Staining of Blood Smear

Romanowsky stain

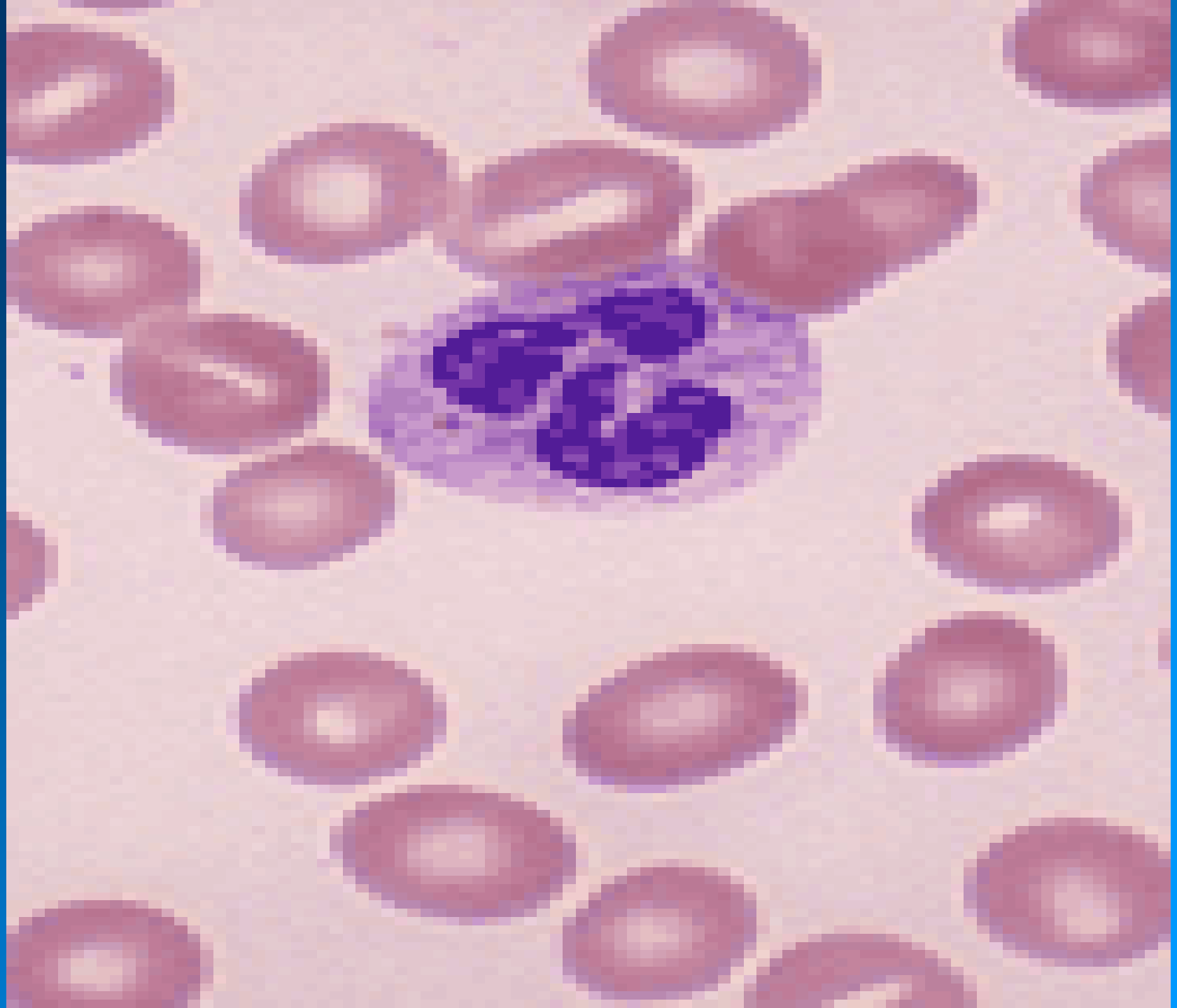
- Romanowsky stain→Eosin Y and Azure B)

