

DIFFERENTIAL LEUKOCYTE COUNT (DLC)

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DYPMCKOP

□ *Aim*

□ *Principle*

□ *Apparatus*

□ *Procedure*


□ *Precaution*

DLC CONTINUE


□ PRINCIPLE:

BLOOD SMEAR IS PREPARED, STAINED WITH LEISHMAN'S STAIN AND CELLS ARE IDENTIFIED UNDER OIL IMMERSION LENS.

APPARATUS:


- 4-5 GLASS SLIDES
 - LANCET/ SPRIT/COTTON
 - LEISHMAN'S STAIN
 - MICROSCOPE
 - CEDAR WOOD OIL
 - DISTILLED WATER
 - STAINING TRAY
- 

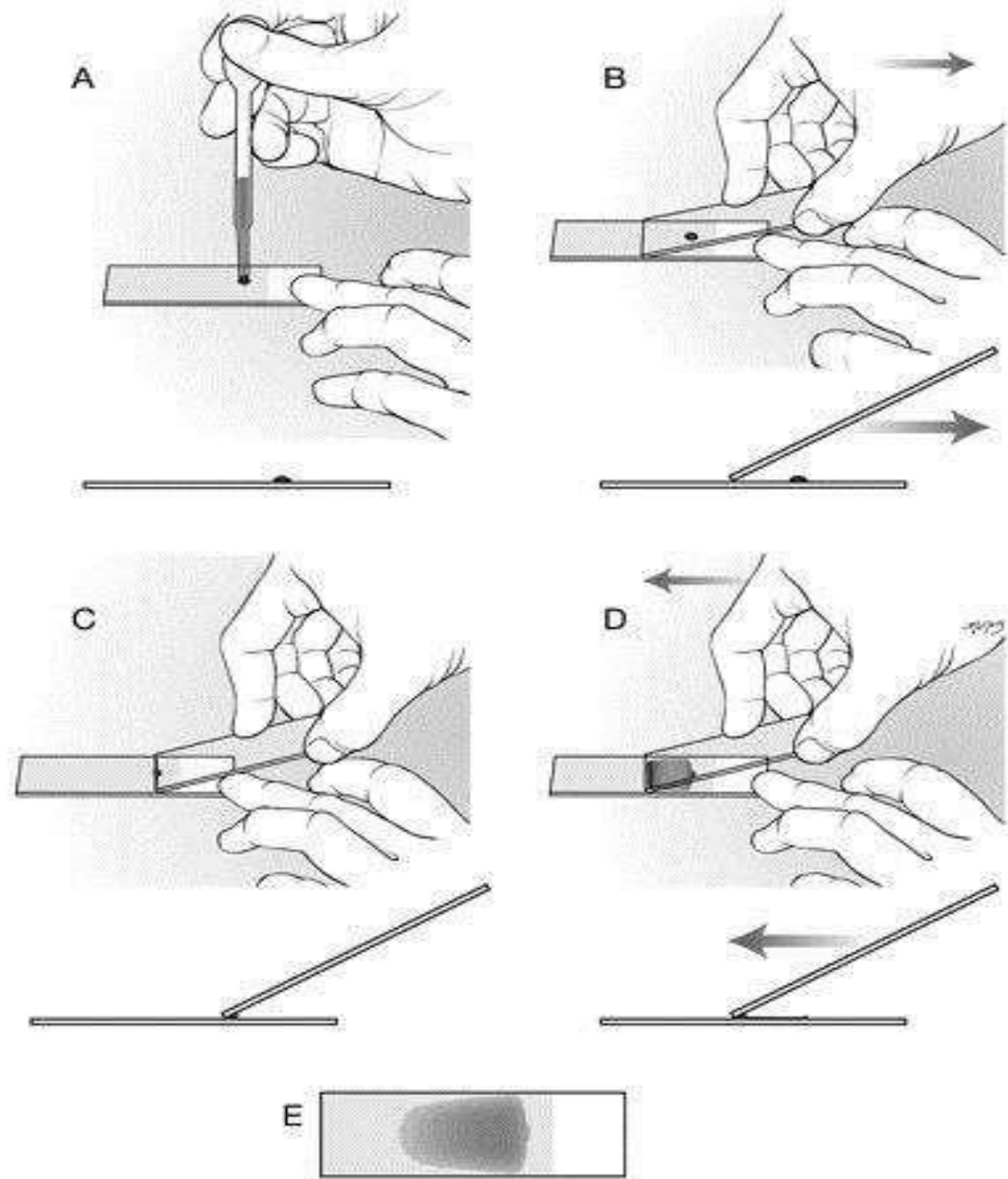
PROCEDURE

- preparation of blood smear
 - staining of blood smear
 - Examination of smear under oil immersion(100x) lens
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Preparation of smear

