BLOOD COLLECTION

Dr. M. VIKRAMATHITHAN,
ASSISTANT PROFESSOR,
PG & RESEARCH DEPARTMENT OF MICROBIOLOGY,
ADHIPARASAKTHI COLLEGE OF ARTS AND SCIENCE,
G.B. NAGAR, KALAVAI.

Blood collection

6

TESTS PERFORMED ON COLLECTED BLOOD

- HEMATOLOGICAL TESTS
- BIOCHEMICAL TESTS
- SEROLOGICAL TESTS
- CULTURAL TESTS

7

SITE

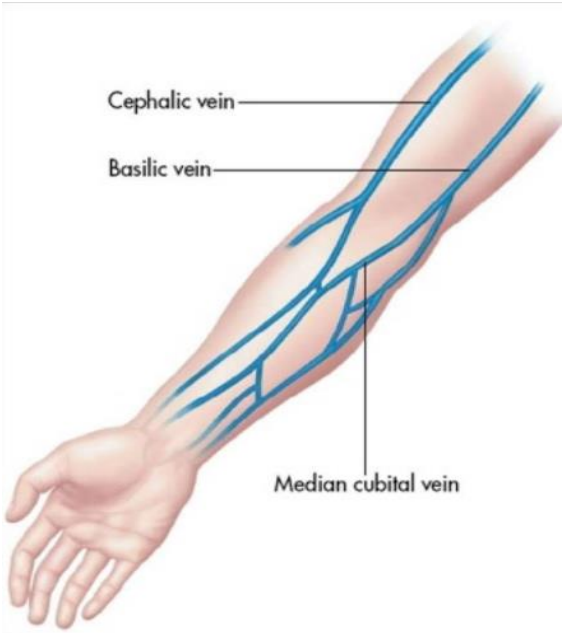
- Blood can be collected from 3 different sources-

1. Capillary
2. Venous (most common)
3. Arterial

VENIPUNCTURE

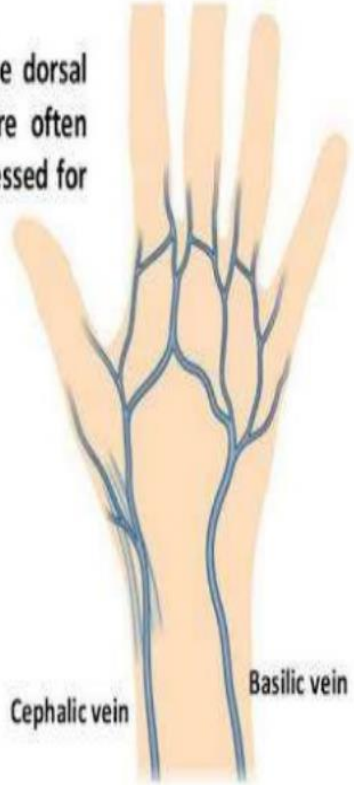
- *Venipuncture* is a routine and common procedure done to collect venous blood directly from the vein.
- **Best site-** Ante-cubital fossa
- In order to do this safely, the phlebotomist must have a basic understanding of the following;

I. Anatomy
II. The criteria for choosing a vein
III. The device used
IV. Skin preparation
V. Personal safety – infection control policy



Common Sites for Venipuncture

Superficial veins on the dorsal aspect of the hand are often visible and can be accessed for numerous procedures.



- **NEEDLES** should not be too fine/ too large/ too long
- Vary from large (16 G) to small (23 G)
  - For adults- 19 or 21 G suitable
  - For children- 23 G
- Ideally should have short shaft (15mm)
- **Butterfly needles-** when blood has to be collected from a very small vein
  - Come in 21, 23, 25 G

COLLECTION OF BLOOD FOR HEMATOLOGICAL EXAMINATIONS:

- Hb, RBC, WBC, DLC, Platelet count, Red Cell Indices, Peripheral Smear

COLLECTION OF BLOOD FOR BIOCHEMICAL EXAMINATIONS:

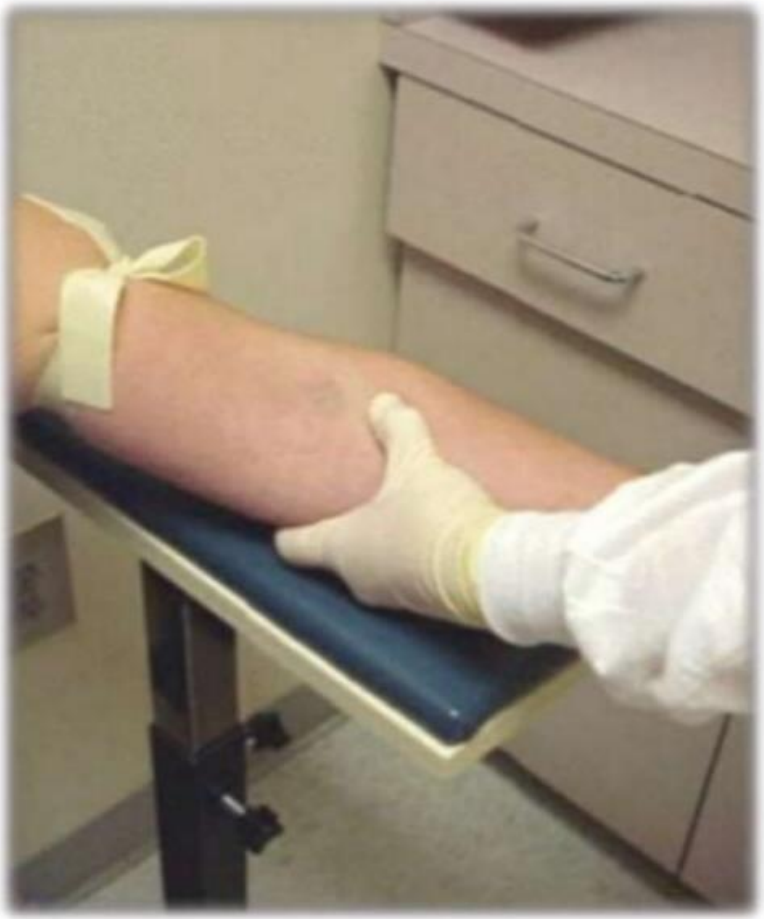
- Fasting conditions are advisable
- Venous blood to be preferred.