  Eosin:Acidic Dye bind to Basic groups (Hb,Granules) → reddish or orange color

  Azure B: Dye bind to nucleic acid & nucleoproteins →Blue-violet color

  Fixation →Methanol<4%water, with 1 hour

  Delay : Adherence of Pro. To slide → Blue background



Romanowsky stain

- Wright
- Wright – Giemsa
- Lishman
- May- grunwald - Giemsa
- jenner

Making blood film

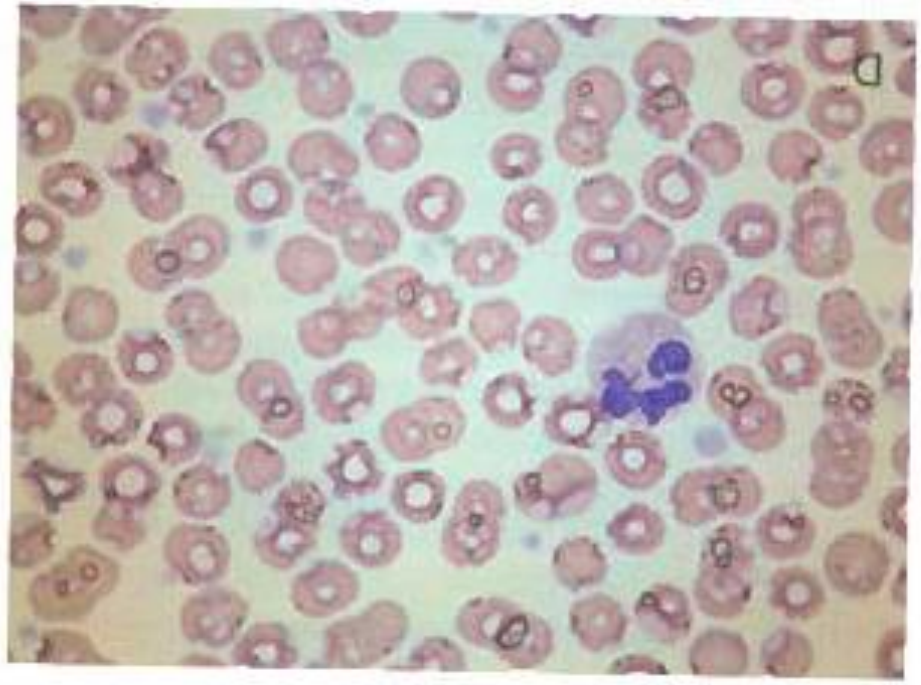
- Blood film can be prepared from fresh blood without anticoagulant or from EDTA anticoagulanted blood.
- blood film should be made on clean glass .
- Clear without any dust

Wedge method

- The most commonly in routine lab
- Method
- Thickness or thinness regulated by
- Amount of blood
- Speed of spreader
- Angle

