MEDICINE
KING SAUD UNIVERSITY

Physiology Team 436

Determination of the Differential & total Leukocyte Count (DLC&TLC)

- This slides contain both (Slides + Handout)
- Procedures in Female slide and handout are the same .Therefore ,we put it as one procedure

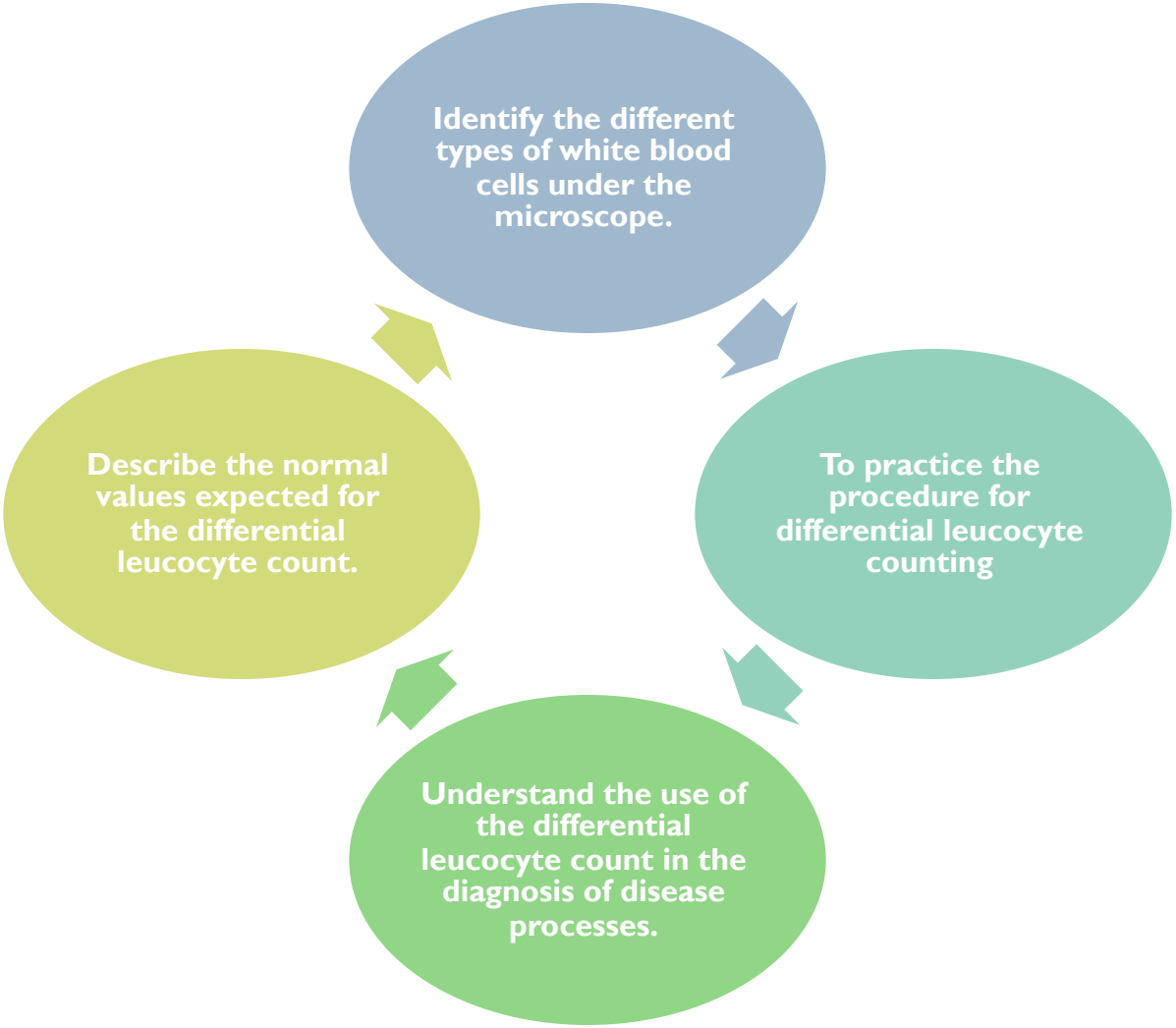
Red: very important.
Green: only found in males' slides.
Purple: only found in females' slides.
Gray: notes.

