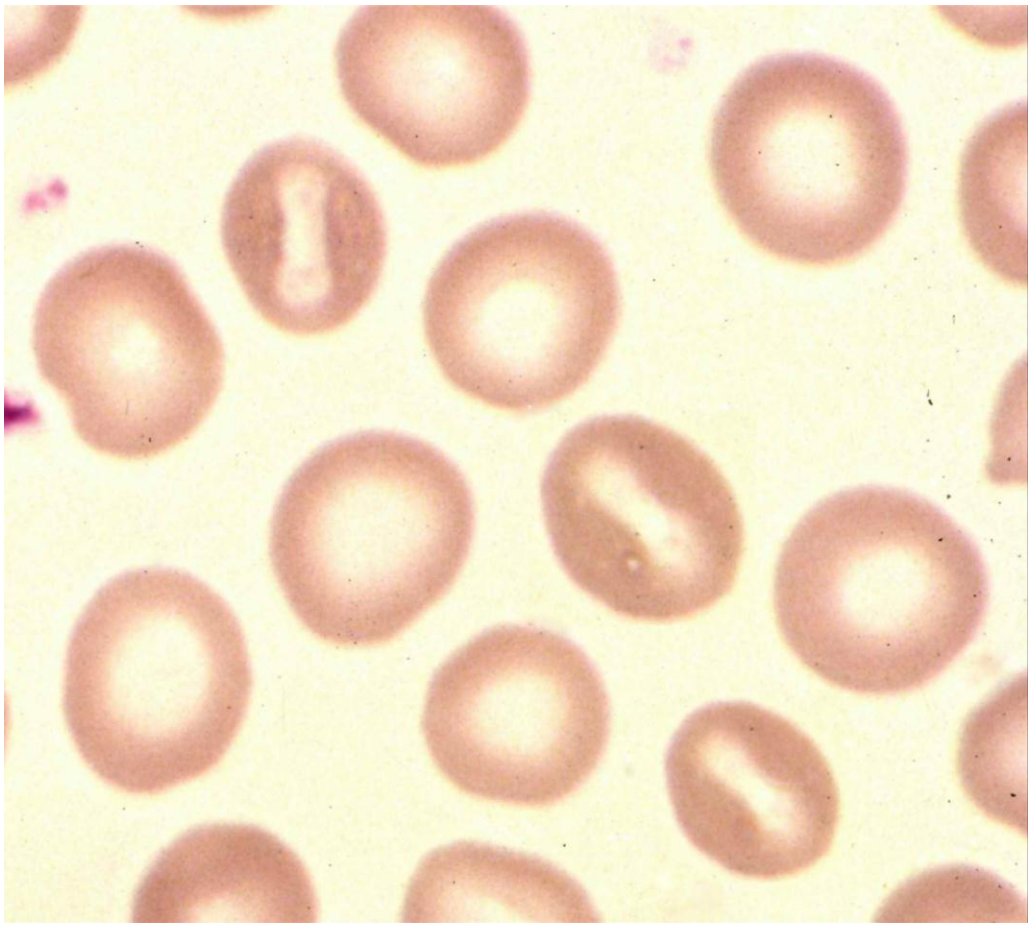
Spherocyte:

- **Absent central palor**
- **look smaller**
- **Hereditary spherocytosis**
- **immune hemolytic anemia**



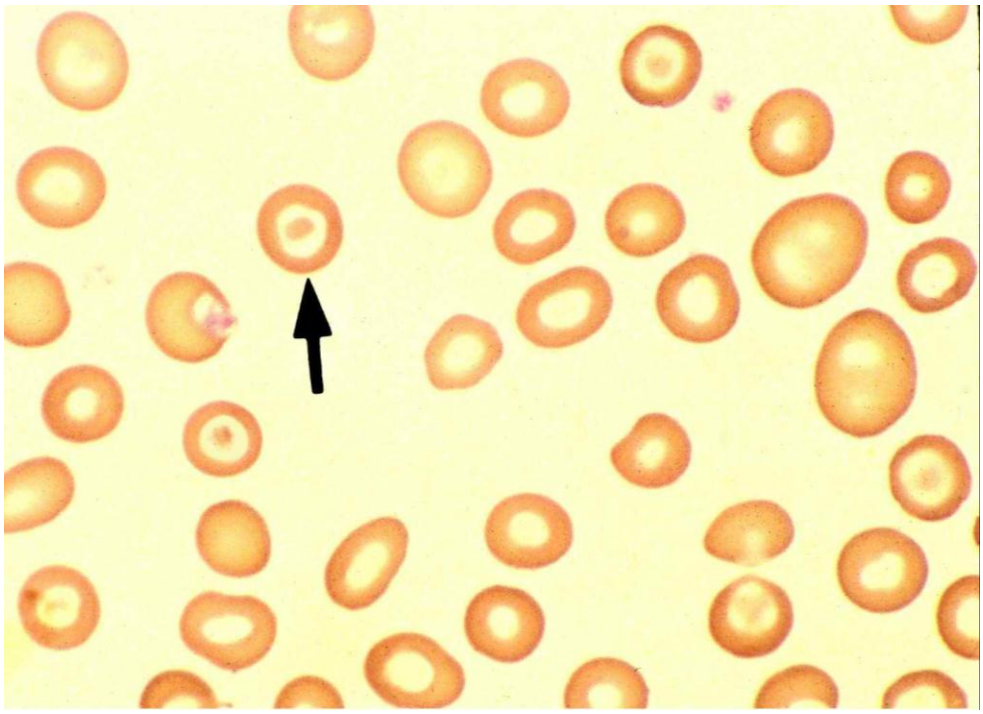
Stomatocyte:

- **Mouth like**
- **Membrane defect**
- **Smear artifact**
- **Hereditary stomatocytosis**
- **Liver disease**



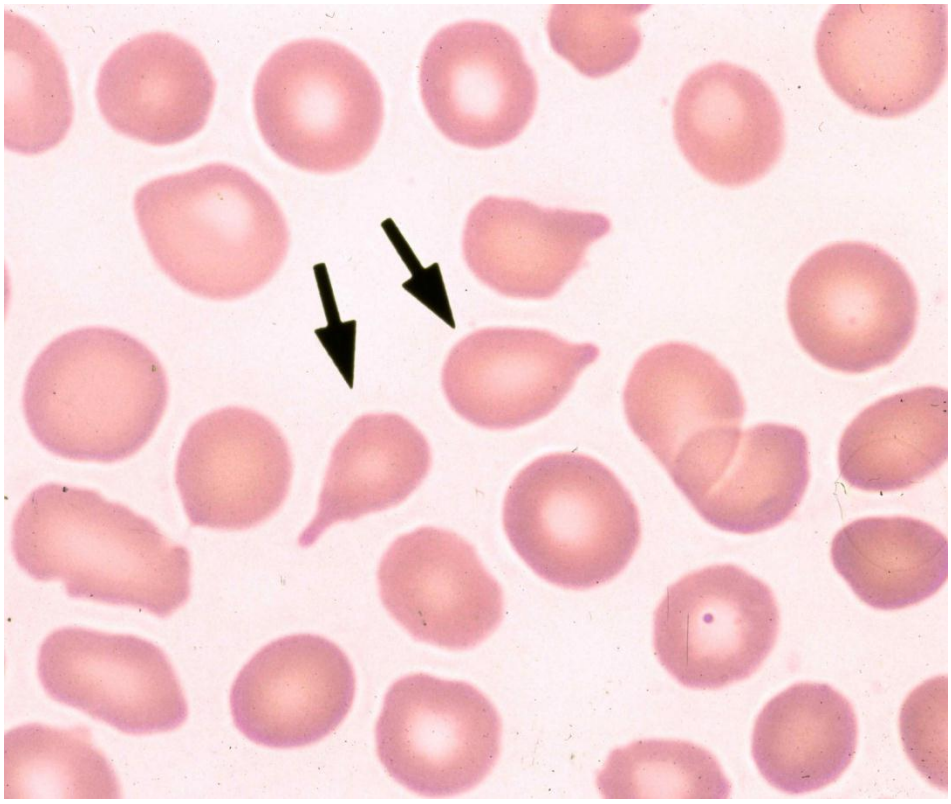
Target cells:

- **Increased redundancy of membrane**
- **hemoglobinopathies**
- **thalassemia**
- **liver disease**



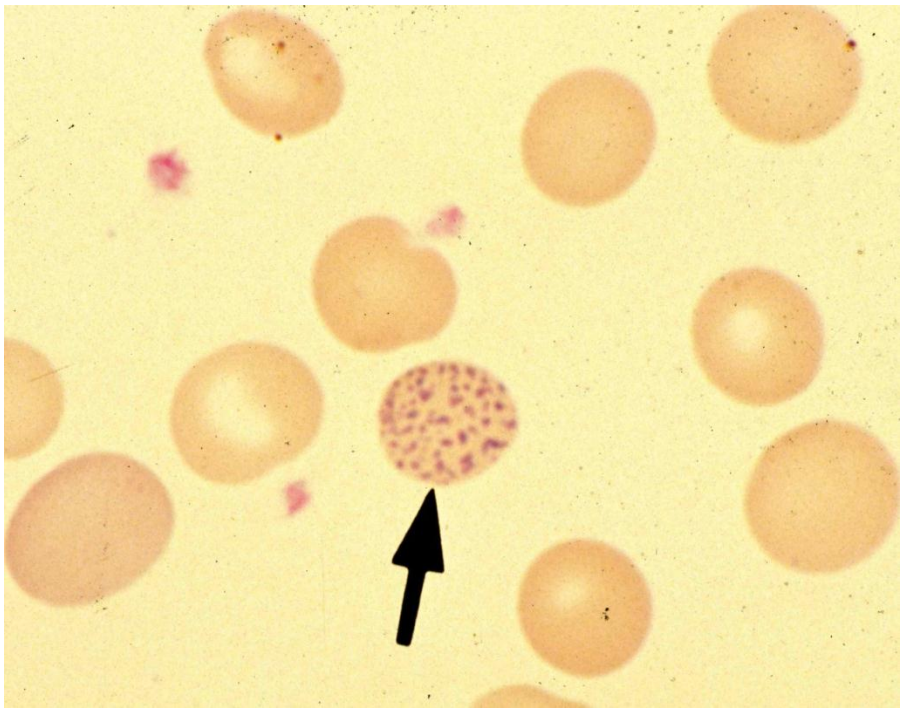
Tear drop cells:

- **Distorted drop shaped**
- **Smear artifact**
- **myelofibrosis**
- **promyeloblastic leukemia**
- **space occupying lesions of marrow**



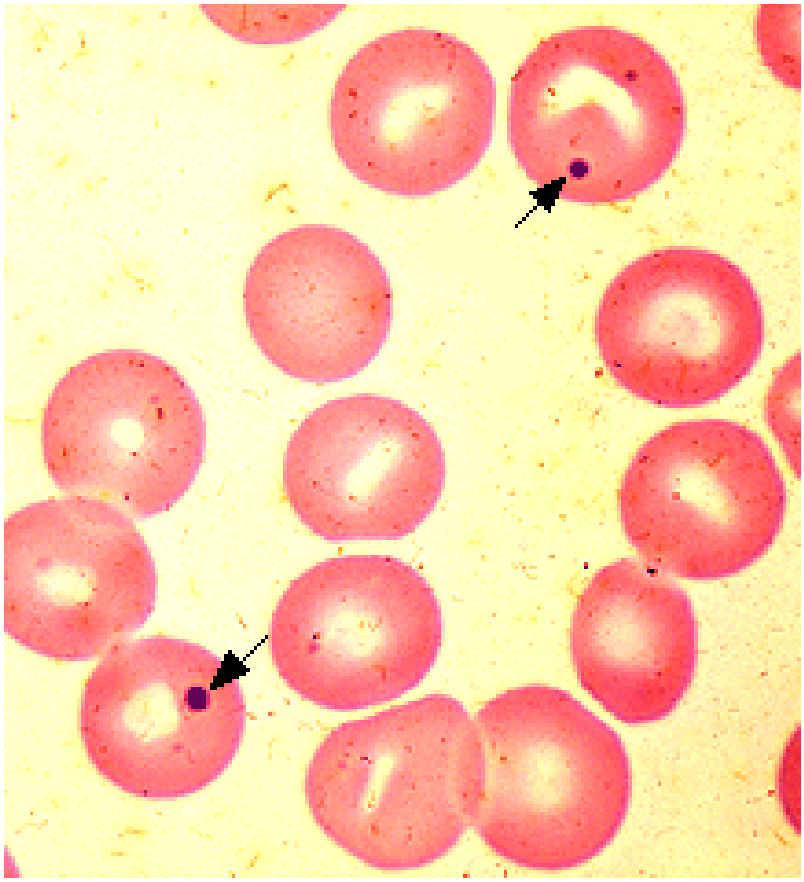
Basophilic stippling:

- **Precipitated RNA**
- **lead or heavy metal poisoning**
- **ETOH abuse**
- **Hemolytic anemia**



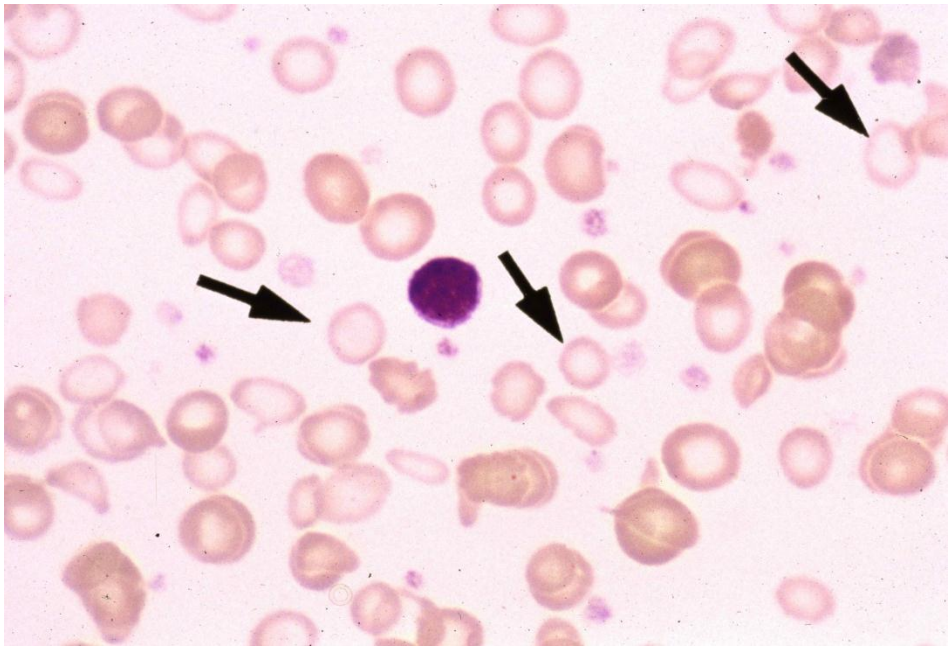
Howell Jolly body:

- **Nuclear remnant - DNA**
- **hemolytic anemia**
- **absent or hypofunction spleen**



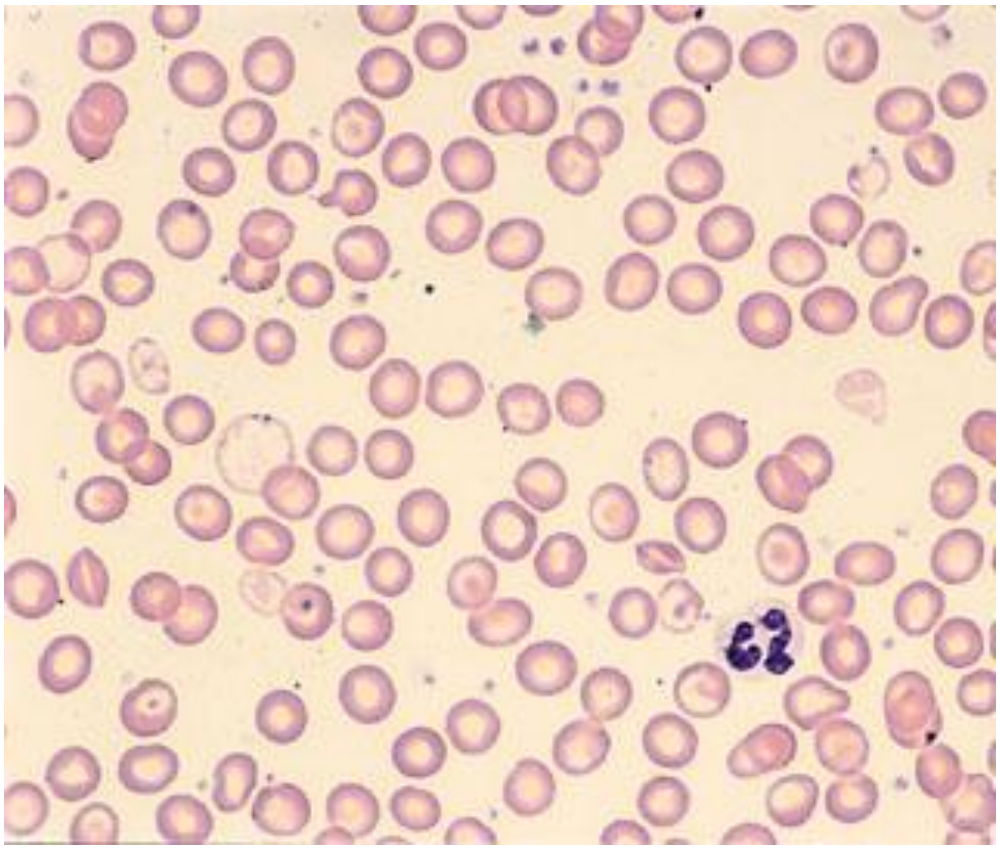
Iron def. Anemia:

- **Low Retic count**
- **High RDW**
- **Due to chronic blood loss**
- **Diet deficiency**



