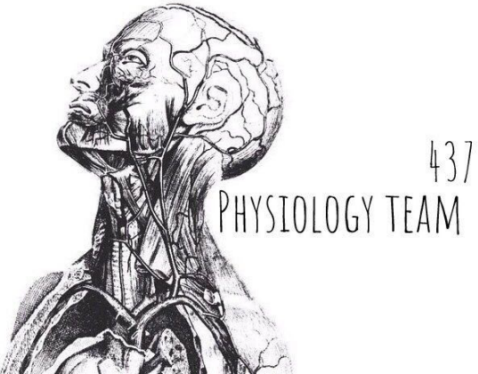
PHYSIOLOGY
PRACTICAL

Total and Differential Leucocytic Count

TLC & DLC



SLIDES + HANDOUT

Red: Important
Green: Notes
Gray: Extra Information
Blue: Only boys slides
Purple: Only girls slides

MED437
KING SAUD UNIVERSITY

Objectives :

- To be able to identify the different types of leukocytes under the microscope.
- To practice the procedure for differential leucocyte counting.
- To know the normal values expected for the differential white cell count.
- To understand the use of the differential white cell count in the diagnosis of disease processes.

*only found in girl's slides

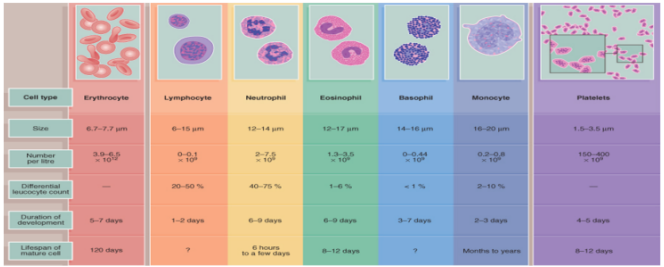
Introduction

*Only found in boy's slides

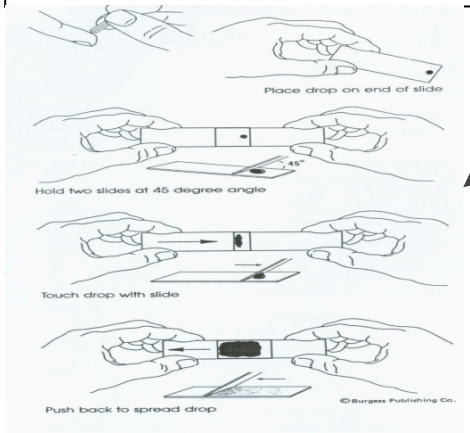
- In the differential leucocyte count (DLC) the percentage of each type of white blood cells in the total leucocyte population is determined

.

- Each type of white cells, performs a different function in the battle against infections
- Each type of infection yields a different white cell picture in the blood .
- The morphology and staining characteristics of each type is peculiar and is responsible for the in specific typing.



Reagents and apparatus:	Procedure
• A microscope with an oil immersion objectives. • Mineral or cedar oil • Various dyes for staining blood films (e.g., Wright's stain and Leishman's stain) can use either stain. • Microscope slides.	• Prepare blood film and stain it with Wright's stain • Examine it under the oil immersion objective lens of the microscope, and identify the different leucocytes(count about 100 cells(



WBCs Count Between 4000-11000

This slide is VERY important

only found in girls's slides

WBCs are classified into

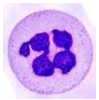
* Based on absorption of color

* any WBC whose cytoplasm Contains dots in it is granular

Granular

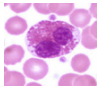
Contain cytoplasmic granules

- Neutrophils



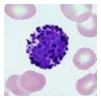
* 3-7 lobes nuclei
Pinkish granules and purplish nuclei

- Eosinophils



* pinkish

- Basophils

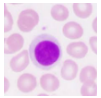


* very dark blue granules

Agranular

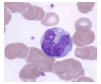
No cytoplasmic granules

- Lymphocytes



* huge nucleus
A little bit of cytoplasm

- Monocytes

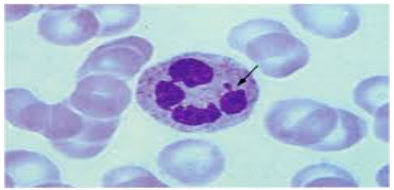


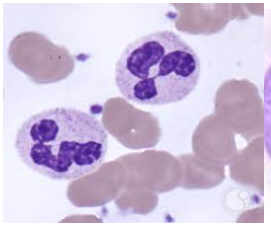
* Often kidney shaped nucleus

clinicals applications in the next slides are about differential count provides clues about certain illnesses. An increase in a specific type of Leukocytes is linked to a certain illness .

