- *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:

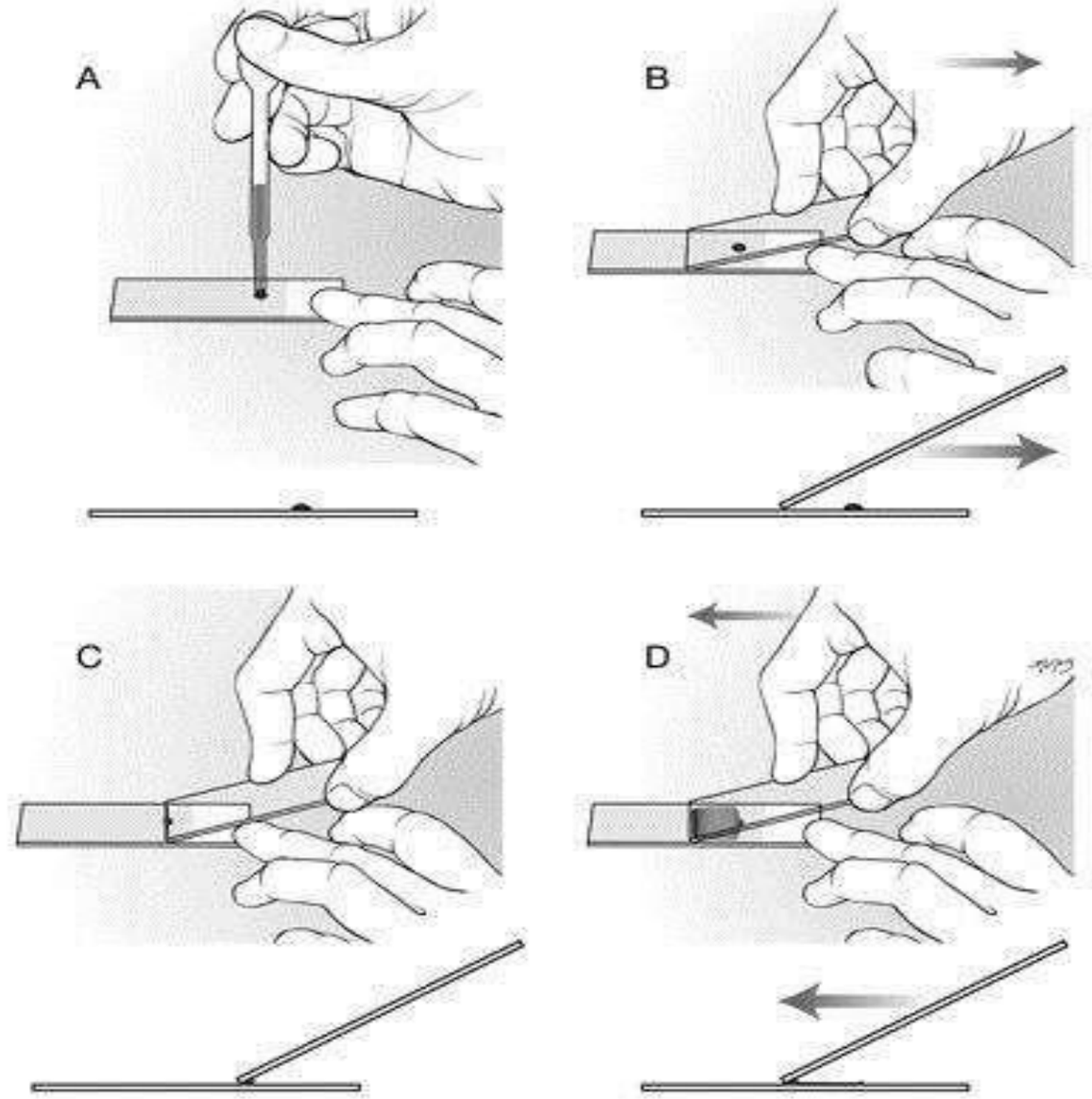
- 4-5 GLASS SLIDES
- LANCET/ SPRIT/COTTON
- LEISHMAN'S STAIN
- MICROSCOPE
- CEDAR WOOD OIL
- DISTILLED WATER
- STAINING TRAY