Physiology Team 436 – Foundation block – Practical
(lecture 2- DCL)

{ يامالك الملك وكلناك أمرنا واستودعناك همومنا
فبشرنا بما يفتح مداخل السعادة إلى قلوبنا}

Lecture: If work is intended for initial studying.
Review: If work is intended for revision.

Objectives


Identify the different types of white blood cells under the microscope.
To practice the procedure for differential leucocyte counting
Understand the use of the differential leucocyte count in the diagnosis of disease processes.
Describe the normal values expected for the differential leucocyte count.

Introduction

What is Differential Leukocyte Count (DLC) ?

- DLC is a routine test in hospitals which determine the percentage of each type of white blood cells in the total leucocyte population.

- Each type of WBCs performs a different function against infections and each type of infection yields a different white cell picture in the blood.

- Each type of WBCs has unique morphology and staining characteristics, which is responsible to specify the type of the cells.

النسب الحمراء هي بملزمة البنات
واللي الدكتورة حرصت عليها

Types of WBC

Never Let Monkey Eat Banana
Neutrophil , Lymphocyte , Monocyte , Eosinophil , Basophil
Most common → less common

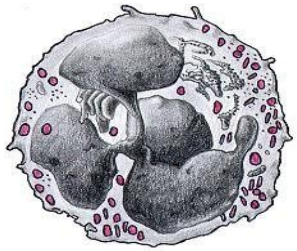
كرات الدم البيضاء ذات النواة المتعددة الأشكال.

Agranular = A means not

1. Granular (polymorphnuclear PMN)

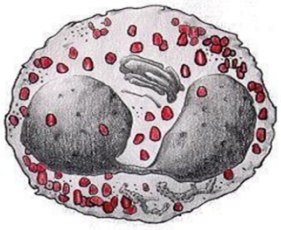
Neutrophil

Most commonly seen white blood cells in the circulating blood (40/50 -70)%



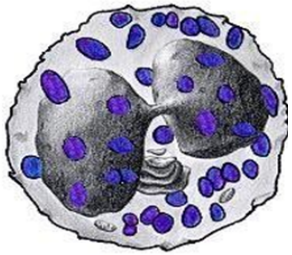
Eosinophil

Less common in the blood stream (1-6%). Some sources say (1–3 %)



Basophil

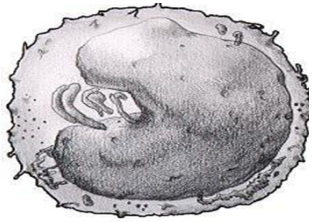
The rarest of all white blood cells found in the blood (0-1%). Or (0.4–1%)



2. A Granular

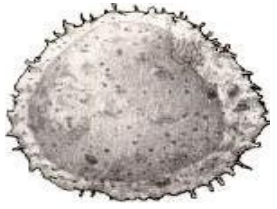
Monocytes

About 4-6% or 5-10% of the blood cells



Lymphocyte

About 25-35% of the blood cells



-Granular: is categorized by: COLOR

-A granular: is categorized by SIZE.

Numbers may vary from one source to another

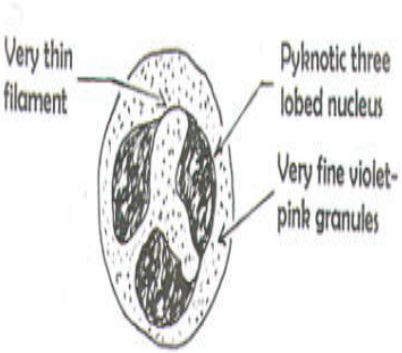
Neutrophils (10-16um)

