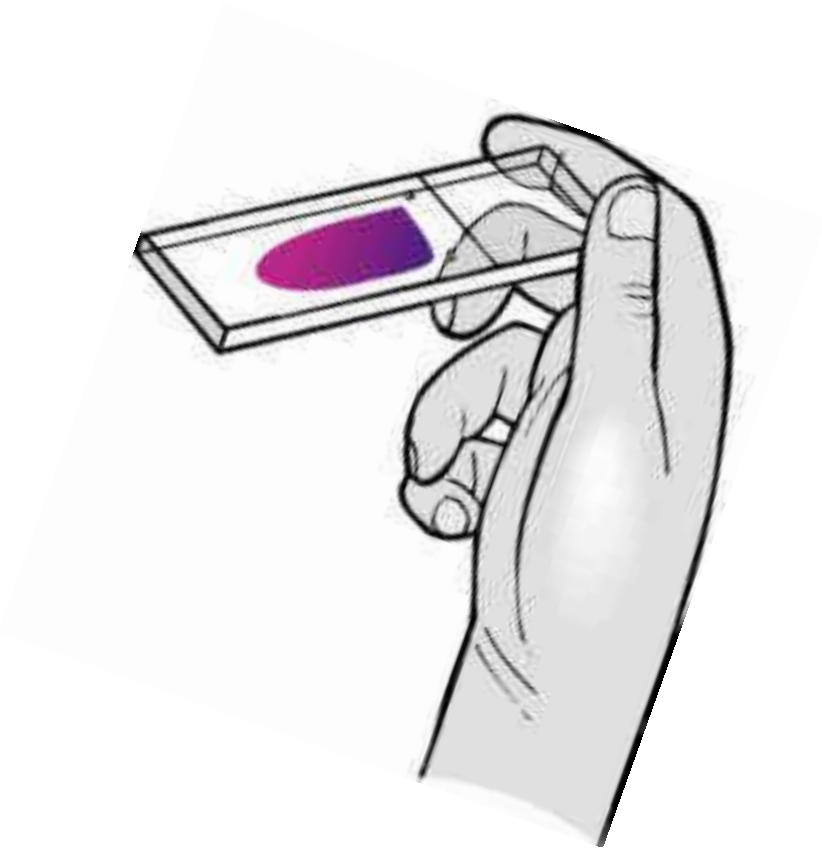
White Blood Cell Differential Count



Anvari_ph@yahoo.com

Three basic steps to make blood smear

1. Preparation of blood smear.
2. Fixation of blood smear.
3. Staining of blood smear.

Equipments for blood smear

- Spreaders
- Clean slides
- Blood capillary tube or micropipette 10 µL
- Fresh blood

Specimen:

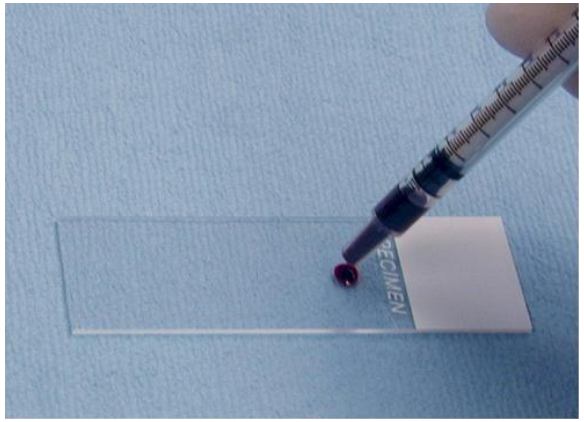
- Peripheral blood smear made from EDTA-anticoagulated blood.

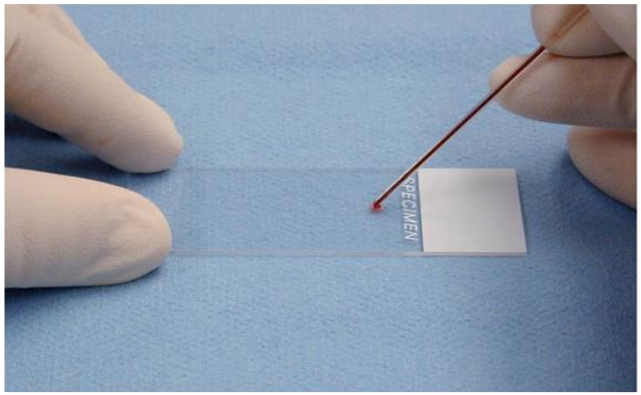
- Blood smears can also be made from finger stick blood directly onto slide.

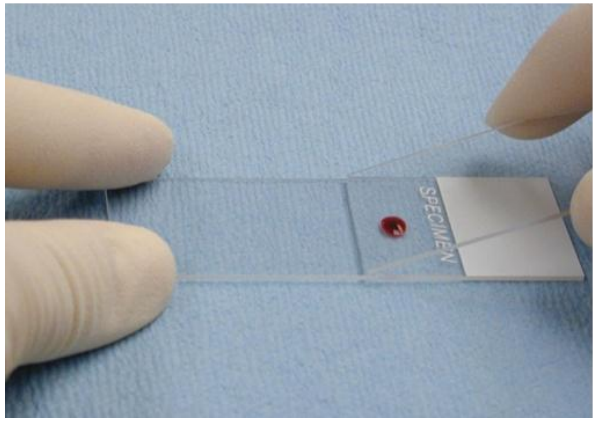
Procedure:

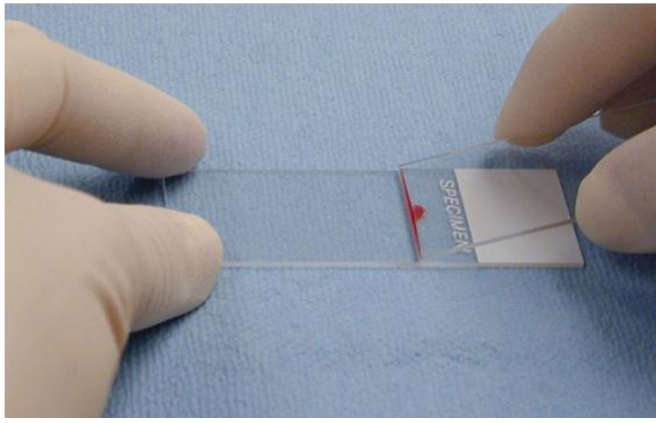
1. Fill a capillary tube three-quarter full with the anticoagulated specimen.
2. Place a drop of blood, about 2 mm in diameter approximately an inch from the frosted area of the slide.
3. Place the slide on a flat surface, and hold the narrow side of the non frosted edge between your left thumb and forefinger.
4. 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.
5. Hold the spreader slide at a 30° angle, and draw it back against the drop of blood.

Steps for Blood Film





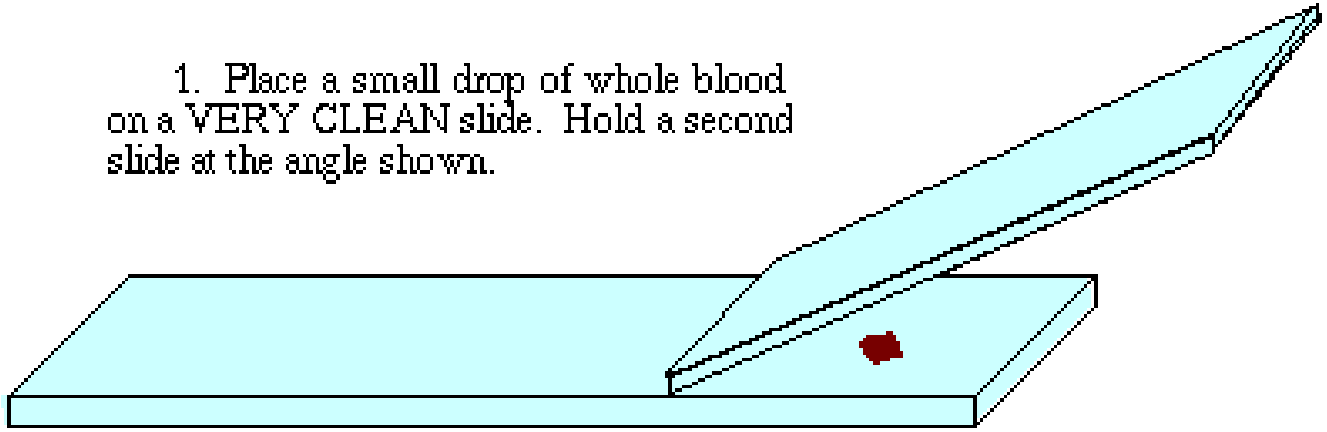




Procedure:

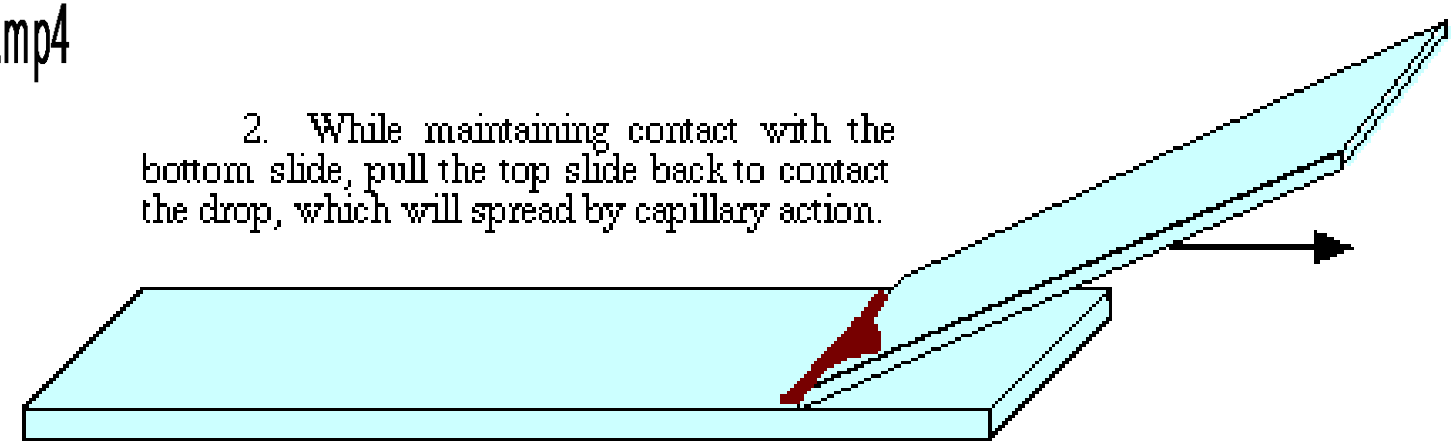
6. Allow the blood to spread almost to the edges of the slide.
7. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
8. Label the frosted edge with patient name and date.
9. Allow the blood film to air-dry completely before staining. (Do not blow to dry. The moisture from your breath will cause RBC artifact).

1. Place a small drop of whole blood on a VERY CLEAN slide. Hold a second slide at the angle shown.

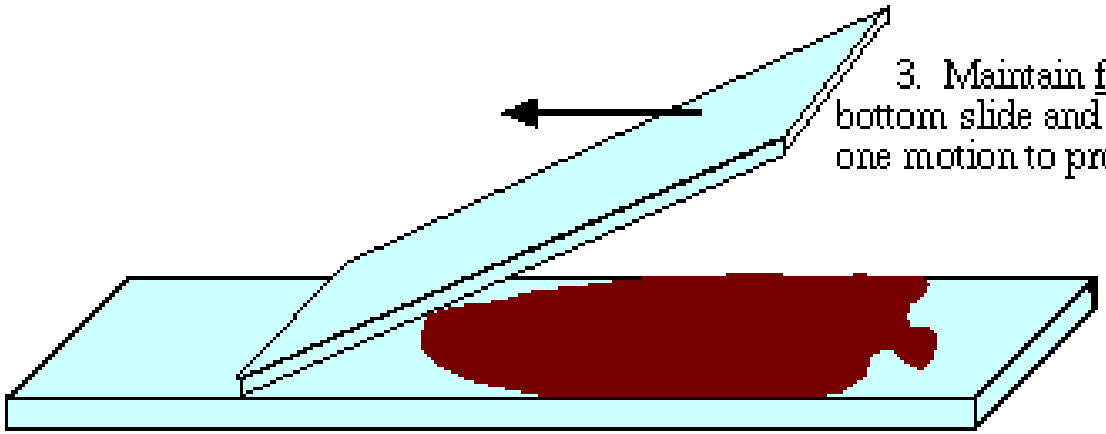


تهيه اسمير خوني.mp4

2. While maintaining contact with the bottom slide, pull the top slide back to contact the drop, which will spread by capillary action.