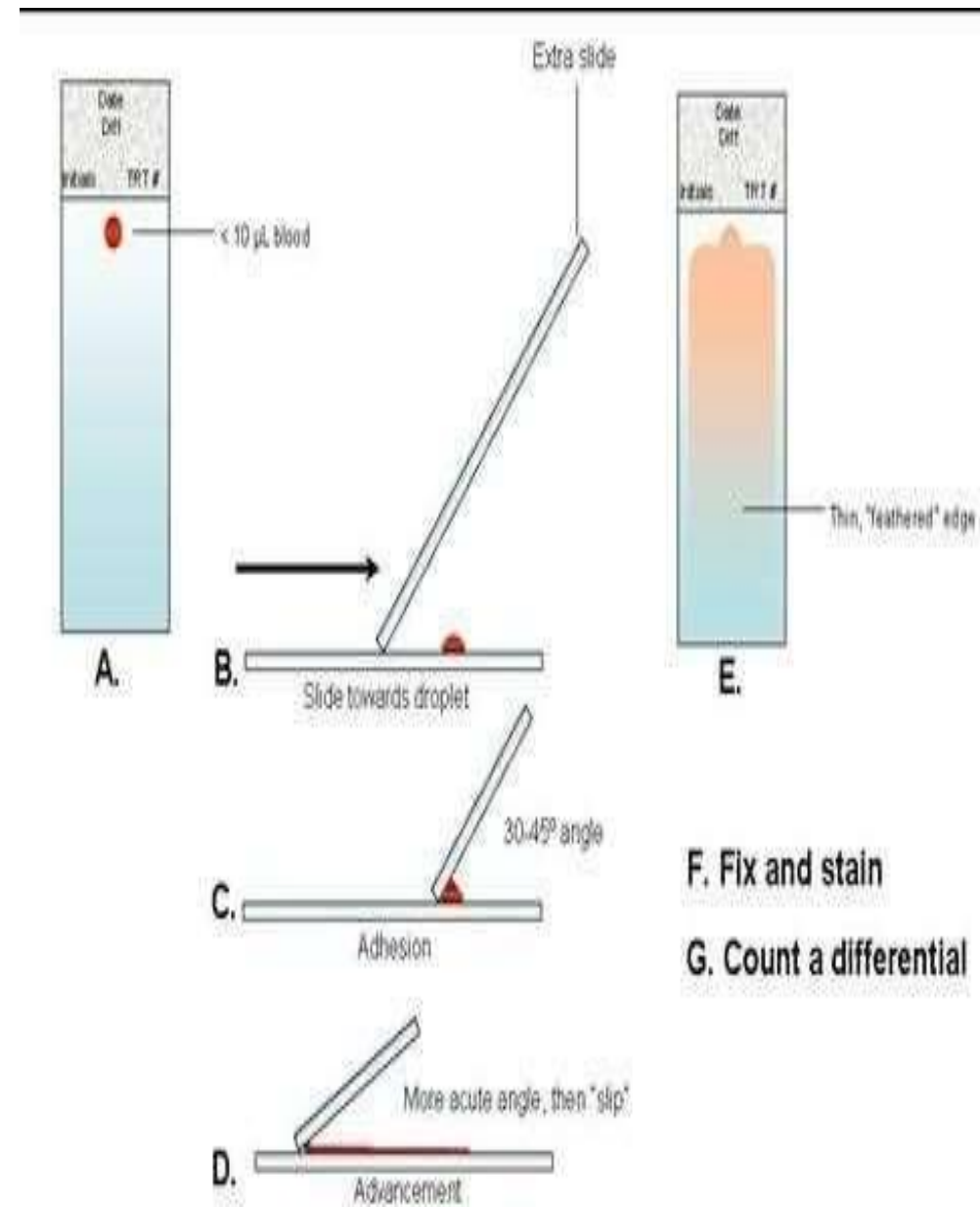
□ *selection of spreader*

- which has smooth edge, without coarse or uneven edge should be avoided.
 - wash out the grease or oil, if it present in the glass slides as well as at the edge of the spreader slide.
 - we have to use oil and grease free slides.
- 



A good smear has following characteristics

- tongue shaped (**head ,body, tail**)
- should cover 2/3rd of the slide
- Should not be thick (single cell thickness)
- should not have marks or blank spaces in the smear.





A



B



C



D



E



F



G



H

staining of blood smear

Leishman's stain:

- Belongs to *Romanowsky* group of stain.
- Contains acidic and basic dye.

Composition:

- **Methylene blue**- basic dye, positively charged and stains negatively charged [acidic] particles (stains nucleus of WBCs, the cytoplasm and basophilic granules)
- **Eosin** –acidic dye, negatively charged and stains positively charged (basic) particles (stains eosinophilic granules / RBCs)
- **Acetone free methyl alcohol**-(*fixative* fix the smear to the slide)

OTHER STAINS

- ***WRIGHT STAIN***


- ***FIELD STAIN***

STAINING THE SMEAR

- MAKE SURE THE SLIDE IS DRY
- POUR THE LEISHMAN'S STAIN DROP BY DROP TILL IT COVERS ENTIRE SMEAR (8-10 DROPS)
- NOTE THE TIME ALLOW FOR 1-2 MINs (its known as **FIXATION TIME**)
- ADD DOUBLE THE AMOUNT DROPS OF DISTILLED WATER
- WAIT FOR 6-8 MINS (**FORMATION OF CATIONS AND ANIONS OF BASIC AND ACIDIC DYE REPECTIVELY**) its knows as **STAINING TIME**.

