

Peripheral Blood Film Reading and Examination.

MLS 332 Dec 2023

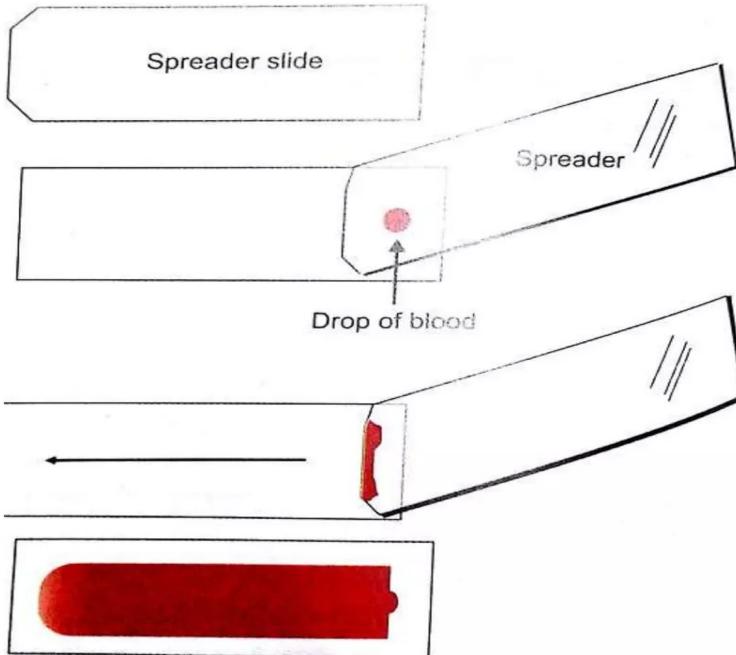
Why do we examine blood film

- To evaluate for anaemia
- To evaluate thrombocytopenia/thrombocytosis
- To identify abnormal cells (blasts/abnormal promyelocytes/atypical lymphoid)
- Infections like malaria and microphiliaria
- Inclusions like basophilic strippling, howell-jolly bodies, cabot ring
- To estimates the cell count
- Proportions of the different type of WBC
- Assess the morphology of blood cells

Peripheral Smear Preparation

- Smear should be made within 1 hour of blood collection from EDTA specimen
- Can be made from finger prick directly into slide

SMEAR PREPARATION



1. Place a drop of blood, about 2-3 mm in diameter approximately 1 cm from one end of slide.
2. Place the slide on a flat surface, and hold the other end between your left thumb and forefinger.
3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
4. Hold the spreader slide at a 30°- 45 angle, and draw it back against the drop of blood
6. Allow the blood to spread almost to the edges of the slide.
7. Push the spread forward with one light, smooth moderate speed. A thin film of blood in the shape of tongue.
8. Label one edge with patient name, lab id and date.
9. The slides should be rapidly air dried by waving the slides or using an electrical fan.

Characteristics of A Good Smear

- 1. Good smear is tongue shaped with a smooth tail.**
- 2. Does not cover the entire area of the slide.**
- 3. Has both thick and thin areas with gradual transition.**
- 4. Does not contain any lines or holes.**

The thickness of the smear

Is determined by:

1. The angle of the spreader slide. (the greater the angle, the thicker and shorter the smear).
2. Size of the blood drop.
3. Speed of spreading

Common causes of a poor blood smear

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 entire edge of the spreader slide against the slide while making the smear.
4. Failure to keep the spreader slide at a 30° angle with the slide

- Slide fixing and staining

Blood films are stained using romanowsky stains

- Leishman stain
- Field's stain
- Automated slide stainer

Principle

- Leishman's stain is a polychromatic stain
- Components:
 - Methanol: fixes cells to slide
 - Methylene blue stains RNA,DNA: blue-grey color
 - Eosin stains hemoglobin, eosin granules orange-red color
 - Eosin + Methylene Blue = thiazine eosinate complex
- The complex will not stain any color unless a buffer is added: 0.05M sodium phosphate (pH 6.4) and aged distilled water (pH 6.4-6.8)

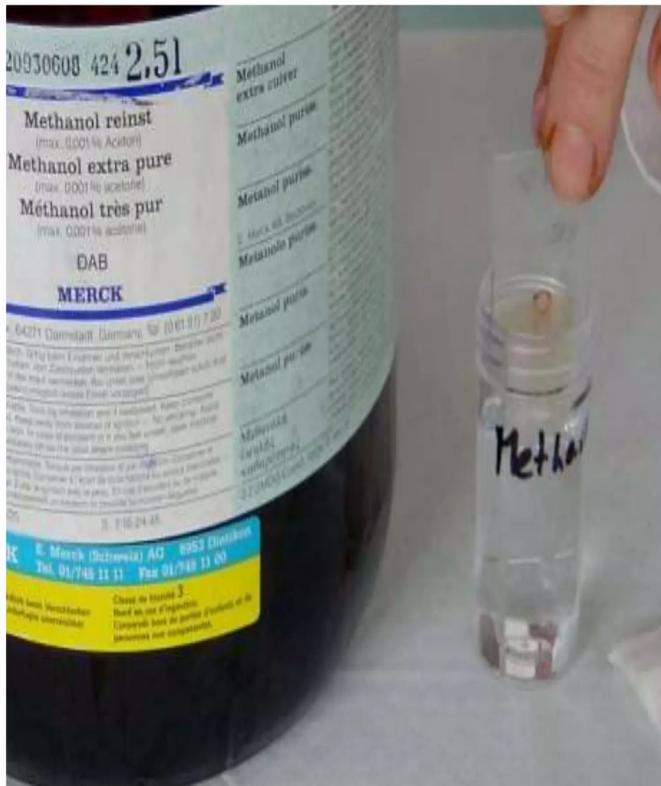
Staining Procedure

- Thin smear are air dried.
- Flood the smear with stain.
- Stain for 1-5 min.
 - Experience will indicate the optimum time.
- Add an equal amount of buffer solution and mix the stain by blowing an eddy in the fluid.
- Leave the mixture on the slide for 10-15 min.
- Wash off by running water directly to the centre of the slide to prevent a residue of precipitated stain.
- Stand slide on end, and let dry in air.

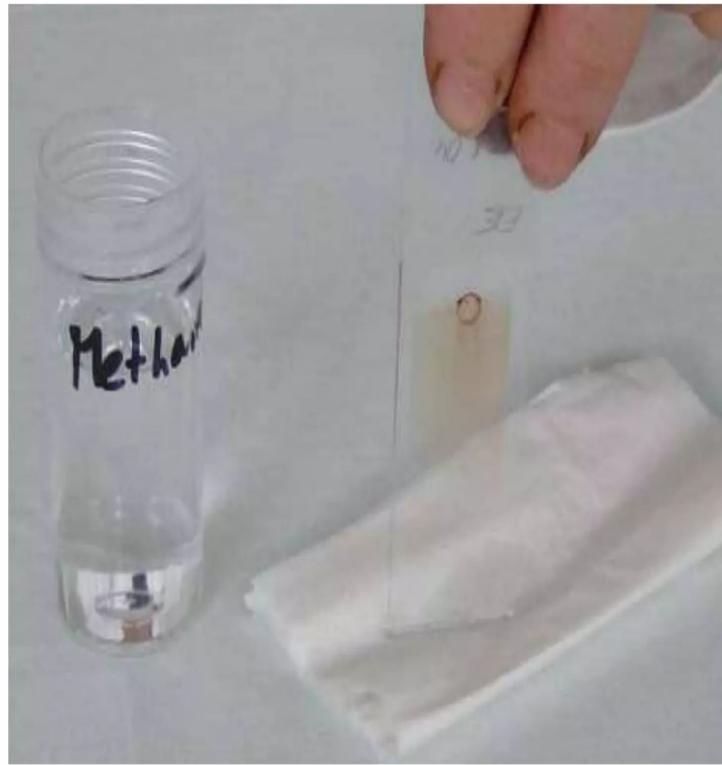
Rapid staining method- Field's stain

- **Advantage**
 - Fast, convenient and takes about 1 minute
 - Cost effective

- **Components**
 - Methanol
 - Solution B- contains eosin
 - Solution A- contains methylene blue



Dip in methanol to fix the smear for 1 minute



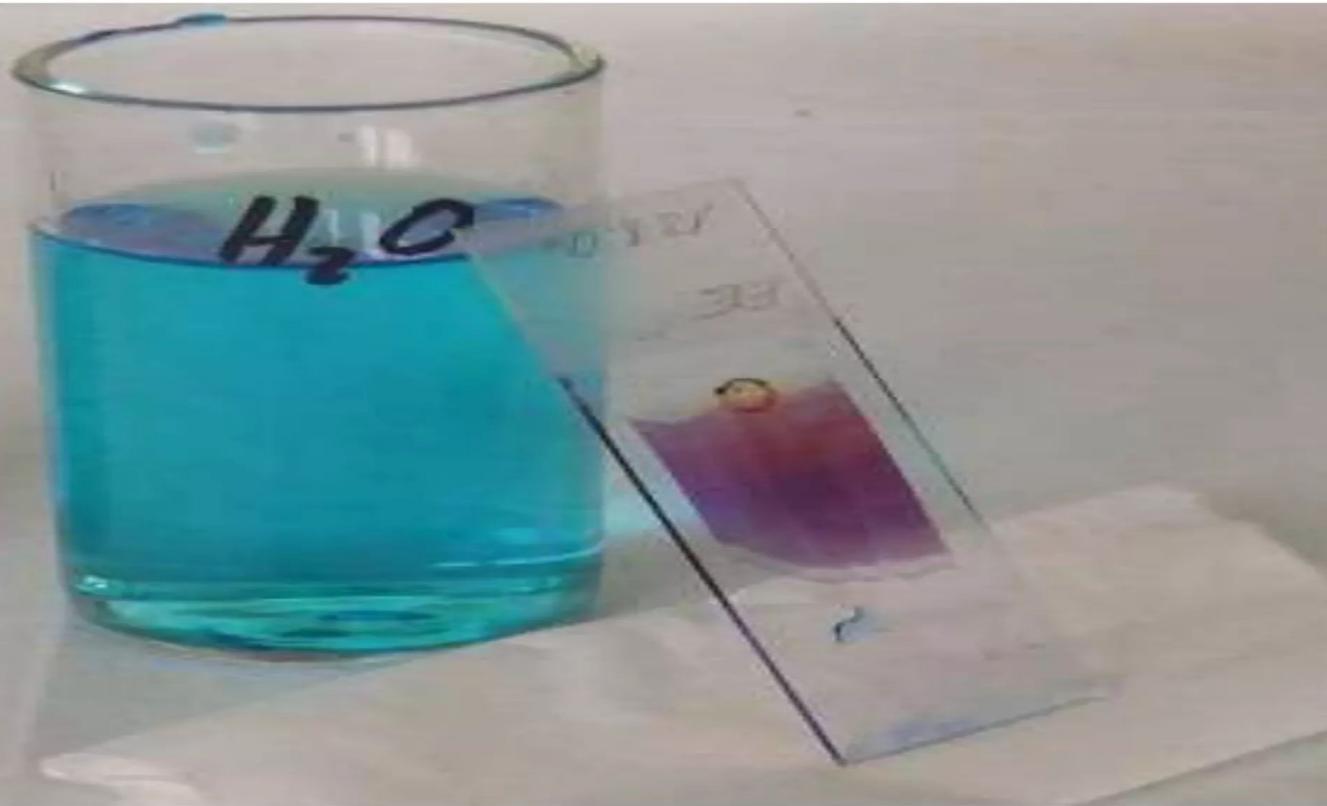
Dry microscopic slide on filter paper



Immerse slide in Field's stain A (Methylene blue) for 10 seconds



Immediately wash with tap water!



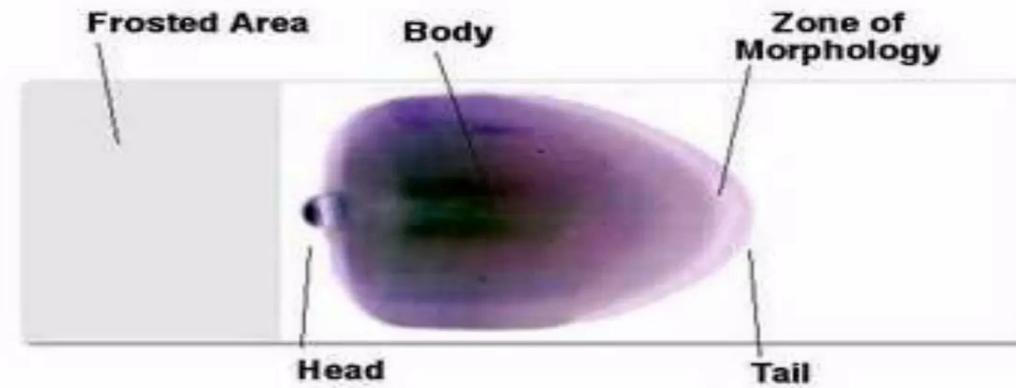
Dry thin films

Automated Slide Stainers

- It takes about 5-10 minutes to stain a batch of smears
- Slides are just automatically dipped in the stain in the buffer and a series of rinses
- Disadvantages
 - Staining process has begun, no STAT slides can be added in the batch
 - Aqueous solutions of stains are stable only for 3-6 hours

Characteristics of a Good Smear

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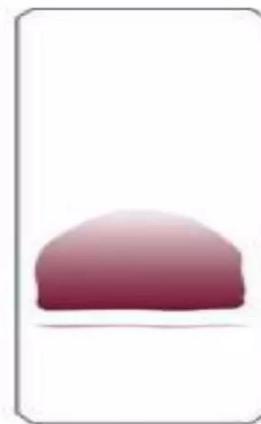
A