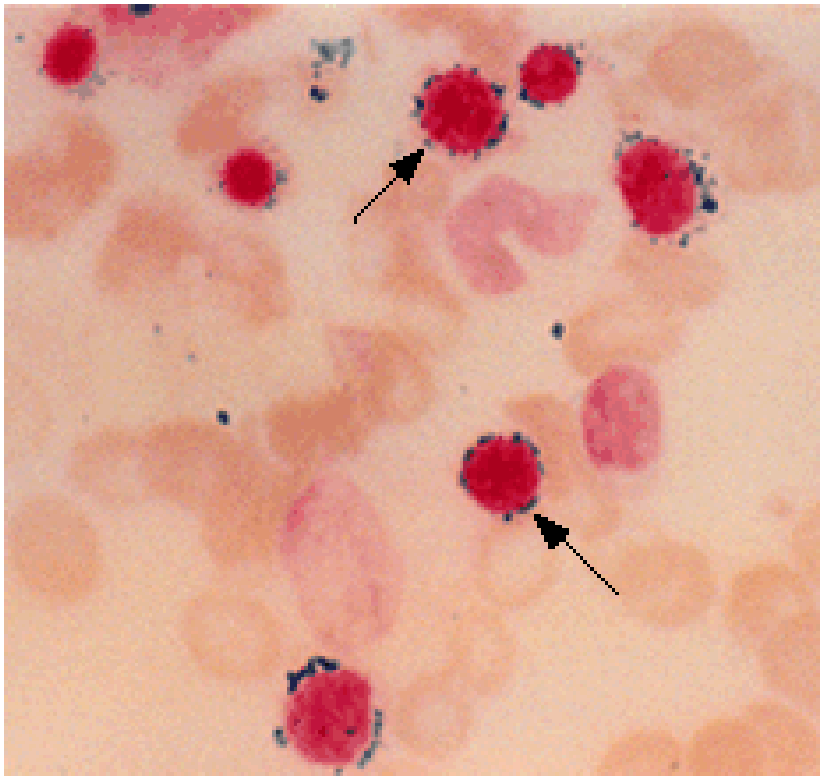
Thalassemia:

- **Normal to inc. RPI**
- **Normal RDW**
- **Target cells**
- **confirm w/ Hgb electrophoresis**



Sideroblastic anemia:

- **Accumulation of mitochondrial iron in erythroblasts**
- **Hereditary**
- **Drugs - lead, zinc, alcohol, chloramphenicol, cycloserine,**
- **Confirm by BM Bx**



Megaloblastic Anemia:

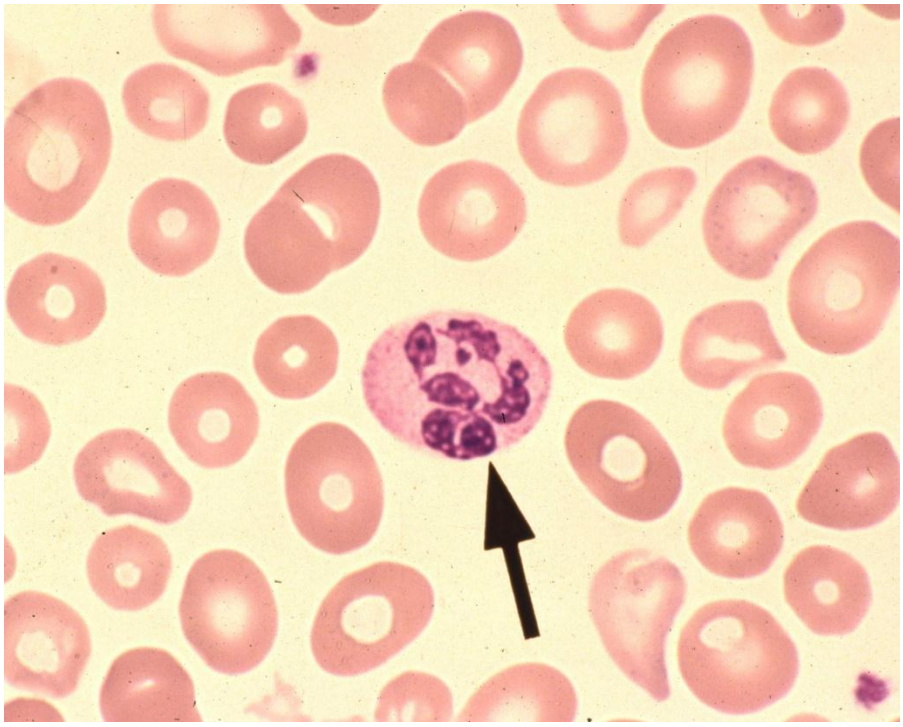
<u>Smear</u>

- **Macro-ovalocytic**
- **Polychromasia**
- **Hypersegmented neutrophil**

<u>Other Labs</u>

- **Homocysteine – Folate def.**
- **Methylmalonic acid – B12 def.**
- **Intrinsic Factor Ab test – very specific for pernicious anemia but only 50% sensitive**
- **Parietal cell AB test – quite sensitive (90%) but not specific**

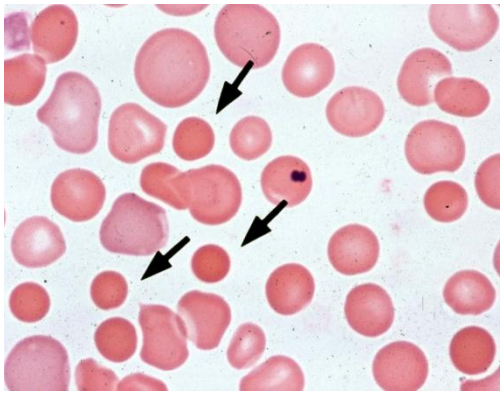
- **Schilling test**



Coombs' Negative Hemolytic Anemia Membrane Defects:

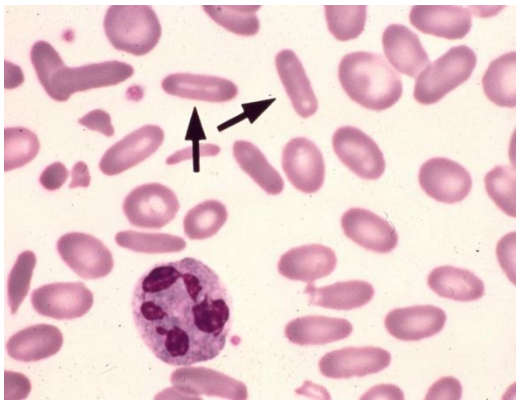
<u>Spherocytosis</u>

- **Common among Northern European**
- **Autosomal dominant**
- **Decreased spectrin**
- **Osmotic fragility test**
- **Autohemolysis test**



Elliptocytosis

- **90% with no clinically significant hemolysis**
- **Abnormal membrane protein**

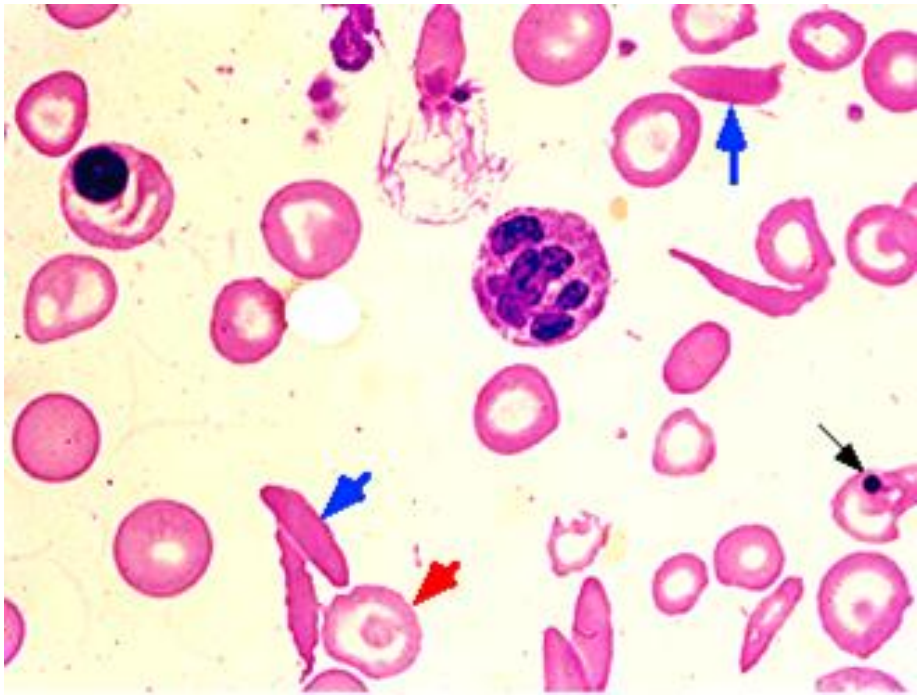


Coombs' Negative Hemolytic Anemia Hemoglobinopathy:

HbS disease

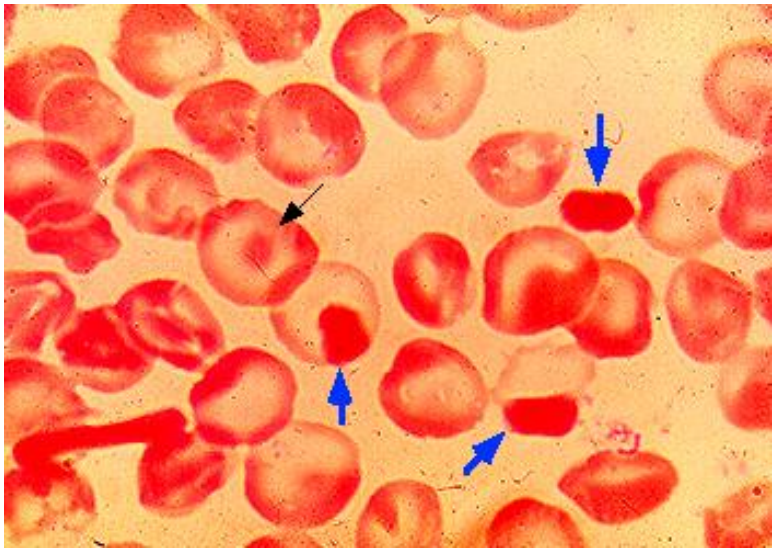
- **Valine substitution for Glutamic acid at the 6th position of b-chain**
- **Sickle crises**
- **Severe anemia**
- **Screening test - Na Metabisulfite solubility**