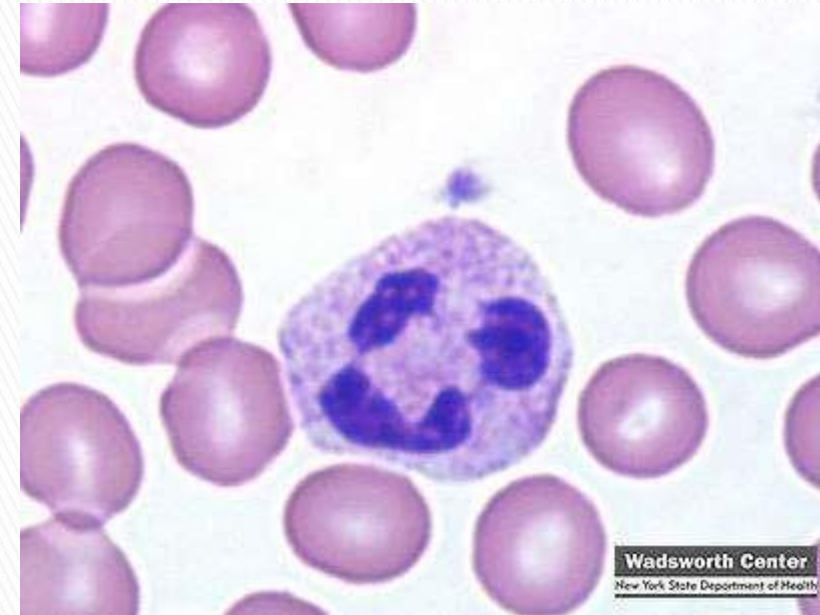
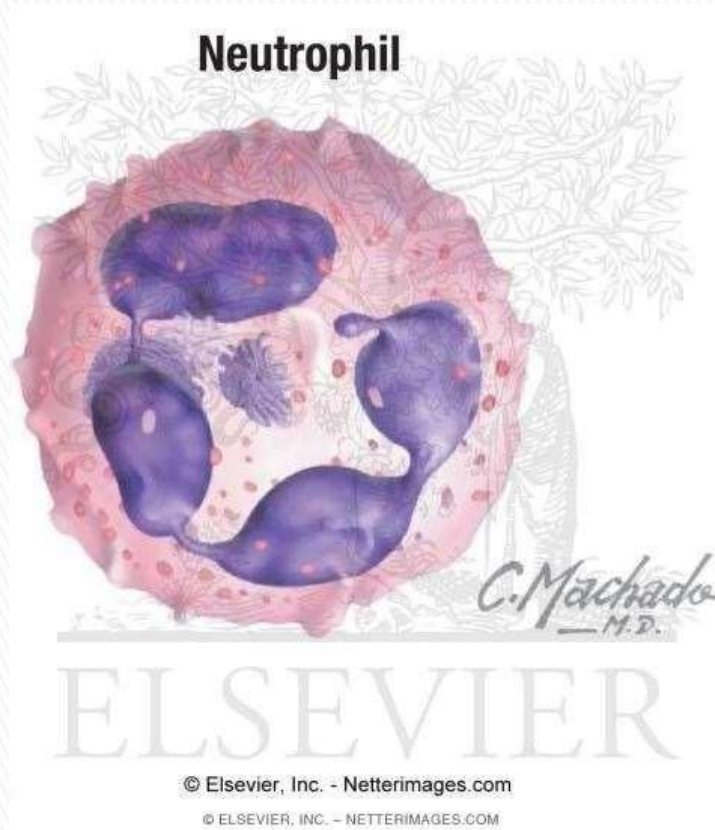
INDICATIONS/USES

(PERIPHERAL BLOOD SMEAR)

- ❑ **TO DETERMINE THE DLC- (GRANULOCYTES /AGRANULOCYTES)**
 - ❑ **TO STUDY THE MORPHOLOGY OF RBCS**
 - ❑ **DETECT THE PRESENCE OF PARASITES LIKE *MALARIA*, *FILARIA***
 - ❑ **SEX DETERMINATION CAN BE DONE BY IDENTIFICATION OF *BAR BODY***
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EXAMINATION OF SMEAR UNDER OIL IMMERSION LENS (100X)

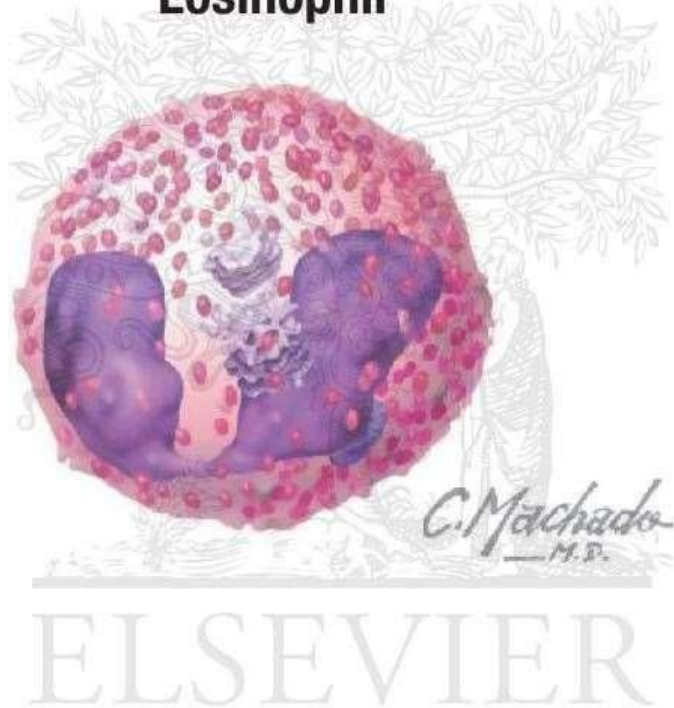
- FOCUS UNDER HIGH POWER (100X)
- PUT ONE DROP OF *CEDAR WOOD OIL*