Neutrophils

40-70%
50-70%
of WBC


Erythrocytes and a neutrophil.
Wright's (Oil)



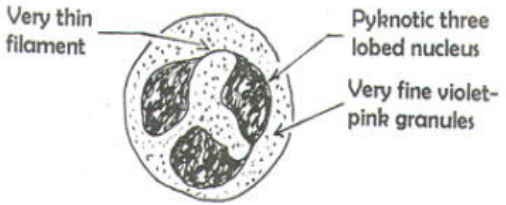
Clinical application

- **Neutrophilia**:increasing in neutrophils > acute bacterial and fungal infections (pyogenic illness(

Function:

- Active phagocytes
- Number increases
- Short term or acute infection

*It is the most common type of blood cell circulating blood

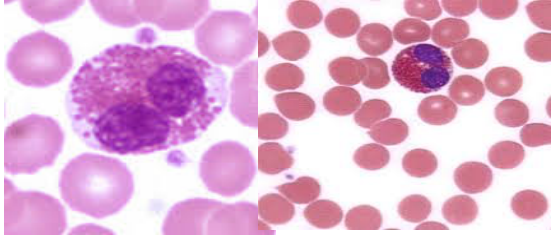


*Prominent feature: Multi-lobed nucleus

Diameter/μm	Nucleus	Cytoplasm	Cytoplasmic Granules
• 14-10	• Blue-violet • 6-2lobes (multilobed complex\nucleus) connected by thin strand of nucleoplasm .	• Slate blue in color	• Fine granules which are difficult to see closely-packed violet-pink (neutral), with various stains such as Wright's stain. • Small granules

Eosinophils

1 - 4/6%
1-3%
of WBC



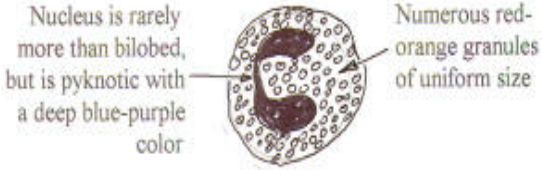
Clinical application:

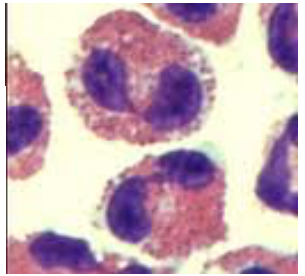
- **Eosinophilia** :increasing in eosinophils > Allergy and parasitic infections

Function:

- Kill parasitic worms
- Increase during allergy attacks

*It is less common in bloodstream than neutrophils



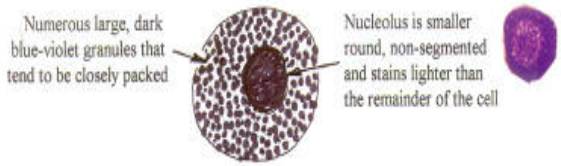


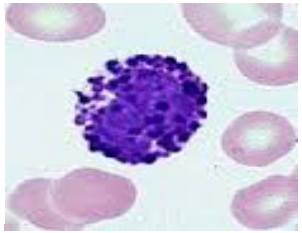
*Prominent feature: Coarse pink/red granules

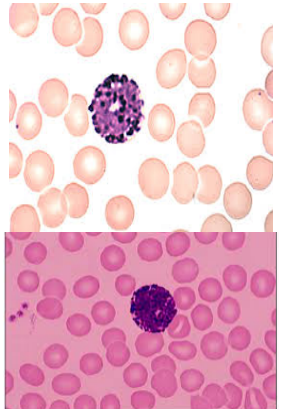
Diameter/μm	Nucleus	Cytoplasm	Cytoplasmic Granules
• 15-10	• 3-2 lobes • Dumbbell-shaped • often bilobed purplish • stained blue-red	• Eosinophilic • Light pink red • Granular	• Large • prominent , • red • Coarse

Basophils

0-1 %
0.4- 1%







Clinical application:

- **Basophilia** :increasing in basophils > allergy and malignancy

Function:

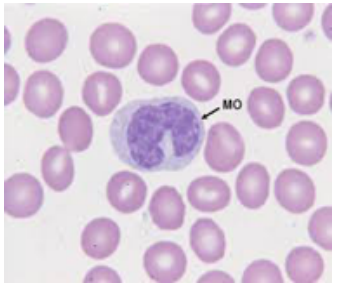
- Contain histamine (vasodilator chemical) which is discharged at site of inflammation
- These large granules contain/secrete heparin and histamine
- The rarest of all white blood cells found in the blood .