- **Hgb electrophoresis**



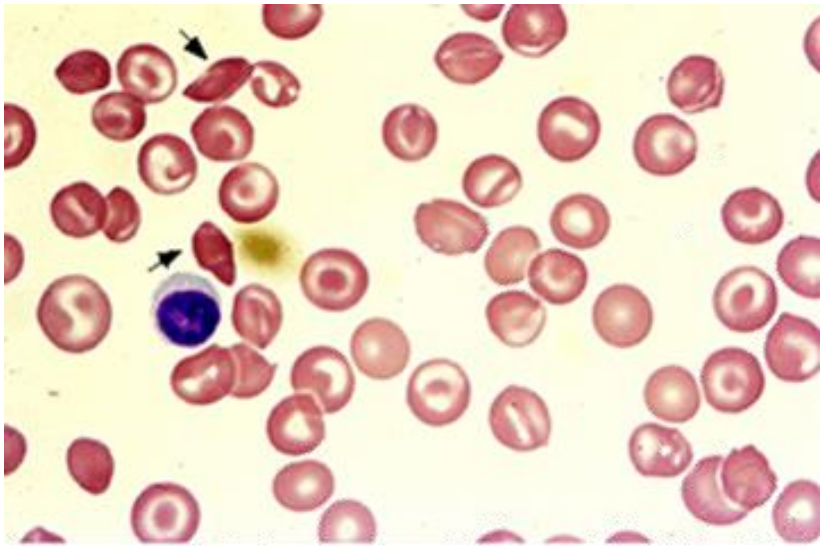
<u>HbC disease</u>

- **Mild hemolysis**
- **Splenomegaly**
- **Lysine substitution**
- **HbC crystals “bar of gold”**
- **Hgb electrophoresis**



HbSC disease

- **Sickle and SC crystals "Washington monument"**
- **Less crises**
- **More retinopathy/aseptic necrosis**



Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: ……..**.....****.....**

Hb F estimation:

Hb F may be estimated by several methods based on its resistance t denaturation at alkaline PH. by:

1. High – performance liquid chromatography (HPLC)
2. An immunological method
3. Alkaline denaturation method

Alkaline denaturation method:

Principle:

To measure the percentage of Hb F in a mixture of haemoglobins sodium hydroxide is added to a lysate and after a set of time denaturation is stopped by adding saturated ammonium sulphate the ammonium sulphate lower the PH and precipitate the denature hemoglobin. After filtration the quantity of undenatured (unprecipitated) haemoglobin is calculated as a percentage of the total haemoglobin present in the sample.

Requirement:

1. Saturated ammonium sulphate
2. EDTA blood specimen
3. Sodium hydroxide 1.2 M\L
4. Test tubes
5. Pipettes
6. Filter paper
7. Colorimeter with filter 420 – 540
8. Drabkin reagent

Procedure:

1. Preparation of haemolysate:
    A. Wash EDTA blood 3 times in normal saline (after removal of the plasma and Buffy coat)
    B. Mixed one volume washed blood cells with two volume of distilled water

C. Mixed, centrifuge and harvest pure supernatant without stroma and placed in refrigerator over night to obtain complete haemolysis.

2. Add 0.25 ml lysate to 4.75 ml cyanide solution to make a solution of haemoglobincyanide.
3. Transfer 2.8 ml of the mixture to another test tube
4. Blow in 0.2 ml of the NaOH and mix for 2 – 3 sec
5. After exactly 2 min below in 2 ml saturated ammonium sulphate solution and mix, leave tube to stand for 5 – 10 min at room temperature
6. Filter through the filter paper using a clean test tube to collect the filtrate.
7. To measure the total haemoglobin transfer 0.4 ml o the haemoglobincyanide solution rom step 2 into another tube and add 13.9 ml o water
8. Read the absorbance of the alkali resistance haemoglobin and total haemoglobin
9. Calculate the percentage of alkali Hb using the formula:
   Alkali Hb = A of resistance Hb\ A of total Hb * 100

Normal range for adult:

0.2 – 1 %

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: ……..**.....****.....**

Osmatic fragility test (oft):

Principle:

Small volumes of are mixed a large of buffered saline solution of concentration. The fraction of red cells lysed at each saline concentration is measured calorimetrically. The test is normally carried out at room temperature.