PROCEDURE

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

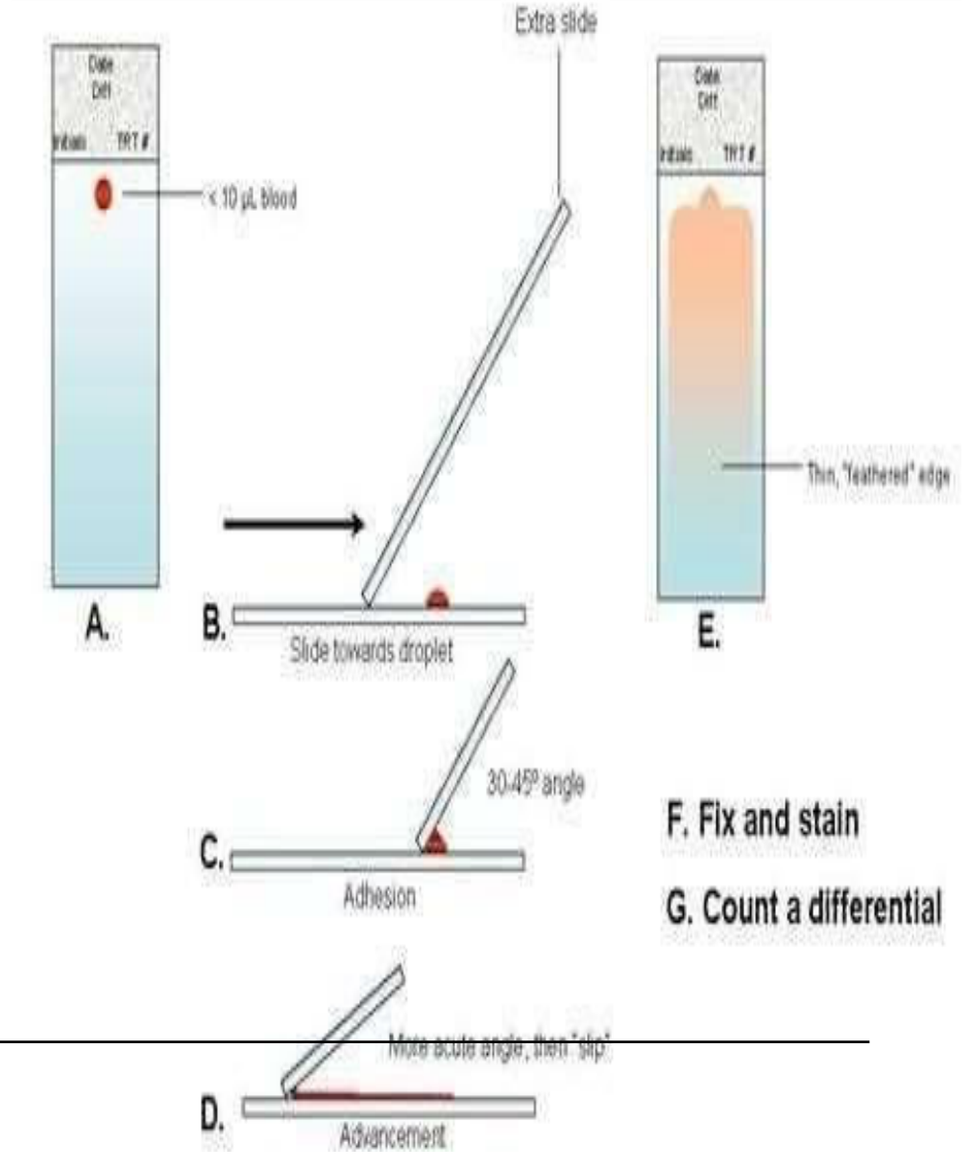
Preparation of smear

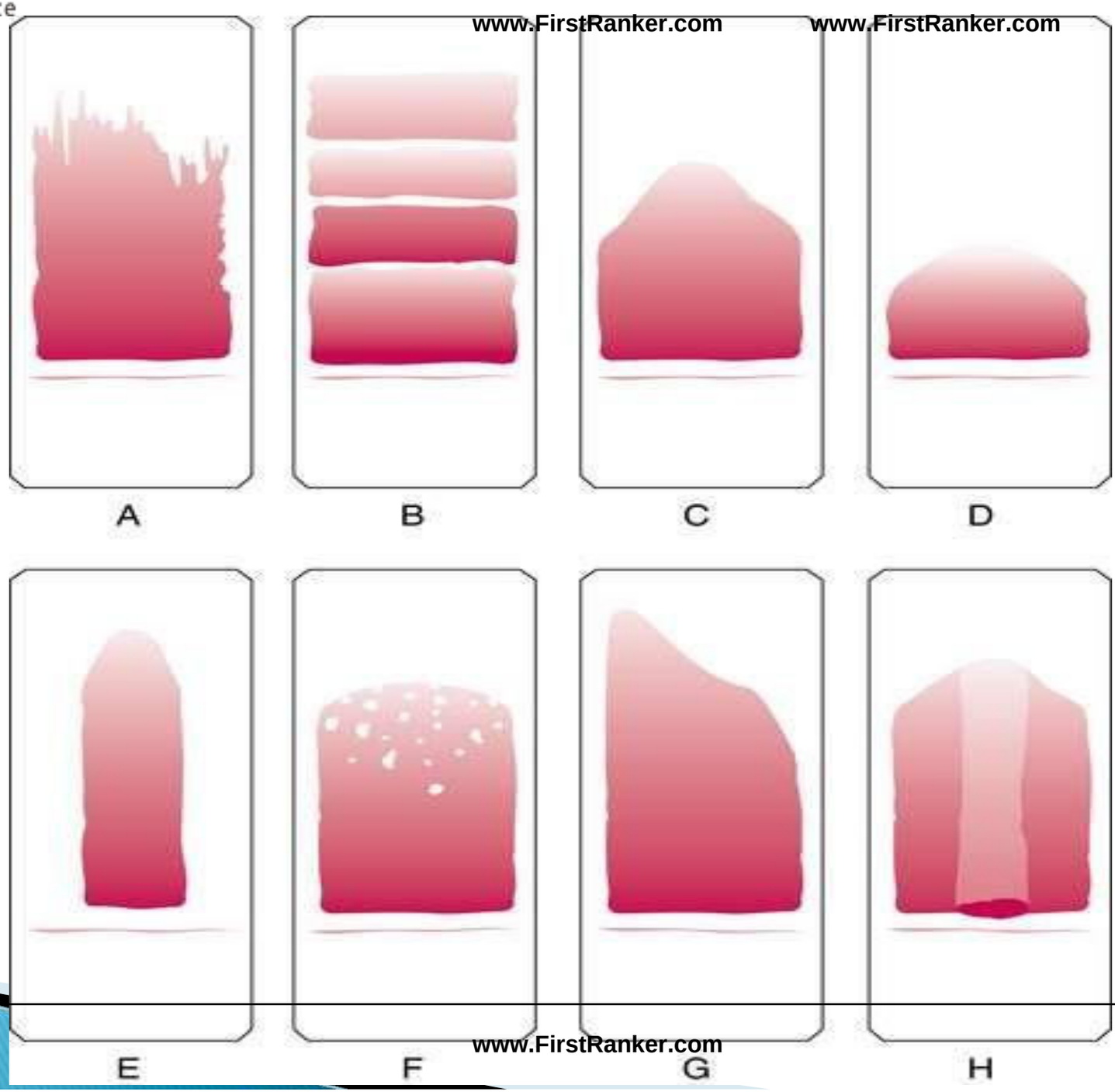
- ***selection of spreader***
  - which has smooth edge, without coarse or uneven edge should be avoided.
  - wash out the grease or oil, if it present in the glass slides as well as at the edge of the spreader slide.
  - we have to use oil and grease free slides.