3. Maintain <u>firm</u> contact with the bottom slide and push the top slide in one motion to produce the smear.



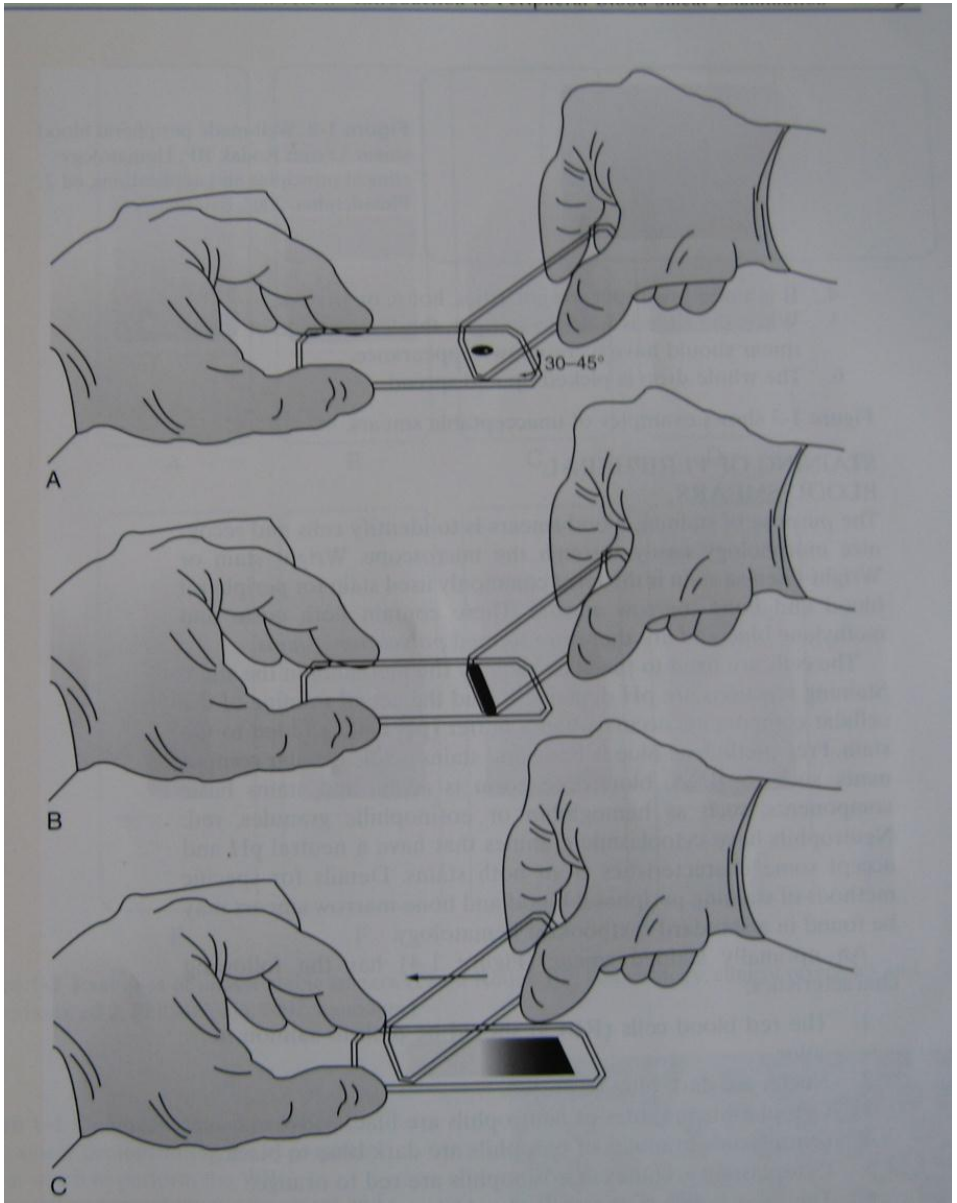
PERIPHERAL BLOOD SMEAR

Wedge technique of making PBS

A. Correct angle to hold spreader slide

B. Blood spread across width of slide

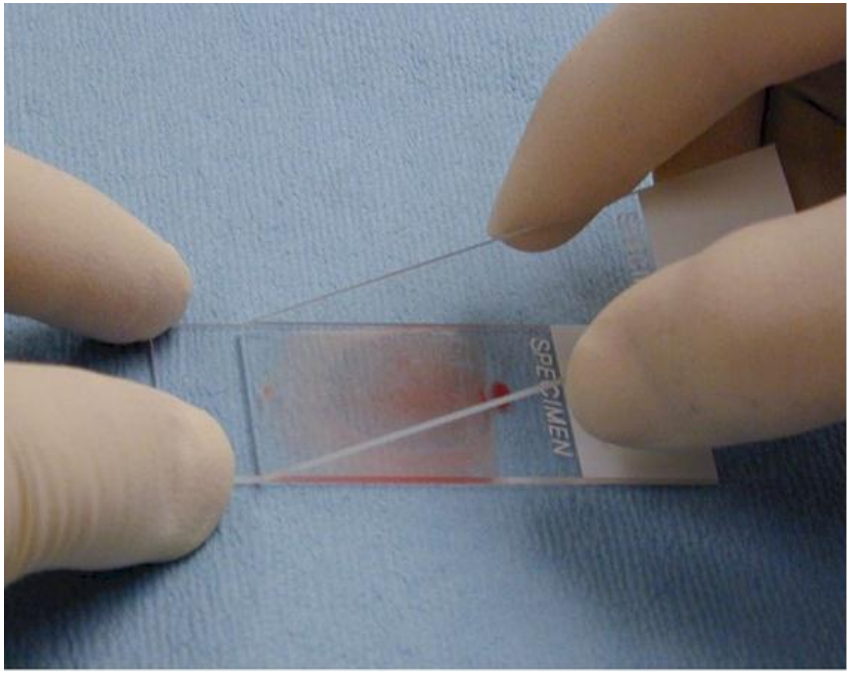
C. Completed wedge smear

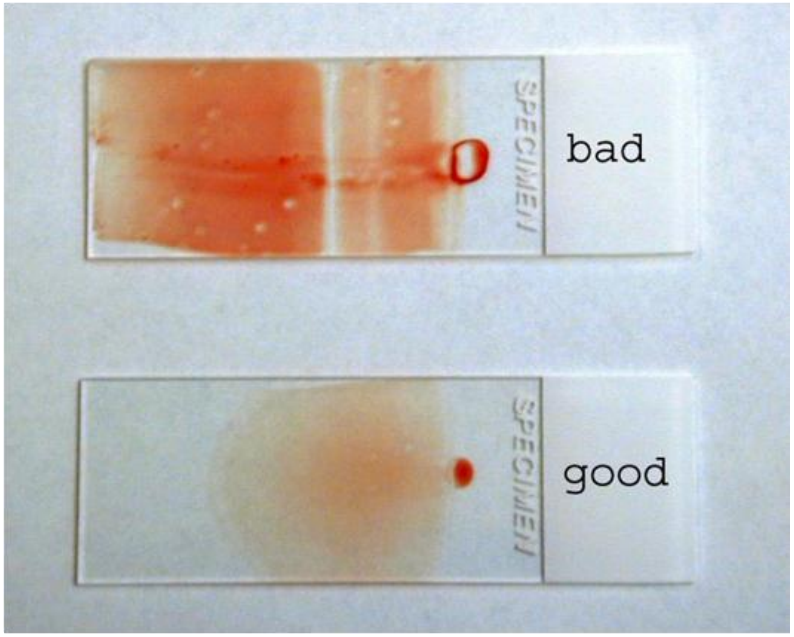


Characteristics of A Good Smear:

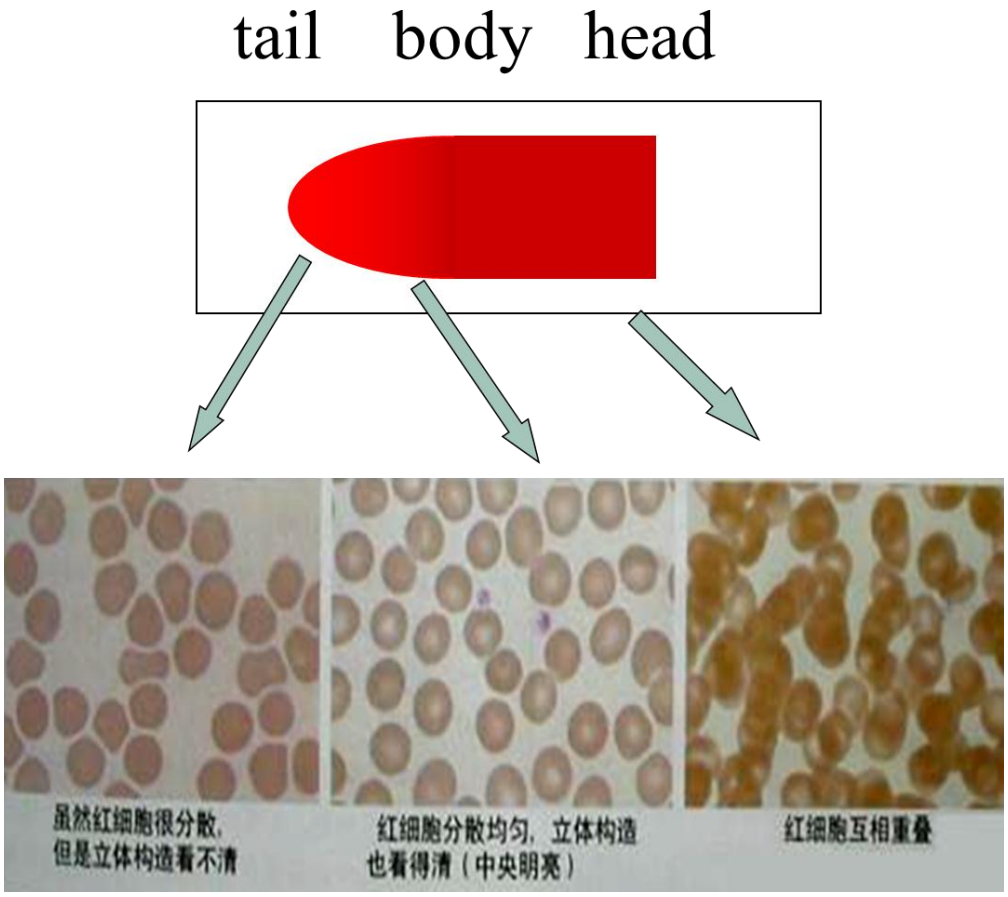
1. A good blood film preparation will be thick at the drop end and thin at the opposite end.
2. The blood smear should occupy the central portion of the slide (around ¾ of the slide).
3. The blood smear should not touch the edges. except for point of application.
4. Should be margin free (Lateral edges of the smear should be visible)
5. About two thirds to three fourths of the slide is covered by the smear
6. It is smooth without irregularities, holes, or streaks
7. When the slide is held up to light, the featheredge of the smear should have a “rainbow” appearance
8. The whole drop is picked up and spread

The shape of blood film





The shape of blood film



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.
5. Failure to push the spreader slide completely across the slide.
6. Irregular spread with ridges and long tail: Edge of spreader dirty or chipped; dusty slide.
7. **Holes in film:** Slide contaminated with fat or grease and air bubbles.
8. **Cellular degenerative changes:** Delay in fixing, inadequate fixing time or methanol contaminated with water.

Factors that affect the smear include

- ✓ blood drop size
- ✓ angle of the slide used to spread
- ✓ Speed of spreading

— Making an acceptable slide takes lots of practice!

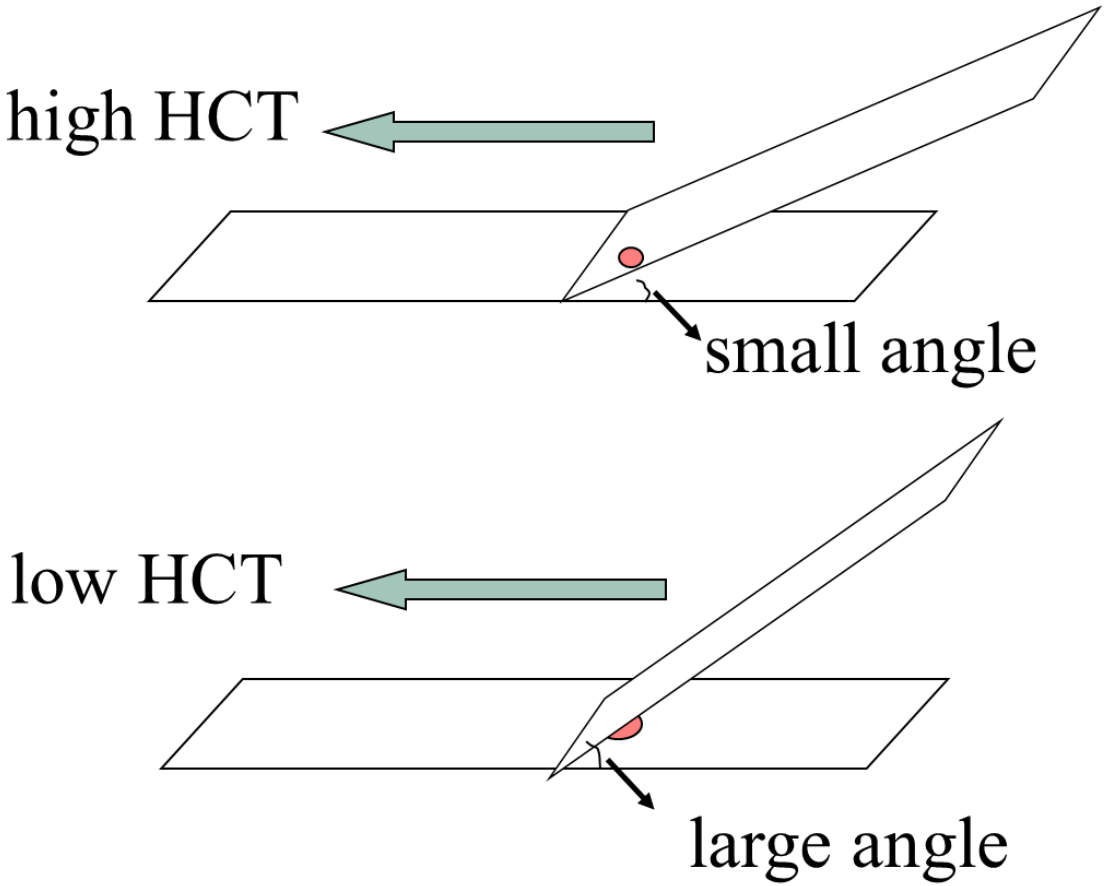
✓ As soon as the drop of blood is placed on the glass slide, the smear should be made without delay. Any delay results in an abnormal distribution of the white blood cells.