SYRINGE

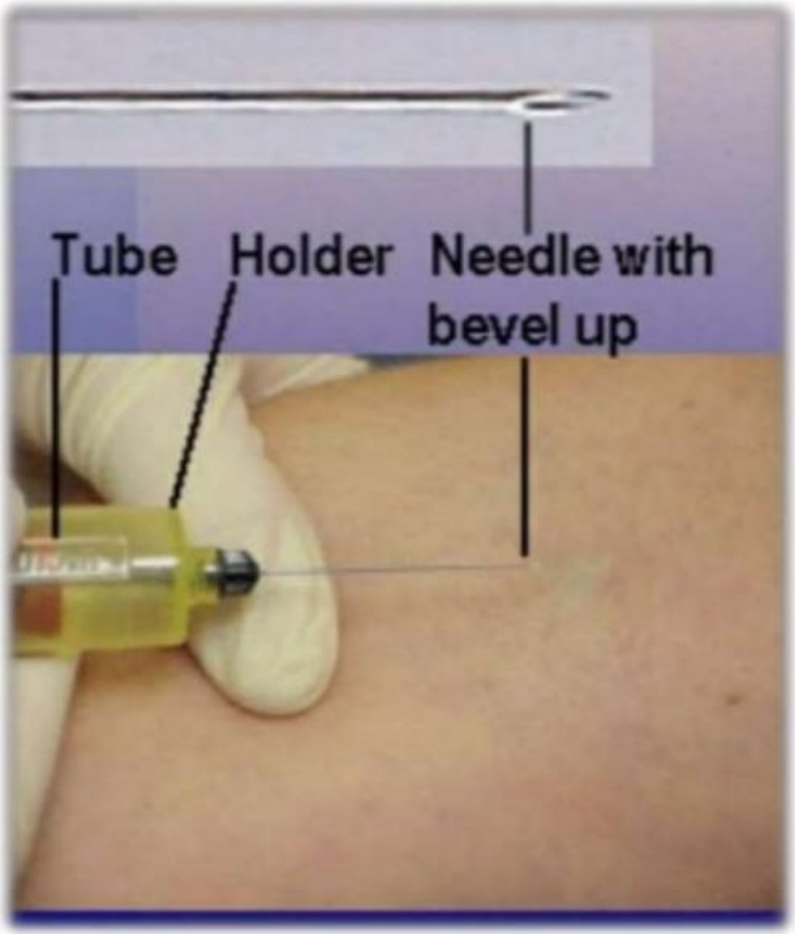
TOURNIQUET

BUTTERFLY NEEDLE

Hold and position vein in place



Insert needle with the bevel up



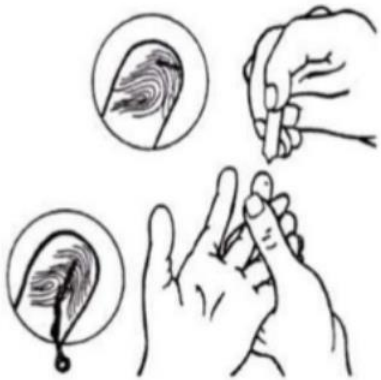
SKIN PREPARATION

- Skin cleansing with an alcohol swab.
- Asepsis should be maintained.
- The two main sources of microbial contamination are:
  a) The hands of the phlebotomist
  b) The skin of the patient
- Good hand washing and drying techniques. If hand washing facilities are unavailable, an alcohol based hand wash solution is an acceptable substitute

SKIN PUNCTURE TECHNIQUE

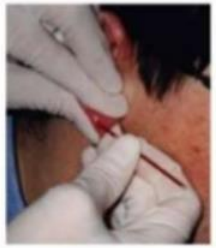
- Select an appropriate puncture site
  - For infants <12 months- Lateral/ Medial plantar heel surface
  - For infants >12 months, children, adults- Palmar surface of last digit of second/third/fourth finger
- Warm the puncture site- arterial enriched blood
- Cleanse the site
- Make puncture with sterile lancet perpendicular to skin surface
- Discard first drop of blood by wiping it away