B



C



D



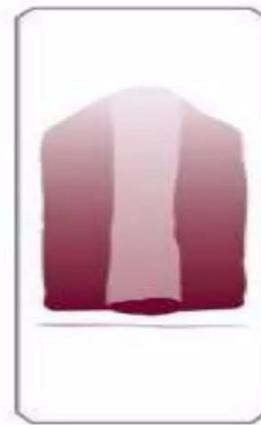
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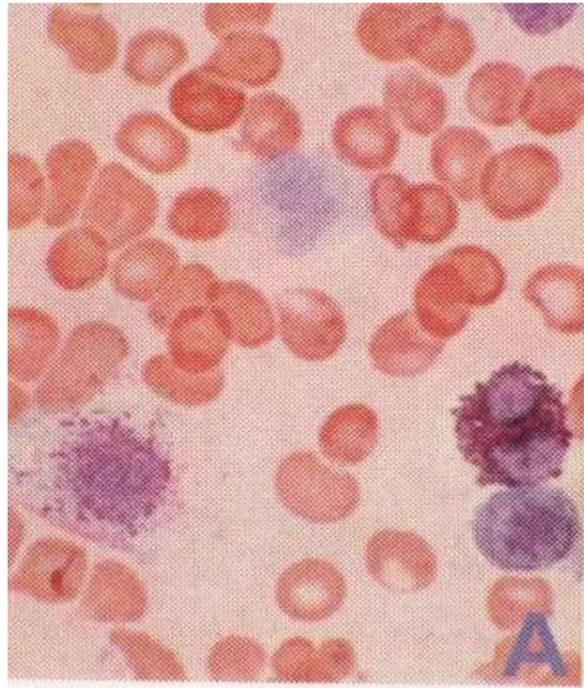
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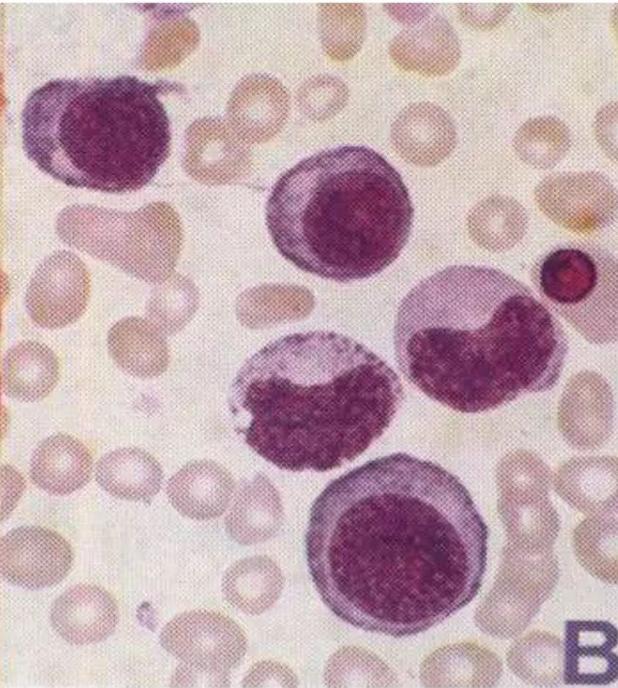
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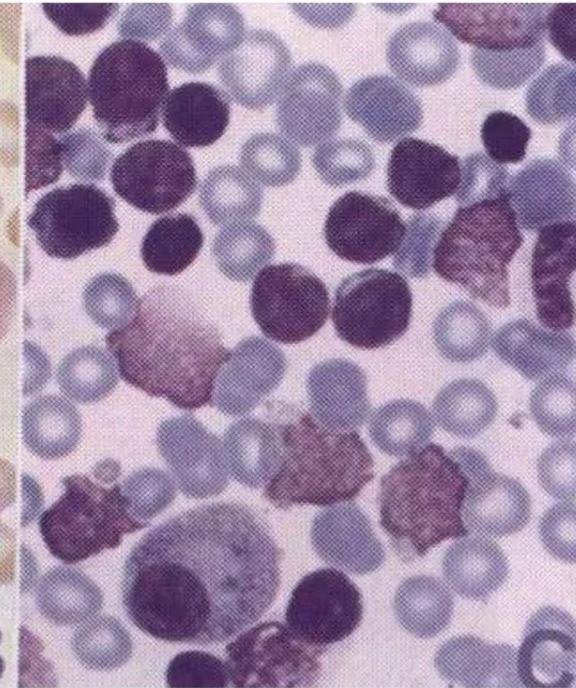
H



TOO ACIDIC



SUITABLE



TOO BASIC

Causes and corrections

- **Too acid stain:**
 - Insufficient staining time
 - Prolonged buffering or washing
 - Old stain
- **Correction:**
 - Lengthen staining time
 - Check stain and buffer pH
 - Shorten buffering or wash time

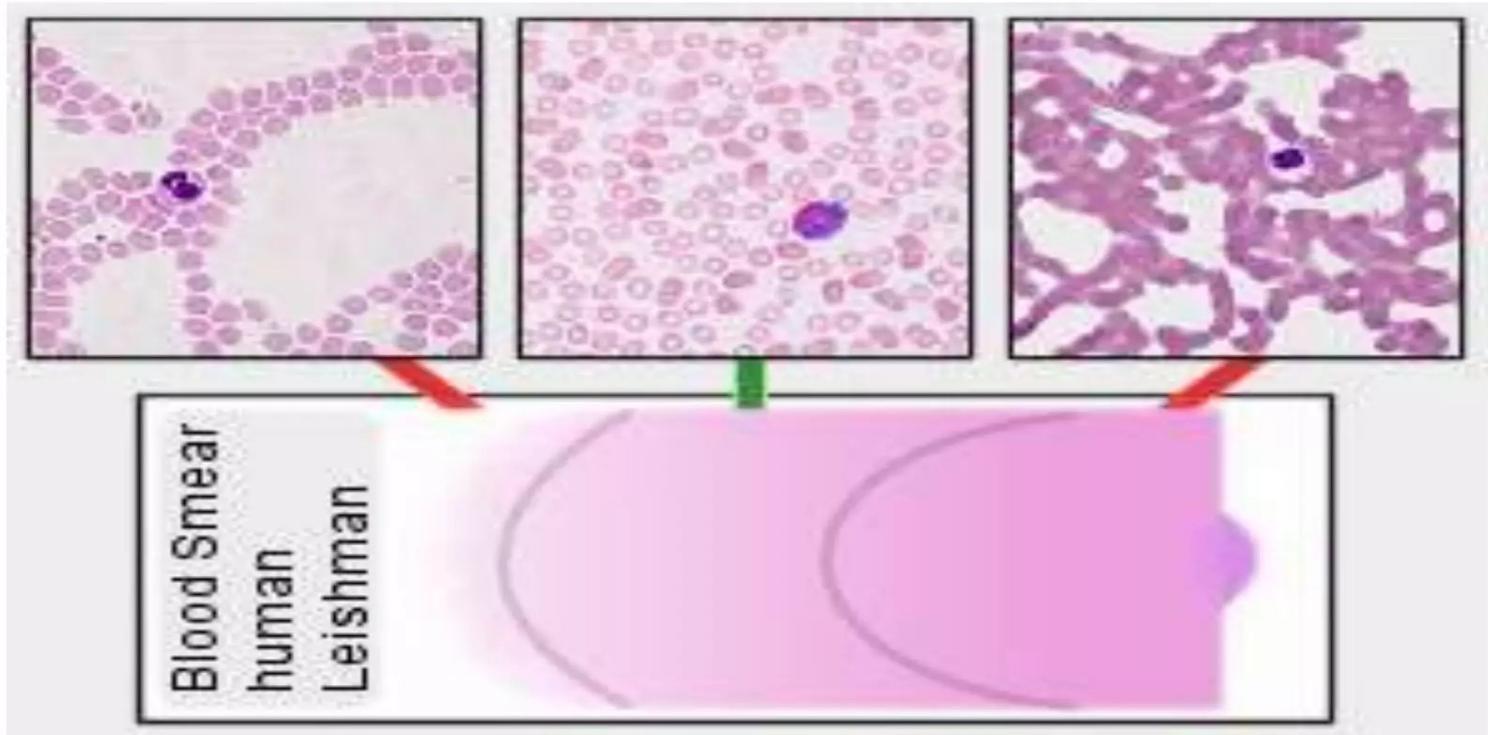
Causes and corrections

- **Too alkaline stain**
 - Thick blood smear
 - Prolonged staining
 - Insufficient washing
 - Alkaline pH of stain components
- **Correction**
 - Check pH
 - Shorten stain time
 - Prolong buffering time

Features of a well-stained PBS

- Macroscopically
 - Color should be pink to purple
- Microscopically
 - RBCs: orange to salmon pink
 - WBCs
 - Nuclei is purple to blue
 - Cytoplasm is pink to tan
 - Granules is lilac to violet
 - Eosinophil: granules orange
 - Basophil: granules dark blue to black

Zones of a slide

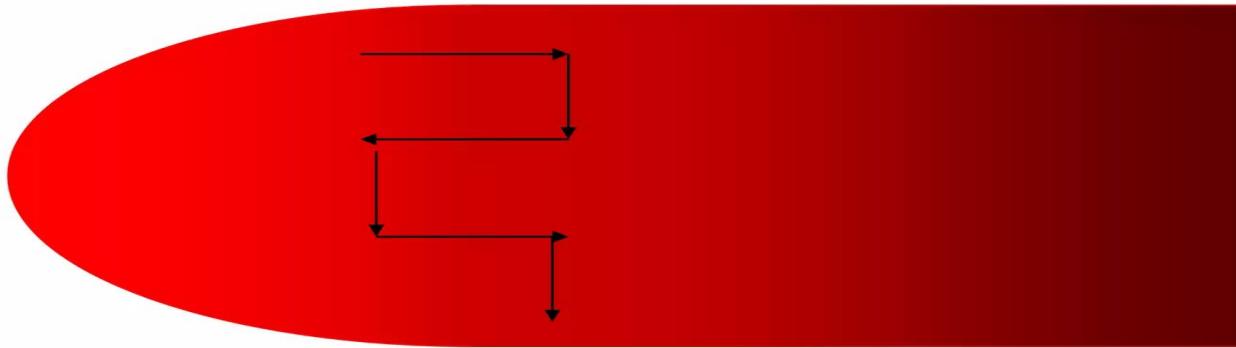


10x Objective

- Assess overall quality of the smear i.e feathery edge, quality of the color, distribution of the cells and the lateral edges can be checked for WBC distribution
- Snow-plow effect: more than 4x/cells per field on the feathery edge: Reject
- Fibrin strands: Reject

Total Leucocyte Count

- 40x Objective
 - Use dry without oil
 - Choose a portion of the peripheral smear where there is only slight overlapping of the RBCs.
 - Count 10 fields, take the total number of white cells and divide by 10.
 - To do a WBC estimate by taking the average number of white cells and multiplying by 2000.
 - Normal leucocyte count ranges from 4000 to 11000/ μ l



Observe one field and record the number of WBC according to the different type then turn to another field in the snake-like direction

To avoid repeat or miss some cells

Manual Differential Counts

- These counts are done in the same area as WBC and platelet estimates with the red cells barely touching.
- This takes place under $\times 100$ (oil) using the zigzag method.
- Count 100 WBCs including all cell lines from immature to mature.
- Expressed as percentage.
- Absolute number of cells/ μl = % of cell type in differential \times white cell count

Nucleated Red Blood Cells (nRBCs)

- If 10 or more nucleated RBC's (nRBC) are seen, correct the TLC
- Corrected WBC Count = $\text{WBC} \times 100 / (\text{nRBC} + 100)$

Example:

If WBC = 5000 and 10 nRBCs have been counted

Then $5,000 \times 100 / 110 = 4545.50$

The corrected white count is 4545.50.

WBCs in PBS

- **GRANULOCYES**

Neutrophils (polymorphonuclear leucocytes)

Eosinophils

Basophils

- **AGRANULOCYTES**

Lymphocytes

Monocytes

normal

mild-moderate left shift

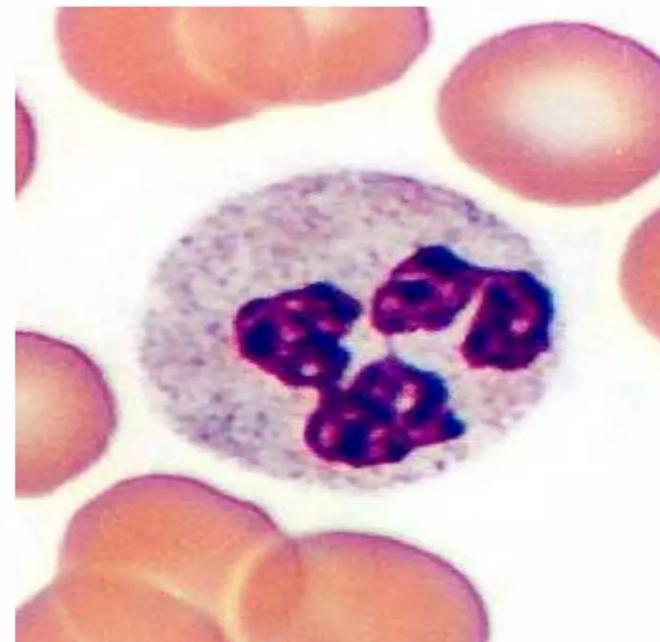
severe left shift



Increasing Neutrophil Maturity

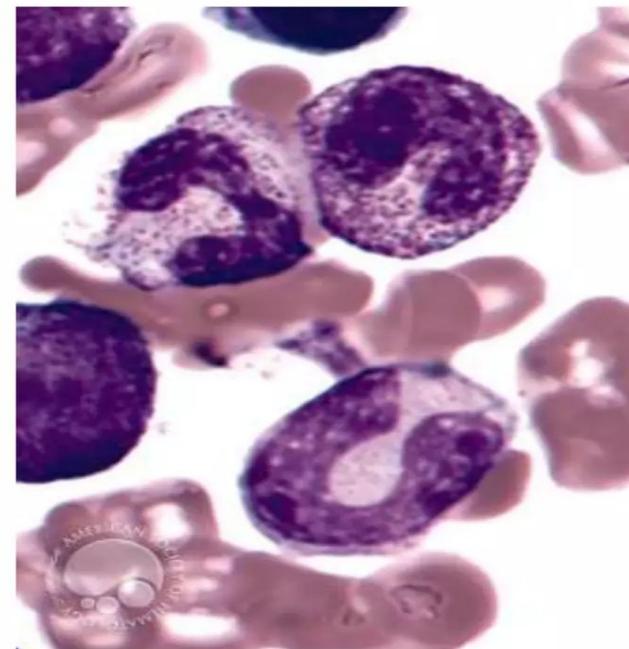
Polymorphonuclear neutrophils

- The terminal stage of development measuring **12-14 µm** in diameter
- Characterised by a **lobulated** nucleus
- **Two to five lobes** of clumped chromatin linked by a thin chromatin strand
- The cytoplasm contains **fine azurophilic granules**