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.





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**.

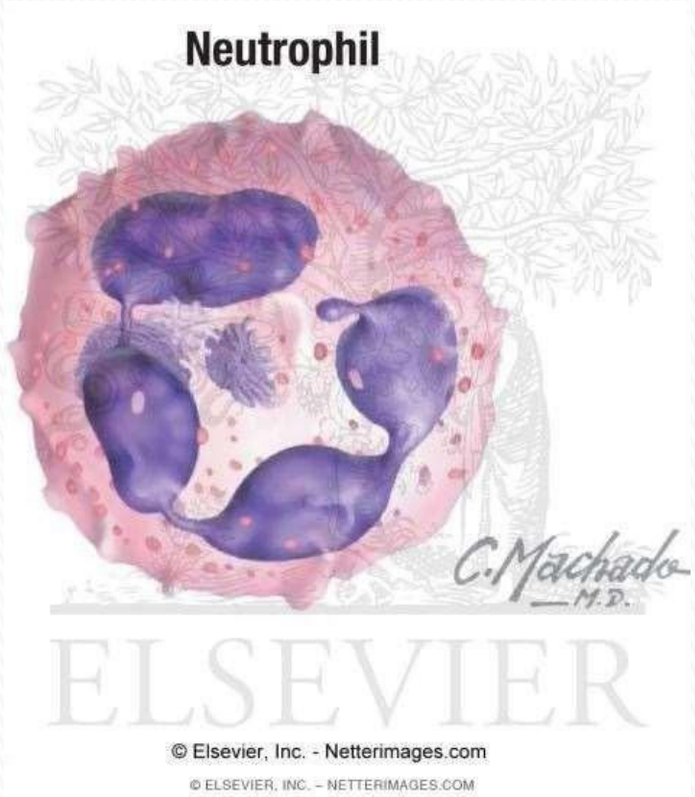
INDICATIONS/USES

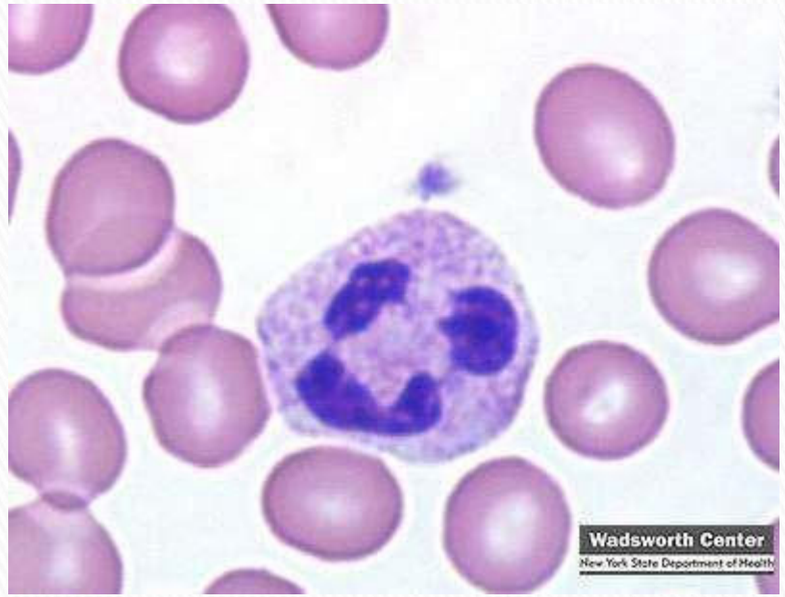
(PERIPHERAL BLOOD SMEAR)