LANCET

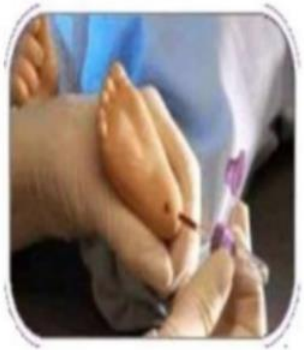


SKIN PUNCTURE

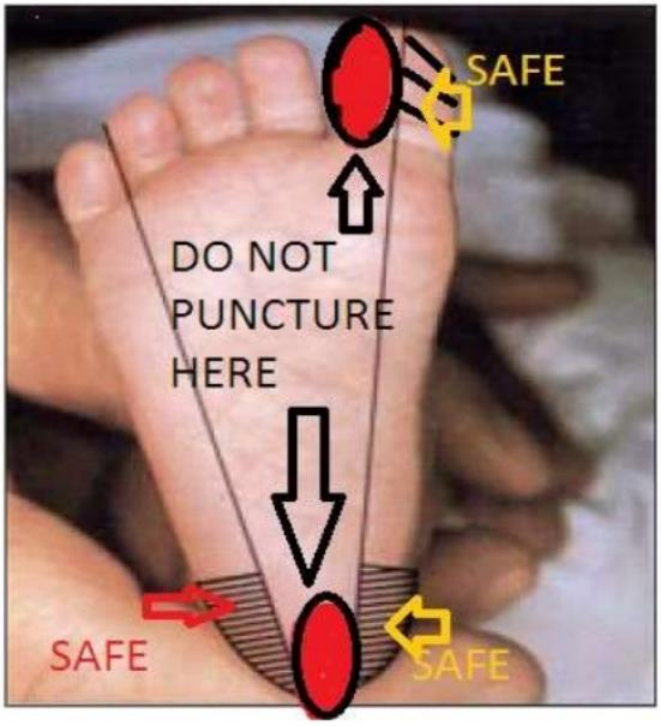
EAR LOBULE



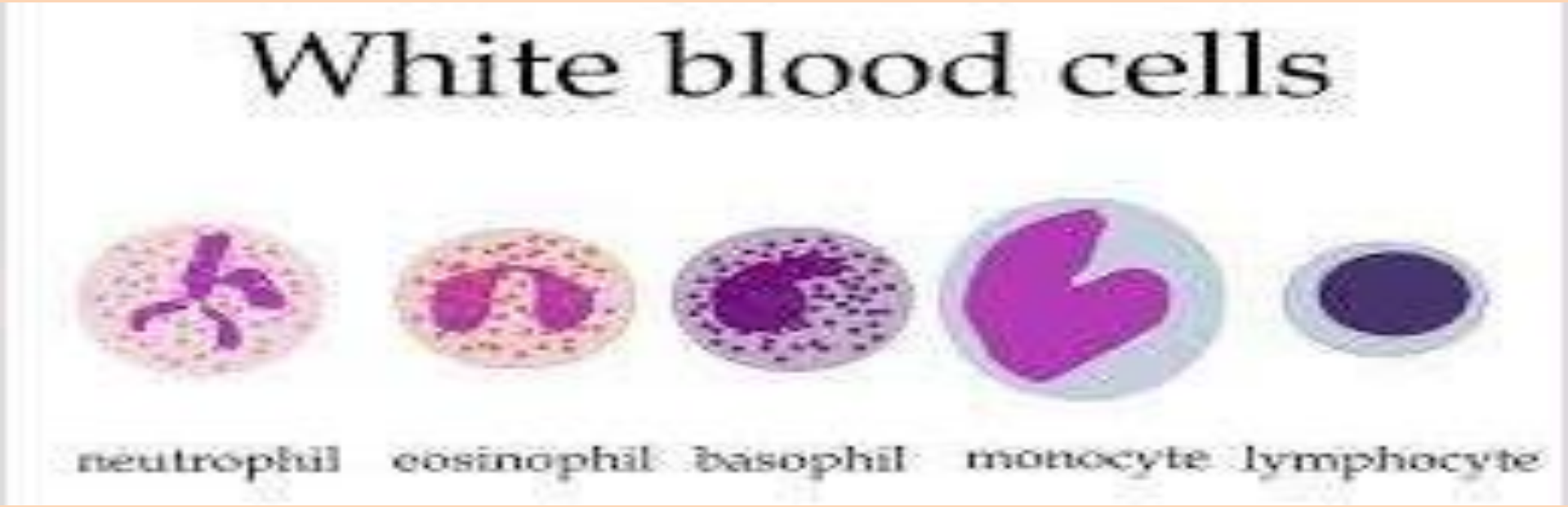
HEEL PULP:



Automatic lancet device



DIFFERENTIAL LEUKOCYTE COUNT



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Learning Objectives

- Introduction
- Identification of WBCs
- Differential Counting of WBCs
- Pathologic variations in DLC

Introduction

- **DLC** → Relative proportion of different leukocytes expressed as percentage.

USES:

- To support the diagnosis of infectious, inflammatory or allergic disorders.
- Diagnosis of malignant blood disorders

Learning Objectives

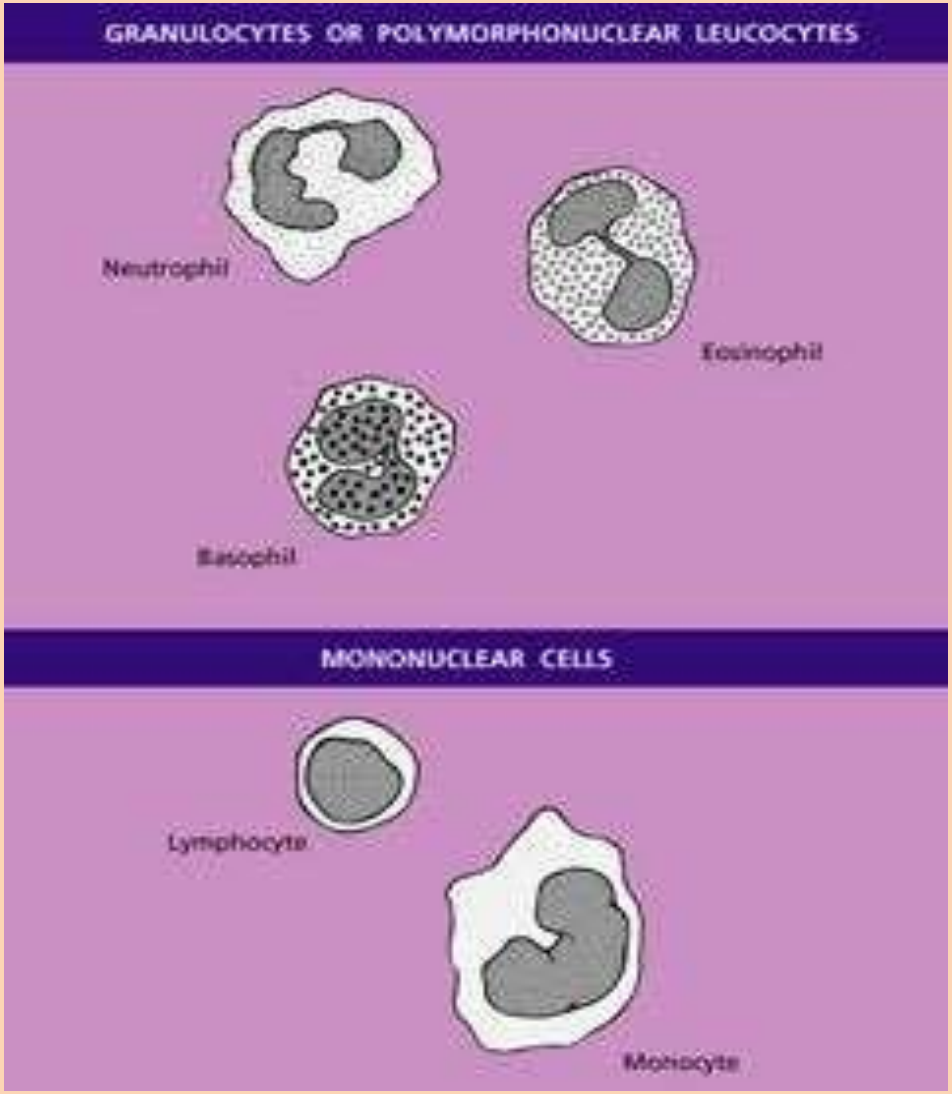
- Introduction
- Identification of WBCs
- Functions
- Pathologic variations in DLC