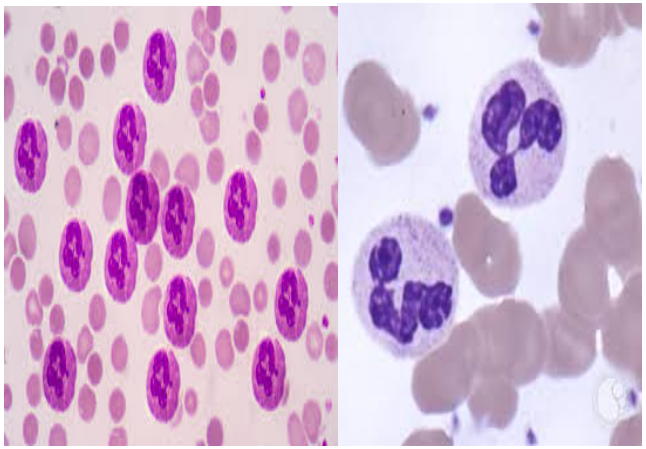
Some sources say 10-14 um

Clinical application :

- It will increase in acute bacterial or fungal infections. (pyogenic illness)

Nucleus	Cytoplasm:	Cytoplasmic granules
• blue-violet • Complex multi-lobed nucleus (from 2 to 6 lobes) connected by chromatin threads. • Seen clearly through cytoplasm.	• slate-blue in color	• Small • Fine, closley-packed violet(purple)-pink. • Not seen seperately • Give ground-glass appearance • Do not cover nucleus





They have small cytoplasmic granules take a neutral (purple or pink) color with various stains such as **Wright's stain**.

Any thing with this color is from male slide (from the schedule that we put it as summary in slide no 13-14)

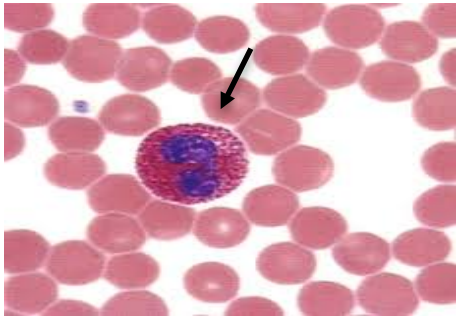
Numbers are vary from source to other

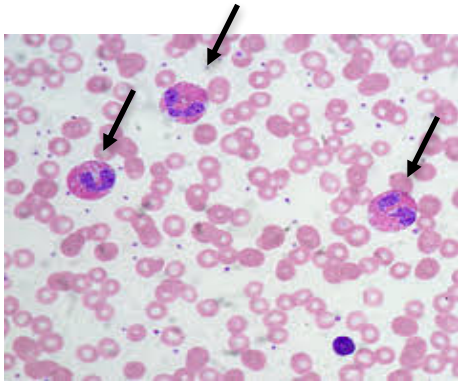
Eosinophils (10-15um)

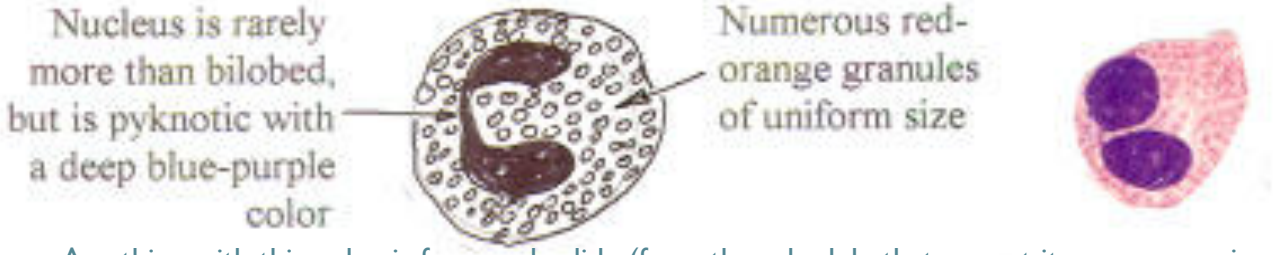
Clinical Application :

- It will increases in parasitic infections and allergies.