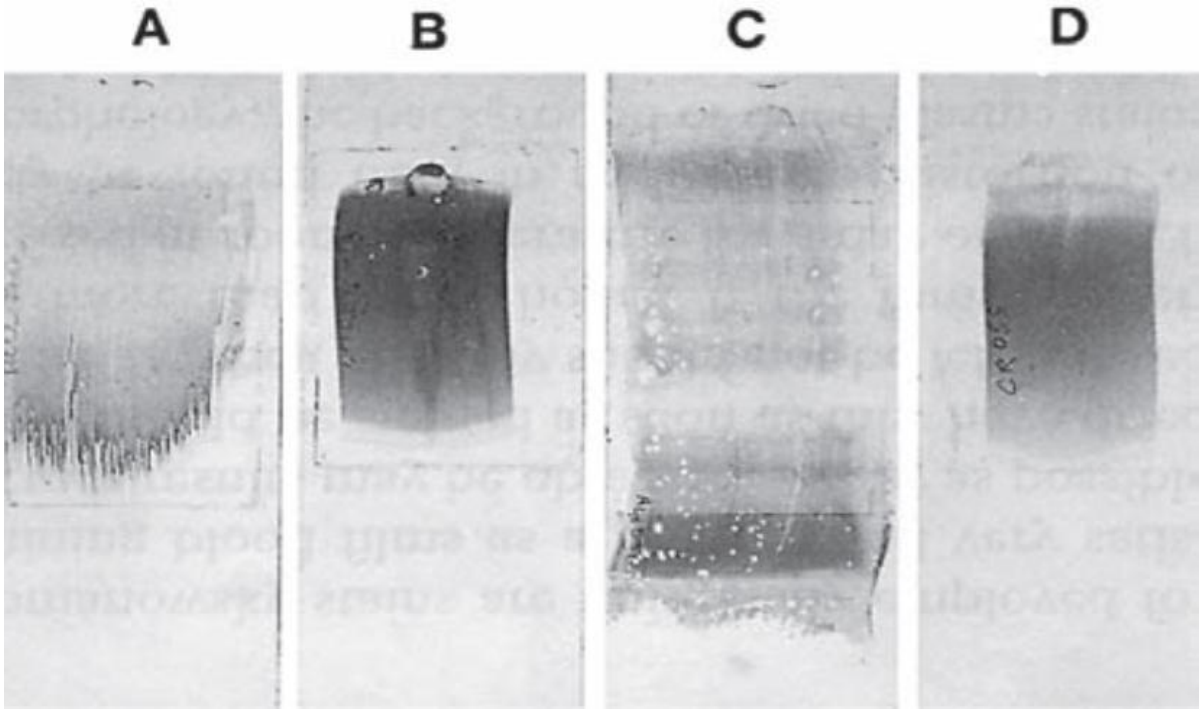
The thickness of the spread notes

1. If the hematocrit is increased, the angle of the spreader slide should be decreased.

2. If the hematocrit is decreased, the angle of the spreader slide should be increased.

The thickness of the spread notes





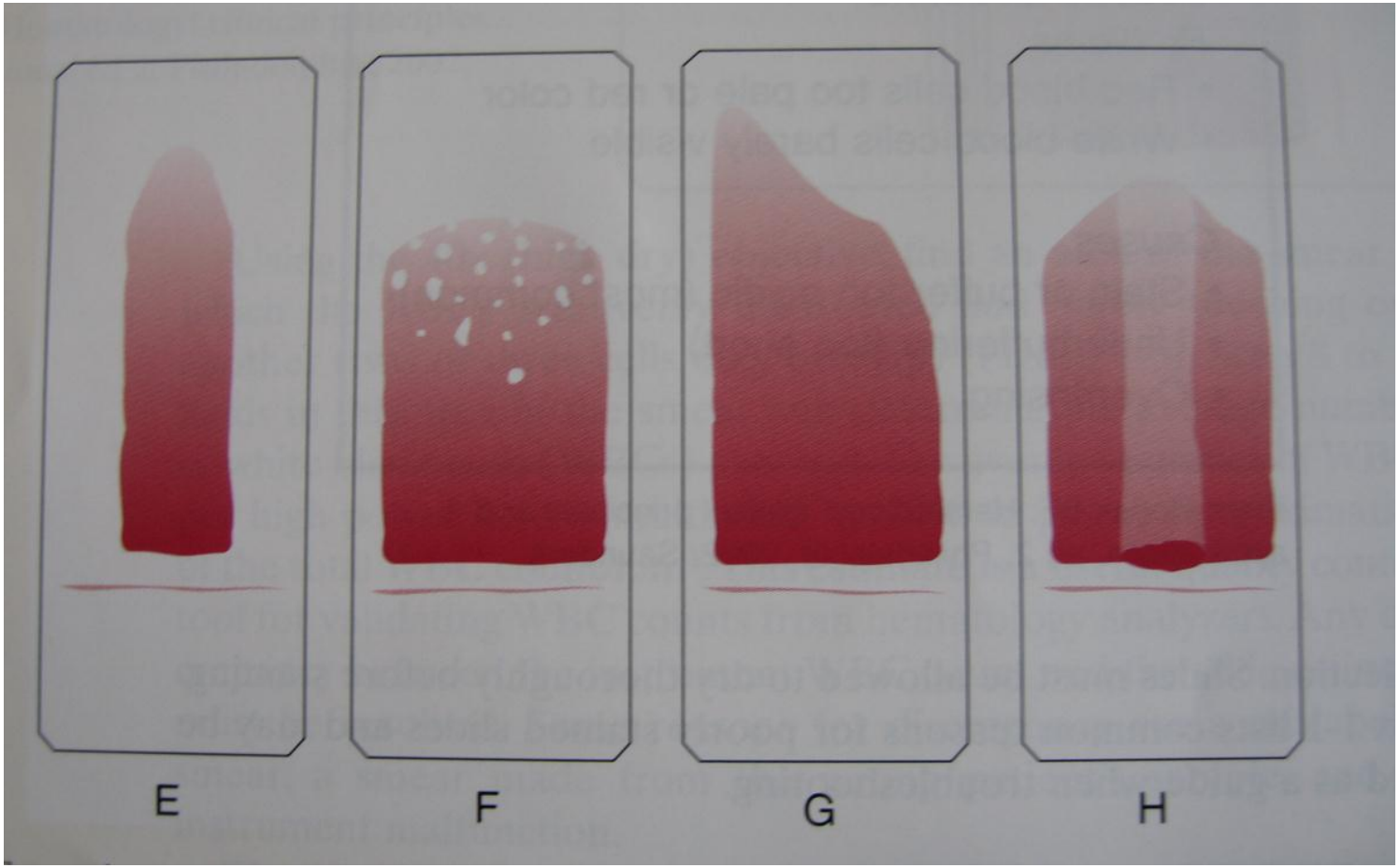
A: Blood film with jagged tail made from a spreader with a chipped end.

B: Film which is too thick

C: Film which is too long, too wide, uneven thickness and made on a greasy slide.