10-14
NUCLEUS HAVING 2-5 LOBES
PURPLE/PINK IN COLOUR

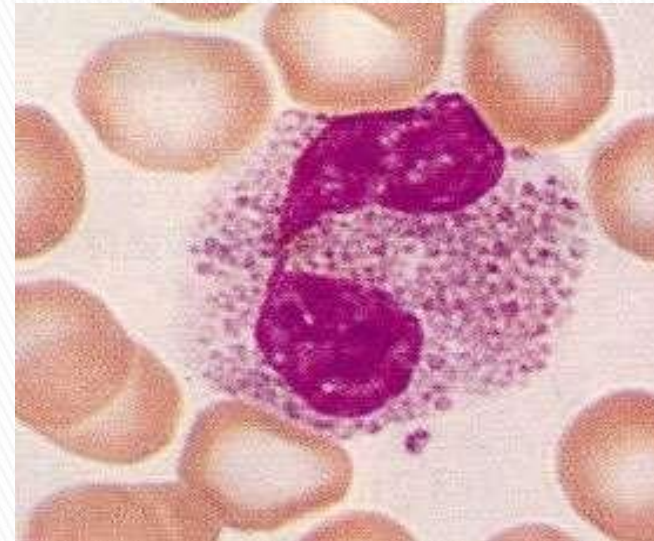
CYTOPLASM PINK IN COLOUR
HAVING FINE PINK OR PURPLE GRANULES

Eosinophil



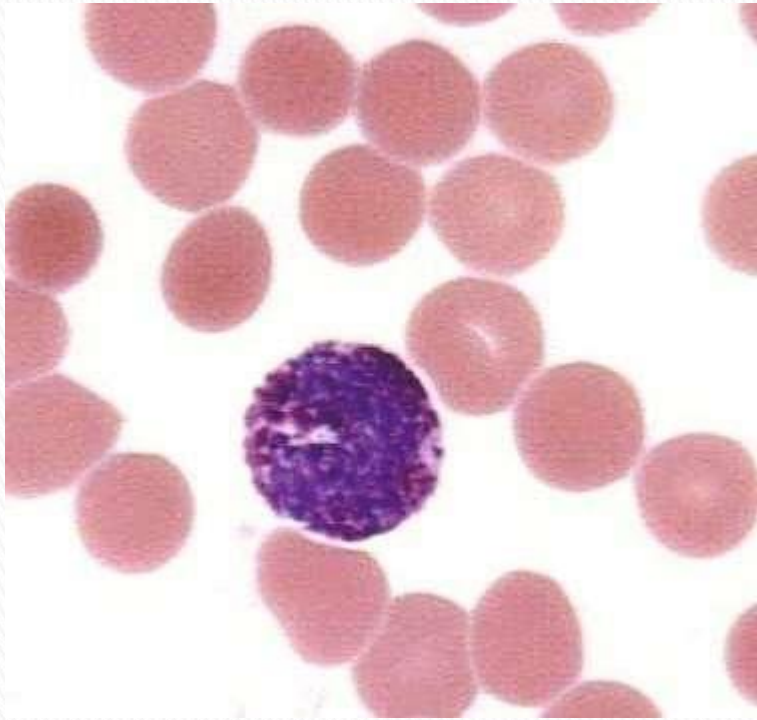
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**10-14 MICRON/ NUCLEUS BILOBED PURPLE
BLUE COLOUR**