- **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***

EXAMINATION OF SMEAR UNDER OIL IMMERSION LENS (100X)

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

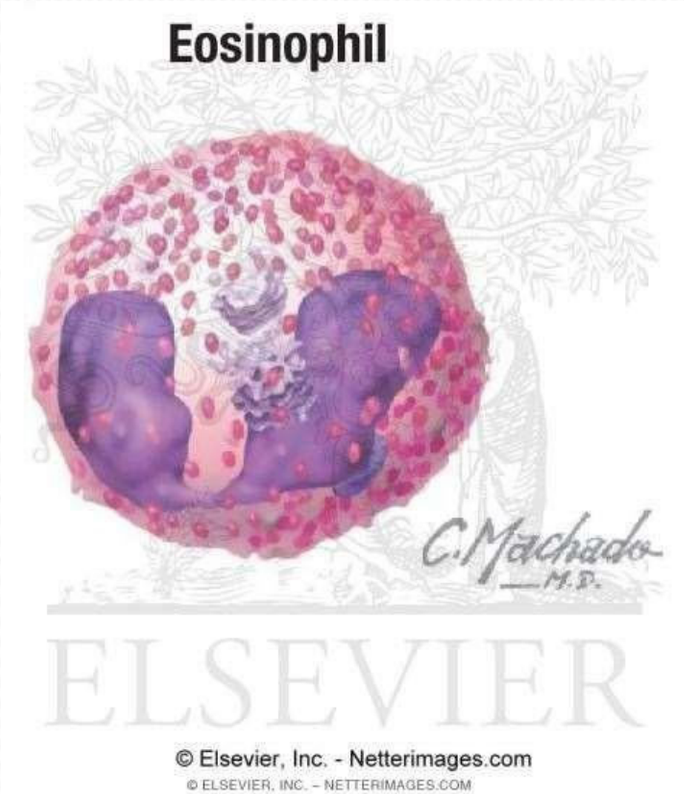


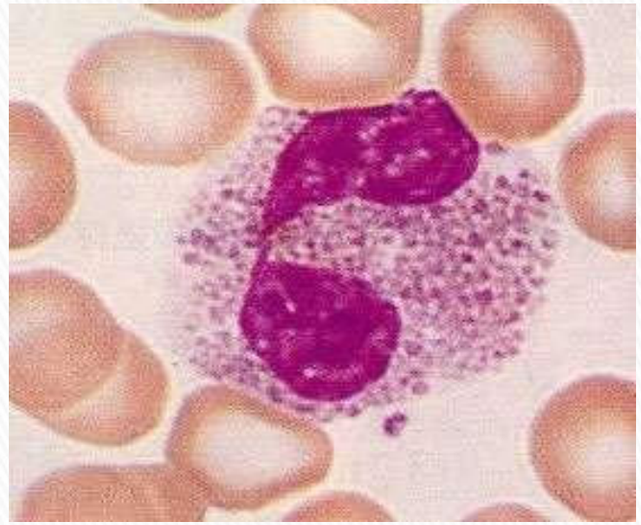


10-14

NUCLEUS HAVING 2-5 LOBES
PURPULE/PINK IN COLOUR

CYTOPLASM PINK IN COLOUR
HAVING FINE PINK OR PURPLE GRANULES

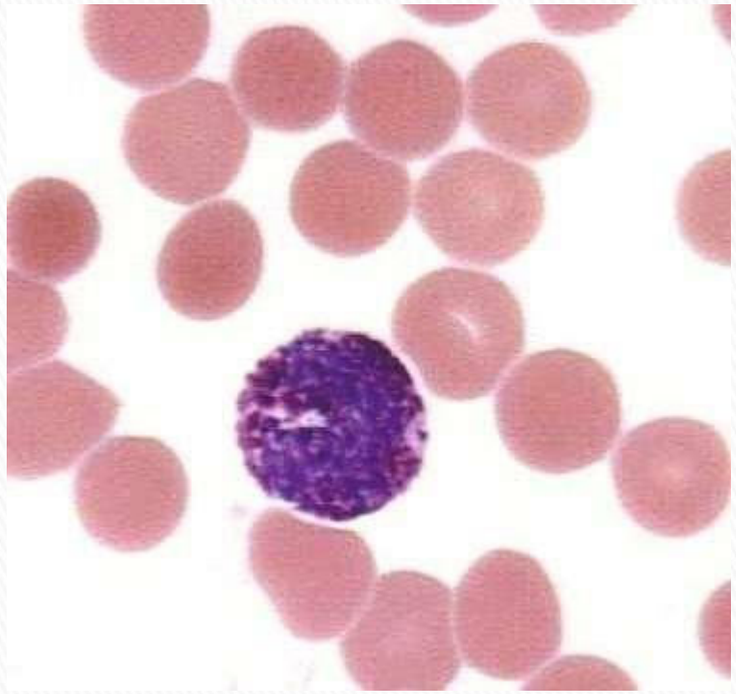




10-14 MICRON/ NUCLEUS BILOBED PURPULE BLUE COLOUR