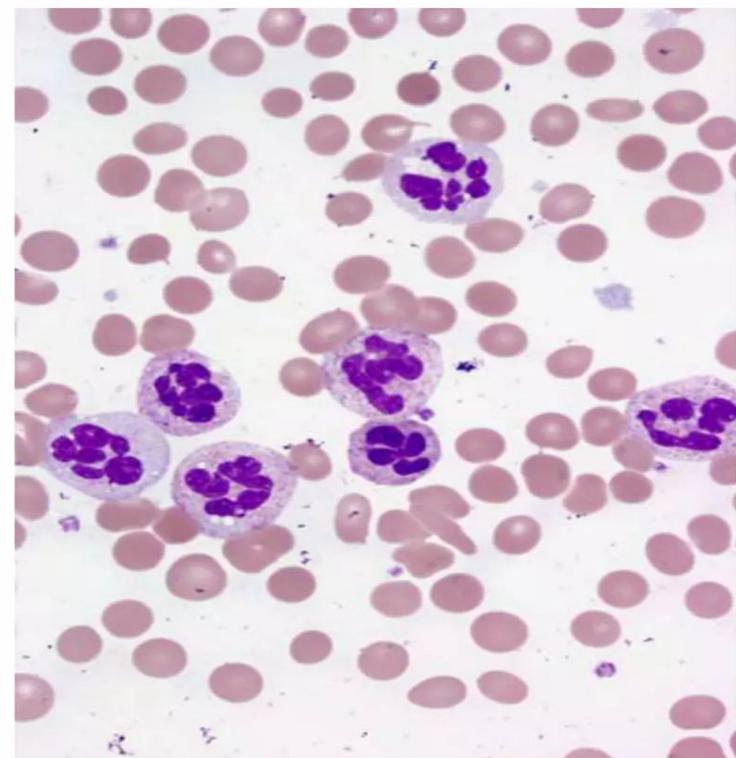
Band/stab forms

- Cells without complete formation of nuclear lobes are classified as band forms
- When **degree of indentation is more than 50%** of the nuclear diameter
- Measure **10-16 µm**
- Plentiful pink cytoplasm with granules
- **Sausage shaped or band shaped nucleus**



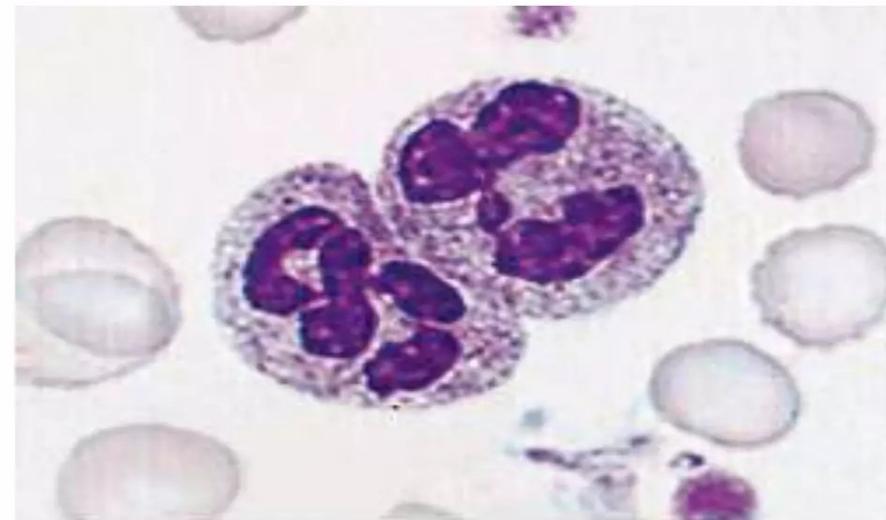
Hypersegmented neutrophils

- Presence of even a single neutrophils with six or more lobes or the presence of more than 5% of neutrophils with five lobes.
- Seen in Megaloblastic anemia
 - Uraemia
 - Drugs-cytotoxic treatment with methotrexate
 - Hydroxycarbamide
 - Myelokathexis



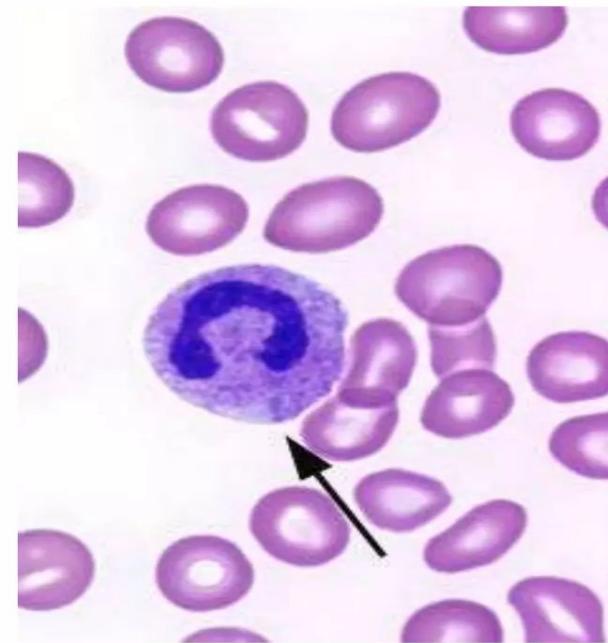
Granules

- Toxic granulation- increase in staining density and number of granules
- Seen with
 - Bacterial infections
 - Burns
 - Administration of G-CSF, GM-CSF



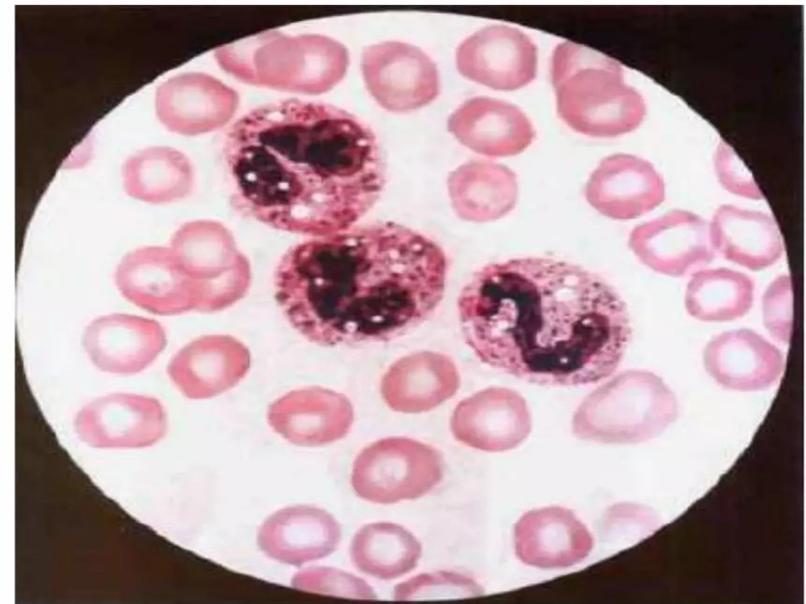
Dohle Bodies

- Small, round or oval, pale blue-grey structure
- Found at periphery of neutrophil
- Contains Ribosomes and Endoplasmic reticulum
- Seen in
 - Bacterial infection
 - Inflammation
 - Administration of G-CSF
 - During pregnancy



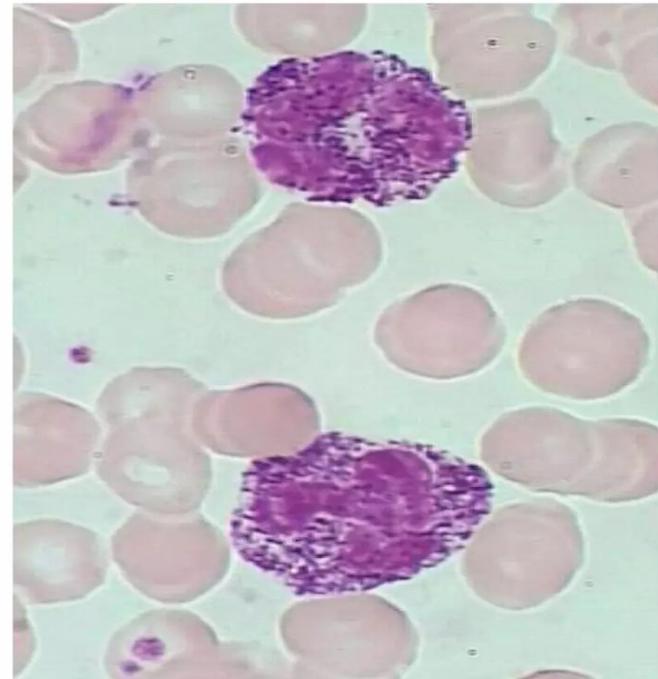
Vacuoles in neutrophils

- In fresh blood smear vacuoles seen in severe sepsis
- Indicative of phagocytosis
- As an artifact with prolonged standing



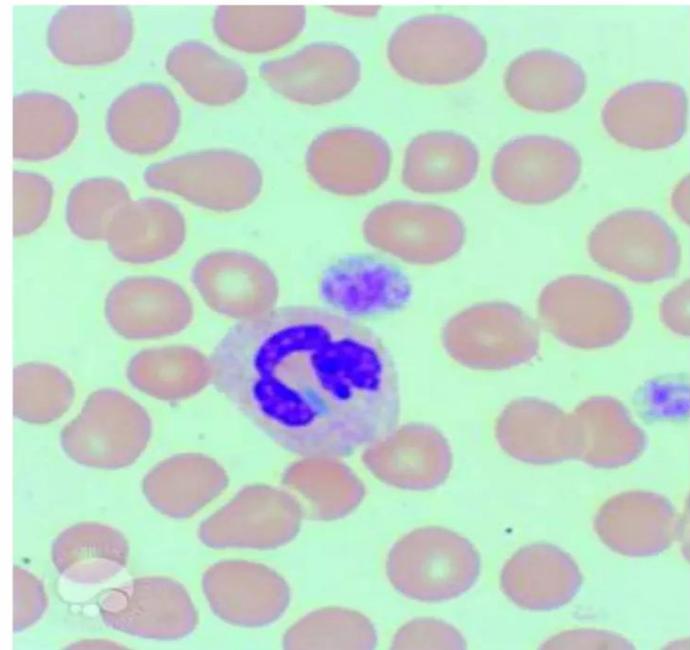
Alder–Reilly anomaly

- This abnormality is commonly seen in mucopolysaccharidoses such as Hurler's and Hunter's syndrome.
- Granules are large,
 - Discrete,
 - Stain deep red
 - May obscure the nucleus
 - Neutrophil function is normal



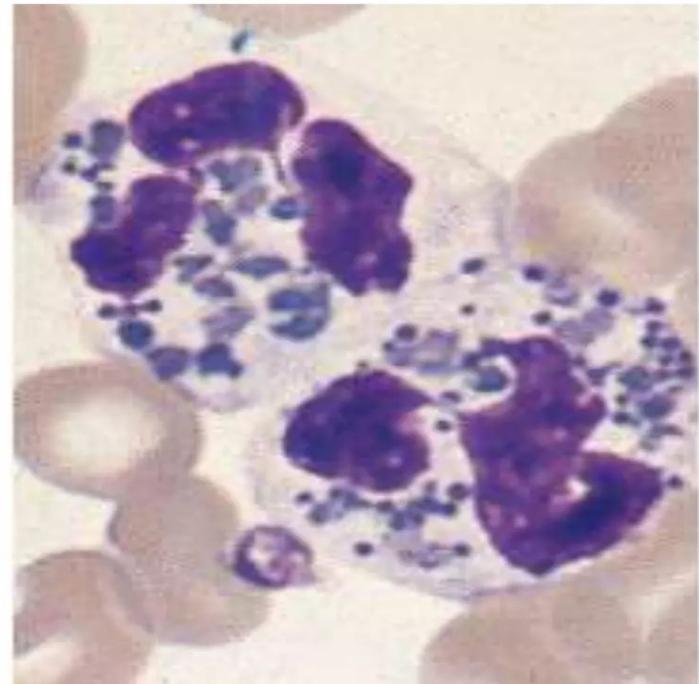
May–Hegglin anomaly

- Autosomal dominant inheritance
- Triad of thrombocytopenia, giant platelets, and Döhle body-like inclusion bodies in granulocytes
- MYH-9 gene