Nucleus	Cytoplasm	Cytoplasmic granules
1. Blue-violet . 2. 2-3 lobes. often is a dumbbell-shaped nucleus (bi-lobed). Lobes connected by thick or thin chromatin band. 3. Seen clearly through cytoplasm.	• Eosinophilic • Light pink/red • Granular	• Large , prominent , coarse , red to orange (eosinophilic) granules • uniform size • Seen seperatley • Do not cover the nucleus







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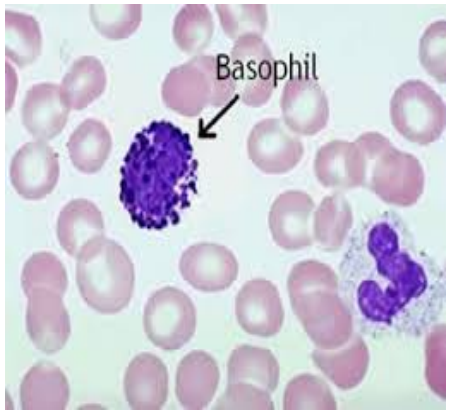
Numbers are vary from source to other

Basophils (10-15um)

- **Clinical application :** It will increase in allergies and malignancies.

Nucleus	Cytoplasm	Cytoplasmic granules
• Blue-violet. • Not clearly Seen through cytoplasm because it is covered by large granules. (somewhat hidden) • Irregularly shaped; may be S shaped • rarely (bi-lobed).	• Basophilic • Bluish • Granular	• Basophilic • Large, very coarse • Variable size • Deep purple • Seen seperatley • Completely fill the cell, cover the nucleus

granules contain Heparin (an anticoagulant). and Histamine, which increases the permeability of capillary walls.



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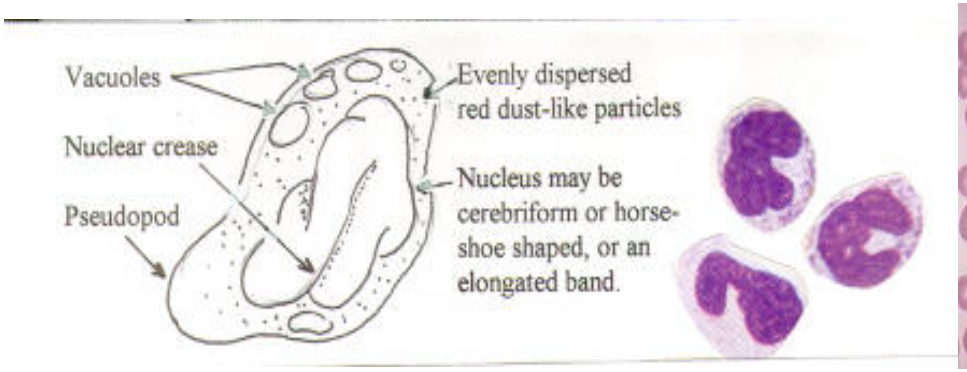
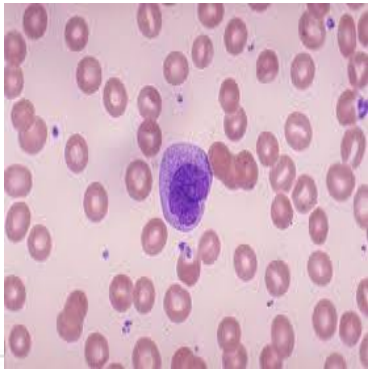
Numbers are vary from source to other

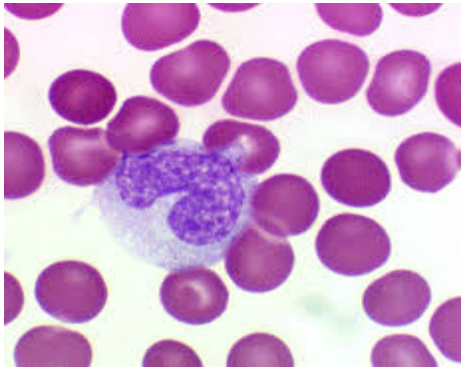
Monocytes

- It is the largest of all white blood cells in size (12-20um).
- **Clinical application :** it will increase in chronic infections.