White blood cells

Granulocytes are of three types named according to their staining characteristics in blood films. They are

- Neutrophils
- Eosinophils
- Basophils.

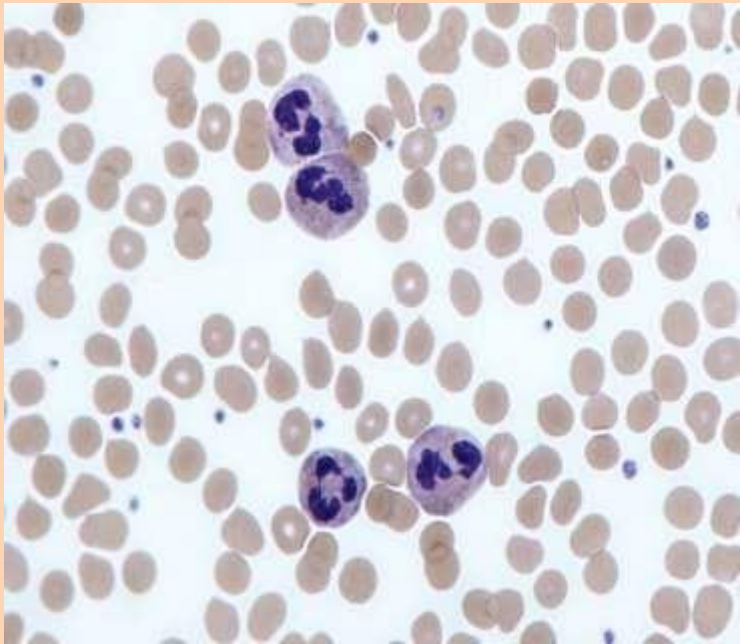
- Agranulocytes/Mononuclear cells are divided into
- Lymphocytes
- Monocytes.



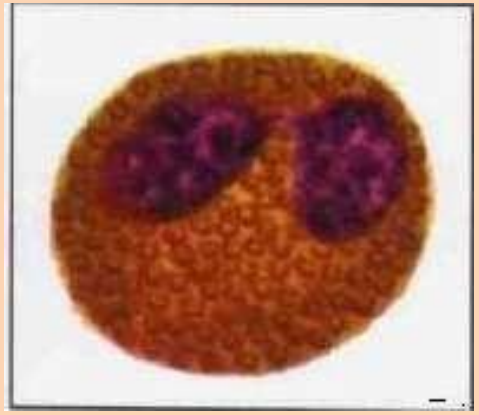
Granulocytes

Polymorph (Neutrophil)