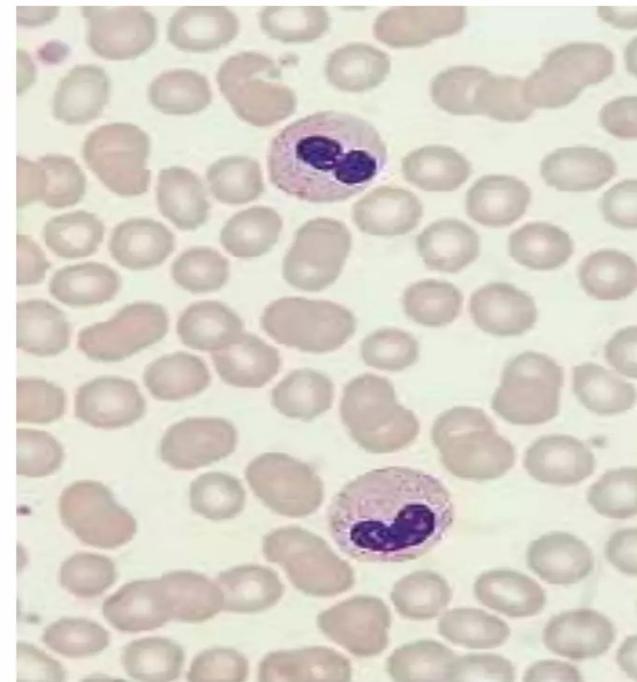
Chédiak-Higashi Syndrome

- Rare autosomal recessive disease
 - Immune deficiency,
 - Poor resistance to bacterial infections,
 - Oculocutaneous albinism,
 - Bleeding tendency,
 - Multiple neurologic abnormalities
- Giant peroxidase-positive lysosomal granules in granulocytes



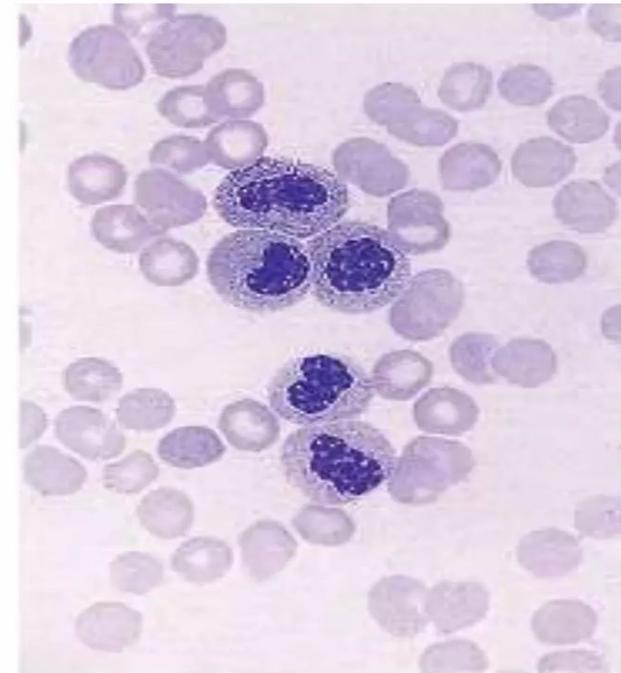
Pelger–Huët Cells

- Pelger–Huët anomaly
- Benign inherited condition.
- Neutrophil nuclei fail to segment properly.
- Majority of circulating neutrophils have only two discrete equal-sized lobes connected by a thin chromatin bridge.



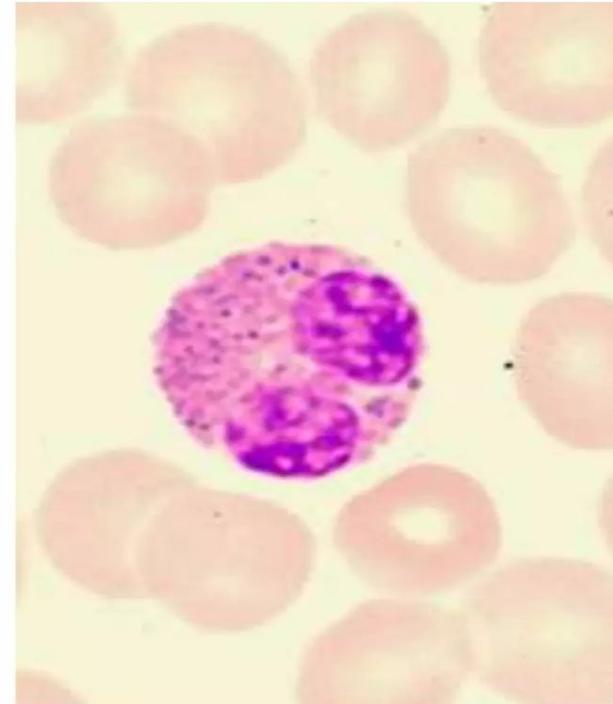
Pseudo-Pelger cells

- Pseudo-Pelger cells or the acquired Pelger–Huët anomaly
- Acquired condition
- Morphologically similar to Pelger–Huët anomaly
- Seen in
 - Myelodysplastic syndromes,
 - Acute myeloid leukaemia with dysplastic maturation,
 - Occasionally in chronic myelogenous leukaemia



Eosinophils

- They are slightly larger than a segmented neutrophil measuring **12-16 µm**
- **Two nuclear lobes** are generally present giving the nucleus a **spectacle shape**
- The cytoplasm has a pale hue and has **numerous dense orange red**

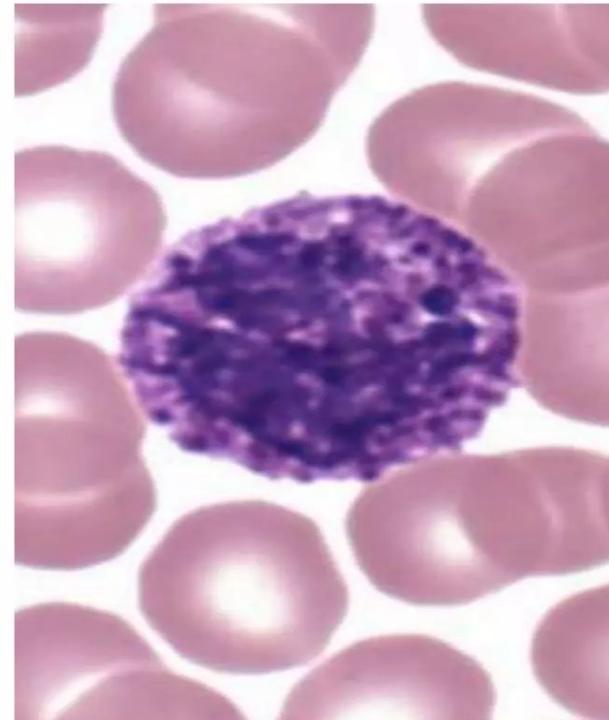


Abnormalities in Eosinophils

- Increase in the absolute eosinophil count in the peripheral blood
- Mild eosinophilia-
 - Allergic conditions hay fever, Asthma, eczema
- Severe eosinophilia-
 - Parasitic infection
 - Reactive eosinophilia
 - Eosinophilic leukaemia
 - Idiopathic hypereosinophilic syndrome

Basophils

- Basophils have a diameter of **10-14 µm**
- Lobulated nucleus
- Distinguished by their **large, coarse, purplish-black granules**
 - Fill the cytoplasm
 - Obscure the nucleus
- Granules are rich in histamine, serotonin and heparin

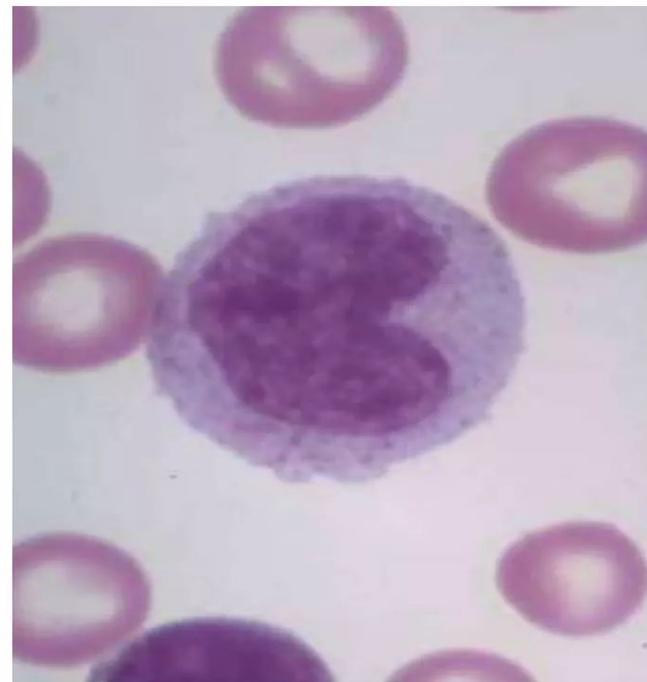


Basophilia

- Myeloproliferative disorders (e.g., chronic myelogenous leukemia)
- IgE mediated Hypersensitivity reactions
- Mastocytosis
- Ulcerative colitis
- Hypothyroidism

Monocytes

- Monocytes are **10 to 11 µm**
- The nucleus is **large** and oval or **indented** and centrally placed.
- The nuclear chromatin is delicate
- The cytoplasm is abundant, is **gray or light blue-gray** and contains numerous vacuoles
- The granules resemble fine dust and give the bluish cytoplasm a ground-glass appearance

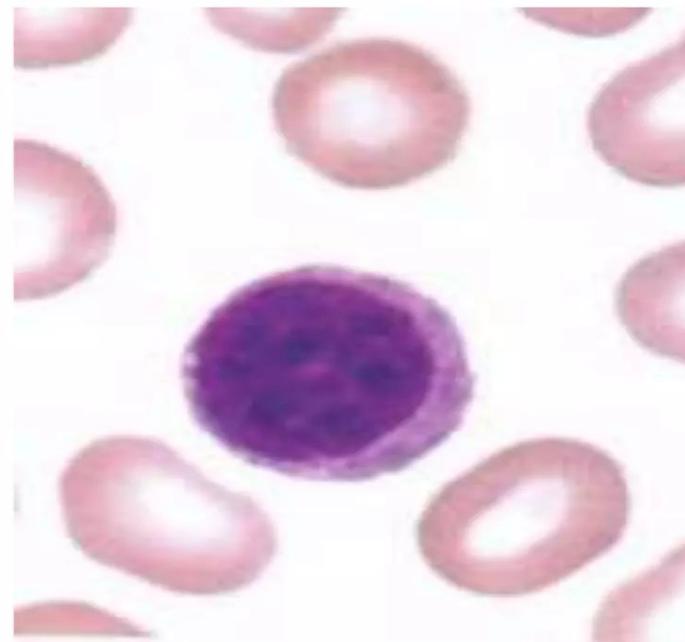


Monocytosis

- Chronic infections and inflammatory conditions such as
 - Malaria,
 - Typhoid,
 - Bacterial endocarditis,
 - Kala- azar
 - Tuberculosis
 - Crohn's disease
 - Infectious Mononucleosis
- Hematolymphoid malignancies
 - Acute myelomonocytic leukaemia (AML M4),
 - Acute monocytic leukaemia (AML M5),
 - Myeloproliferative neoplasms,
 - Chronic myelomonocytic leukaemia,
 - Myelodysplastic syndrome

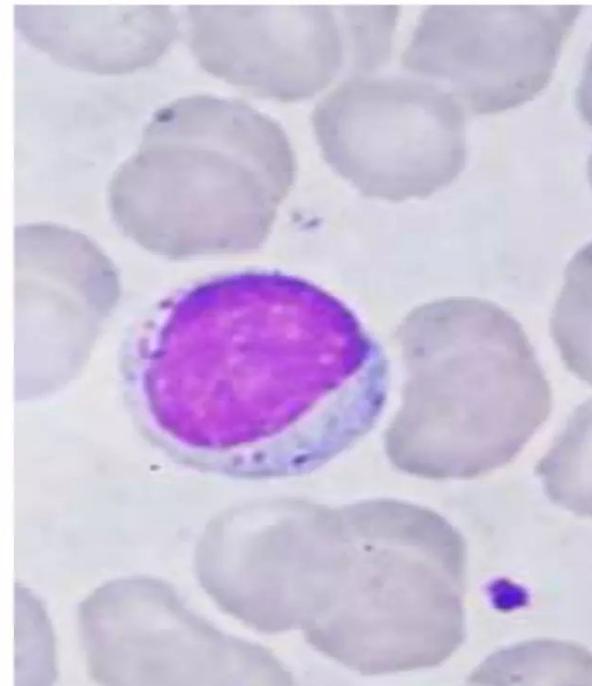
Small lymphocyte

- Measuring 9-12 μm
- Smaller than granulocytes
- Cytoplasm in the form of a **thin rim around the nucleus**
- Round or slightly indented nucleus
- **Heavily clumped deeply staining chromatin**



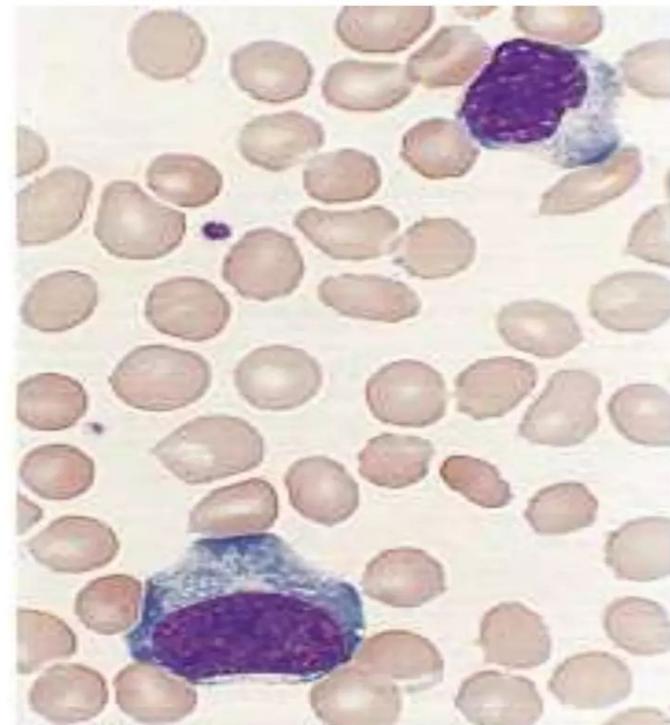
Large lymphocyte

- Measuring **12-15 µm**
- Round in outline
- Nucleus is round to slightly indented with clumped chromatin
- Cytoplasm is **more abundant** than lymphocyte and is pale blue in color