Optimal blood smear characteristic

- minimum 2.5 cm in length terminating at least 1 cm from the end of the slide
- Gradual Transition in thickness from thick to thin area ending in a Square or straight edge
- No streaks , waves , or troughs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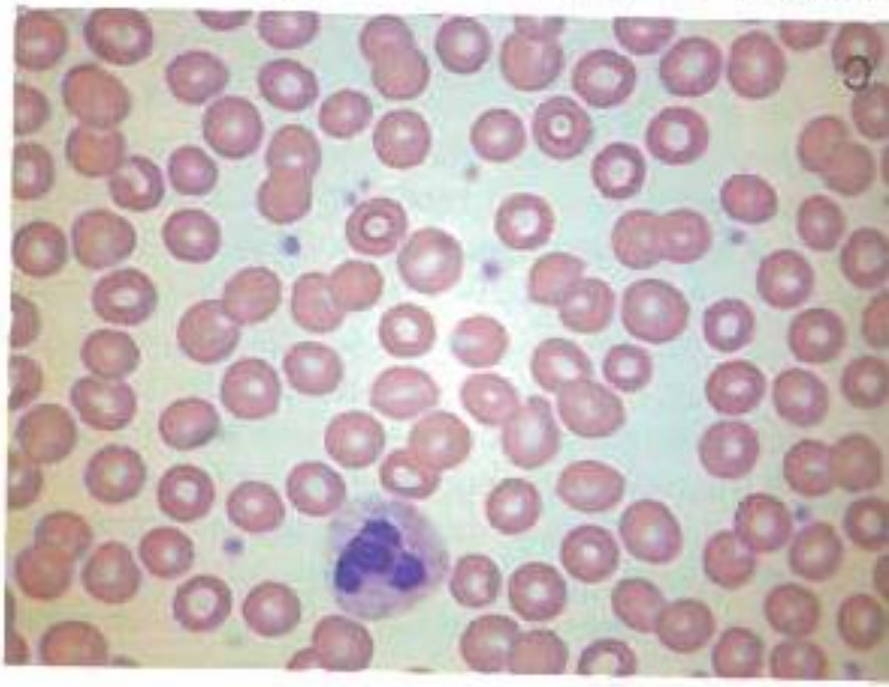