Nucleus	Cytoplasm:	Cytoplasmic granules
• Pale blue-violet, • Large single • May be indented horseshoe-shaped, or kidney-shaped (can appear oval or round if seen from the side)	• slate-blue in color. • Abundant • "frosty" • Amount may be larger than that of nucleus	•do not contain any granules.







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Numbers are vary from source to other

Lymphocytes

General characteristics:

- Deep blue-violet
- Small, spherical cells with large, round nucleus in each of them.
- The Nucleus occupies most of the volume of the cell, leaving only a thin crescent rim of Cytoplasm around it.
- The cytoplasm of these cells does not contain any granules.
- It will increase in acute viral infections (infectious mononucleosis) and malignancies .

Large Lymphocytes

Small Lymphocytes

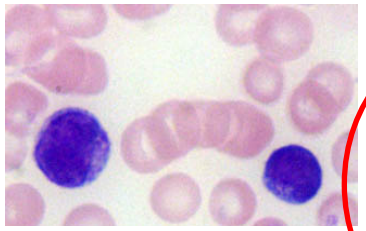
Small and Large Lymphocytes are explained in next slide

Any thing with this color is from male slide (from the schedule that we put it as summary in slide no 13-14)

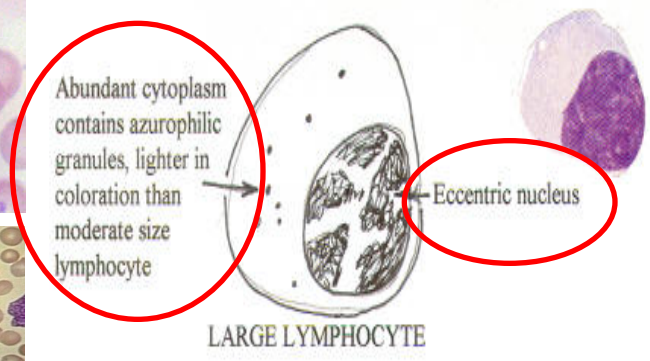
Large and small Lymphocytes (male slide)

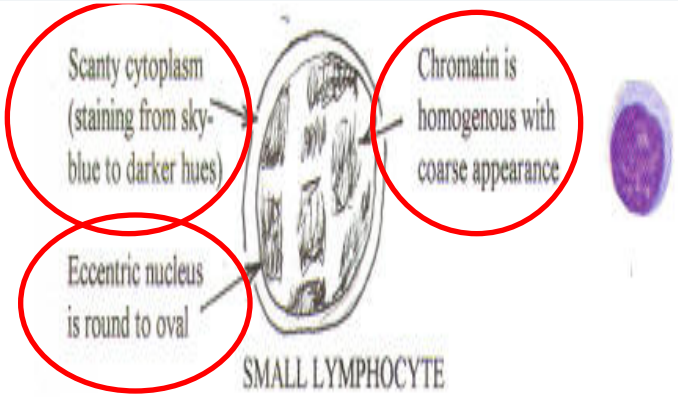
	Large lymphocyte	small lymphocyte
Nucleus	• Deep blue-violet • Single, large, round or oval, almost fills cell. • May be central or eccenric	• Deep blue-violet • Single, large, round, almost fills cell. • Condensed lumpy chromatin, gives "ink spot" appearance.
Cytoplasm	• Large crescent of clear, light blue cytoplasm • Amount larger than in small lymphocyte	• Hardly visible • Thin crescent of clear, light blue cytoplasm
Cytoplasmic granules	No visible granules	
Exist and Size	(5-10%) , (10-15 um)	(20-40%) (7-9 um)

Large

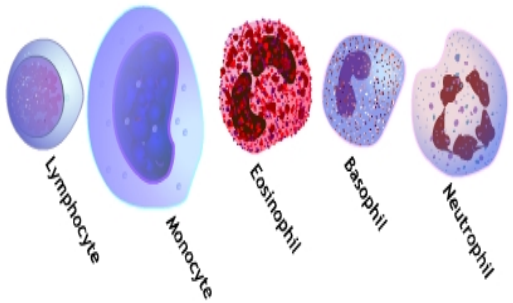


Small





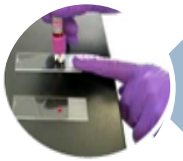
DLC Equipment & Procedures :
DIFFERENTIAL LEUCOCYTE COUNT