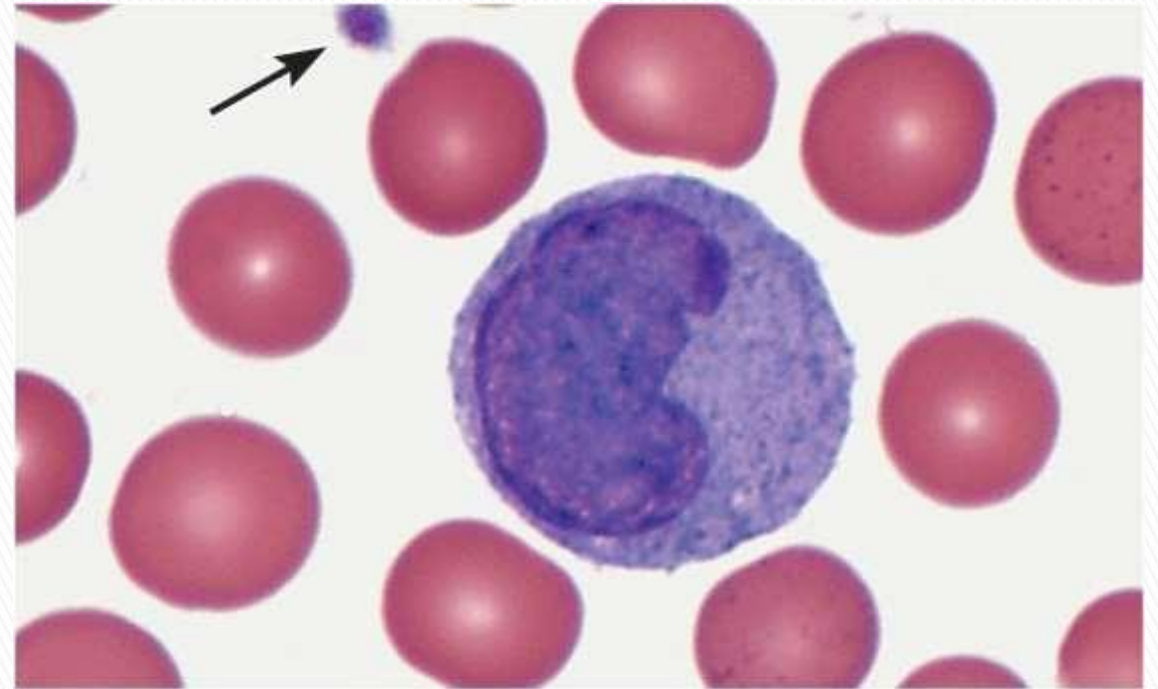
**COARSE ORANGE TO RED IN COLOUR
GRANULES/ CYTOPLASM PINK COLOUR**

BASOPHIL



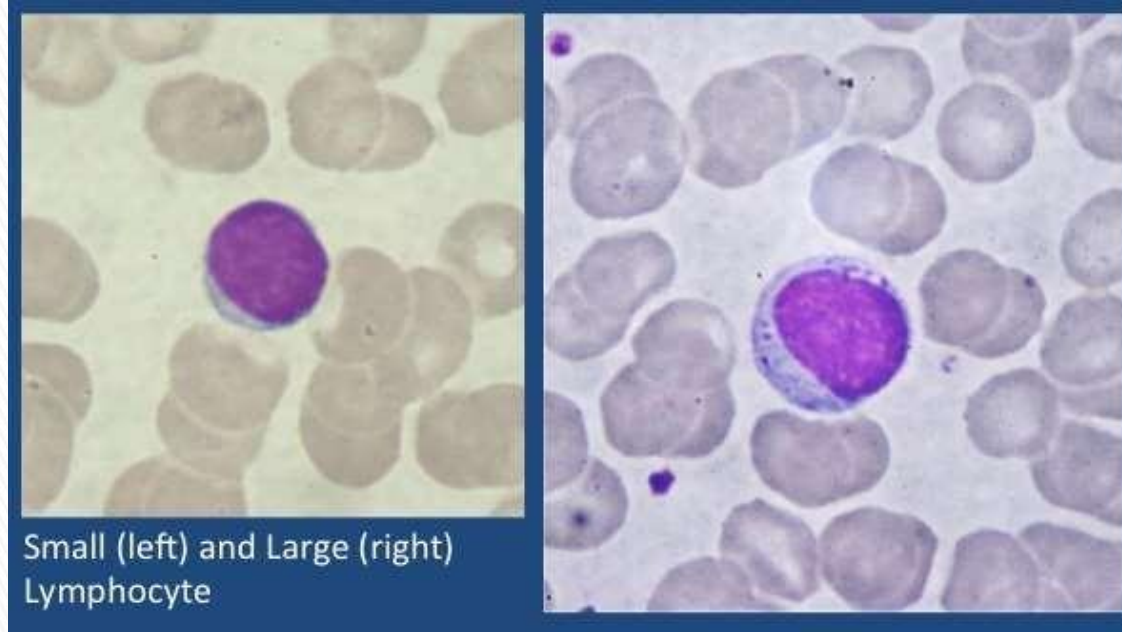
8-10 /NUCLEUS 2-3 LOBES/NOT PROPERLY
VISIBLE BECAUSE OF GRANULES/
COARSE BLUISH BLACK GRANULES OVERLYING
THE NUCLEUS

MONOCYTE



18-22/NOTCHED OR OVAL OR HORSE SHOE SHAPE
NUCLEUS
NONE OR MODERATE COARSE AZURE GRANULES
CYTOPLASM SKY BLUE

LYMPHOCYTE



SMALL LYMPHOCYTE

7-10

NUCLEUS LARGE ROUND FILLS THE WHOLE CELL

SKY BLUE THIN RIM

SKY BLUE/NONE OR MODERATE GRANEULES

LARGE LYMPHOCYTE

10-14

SMALL ROUND NUCLEUS


CYTOPLASM SKY BLUE

GRANEULS NONE OR FEW

CELL COUNT

CELLS	SIZE	NUCLEUS	CYTOPLASM	GRANULES
NEUTROPHIL	10-14	2-5 LOBES PURPULE/PINK	PINK	FINE PINK OR PURPLE GRANULES
EOSINOPHIL	10-14 MICRON	BILOBED PURPULE BLUE COLOUR	PINK	COARSE RED IN COLOUR
BASOPHIL	8-10	2-3 LOBES/NOT PROPERLY VISIBLE BECAUSE OF GRANULES	PALE PINK	COARSE BLUISH BLACK GRANULES OVERLYING THE NUCLEUS
LARGE LYMPHOCYTE	10-14	SMALL ROUND NUCLEUS	SKY BLUE	NONE OR FEW
SMALL LYMPHOCYTE	7-10	LARGE ROUND FILLS THE WHOLE CELL	SKY BLUE THIN RIM	SKY BLUE/NONE OR MODERATE GRANEULES
MONOCYTE	18-22	NOTCHED OR OVAL OR HORSE SHOE SHAPE	SKY BLUE	NONE OR MODERATE COARSE AZURE GRANULES