COARSE ORANGE TO RED IN COLOUR GRANULES/ CYTOPLASM PINK COLOUR

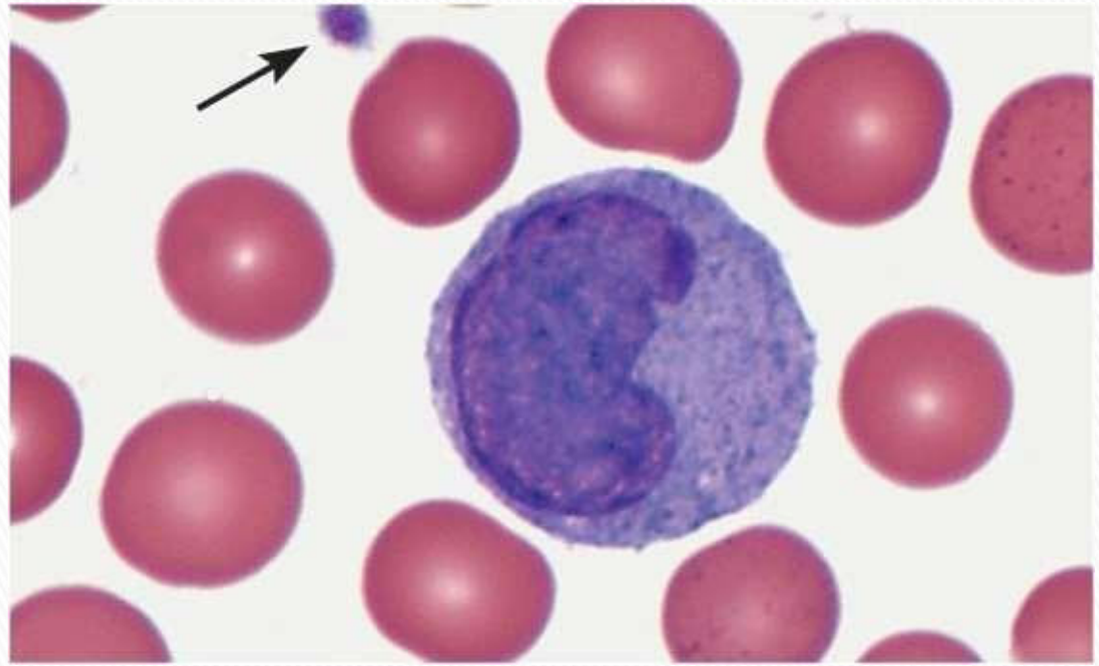
BASOPHIL



8-10 /NUCLEUS 2-3 LOBES/NOT PROPERLY VISIBLE BECAUSE OF GRANULES/

COARSE BLUISH BLACK GRANULES OVERLYING THE NUCLEUS

MONOCYTE

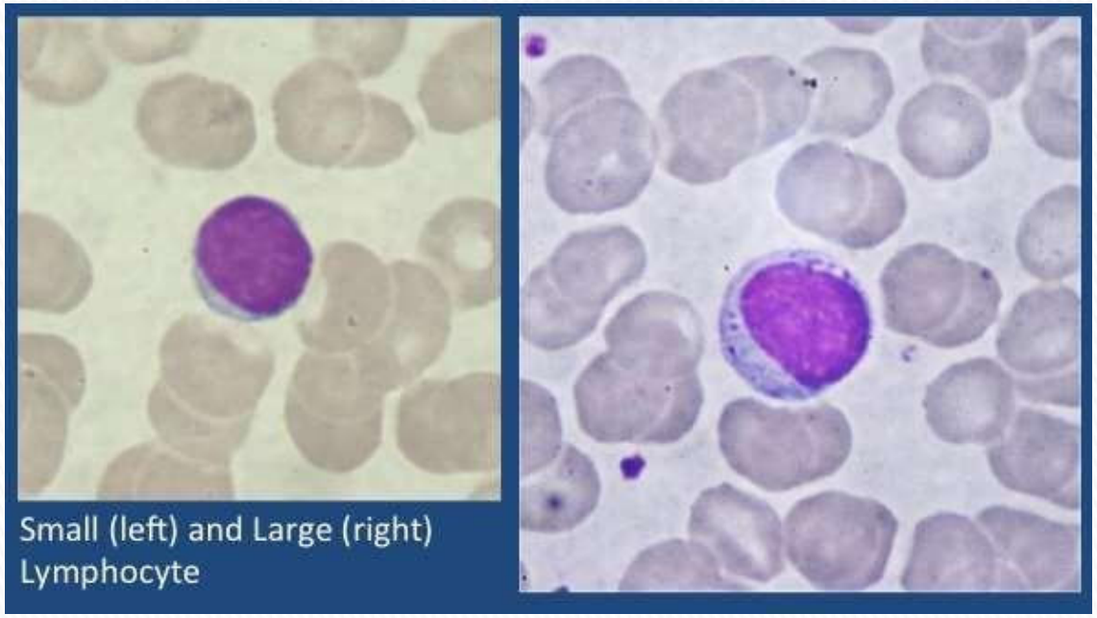


18-22/NOTCHED OR OVAL OR HORSE SHOE SHAPE NUCLEUS

NONE OR MODERATE COARSE AZURE GRANULES

CYTOPLASM SKY BLUE

LYMPHOCYTE



Small (left) and Large (right) 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%
- LYPHOCYTE 20-40%