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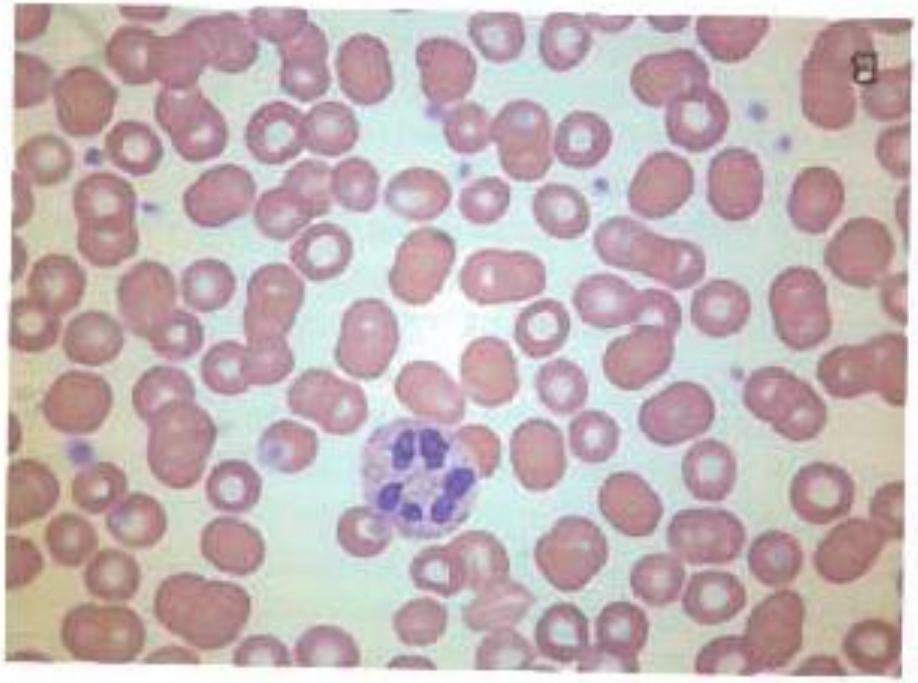
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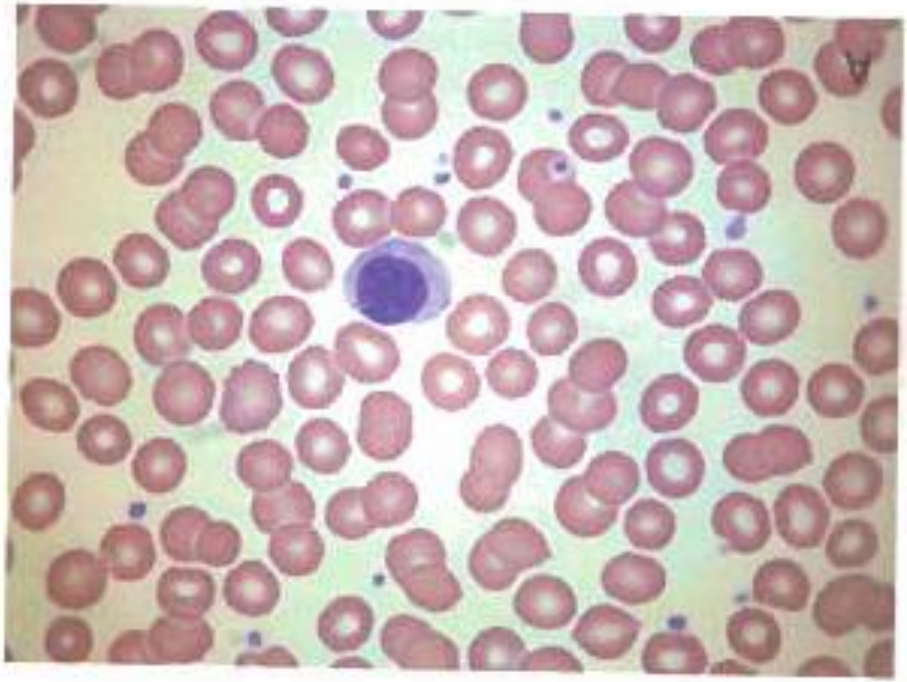
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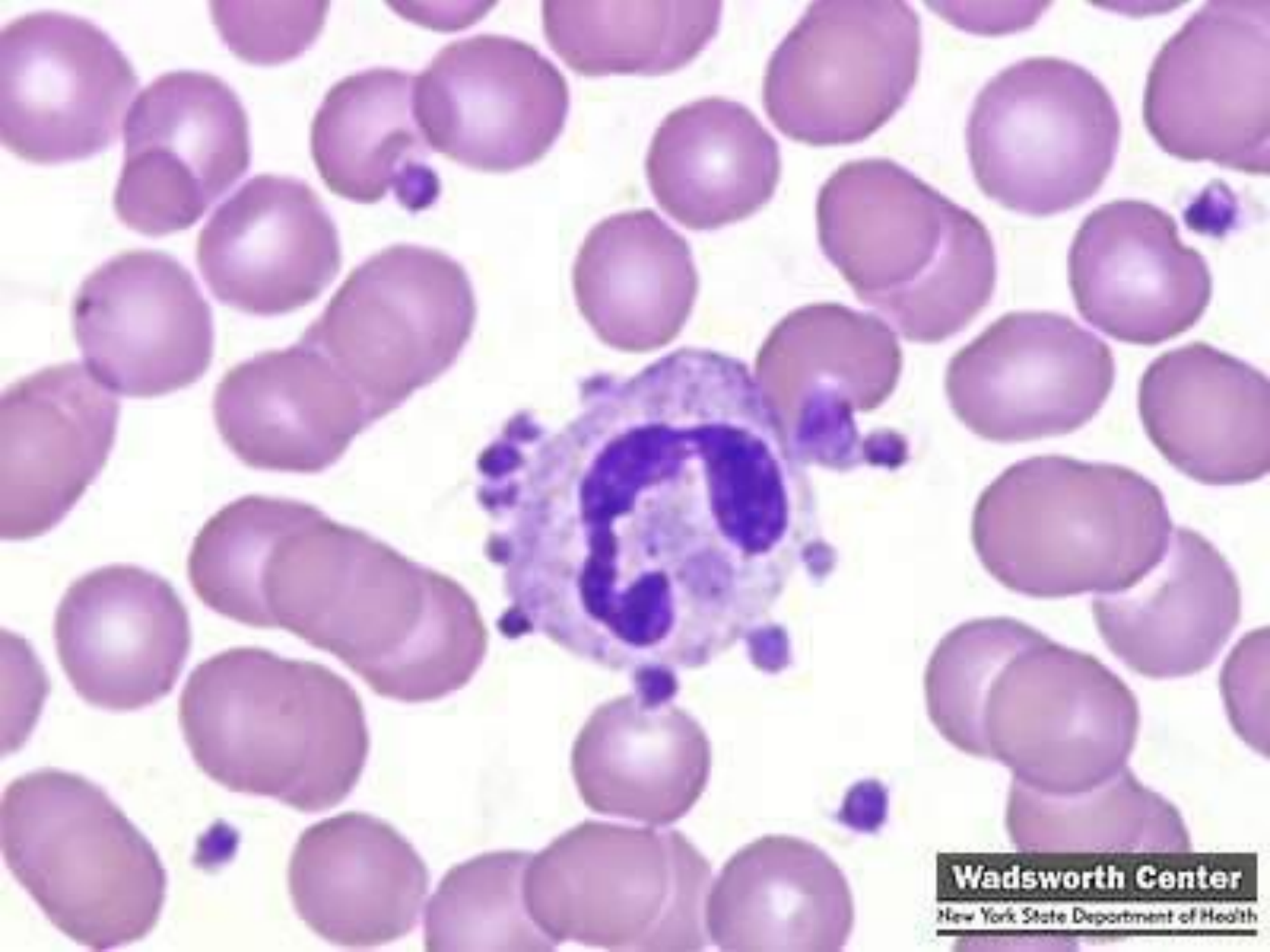
Wadsworth Center
New York State Department of Health