Reactive lymphocytes (Downey cells)

- Have slightly larger nuclei with more open chromatin
- Abundant cytoplasm that may be irregular. (scalloping/skirting RBCs)
- Seen in
 - Infectious



Summarizing WBC parameters

- STEP 1
WBC increased : leukocytosis
WBC decreases: leukopenia
- STEP 2
Relative differential count
- STEP 3
Absolute Cell Counts
- STEP 4
Examination for immature cells
Young cells should not be seen in the peripheral blood smear
Immature cells: possess a nucleus
 - do not lyse during testing
 - can be counted as WBC and falsely elevateWBC results

- **White Blood Cells.**

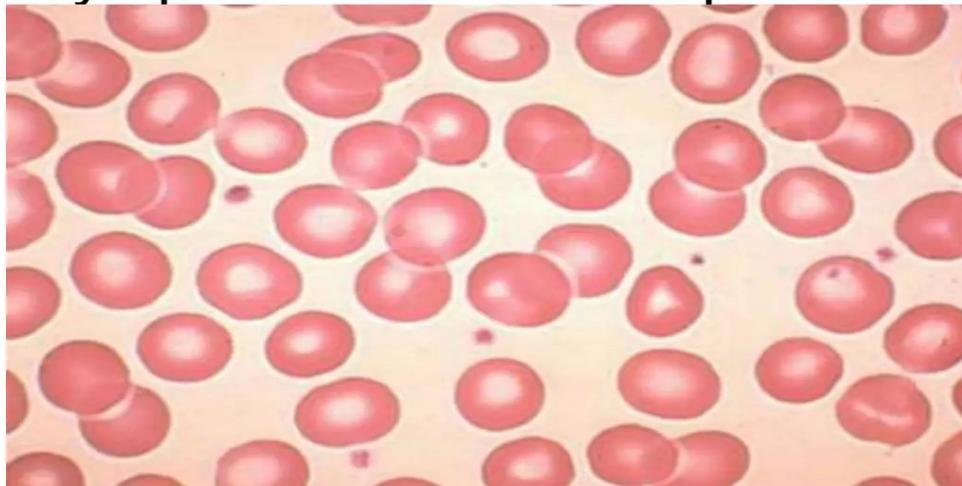
1. Check for even distribution and estimate the number present (also, look for any gross abnormalities present on the smear).
2. Perform the differential count.
3. Examine for morphologic abnormalities.

RBC morphology

- Scan area using $\times 100$ (oil immersion).
- Observe 10 fields.
- Red cells are observed for
 - Size,
 - Shape,
 - Hemoglobin content,
 - Inclusions
- Abnormal morphology
 - Note that red cell morphology must be scanned in a good counting area

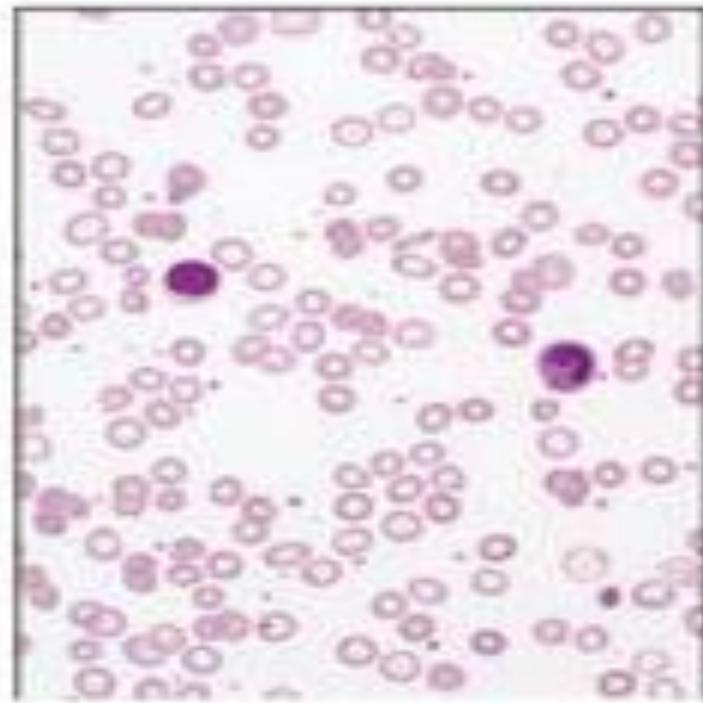
RBC

- In the blood from healthy person RBCs are
 - Circular , Homogenous disc nearly of uniform size (7–8 μm)
 - Deep pink cytoplasm with Central pallor <1/3rd



Hypochromia

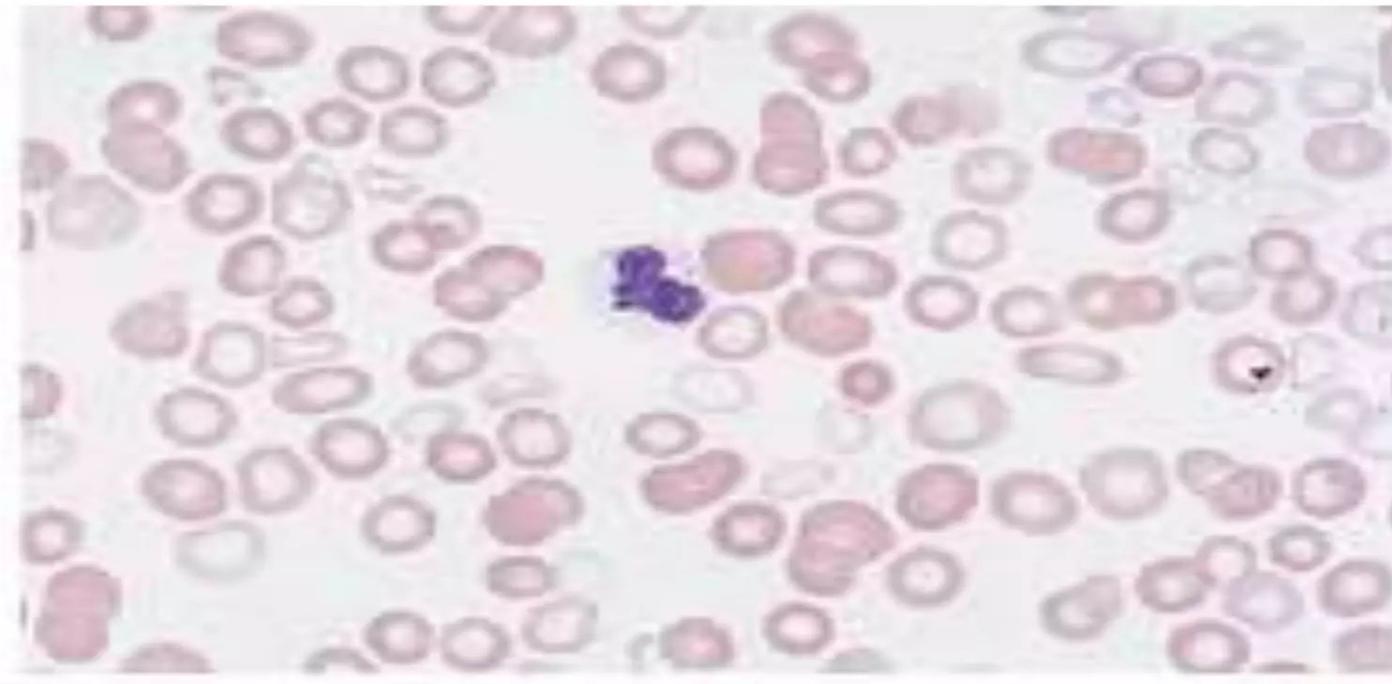
- Decrease in hemoglobin content of RBC
- Increase in central pallor(>1/3rd)
- Decrease in MCH and MCHC
- Seen in various anaemias



Dimorphic anaemia

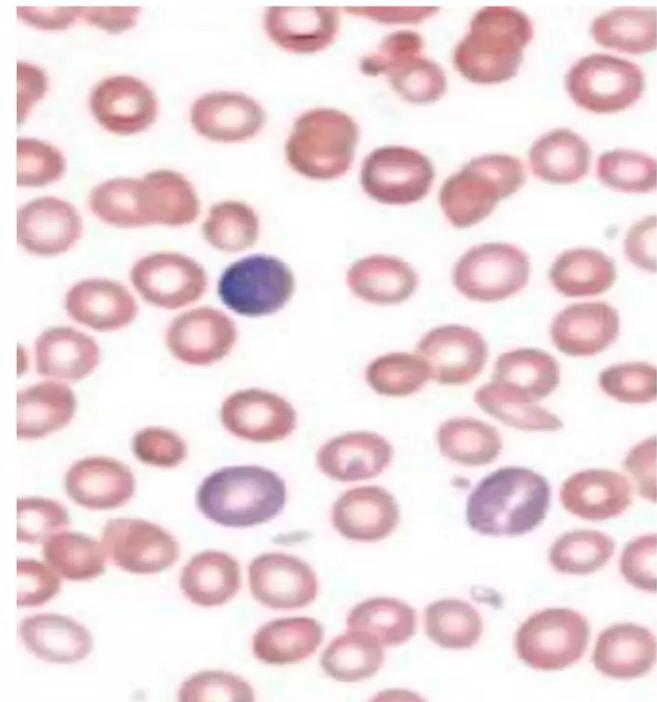
- Presence of anisocytosis and anisochromia in the same film.
- Seen in
 - Coexistence of iron deficiency and megaloblastic anaemia
 - Sideroblastic anemia
 - Some weeks after iron therapy for iron deficiency anemia
 - Hypochromic anemia after transfusion with normal cells

Dimorphic blood picture



Polychromatophilia

- Blue grey tint of red cells
- Due to presence of residual RNA in young cells.
- Larger than normal and may lack central pallor
- Implies Reticulocytosis and therefore marrow

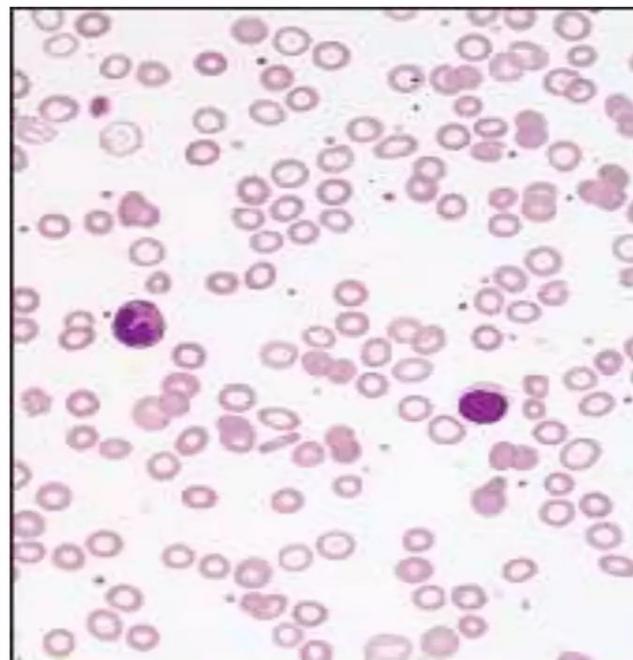


Variation In Size

- Anisocytosis- Variation in size of the red blood cells
- Normal MCV is ~80-100 fl
- Microcytes (MCV <80 fl)
- Macrocytes (MCV >100fl)
- Anisocytosis is a feature of most anemias.

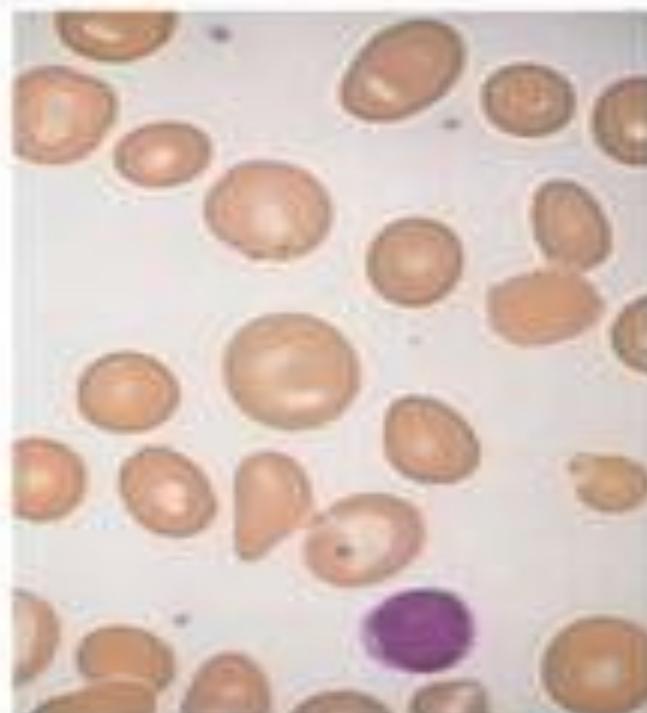
Microcytes

- Size of RBC is reduced (<80fl)
- Seen when hemoglobin synthesis is defective
 - Iron deficiency anemia
 - Thalassemia
 - Anemia of chronic disease
 - Sideroblastic anemia



Macrocytes

- When MCV of RBC is increased(>100fl)
- Seen in
 - Vit B12 and folate deficiency
 - Alcoholism
 - Hepatic disease
 - Haemolytic states
 - Hypothyroidism
 - Following treatment with chemotherapeutic drugs