- MONOCYTE 2-8%
- EOSINOPHIL 1-4%
- BASOPHIL 0-1%

METHOD OF COUNTING

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

N-NEUTROHIL
E-EOSINOPHIL
B-BASOPHIL
L- LYMPHOCYTE
M-MONOCYTE

OBSERVATION

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

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

ARNETH COUNT

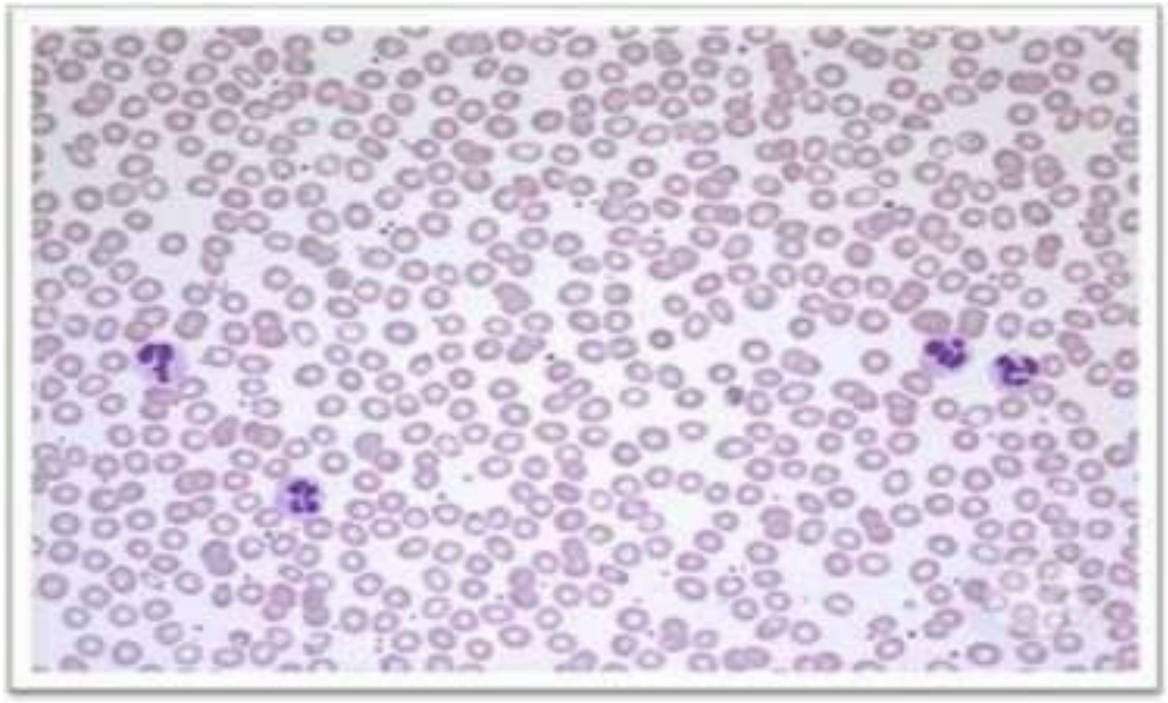
- DETERMINATION OF THE PERCENTAGE DISTRIBUTION OF DIFFERENT TYPES OF NEUTROPHILS ON THE BASIS OF NUMBER LOBES IN THEIR NUCLEUS