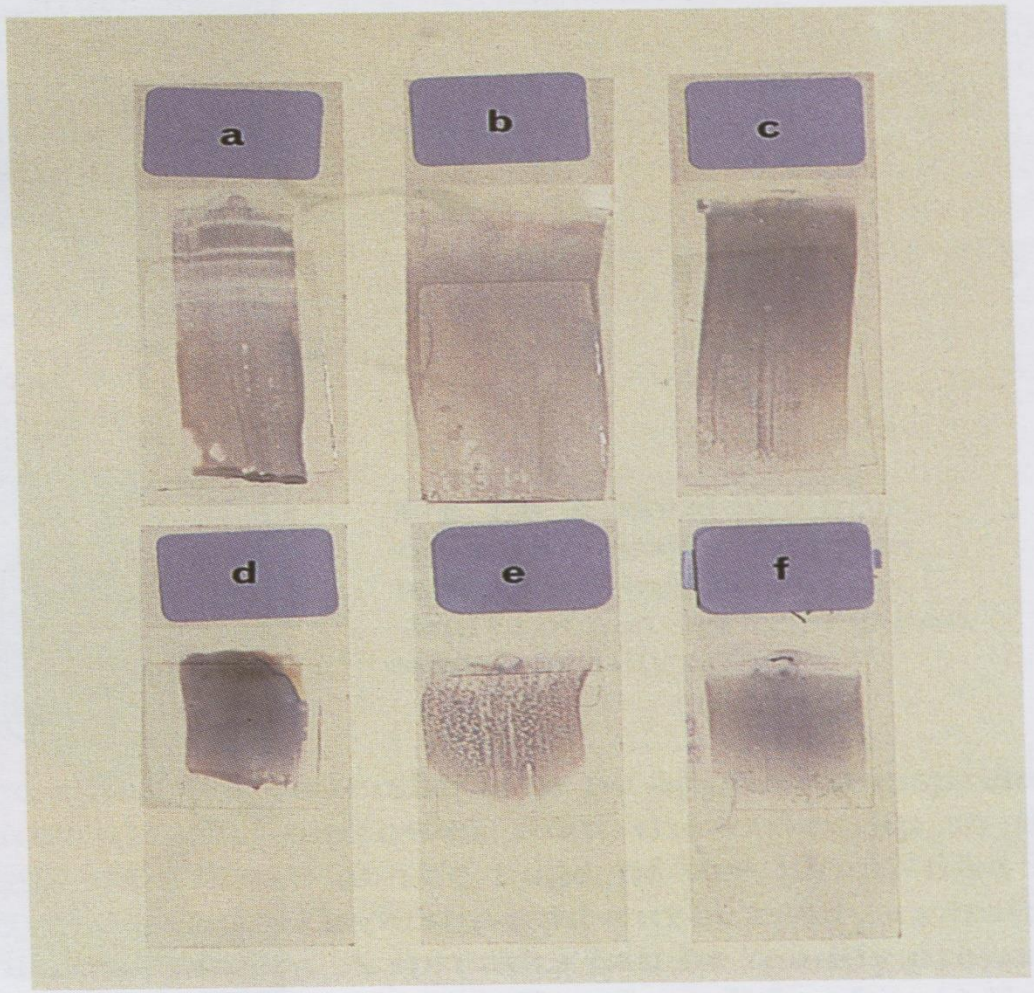

Fig. 1.11 Unsatisfactory and satisfactory blood films: (a) uneven pressure has produced ridges; (b) too broad and too long—the edges and the tail of the film cannot be examined adequately; (c) too long and streaked by an uneven spreader; (d) too thick and short due to the wrong angle or speed of spreading; (e) even distribution of blood cells has been interrupted because the slide was greasy; and (f) satisfactory.

Sources of error in preparation of a blood smear

problem	*Resolution*
Presence of crenated erythrocyte	Dry smear quickly and thoroughly
Thin smear due to anemia	Increased spreader slide angle and increased push speed
Thick smear due to polycythemia	Decrease spreader slide angle and decrease push speed
Presence of agglutinated erythrocytes associated with cold agglutinin disease	Warm blood in 37°C for 15 min prior to preparing smear
Increased viscosity associated with multiple myeloma	Decrease spreader slide angle and decrease push speed

Spinner

- Blood films that combine the advantages of easy handling of the wedge slide & uniform distribution of cells of the coverglass reparation
- Method
- Advantages: minimal exposure to biohazardous , increased optimal counting area

Reference method

- Pure Azure B (260mg/100ml methanol)
- Pure eosin y (130 mg/100ml methanol)
- 1 part Azure B + 1 part eosin y +10 part sorensens phosphate buffer 66mmol/l ph= 6.8
- 10 min
- washing

Characteristic of aproperly stained blood smear

Type of evaluation	*Characteristic*
Macroscopic	Smear is pinkish purple in color