NORMAL DLC

- NEUTROPHIL 50-70%
 - LYMPHOCYTE 20-40%
 - MONOCYTE 2-8%
 - EOSINOPHIL 1-4%
 - BASOPHIL 0-1%
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METHOD OF COUNTING

- DRAW 100 SQUARES
- IDENTIFY THE VARIOUS CELLS ENTER FIRST LETTER

N-NEUTROPHIL

E-EOSINOPHIL

B-BASOPHIL

L-LYMPHOCYTE

M-MONOCYTE

OBSERVATION


- NEUTROPHIL= ? %
- EOSINOPHIL = ? %
- LYMPHOCYTE= ? %
- MONOCYTE = ? %
- BASOPHILS = ? %

- ABSOLUTE NEUTROPHIL = $\frac{\text{WBC count} \times \text{neutrophil \%}}{100}$ -- cumm

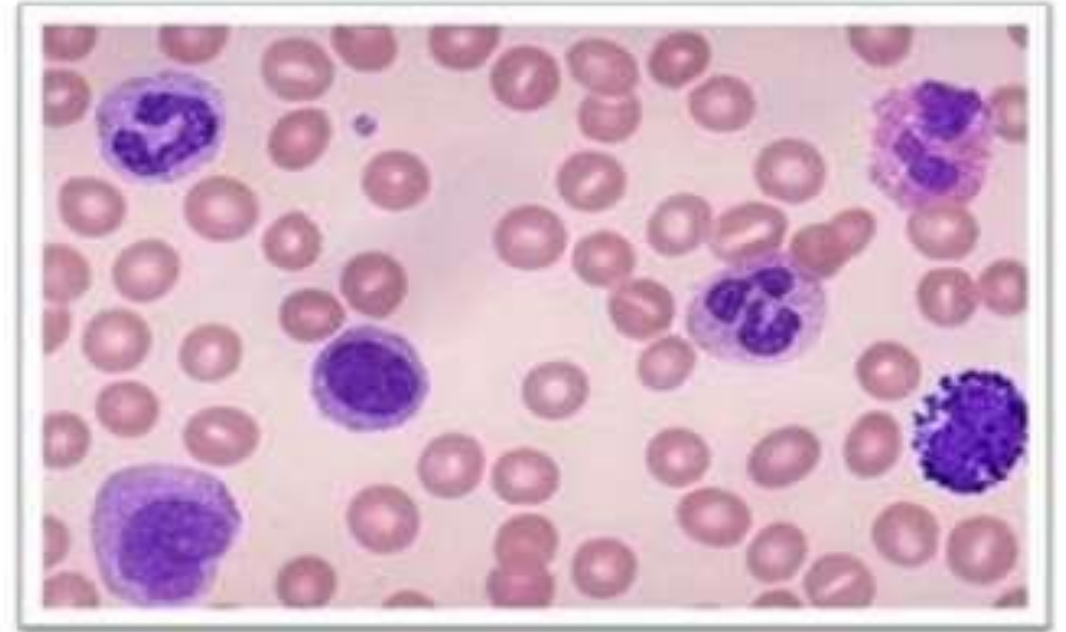
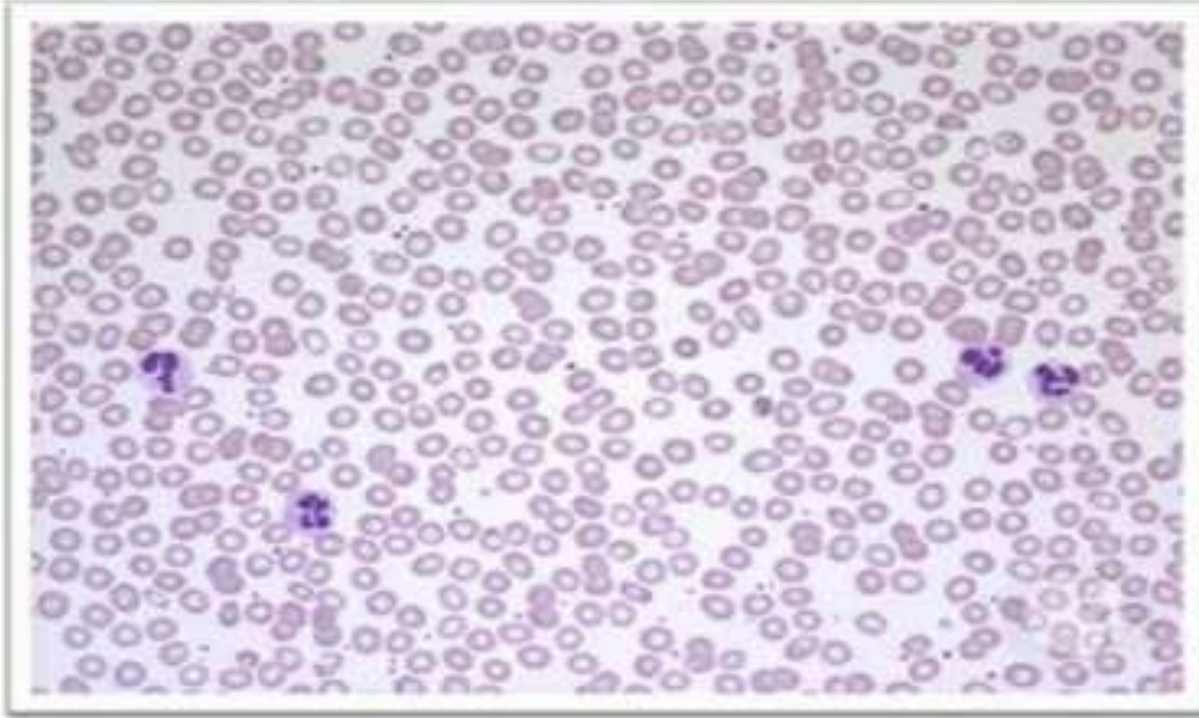
ARNETH COUNT