D: A well-made blood film

Examples of unacceptable smears

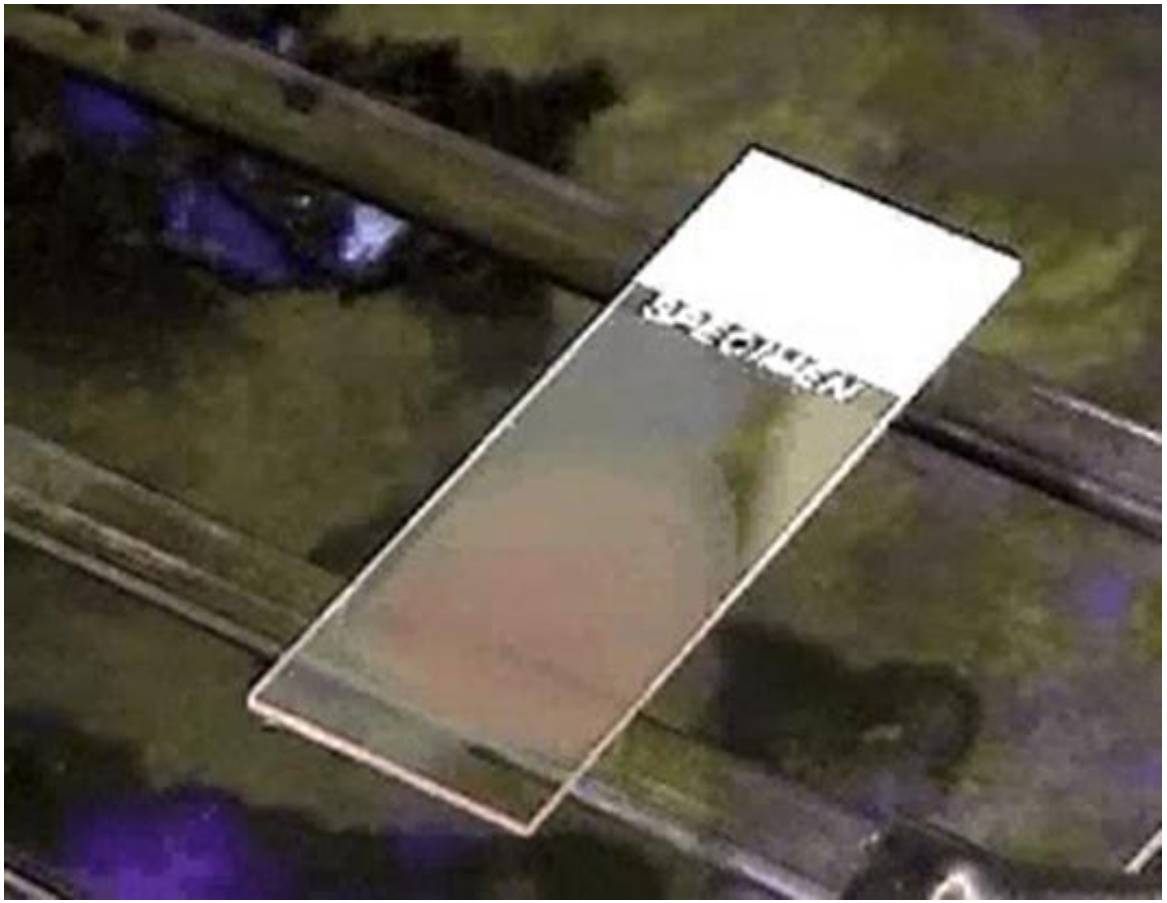


Blood Smear Preparation importance:

- Conditions which produce changes in the appearance of blood cells and differential white cell count.

- Also can provide rapidly and at low cost, useful information about a patient's condition.

Fixation



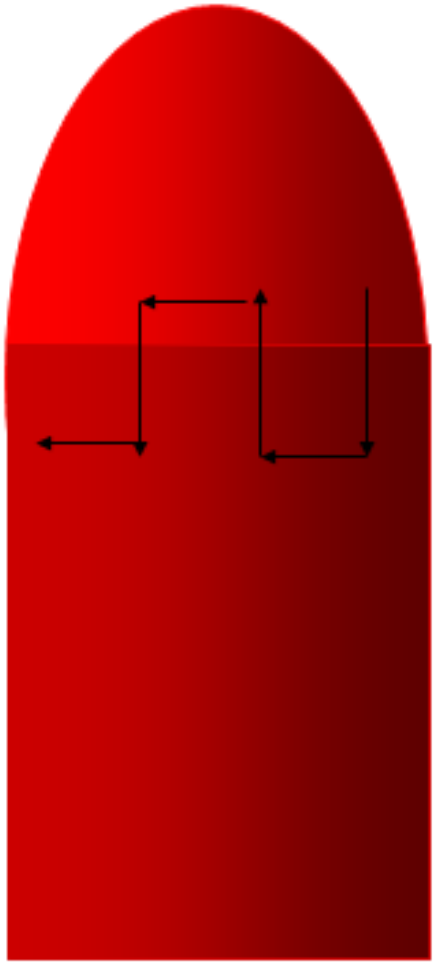
Staining of PBS

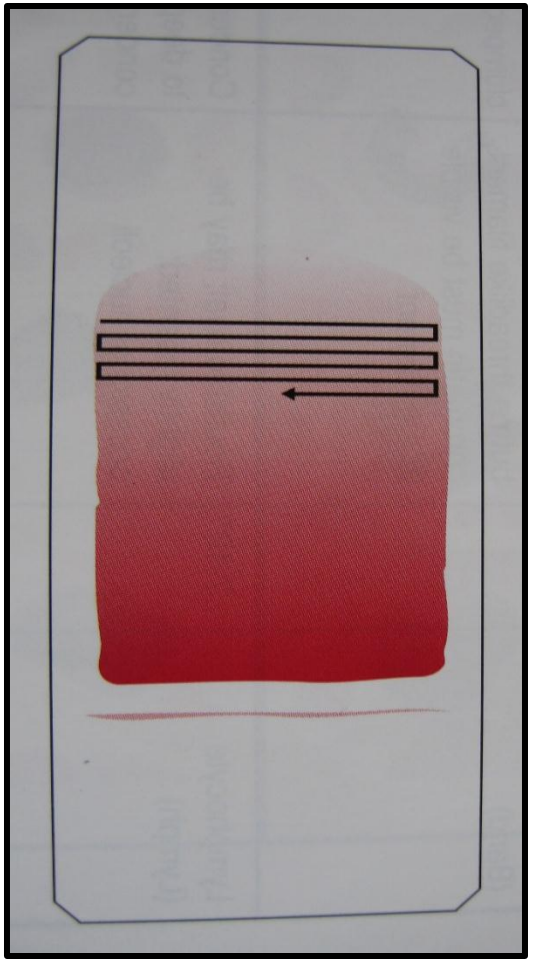
- Purpose of staining is to identify cells and recognize morphology easily through the microscope.

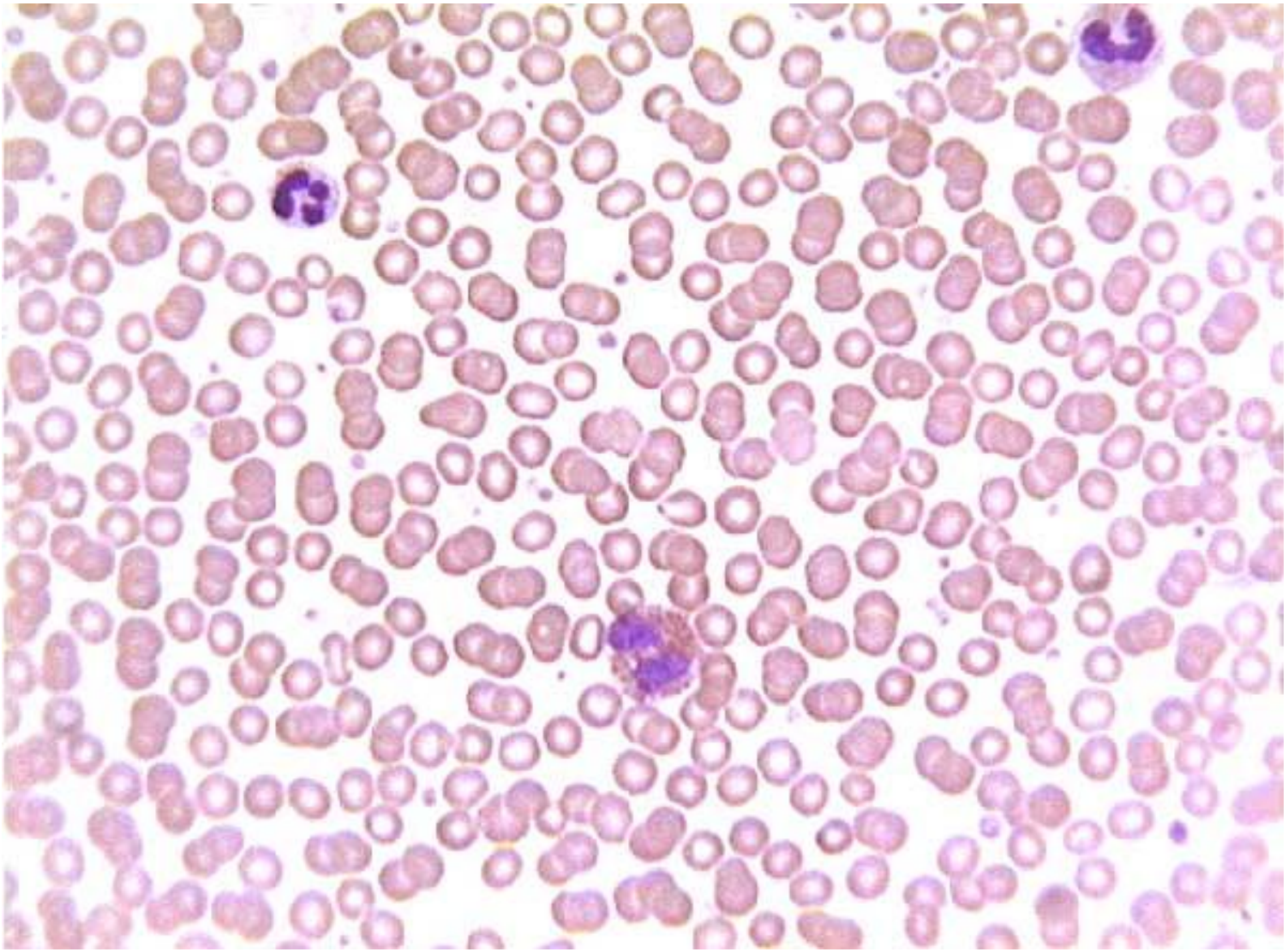
- The use of polychrome methylene blue and eosin Y, which are now used in the Wright-Giemsa Stain Solution, was developed by Romanowsky in 1891.