Equipment

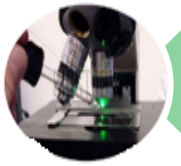
- Light Microscope with an Oil Immersion Object
- Mineral or Cedar wood Oil
- Various dyes for staining blood films : Wright's Stain & Leishman's stain
- Microscope Slides

Procedures



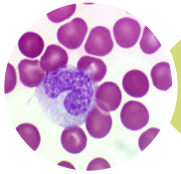
Using various dyes + microscope slide

1.Prepare a stained blood film with the help of Wright's stain

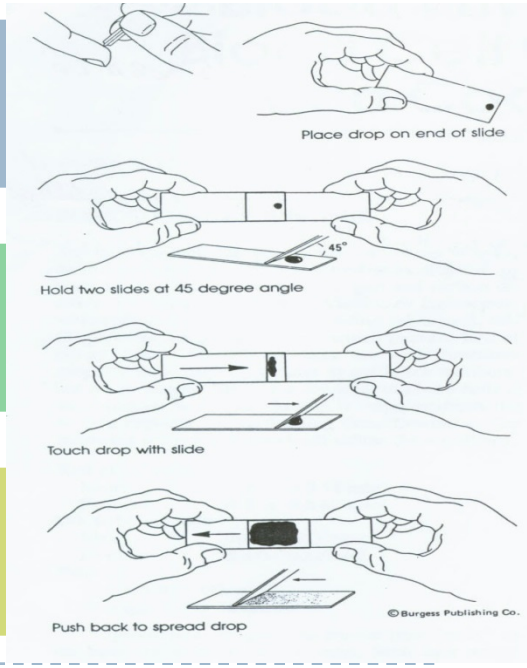


Using microscope with an oil immersion objectives + Mineral or Cedar wood oil

2.Set the stained blood film under the oil immersion objective lens in a light microscope