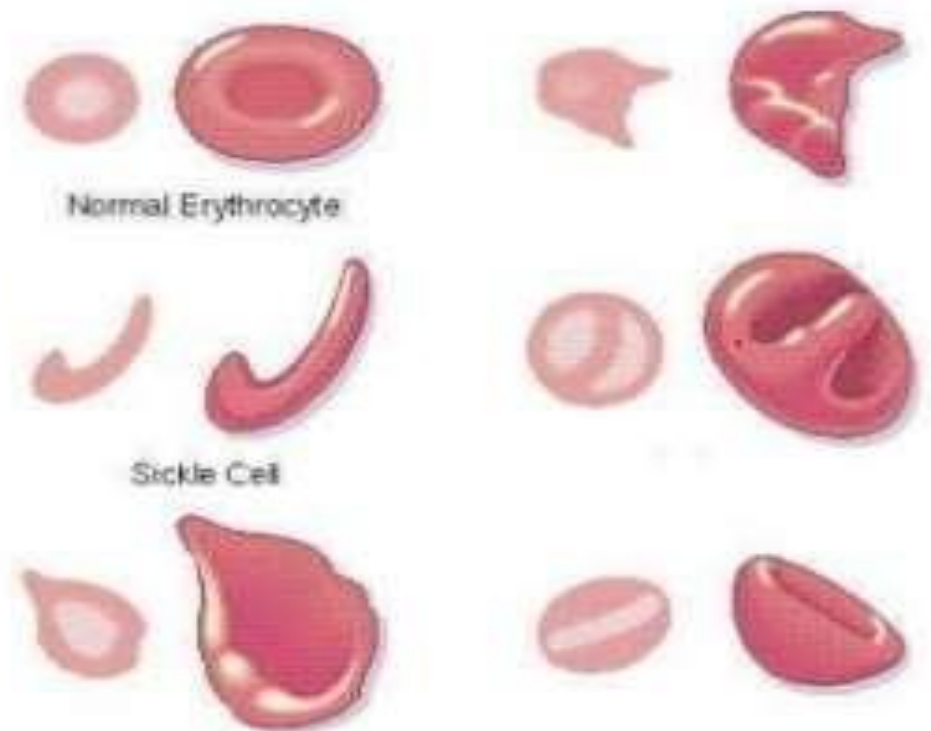
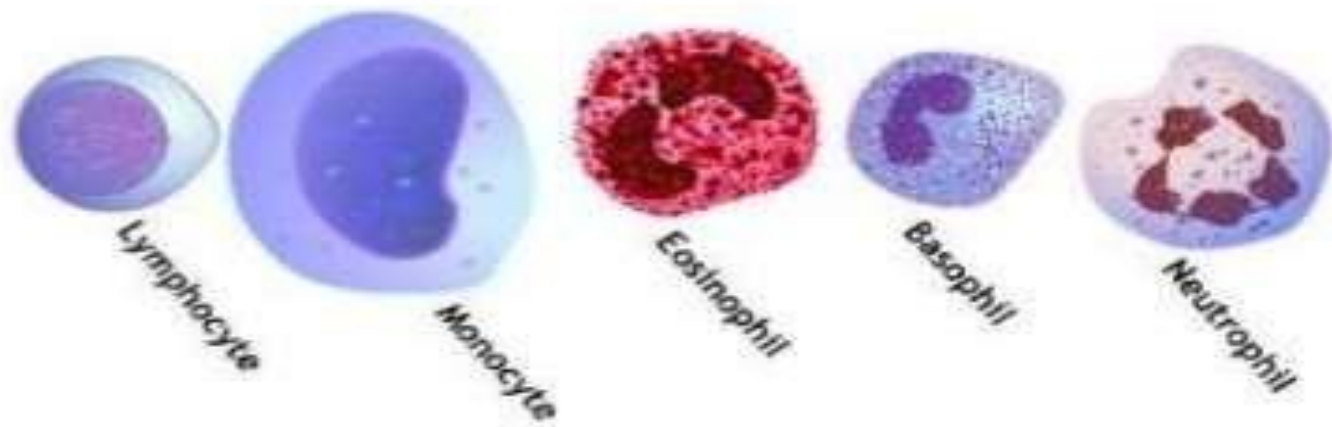
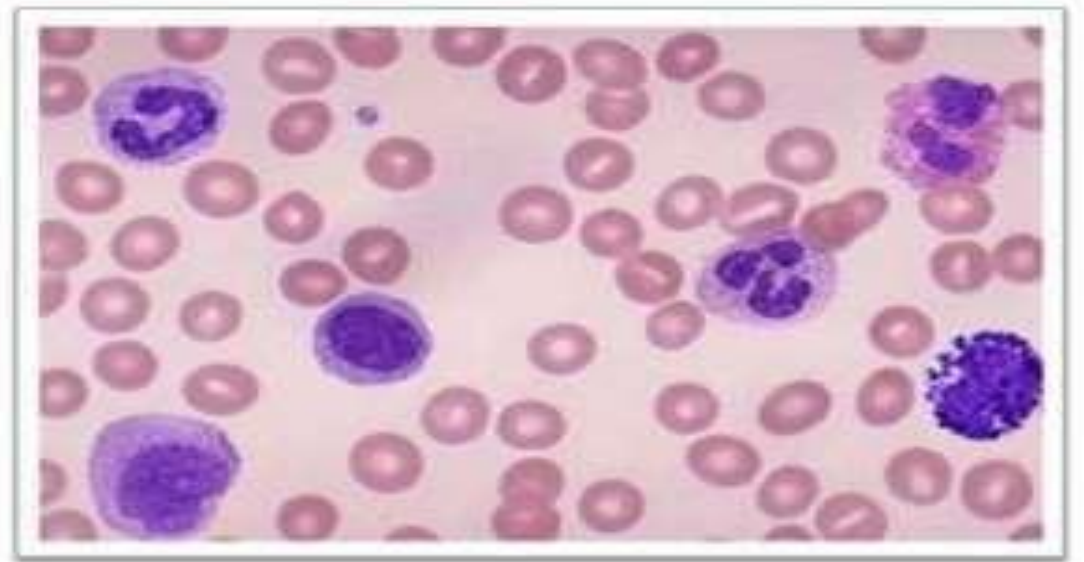
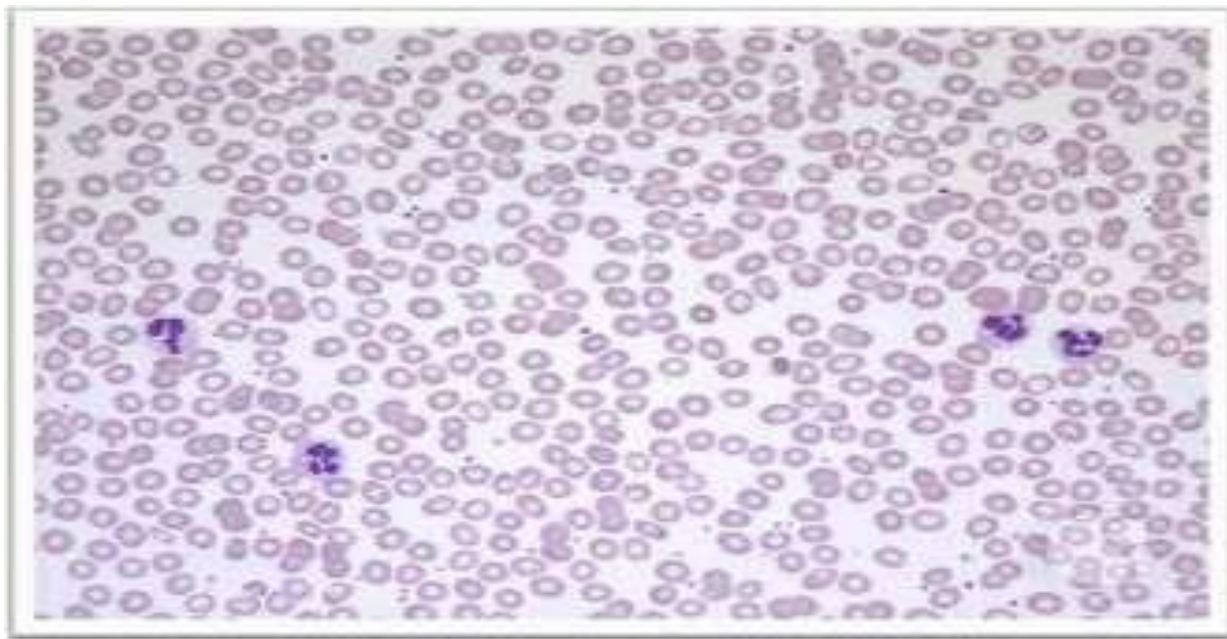
- DETERMINATION OF THE PERCENTAGE DISTRIBUTION OF DIFFERENT TYPES OF NEUTROPHILS ON THE BASIS OF NUMBER LOBES IN THEIR NUCLEUS

- Normal count
 - N1 - 2-10%
 - N2 - 20-30%
 - N3 - 40-50%
 - N4 – 10-15%
 - N5 – 2-5 %

- **SHIFT TO LEFT** –MORE YOUNGER CELLS (REGENARATIVE SHIFT)
 - **SHIFT TO RIGHT** - MORE OLDER CELLS (DEGENARATIVE SHIFT)
- 

Try to Identify the Cell and Their Name





Reference

- **Textbook of Practical Physiology by**
 - **A.K.Jain**
 - **C.L. Ghai &**
 - **G.K.Pal**
 - **Net Source for images**

THANKYOU..