- Uses Wright stain or Wright-Giemsa stain which contain both eosin and methylene blue → polychrome stain

“Battlement” pattern for performing a WBC differential count







Neutrophils – 55-65%

Eosinophils – 1-4%

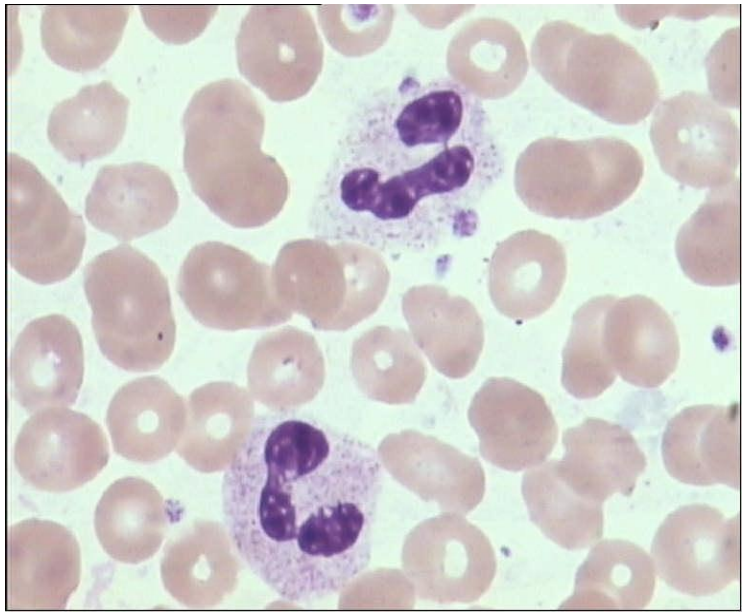
Basophils – 0-1%

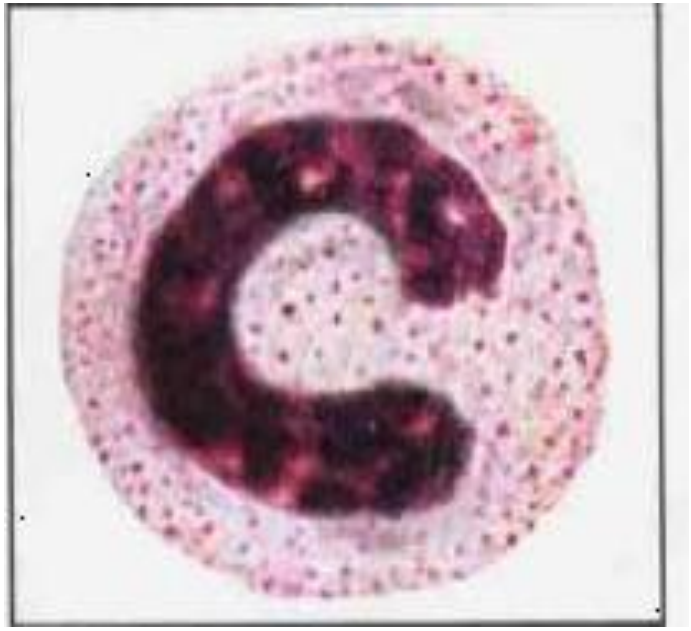
the lymphocytes – 20-40%

the monocytes – 3-8%

Neutrophil

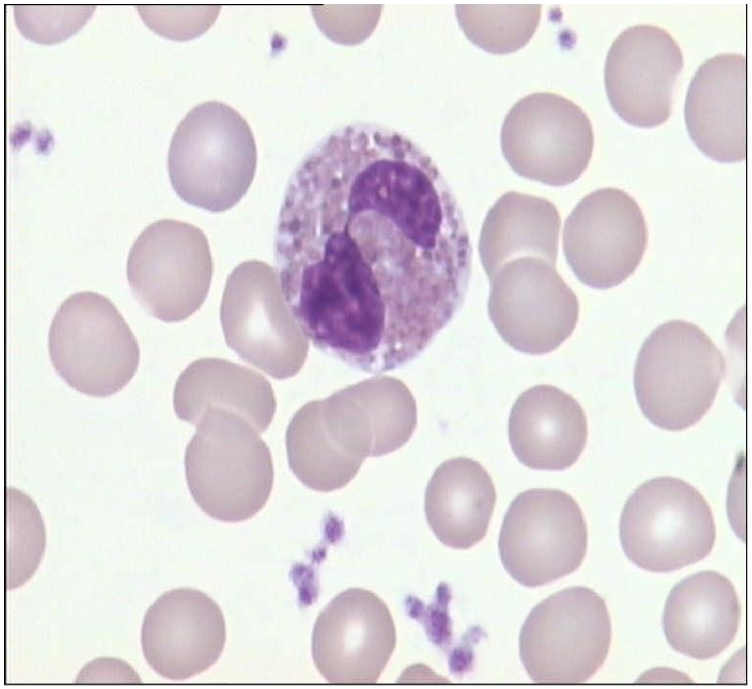
- Diameter:12-16 μm
- Cytoplasm : pink
- Nucleus : dark purple blue





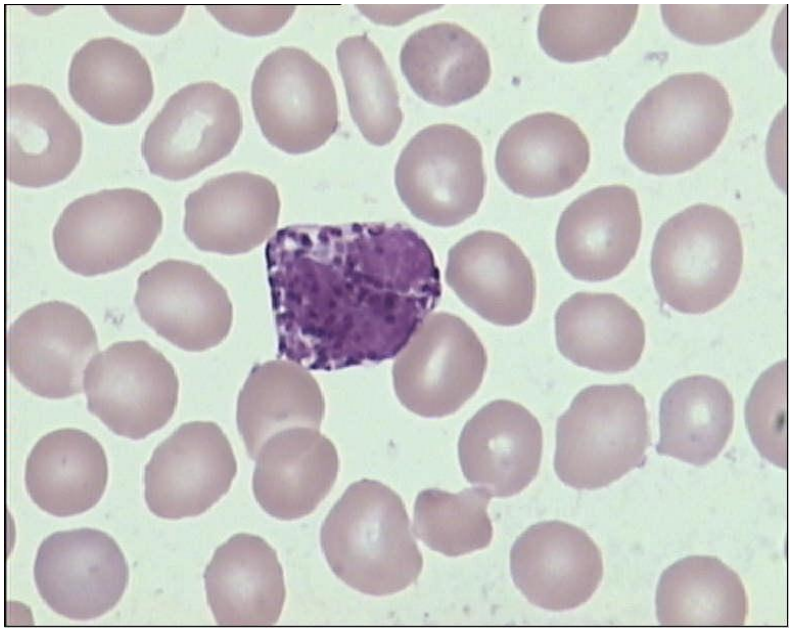
Eosinophil

- Diameter: 14-16 μm
- Cytoplasm : full of granules
- Granules: orange-red
- Nucleus: blue