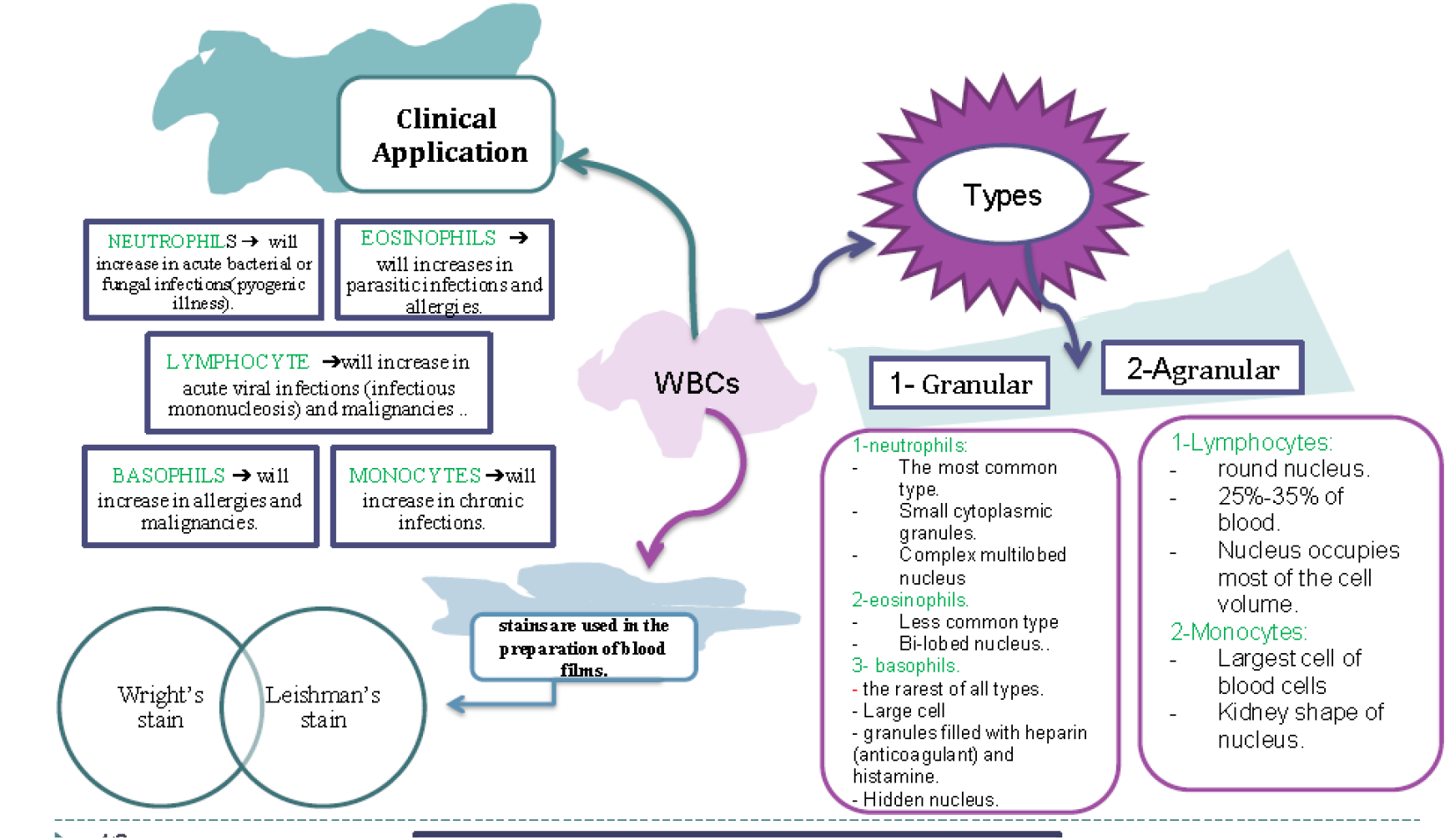


3.Identify various types of white blood cells according to their histological characteristics , count about 100 cells



The diagram above from 435

Mind map



The diagram above from 435

Summary

TABLE 1.2

Appearance of white blood corpuscles in a stained blood film

Cell type	*Diameter (μm)*	*Nucleus*	*Cytoplasm*	*Cytoplasmic granules*
Granulocytes				
Neutrophils (40-70%)	10-14 (1.5-2X a RBC)	• Blue-violet. • 2-6 lobes, connected by chromatin threads. Seen clearly through cytoplasm.	• Slate-blue in color.	• Fine, closely-packed violet-pink. • Not seen separately. • Give ground-glass appearance. • Do not cover nucleus.
Eosinophils (1-6%)	10-15	• Blue-violet • 2-3 lobes, often bi-lobed, lobes connected by thick or thin chromatin band. • Seen clearly through cytoplasm.	• Eosinophilic. • Light pink-red. • Granular.	• Large, coarse. • Uniform-sized. • Brick-red to orange. • Seen separately. • Do not cover nucleus.
Basophils (0-1%)	10-15	• Blue-violet. • Irregular shape, may be S-shaped, rarely bilobed. • Not clearly seen, because overlaid with granules.	• Basophilic. • Bluish. • Granular.	• Large, very coarse. • Variable-sized. • Deep purple. • Seen separately. • Completely fill the cell, and cover the nucleus.

Summary continue

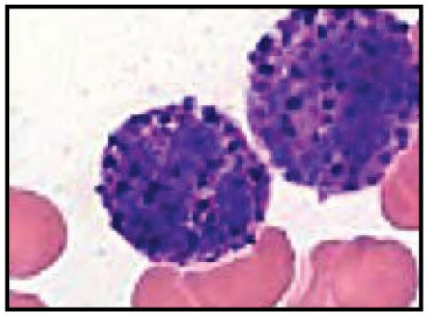
Agranulocytes				
Monocytes (5-10%)	12-20 (1.5-3 X a RBC)	• Pale blue-violet. • Large single. • May be indented horse-shoe, or kidney shaped (can appear oval or round, if seen from the side).	• Abundant. • 'Frosty'. • Slate-blue. • Amount may be larger than that of nucleus.	• No visible granules.
Small Lymphocyte (20-40%)	7-9	• Deep blue-violet. • Single, large, round, almost fills cell. • Condensed, lumpy chromatin, gives 'ink-spot' appearance.	• Hardly visible. • Thin crescent of clear, light blue cytoplasm.	• No visible granules.
Large lymphocyte (5-10½)	10-15	• Deep blue-violet. • Single, large, round or oval, almost fills cell. • May be central or eccentric.	• Large, crescent of clear, light blue cytoplasm. • Amount larger than in small lymphocyte.	• No visible granules.