Requirement:

1. Patient and control heparinized blood
2. Stock saline equal 10 % (10 g in 100 ml DW)
3. Working buffer equal 1 % prepare from the stock saline using RV\O
4. Preparation of serial; dilution of hypotonic saline from working saline with the following concentration:- 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.45, 0.4, 0.35, 0.3, 0.2, 0.1. 0.0
5. Add to each tube 50 micro liter o well – mixed blood
6. Mix genteelly and incubate at RT for 30 min
7. Centrifuge all tubes for 5 min at 1200 rpm
8. Read OD of the test and control supernatant at 540 nm against DW as blank
9. Calculate haemolysis % for each tube compare with tube zero (complete haemolysis)
10. Haemolysis % = OD of test\ OD of complete haemolysis tube * 100
11. Construct the curve with saline concentration in X axis against haemolysis % in Y axis to obtain the MCf

Reference value:

MCf = 0.4 – 0.45

Interpretation:

Decrease fragility less than 0.3 occur in:

1. Thalassemia
2. Sickle cell anaemia
3. Iron deficiency anaemia

4. Liver diseases

Increased fragility more than 0.45 occur in:

1. Hereditary spherocytosis
2. Chemical poisoning
3. Autoimmune haemolytic anaemia
4. Burns

Lab question:

You are provided with blood sample labeled as ( ) you are asked to perform oft and comment on your result:

Tube no	Nacl conc.	Haemolysis %	point
1.	1.0		
2.	0.9		
3.	0.8		
4.	0.7		
5.	0.6		
6.	0.5		
7.	0.45		
8.	0.4		
9.	0.4		
10.	0.35		
11.	0.3		
12.	0.2		
13.	0.1		
14.	0.0	100 %	(0.0 – 100)

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Date: …….**.....****.....**

Direct and Indirect antiglobulin (coombs) test:

Direct coombs test:

Normally, red blood cells are not sensitized in vivo.

Red blood cells may become sensitized in some clinical condition includes:

1. Autoimmune hemolytic anaemia
2. Hemolytic diseases of the new born
3. Transfusion reaction

Principle:

Anti – human globulin reagent is used to detect the presence of antibody or complement directly on an RBCs due to in vivo sensitization.

Materials:

1. Test tubes
2. 5 % cell suspension in saline
3. Poly specific AHG and\ or mono specific AGH

Method:

1. Patients red cells are first washed 3 times with normal saline to remove the unbound antibodies, then add 2 drops to the test tube.
2. Add 2 drops of AGH reagent
3. Centrifuge the tube at 1000 rpm or 30 sec
4. Record the result

Interpretation of the result:

1. Positive agglutination with AGH reagent indicate that IgG antibodies or complement molecules or both are bound to the patients red cell
2. No agglutination after the addition of AGH reagent is interpreted as a negative DAT

Indirect coombs test:

Aim:

Is used to detect in vitro sensitization of red blood cells.

Principle:

The purpose o indirect antiglobulin test is to detect in vetro sensitization of red cells. This is done when sensitization does not lead to direct agglutination (this occur when there are too few antigens on the red blood cells, or too few antibodies in the serum those antibodies are mainly in the IgG class)

Materials:

1. 5 % cell suspension
2. Serum to be tested
3. Glass test tubes
4. Anti – human globulin

Method:

Stage one:

1. In a tube, mix 3 – 4 drops of serum with one drop of cells (O polled cells)
2. Incubate at 37 C for 45 min
3. Wash at least 3 times in saline to remove unbound antibody or complement

Stage two:

1. Add to the washed cells 2 drops of AHG
2. Centrifuge the tube at 1000 rpm for 30 sec
3. Record the result

Interpretation of the result:

1. Any agglutination is interpreted as positive result, and that means the red cells are sensitized by unexpected antibody or complement present in the patient serum.
2. No agglutination interpreted as negative result.

Uses o indirect antiglobulin test (IAT):

1. Cross match
2. Antibodies detection
3. Antibodies identification
4. Student's findings (measurements or observations):
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5. comments and interpretation:
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6. Evaluation (carried out by the instructor):
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7. Name and signature of the instructor:
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8. Date: ……..\.....\.....

Acidified serum lysis test

(Hams test)

Principle:

The patient red cells are exposed at 37 C to the action of normal or the patient's own serum that suitably acidified to the optimum PH for lysis (PH 6.5 – 7.0).

The patient serum is best obtained by defibrination the normal plasma is preferred tom patients serum.

Method:

1. Labeled 3 test tubes as test and 3 tubes as control

Reagent						
fresh normal serum	0.5 ml	0.5 ml	_	0.5 ml	0.5 ml	
Inactivate serum	_	_	0.5 ml	_	_	0.5
HCL	0.05 ml	_	0.05	0.05	_	0.05
Patient cells	0.05 ml	0.05	0.05	_	_	_
Normal cells	_	_	_	0.05	0.05	0.05

2. Mix the content carefully and leave the tubes at 37 C. centrifuge them after 1 hr

Interpretation:

If the cells are from PNH patient they undergo define complete lysis in the acidified serum ant not in the other serum.

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: ……..**.....****.....**