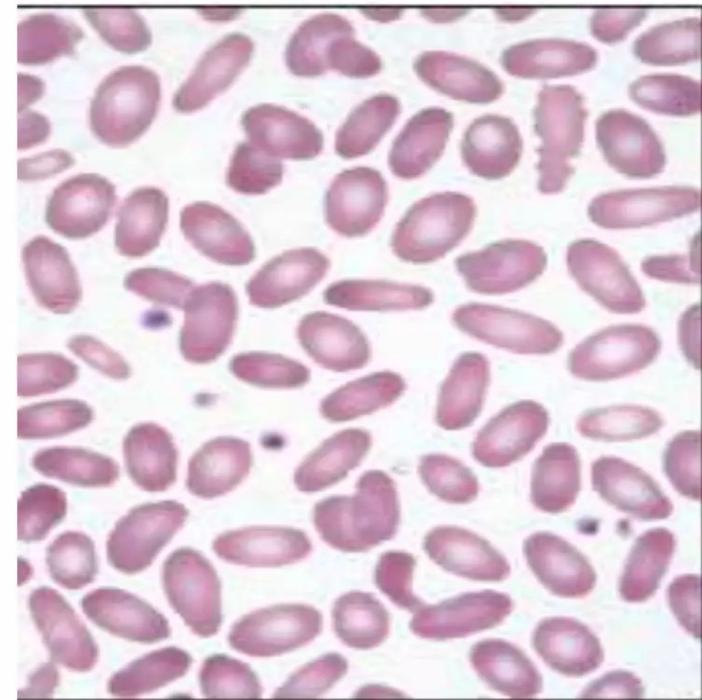


Shape

- Variation in shape is called Poikilocytosis.
- It is of following types-
 - Elliptocytes
 - Spherocytes
 - Target cells
 - Schistocytes
 - Acanthocytes
 - Keratocytes
 - Echinocytes

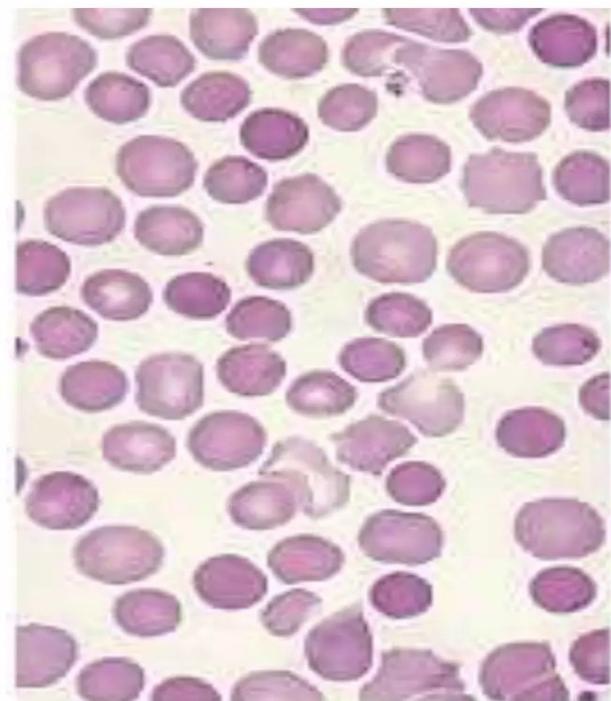
Elliptocytes

- Elliptical in shapes
- Most abundant in hereditary elliptocytosis
- Seen in –
 - Iron deficiency anemia
 - Myelofibrosis with myeloid metaplasia
 - Megaloblastic anemia



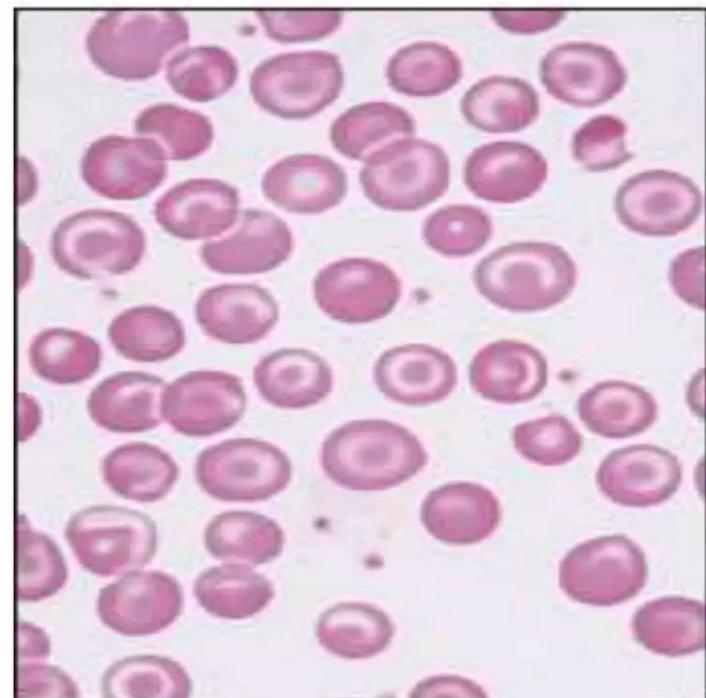
Spherocytes

- Nearly spherical
- Diameter is smaller than normal
- Lack central pale area or have a smaller , eccentric, pale area
- Seen in
 - Hereditary spherocytosis
 - Some cases of autoimmune hemolytic anemia
 - Direct physical or chemical injury



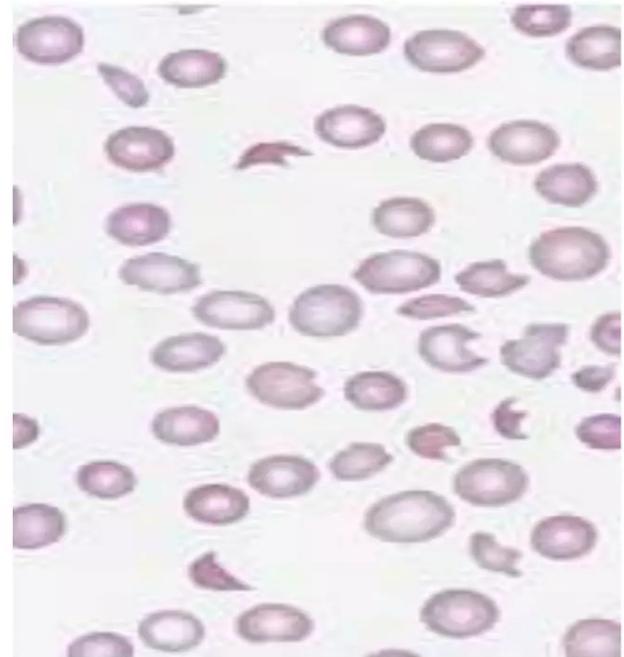
Target cells

- Cells in which central round stained area & peripheral rim of cytoplasm
- Seen in
 - Sickle cell anaemia
 - Thalassemia major
 - Hemolytic anaemias
 - Postsplenectomy



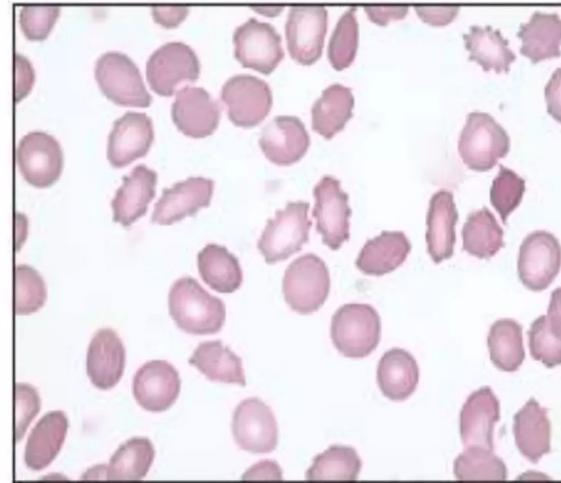
Schistocytes

- These are fragmented erythrocytes.
- Smaller than normal red cells and of varying shape
- Hallmark in the diagnosis of hemolytic anaemias
- Cardiac anaemia
- Microangiopathic hemolytic anaemias



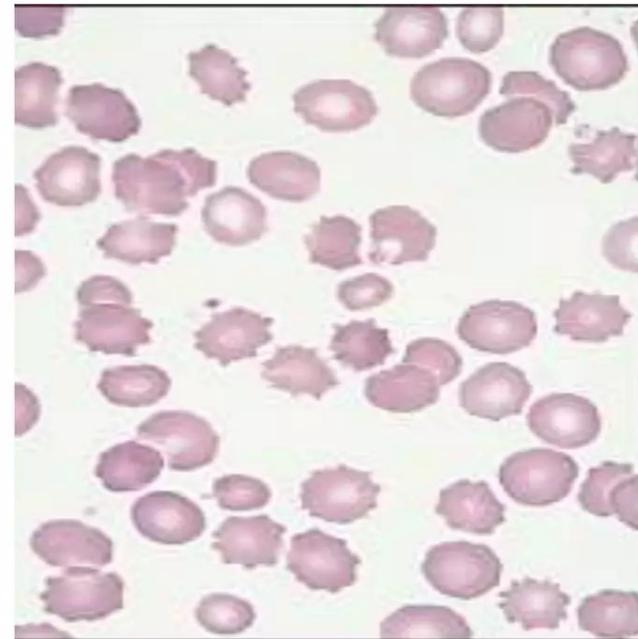
Acanthocytes

- Thorny projections on red cell membrane
- Few, irregular, non-uniform
- Seen in
 - Abetalipoproteinemia
 - Spur cell hemolytic anaemis
 - Hypothyroidism
 - Liver disease
 - McLeod phenotype



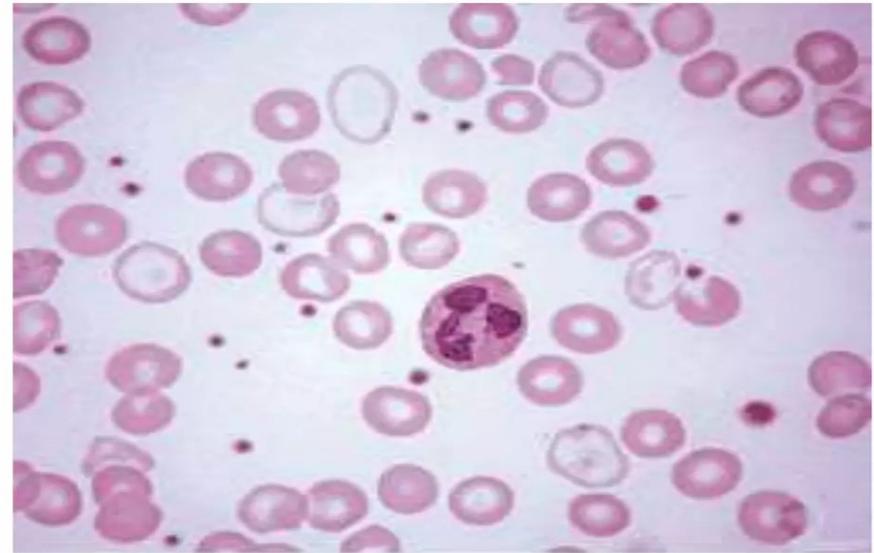
Echinocytes (Burr cells)

- Numerous, short, regular projection
- Commonly occur as an artifact during preparation of film
- Uraemia (Chronic renal disease)
- Liver disease
- Hyperlipidemia



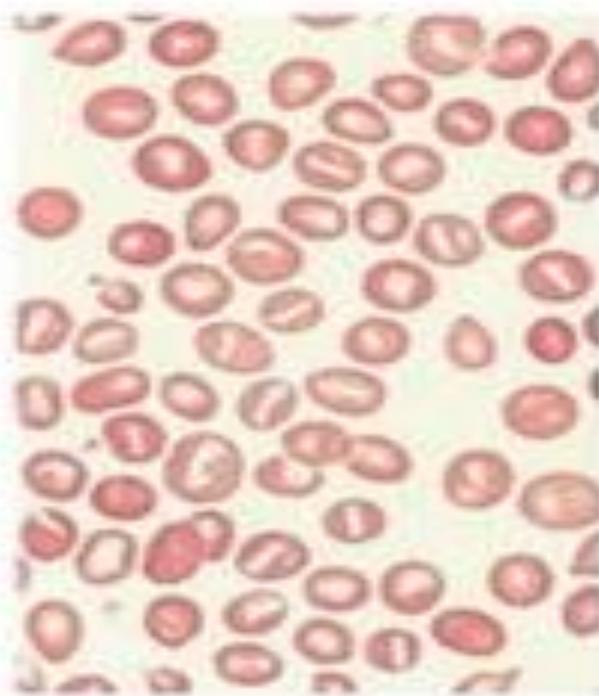
Leptocytes

- Thin red cells with large unstained central area
- Also known as pessary cells
- Seen in
 - Severe iron deficiency anemia
 - Thalassemia



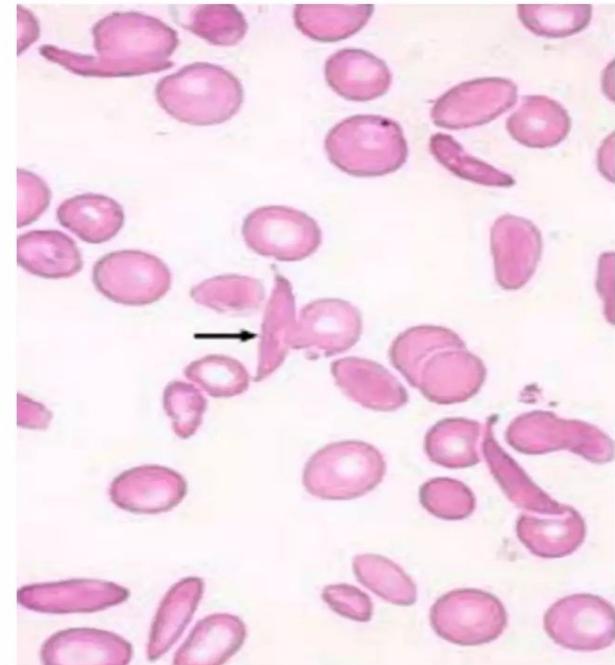
Stomatocytes

- Red cells with central biconcave area appears slit like in dried film.
- Seen in
 - Artifact normally <5%
 - Hereditary >30%
 - Liver disease
 - Alcoholism
 - Myelodysplastic syndromes