Summary

Blood element	% of leukocyte	Size μ	Cytoplasmic staining	Nucleus morphology
Erythrocyte	-	7-8	pink, no granules	none
Neutrophil	50-70	10-12	salmon-colored small granules	Segmented,-2-5 lobed
Lymphocyte	25-35	7-8	Light blue, scant amount, no granules	Single large Oval purple
Monocyte	4-6	16-18	Basophilic, no granules	Large, kidney shaped
Eosinophil	1-3	13-14	Bright red coarse granules	bilobed purplish
Basophil	0-4-1	14-15	Large, basophilic granules	Bilobed bluish black

Examine yourself !

What are these cells ?



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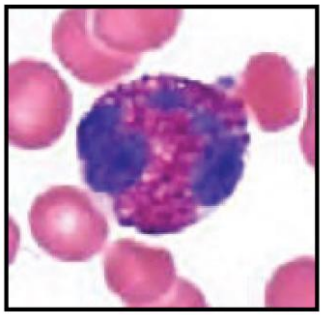

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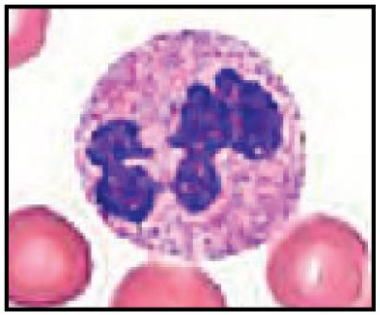

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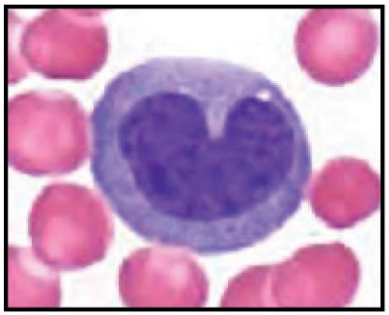

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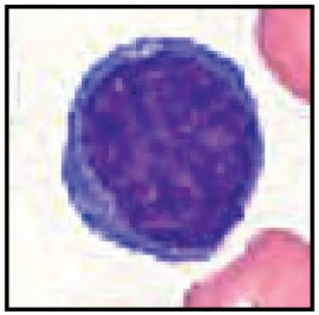

5

- **What stains are used in the preparation of blood films?**

1. Leishman's stain
2. Wright's stain

1. Basophils
2. Eosinophils
3. Neutrophils
4. Monocyte
5. lymphocytes

Video describe WBCs :

https://www.youtube.com/watch?v=Tdx-U8S6ZMk

Examine yourself !

1. **What are the normal values of each different type of white blood cells?**
   - NEUTROPHILS → 50 – 70 %
   - EOSINOPHILS → 1 – 3 %
   - BASOPHILS → 0.4 – 1 %
   - MONOCYTES → 4 – 6 %
   - LYMPHOCYTE → 25 – 35 %

2. **Under what conditions are the percentages of the various types of white blood cells increased?**
   - NEUTROPHILS → will increase in acute bacterial or fungal infections.
   - EOSINOPHILS → will increases in parasitic infections and allergies.
   - BASOPHILS → will increase in allergies and malignancies.
   - MONOCYTES → will increase in chronic infections.
   - LYMPHOCYTE → will increase in acute viral infections and malignancies.

Thank you!

The Physiology 436 Team:

Ruba Ali

Dorrah alhamdi

Nasser Abu Dujeen

Hassan Al Shammari

Team Leaders:

Qaiss Almuhaideb

Lina alwakeel

Contact us:

Physiology436@gmail.com

@Physiology436