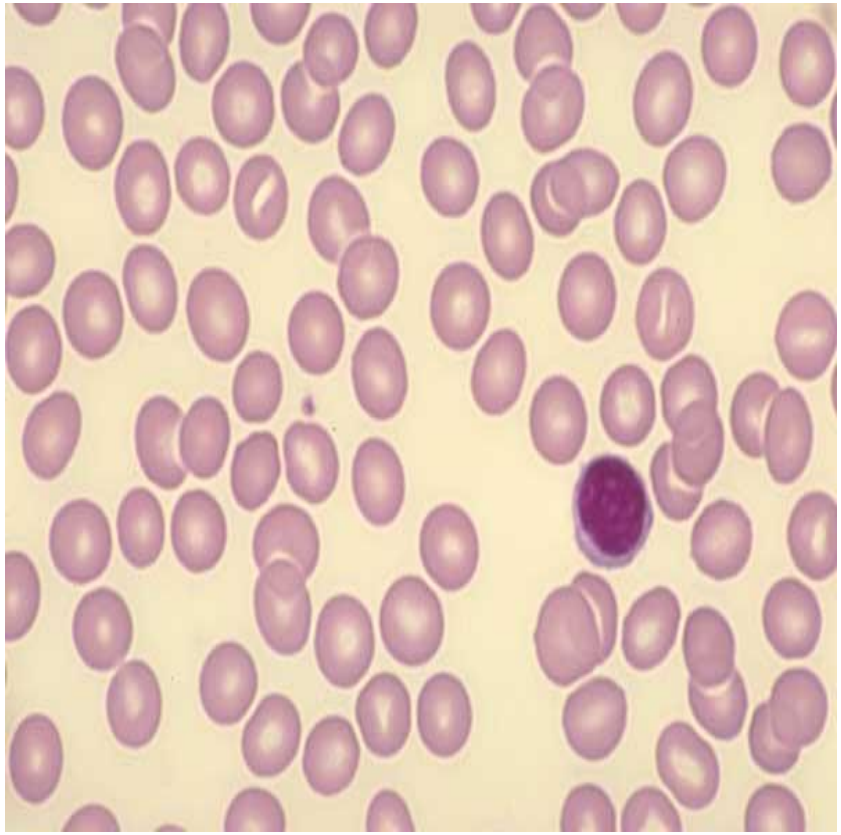


Basophil

- Diameter: 14-16 μm
- Cytoplasm: pink
- Granules: dark blue
- Nucleus: blue

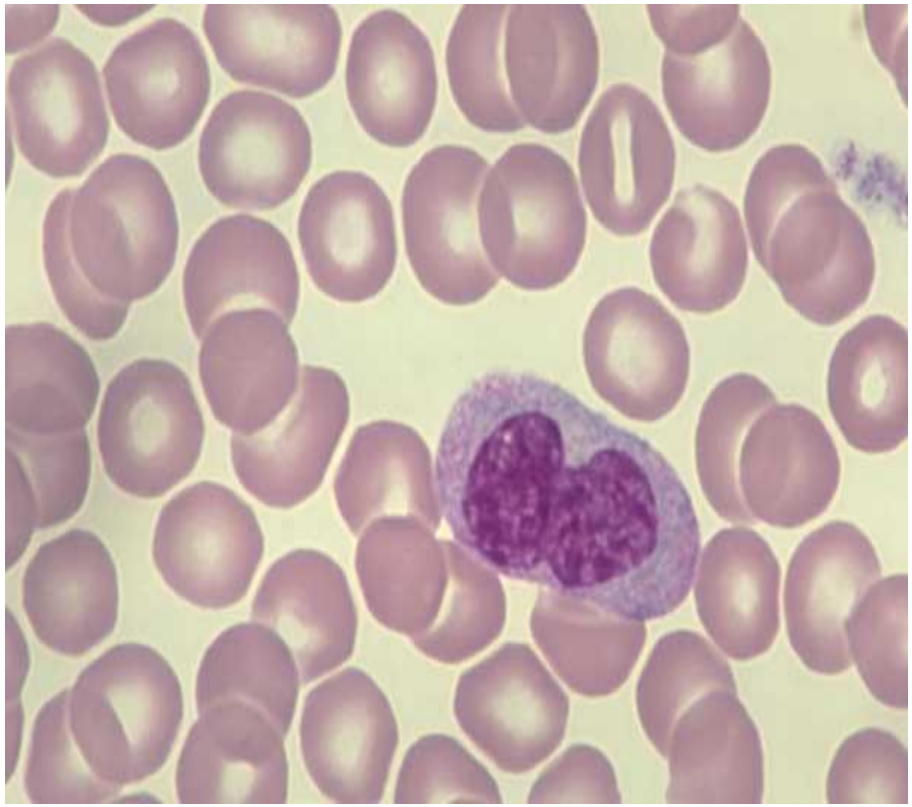


Normal lymphocytes



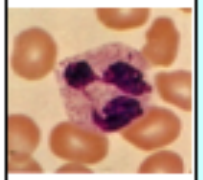
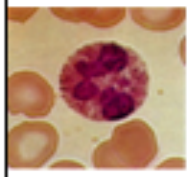
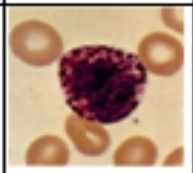
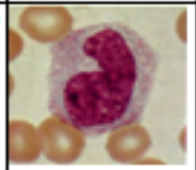
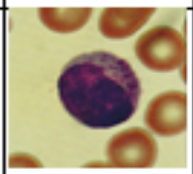
- Lymphocytes are the smallest WBC.
- They have large condensed nucleus, with a scanty pale blue cytoplasm.