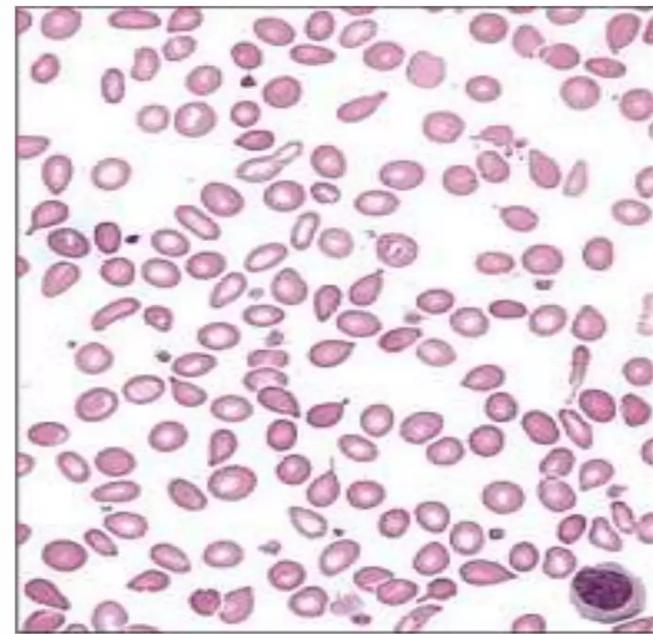
Sickle cell

- Cells are sickle (boat shape) or crescent shape
- Present in film of patient with homozygosity for HbS.
- Usually absent in neonates and rare in patients with high Hb F percentage



Tear drop cells

- Also called dacrocytosis
- One side of cells is tapered and other is blunt
- Seen in
 - Beta thalassemia
 - Post-splenectomy
 - Myelophthisic anaemia
 - Severe iron deficiency

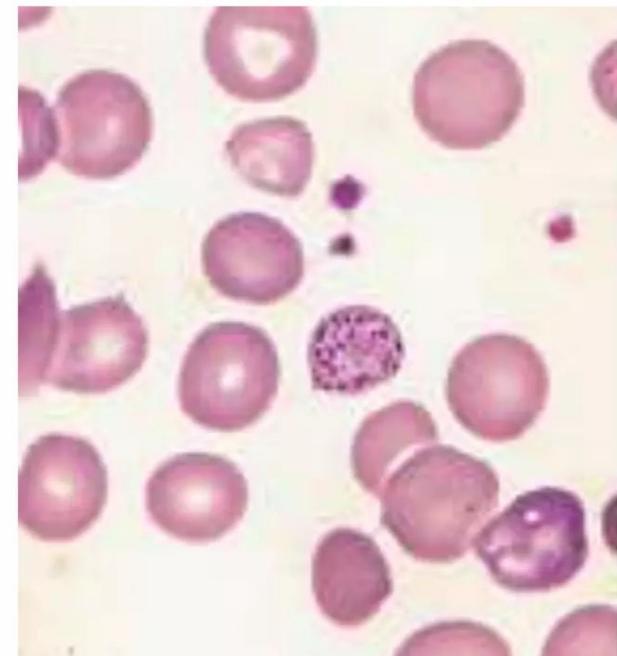


RBC Inclusions

| Name of Inclusion | Content |
|------------------------------|--------------------------|
| Howell-Jolly body | DNA |
| Basophilic stippling | RNA |
| Pappenheimer body | Iron |
| Heinz body (supravital only) | Denatured hemoglobin |
| Crystals | Hemoglobin-C |
| Cabot rings | Mitotic spindle remnants |

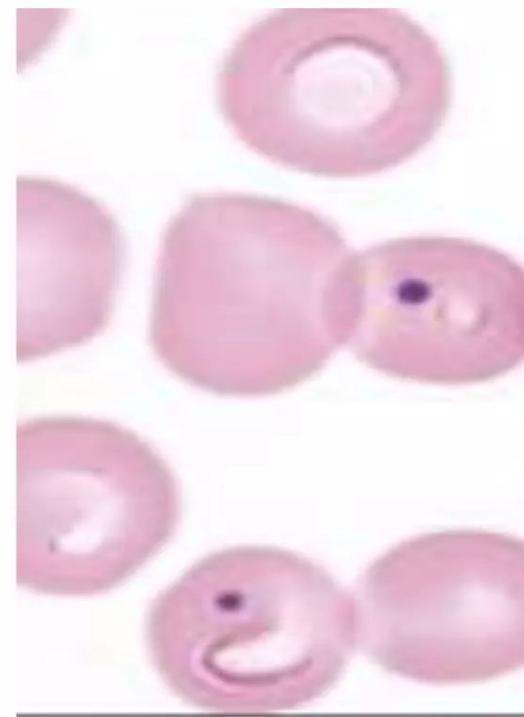
Basophilic Stippling

- Presence of irregular basophilic granules within RBC which are variable in size .
- Stain deep blue with Wright's stain
- Fine stippling seen with
 - Increased polychromatophilia
 - Increased production of red cells.
- Coarse stippling
 - Lead and heavy metal poisoning
 - Disturbed erythropoiesis
 - Megaloblastic anemia
 - Thalassaemia
 - infection
 - liver disease
 - Unstable Hb



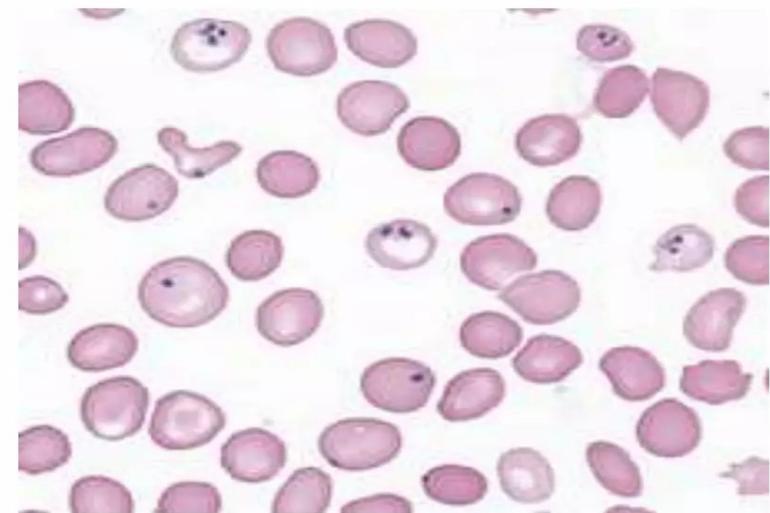
Howell-Jolly Bodies

- Smooth single large round inclusions which are remnant of nuclear chromatin.
- Seen in
 - Megaloblastic anemia
 - Hemolytic anemia
 - Postsplenectomy
 - Abnormal erythropoiesis



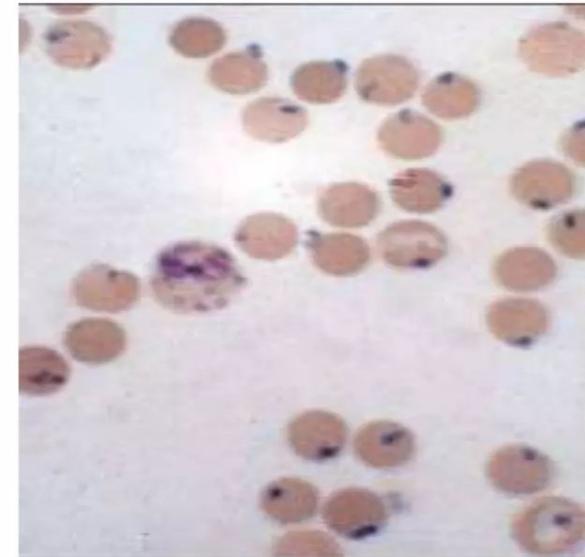
Pappenheimer Bodies

- These are small single or multiple peripherally situated angular basophilic (almost black) erythrocyte inclusions.
- Smaller than Howell–Jolly bodies.
- Composed of haemosiderin.
- Their nature can be confirmed by Perls' stain.
- Seen in
 - Sideroblastic erythropoiesis
 - Hyposplenism
 - Myelodysplastic syndrome



Heinz bodies

- Seen on supravital stains
- Purple, blue, large, single or multiple inclusions attached to the inner surface of the red blood cell.
- Represent precipitated normal or unstable hemoglobins.
- Seen in
- Postsplenectomy
- Oxidative stress
 - Glucose-6-phosphate dehydrogenase deficiency,
 - Glutathione synthetase deficiency
- Drugs
- Toxins
- Unstable hemoglobins



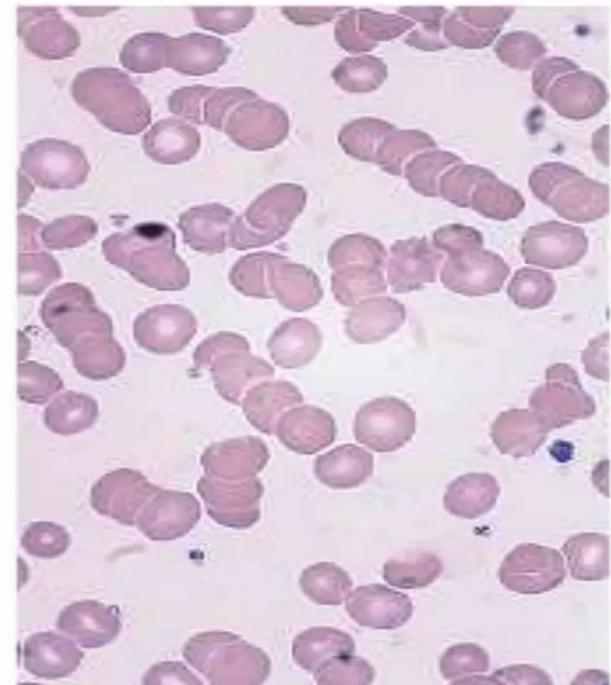
Cabot Rings

- These are Ring shaped, figure of eight or loop shaped
- Red or Reddish purple with Wright's stain and have no internal structure
- Observed in
 - Megaloblastic anaemia
 - Pernicious anemia
 - Lead poisoning



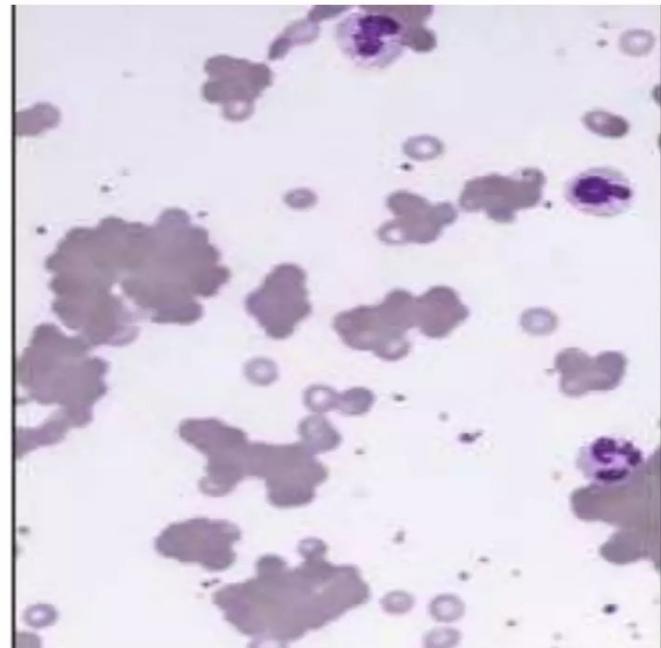
Rouleaux Formation

- Alignment of red cells one upon another so that they resemble stacks of coins
- Occurs in
 - Conditions associated with increased concentrations of globulins and/or fibrinogen
 - Hyperparaproteinemias
 - Waldenstrom's ,macroglobulinemia
 - Multiple myeloma
 - Chronic inflammatory disorders



Agglutination

- It is more irregular and round clumping than linear rouleaux
- Cannot distinguish the outlines of individual RBCs
- Seen with cold agglutinin
 - Anti RBC antibody
 - Autoimmune hemolytic anemia
 - Macroglobulinemia



Summarizing RBC Parameters

- Step 1

Examine Hb and Hct for anemia or polycythemia

If the RBC morphology is normal: Use rule of three to estimate the Hct

- Step 2

MCV: to check and correlate to the morphologic appearance of the cells

- Step 3

Examine MCHC

Describes how well the cells are filled with Hb

Hypochromic, normochromic

2 conditions when MCHC should be evaluated:

1. spherocytosis: slight elevation
2. lipemia/icterus: markedly increase

- Step 4

Examine MCHC

Describes how well the cells are filled with Hb

Hypochromic, normochromic

2 conditions when MCHC should be evaluated:

1. spherocytosis: slight elevation
2. lipemia/icterus: markedly increase

- Step 5

Morphology

1. Size
2. Shape
3. Inclusions
4. Young rbcs
5. Color
6. Arrangement

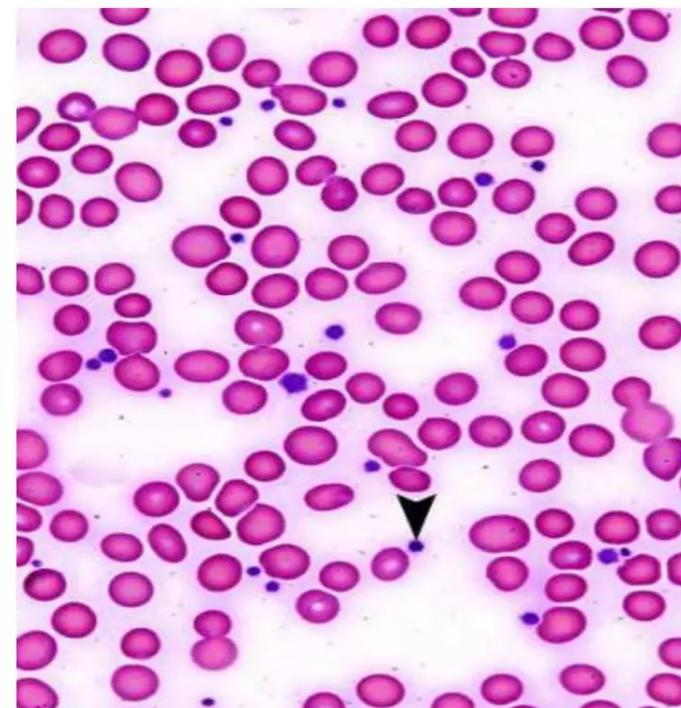
- **Red Blood Cells**, Examine for :
 1. Size and shape.
 2. Relative hemoglobin content.
 3. Polychromatophilia.
 4. Inclusions.
 5. Rouleaux formation or agglutination