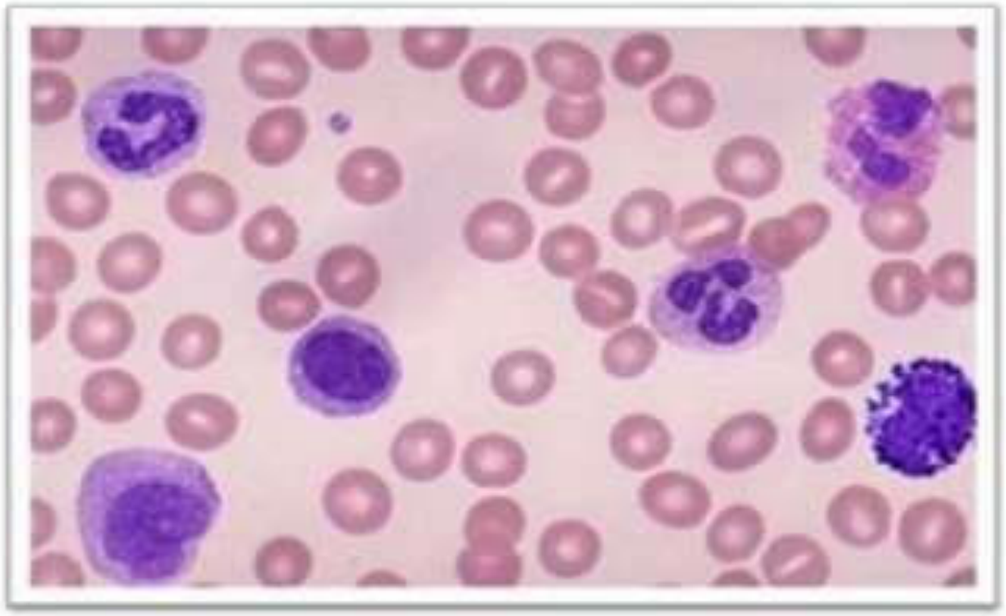
- Normal count

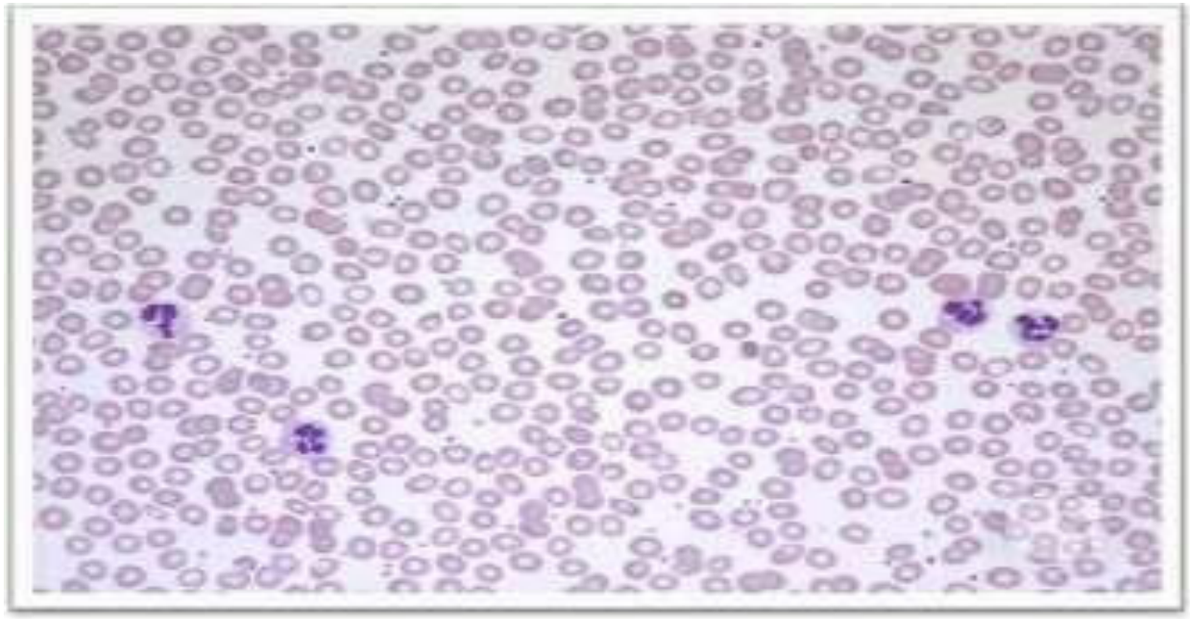
  - N1 - 2-10%
  - N2 - 20-30%
  - N3 - 40-50%
  - N4 – 10-15%
  - N5 – 2-5 %