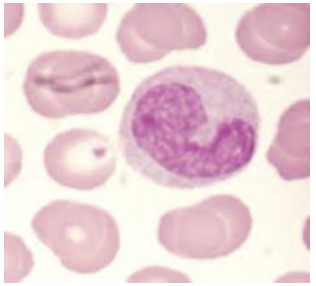
*Prominent feature: Nucleus hidden by blue granules

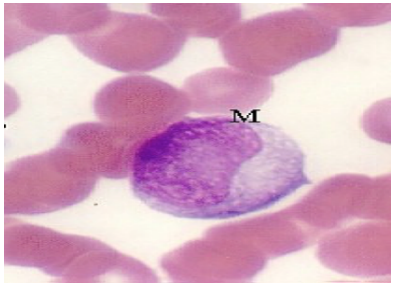
Diameter/µm	Nucleus	Cytoplasm	Cytoplasmic Granules
• 15-10	• Irregular • May be S or U shaped • Not clearly seen • Nucleus hidden behind granule (because overlaid with granules)	• Basophilic • Bluish • Granular • stains dark blue	• Large very coarse • Completely fill the cell • Few large blue purple granules"basophilic granules "

Monocytes

5-10%
4 - 8%
4-6%
of WBCs





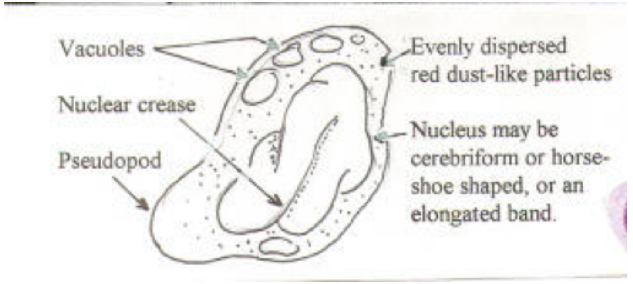


Clinical application:

Monocytosis: Monocyte count will increase with chronic infections

Function:

- Active phagocytes that become macrophages in the tissues
- Increase in number during chronic infections such as tuberculosis

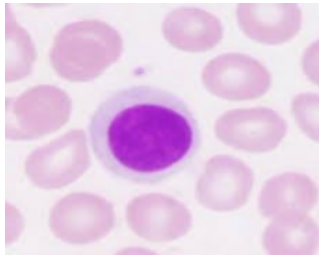


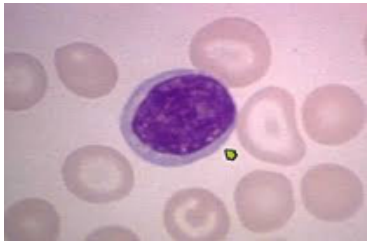
*Prominent feature: Large cell with kidney shaped nucleus (no granules(

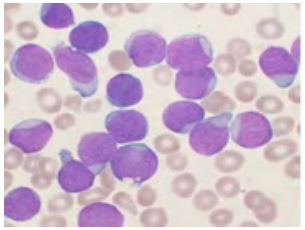
Diameter/μm	Nucleus	Cytoplasm	Cytoplasmic Granules
• 20-15 • The Largest WBC	• Single • Large • Kidney or Horseshoe shaped • dark blue purple nucleus	• Abundant • Basophilic • Glass like texture	• No visible granules

Lymphocytes

20-40\45 %
25 -35%
of WBC







Clinical application:

- **Lymphocytosis**(Lymphocyte count will increase with viral infections (infectious mononucleosis :

Function:

- Part of immune system
- B-Lymphocytes produce antibodies
- T-Lymphocytes involved in graft rejection, fighting tumors and viruses, and activating B-Lymphocytes.

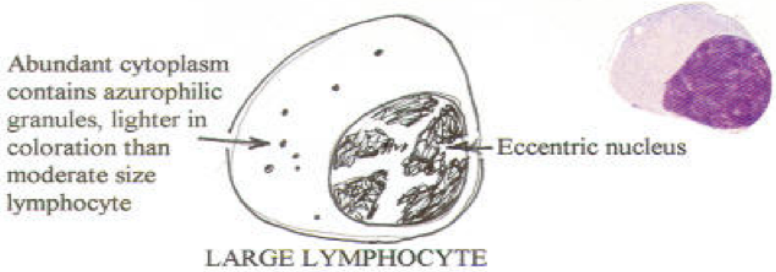
*Prominent feature: Large nucleus taking up most of cell volume and very small cells

Diameter/μm	Nucleus	Cytoplasm	Cytoplasmic Granules
• Small 9-7 • Some large 15-10	• Single • Large (occupies most of cell volume (• Round • dark purple-blue nucleus	• Thin rim (crescent) of cytoplasm • light blue color	• No visible granules