Platelets

- Use the oil immersion lens estimate the number of platelets per field.
- Look at 5-6 fields and take an average.
- Multiply the average by 15,000.
- Platelets per oil immersion field (OIF)
 - <7 platelets/OIF = decreased
 - 7 to 15 platelets/OIF = adequate
 - >15 platelets/OIF = increased

Platelets

- Size - $1\text{-}3\mu\text{m}$
- Normal count – 1.5 to 4.5 lac/cmm
- Non nucleated derived from cytoplasmic fragments of Megakaryocytes
- Have an irregular outline and fine purple red granules



Thrombocytopenia

- **Artifactual thrombocytopenia**

Platelet clumping caused by anticoagulant-dependent immunoglobulin
Platelet satellitism
Giant platelets

- **Decreased platelet production**

Hypoplasia of megakaryocytes
Ineffective thrombopoiesis
Disorders of thrombopoietic control
Hereditary thrombocytopenias

- **Abnormal platelet distribution or pooling**

Disorders of the spleen (neoplastic, congestive, infiltrative, infectious, of unknown cause)
Hypothermia
Dilution of platelets with massive transfusions

- **Increased platelet destruction**

Caused by immunologic processes

Autoimmune

Idiopathic

Secondary: Infections, pregnancy, collagen vascular disorders, lymphoproliferative disorders, drugs, miscellaneous

Alloimmune

Neonatal thrombocytopenia

Posttransfusion purpura

Thrombotic microangiopathies

Disseminated intravascular coagulation

Thrombotic thrombocytopenic purpura

Hemolytic-uremic syndrome

Platelet damage by abnormal vascular surfaces

Miscellaneous

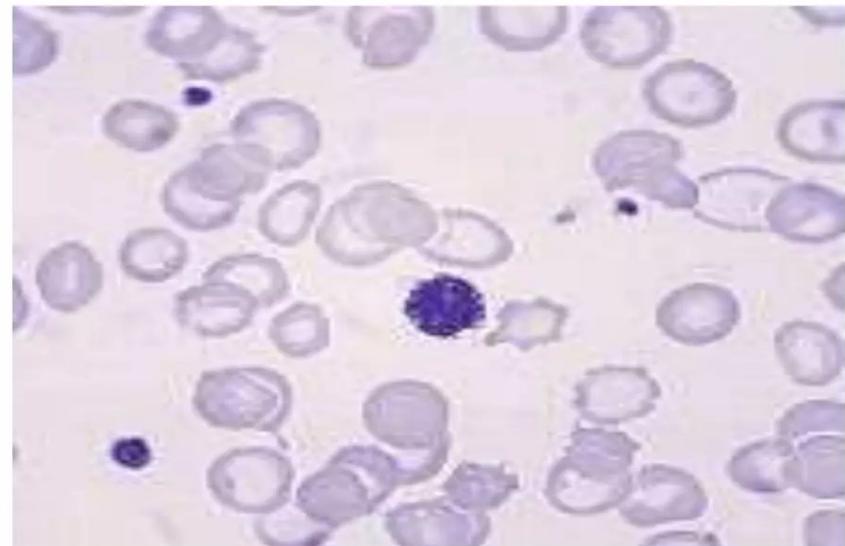
Infection

Thrombocytosis

- Essential thrombocythemia
- CML
- Reactive thrombocytosis
 - Post infection
 - Iron deficiency
 - Inflammation
 - Collagen vascular disease

Platelet morphology: Giant platelets

- Platelets seem to be size of RBCs
- Seen in
 - May –Hegglin anomaly
 - Bernard Soulier syndrome
 - Alport syndrome
 - Storage disorders



Summarizing Platelet Parameters

- Platelet count ($\times 10^9/L$)
- Mean Platelet Volume MPV, fl
- Morphology

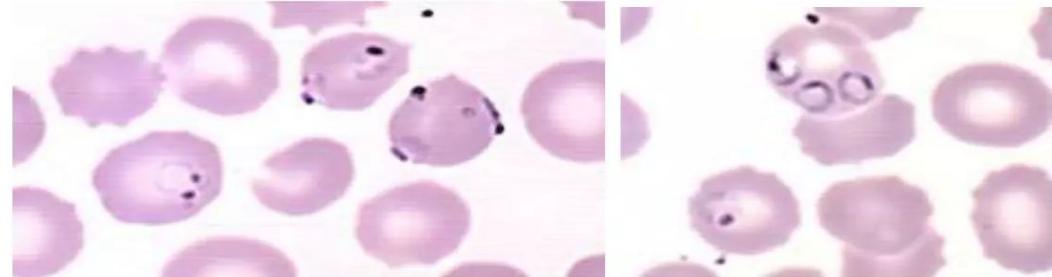
- **Platelets.**
 1. Estimate number present.
 2. Examine for morphologic abnormalities.

Malaria

- Remains the gold standard for diagnosis
 - Giemsa stain
 - distinguishes between species and life cycle stages
 - parasitemia is quantifiable
- Threshold of detection
 - thin film: 100 parasites/ μl
 - thick film: 5 -20 parasites/ μl
- Requirements: equipment, training, reagents, supervision
- Simple, inexpensive yet labor-intensive

Plasmodium falciparum

- Infected rbcs are of normal size
- Multiple infections

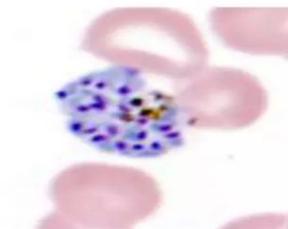
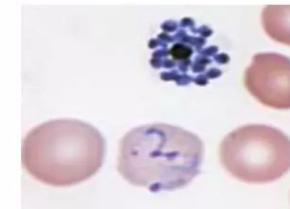
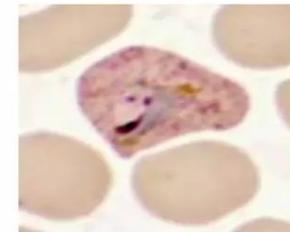


- Gametocytes:
 - Mature
 - Immature
- Immature forms rarely
- Seen in peripheral blood



Plasmodium vivax

- Infected RBCs enlarged and deformed
- Ring forms
 - thick membrane opposite the chromatin dot
- Trophozoites:
 - ameboid;
 - deforms the erythrocyte
- Schizont stage
 - Contains 12-24 merozoites

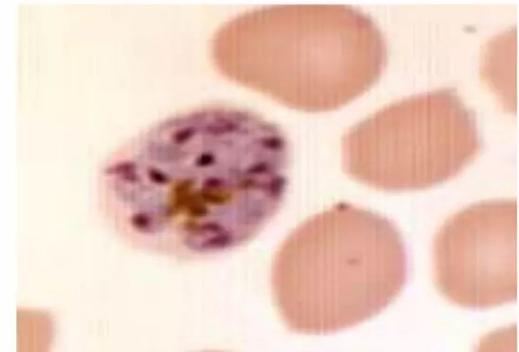
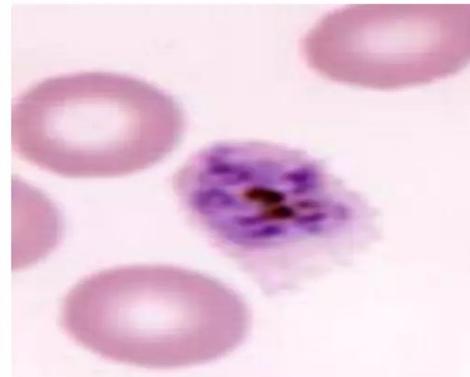


Plasmodium ovale

- Infected erythrocytes
 - Moderately enlarged
 - Fimbriated
 - Oval



- Schizonts:
 - 6-14 merozoites
 - Dark pigment
 - Form rosettes



Plasmodium malariae

Infected erythrocytes: size normal to decreased

- Trophozoite:
 - compact
 - typical band form

- Schizont:
 - 6-12 merozoites;
 - coarse, dark pigment

- Gametocyte:
 - round; coarse,
 - dark pigment



Filariasis

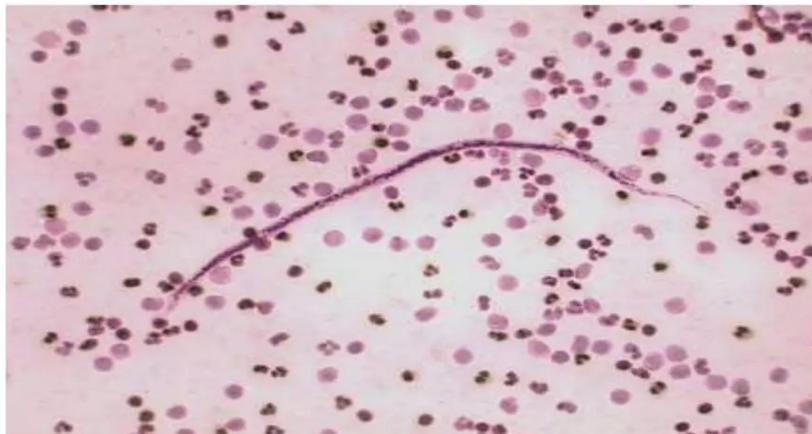
- Causes elephantiasis
- Mainly caused by *Wuchereria bancrofti* and *Brugia malayi*
- Pathology:
 - Due to adult worm obstructing lymphatics.
- Acute: lymphadenitis lymphatic varices
- Chronic: lymphedema, hydrocele, chyluria.

Filariasis

| species | Disease | Geographic distribution | Location of adult in humans | Location of microfilaria | vector | Lab. diagnosis |
|------------------------------------|----------------------------------|--|--------------------------------|-------------------------------|---------------------------|----------------|
| <i>Wuchereria bancrofti</i> | elephantiasis | Tropical and subtropical areas | Lymphatic vessels | Blood (nocturnal periodicity) | mosquitoes | Blood film |
| <i>Brugia malayi</i> | elephantiasis | Asia | Lymphatic vessels | Blood (nocturnal periodicity) | mosquitoes | Blood film+ICT |
| <i>Onchocerca volvulus</i> | Onchocerciasis (river blindness) | Africa, Central and South America, Yemen | Subcutaneous nodules | Skin, eyes, no periodicity | Simulium spp. (black fly) | Skin snip |
| <i>Loa loa</i> | loiasis | Central Africa | Moving in subcutaneous tissues | Blood (diurnal periodicity) | Chrysops spp. (deer fly) | Blood film |

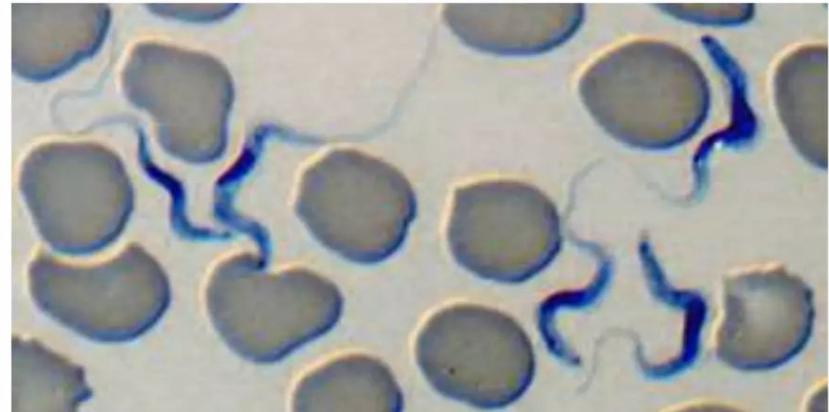
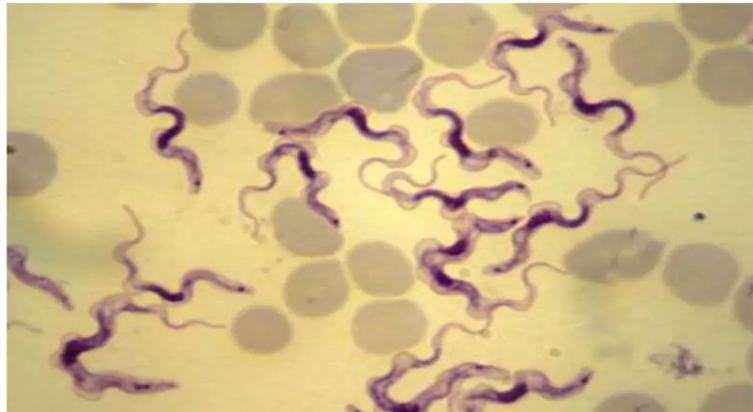
Filariasis

- Detection of microfilariae in blood in early stages of the disease:
- Blood film, Knott's method (concentration of 1 ml of blood)
- Best 10 pm to 2 am (nocturnal periodicity)



Trypanosomiasis

- Causes African Sleeping sickness
- Transmitted by tse tse fly



Babesia

- Caused by species of the intraerythrocytic protozoan Babesia
 - *B. microti*
 - *B. divergens*
- Vector is tick
- Causes a malaria-like sickness
- Maltese cross appearance in erythrocytes