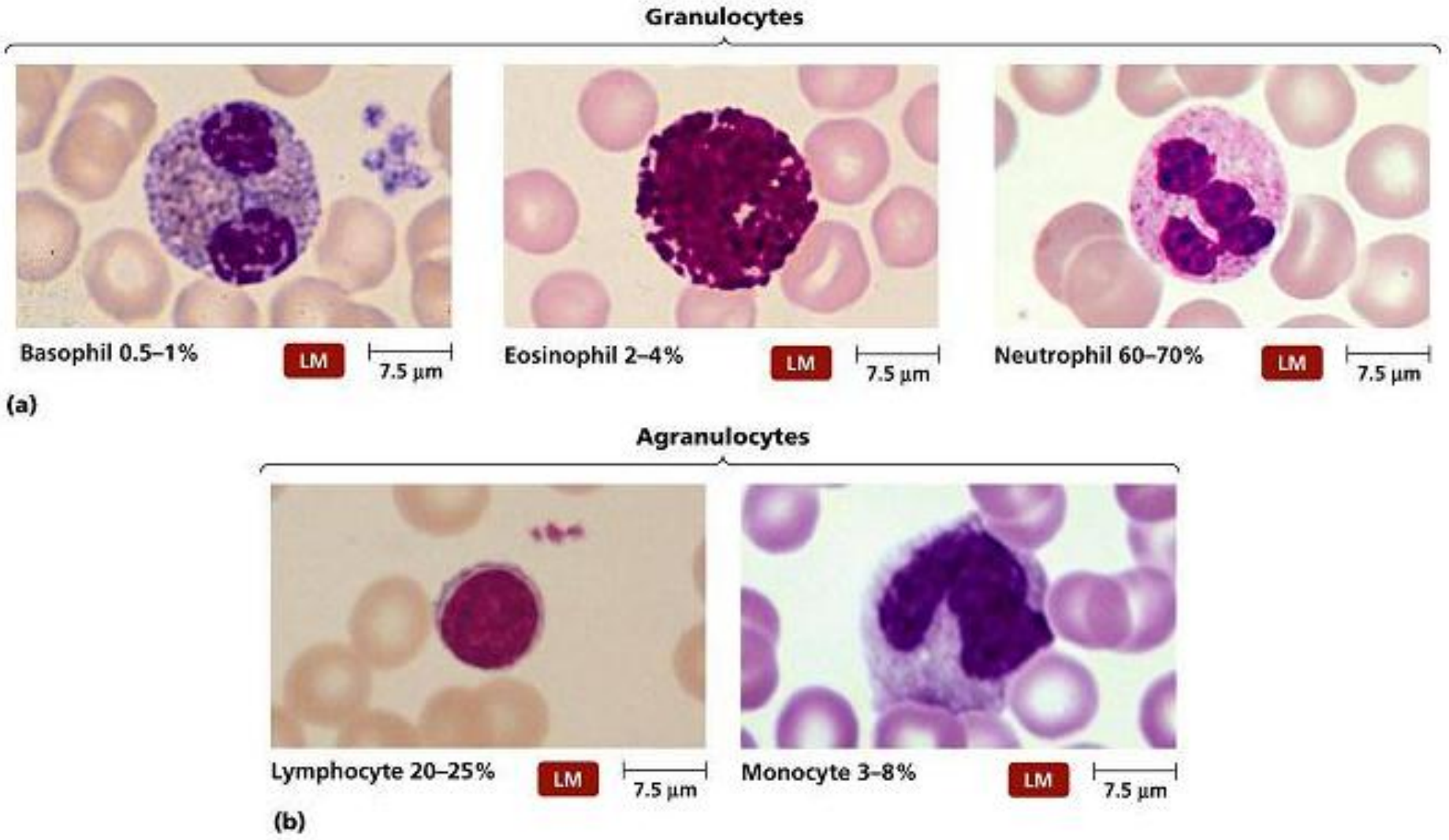
Normal monocyte

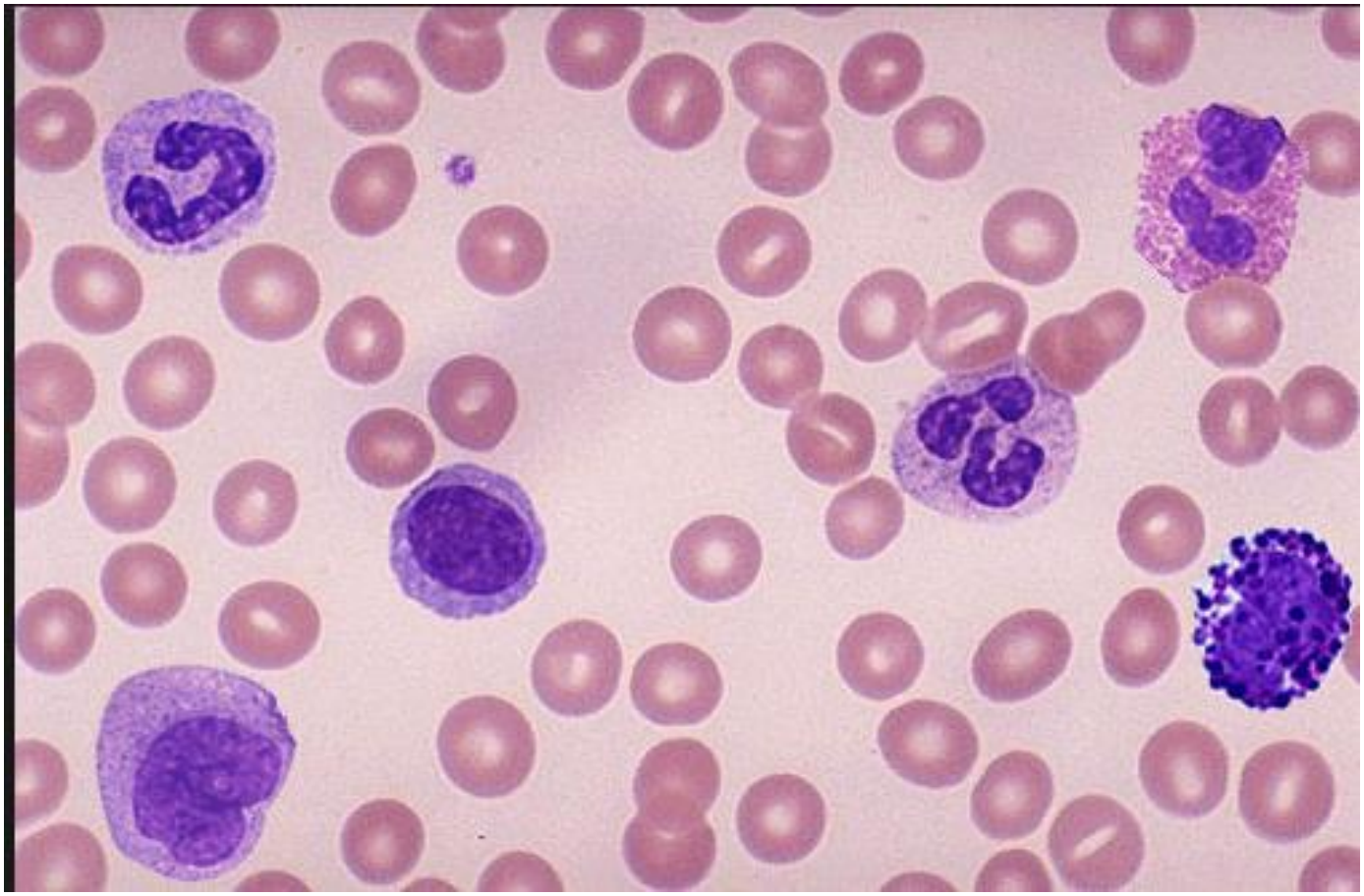


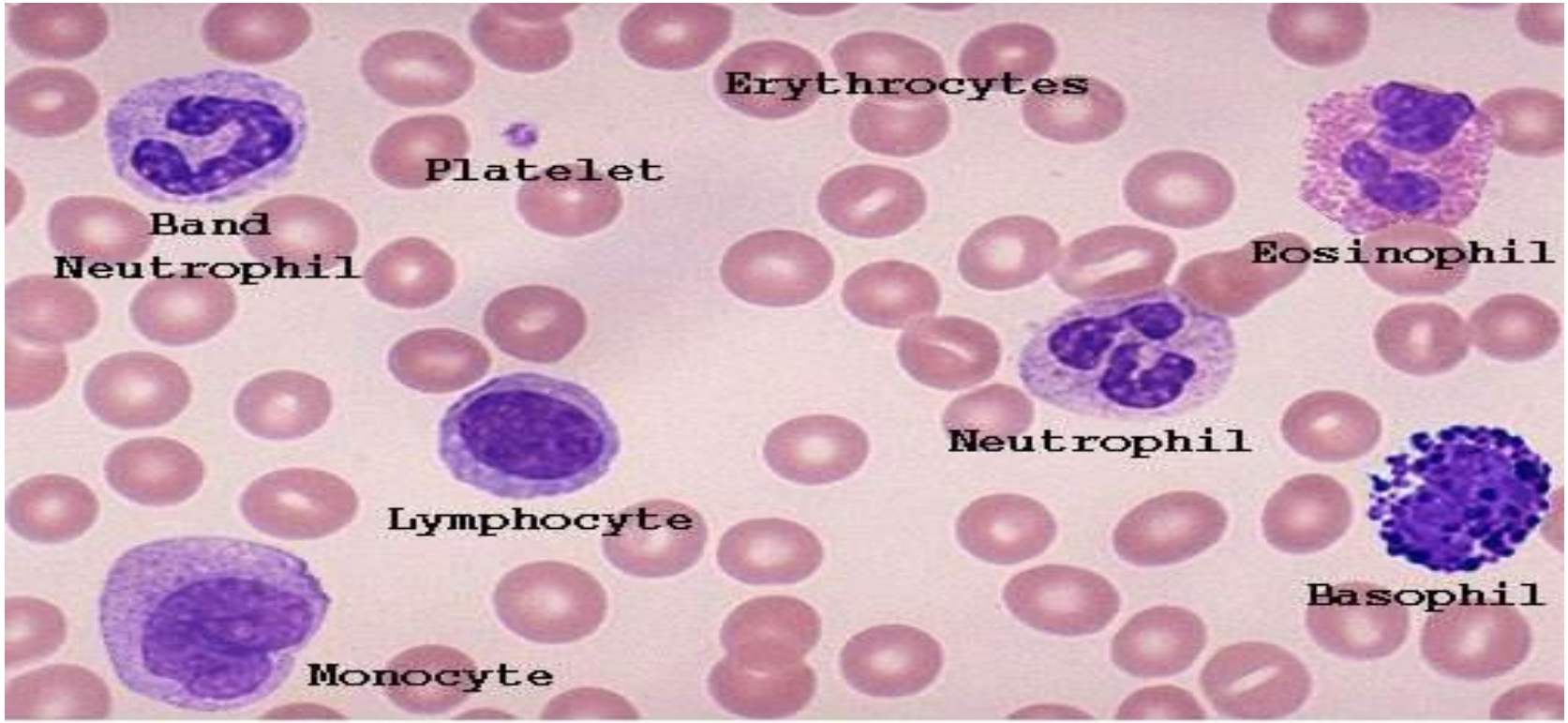
- Monocytes are the largest WBC.
- The cytoplasm is abundant, sky blue in colour.

مشخصات گلبول‌های سفید در فروتی رنگ آمیزی شده

نوع سلول		شکل	قطر µm	مسته	دانه های سیتوپلاسمی
گرانولوسیتها	نوتروفیلها		10-14	2-6 لب. که بوسیله رشته‌های کروماتین به همدیگر متصل شده‌اند و بوضوح در داخل سیتوپلاسم دیده می‌شوند.	دانه‌های مشخص میخکی آبی که یک زمینه شیشه مانند بوجود می‌آورند.
	ائوزینوفیلها		10-15	با لبهای کمتر. اغلب دو لبه که بوسیله رشته‌های کرماتین به همدیگر متصل شده‌اند.	دانه های قرمز یا نارنجی
	بازوفیلها		10-15	لبهای بی شکل. ممکن است دو لبه یا به شکل S باشند. چون گرانولهای تند روی آنها می‌افتند. ممکن است واضح دیده نشوند.	گرانولهای خیلی زیاد که رنگ میخکی تیره بخود می‌گیرند و تمام سلول را پر می‌کنند.
آگرانولوسیتها	منوسیتها		15-20	درشت. خارج مرکزی. ممکن است به شکل تعل اسبی یا کلیوی دیده شود. در نگاه نیمرخ ممکن است بیضی دیده شود.	سیتوپلاسم به مقدار فراوان به رنگ خاکستری-آبی کمرنگ. که گرانول قابل رویت در ان دیده نمی‌شود.
	لنفوسیت کوچک		7-9	درشت. گرد. کاملاً تمام سلول را پر می‌کند. به شدت رنگ آبی به خود گرفته و مثل یک لکه جوهر دیده می‌شود.	یک سیتوپلاسم آبی روشن هلالی شکل که گرانولی در آن دیده نمی‌شود.
	لنفوسیت بزرگ		12-15	درشت. گرد. کاملاً تمام سلول را پر می‌کند و به شدت رنگ آبی به خود می‌گیرد.	سیتوپلاسم به مقدار زیاد به شکل هلالی و به رنگ آبی روشن (بیشتر از لنفوسیت کوچک). گرانول ندارد.


Granulocytes
Basophil 0.5–1%
LM
7.5 µm
Eosinophil 2–4%
LM
7.5 µm
Neutrophil 60–70%
LM
7.5 µm
(a)
Agranulocytes
Lymphocyte 20–25%
LM
7.5 µm
Monocyte 3–8%
LM
7.5 µm
(b)





Wright-stained smear of normal blood (x1000)
The RBC's are biconcave discs stained buff-pink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.