Microscopic	Blood cells are evenly distributed Areas between cells are clear Erythrocytes are orange red Neutrophilic granules are lilac Eosinophilic granules are red orange Lymphocytes cytoplasm is blue Leukocytes nuclei are purple Precipitated stain is minimal or absent

problem	***Potential causes***
Excessively blue or dark stain	Prolonged staining Inadequate washing Too high an alkalinity of stain and / or buffer Thick blood smear
Excessively pink or light stain	Insufficient staining Prolonged washing Too high an acidity of stain and / or buffer
Presence of precipitate	Unclean slides Drying during staining process Inadequate filtration of stain

problem	***Potential causes***
Pale stainig	Old stainig solution overused staining solution Impure dyes High ambient temperature
Blue Background	Prolonged storage before fixation Blood collection into heparinas anticoagulant

Quality Control

رنگ پس از تهیه از نظر آلودگی قارچی و میکروبی وهر گونه رسوب و پارتیکل وهمچنین نحوه رنگ گرفتن سلول های خونی بررسی می گردد.رنگ آمیزی گسترش های خونی روتین نیز هفته ای یک بار توسط مسئول فنی داخلی از نظر موارد فوق بررسی می گردد که بصورت مکتوب ومستند باید در آزمایشگاه قرار گیرد.کیفیت رنگ آمیزی مورد قبول سلول ها مطابق جدول زیر می باشد .

اجزاء سلولی	رنگ
هسته	
کروماتین	بنفش
هستک	آبی روشن
سیتوپلاسم	
اریتروبلاست	آبی تیره
ارتیروسیت	صورتی تیره
رتیکولوسیت	خاکستری-آبی
لنفوسیت	آبی
متامیلوسیت	صورتی
منوسیت	خاکستری-آبی
نوتروفیل	صورتی-نارنجی
پرومیلوسیت	قرمز یا بنفش
بازوفیل	آبی
گرانولها	
پرومیلوسیت (گرانولهای اولیه)	قرمز یا بنفش
بازوفیل	بنفش تیره
ائوزینوفیل	قرمز - نارنجی
نوتروفیل	بنفش
توکسیک گرانول	آبی تیره

GOOD LUCK !