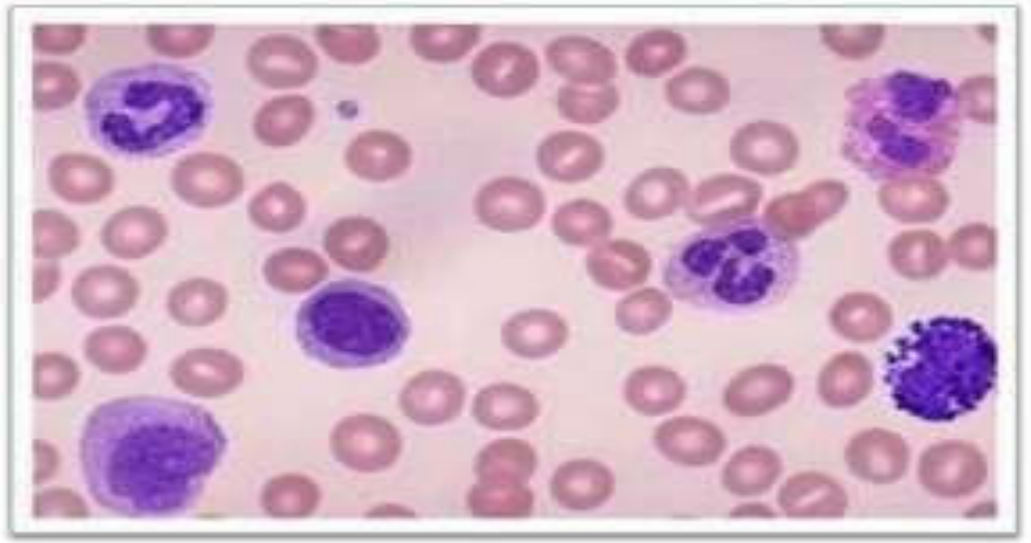
- SHIFT TO LEFT –MORE YOUNGER CELLS (REGENARATIVE SHIFT)
- SHIFT TO RIGHT - MORE OLDER CELLS (DEGENARATIVE SHIFT)

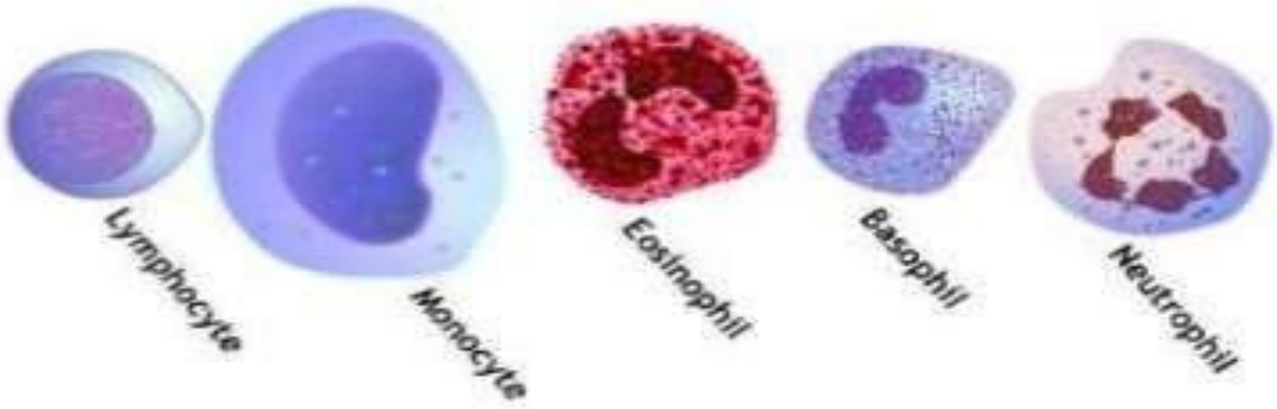
Try to Identify the Cell and Their Name

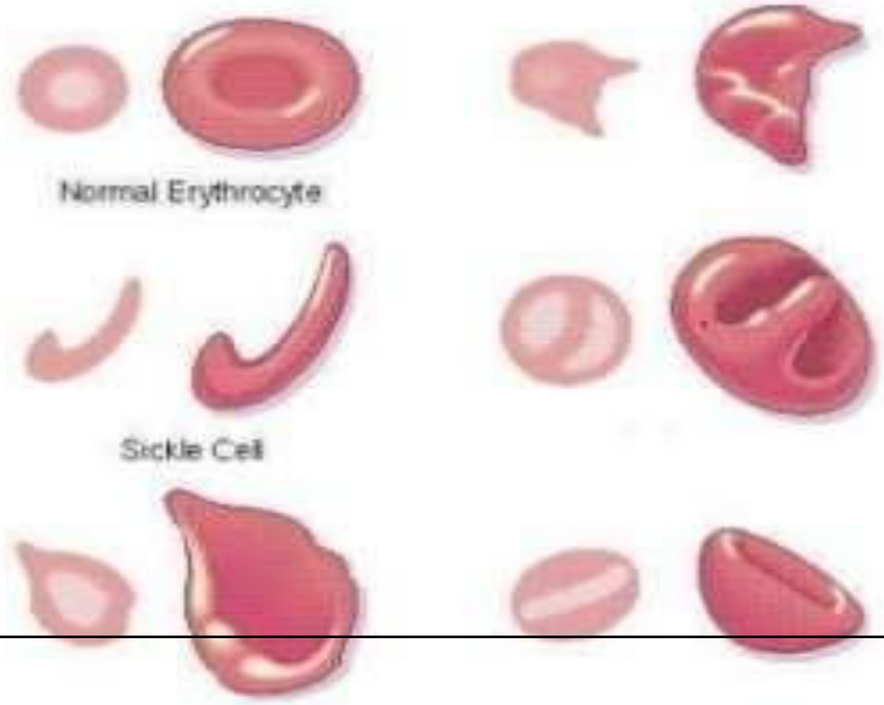












Reference

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