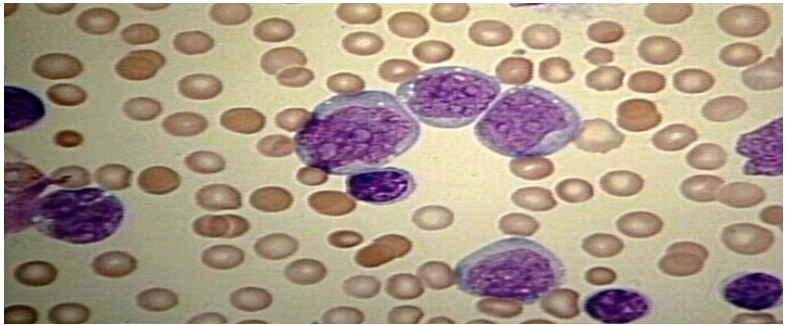
Difference between Small and Large Lymphocytes

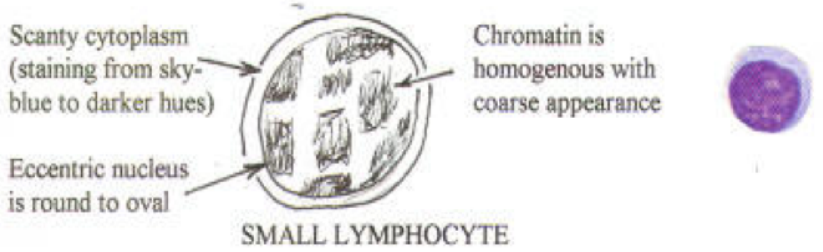
*only in male slides

Large Lymphocytes µm15-10

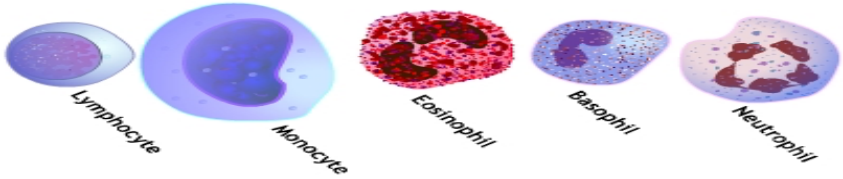


Small Lymphocytes µm9-7





DLC Equipment & Procedures :
DIFFERENTIAL LEUCOCYTE COUNT



EQUIPMENTS:

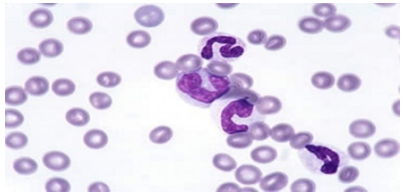
1-electron microscope with an oil immersion objective 2- mineral or cedarwood oil
3- wright's stain 4- microscope slides

PROCEDURES:

A - Prepare a stained blood film with the help of Wright's stain.
"Using various dyes + microscope slide "

B - Set the stained blood film under the oil immersion objective in an electron microscope. "Using microscope with an oil immersion objectives + Mineral or Cedar

C - Identify various types of white blood cells according to their histological characteristics.



*found in the handout

*Values of “% of leukocytes” and “Size” may slightly vary

Summary

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

Questions and problems:

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.

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

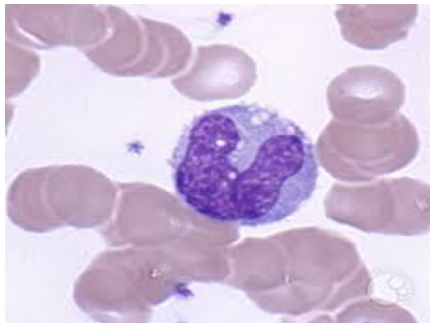
[1 Leishman's stain

[2 Wright's stain

*found in the handout

Identify the following pictures :

1



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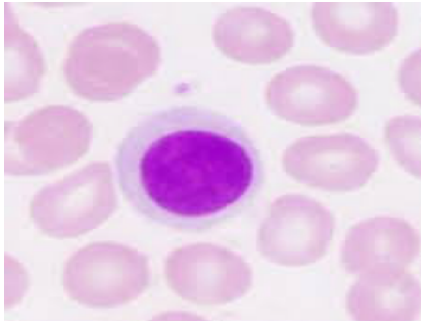

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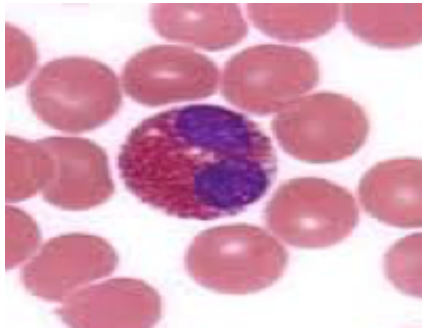

5

-1Basophils
-2Monocytes
-3Lymphocytes
-4Neutrophils
-5Eosinophils

GOOD LUCK!

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Team Leaders:
Reem ALQarni
Tareq ALOmaim

References:
1- Girls' & Boys' slides
2- Handout