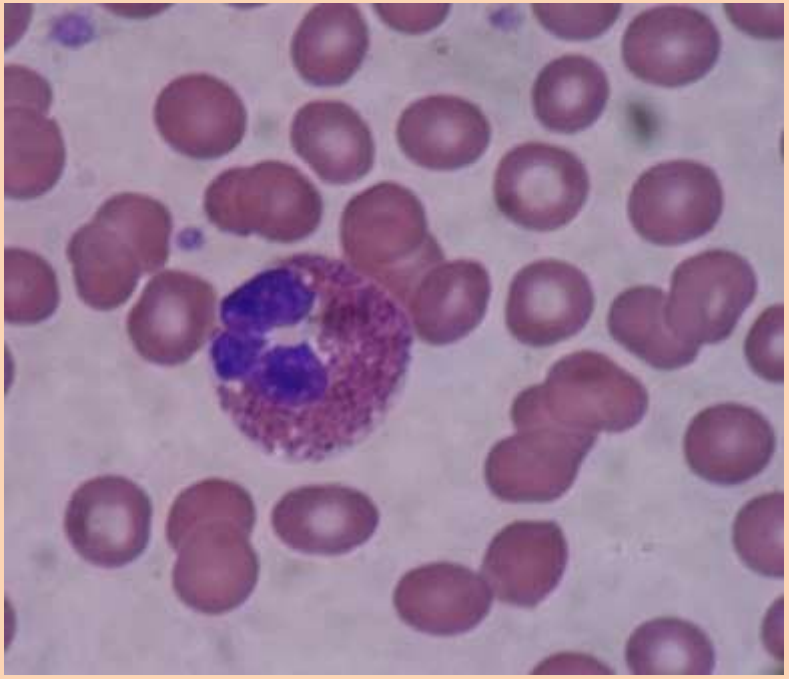
- **Cell diameter :** 12-15 µm
- **Nucleus : 2-5 lobes, clumped chromatin**
- **Cytoplasm : Pink/white granules**
- **Normal % : 40-80**
- **Absolute count per µl : 2000- 7500**



Granulocytes
Eosinophil

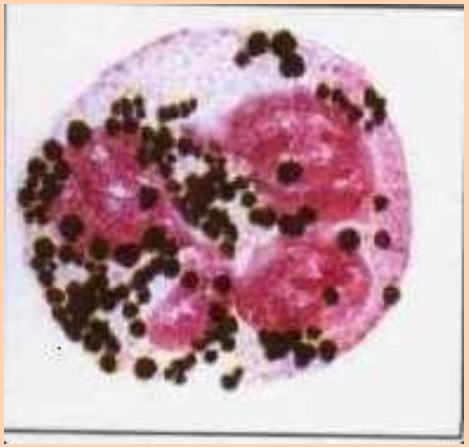


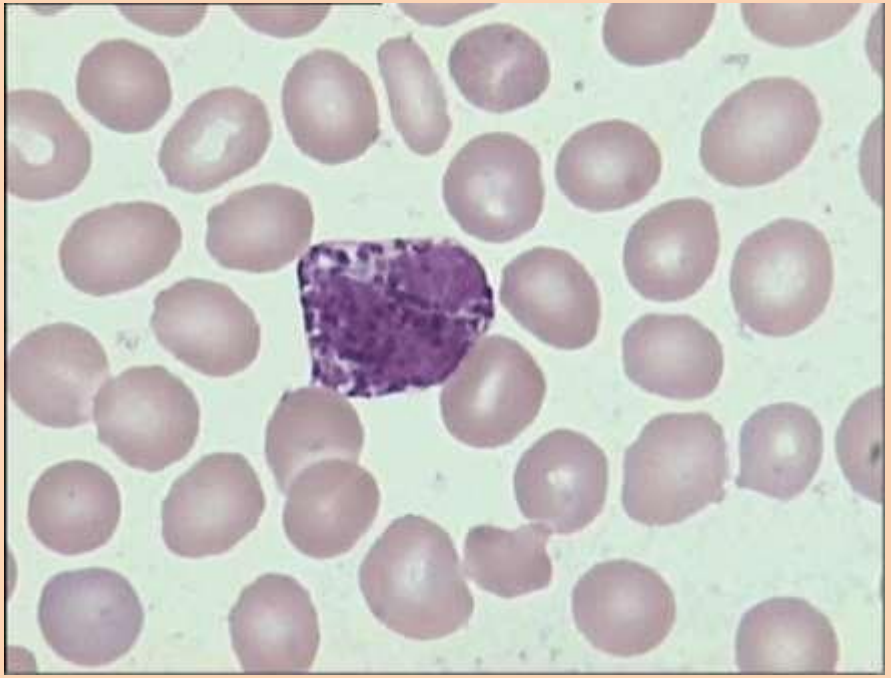
- **Cell diameter :** 12-15 µm
- **Nucleus : Bilobed, clumped chormatin.**
- **Cytoplasm : Coarse grimson red granules.**
- **Normal % : 1-6.**
- **Absolute count per µl : 40-400.**



Granulocytes
Basophil

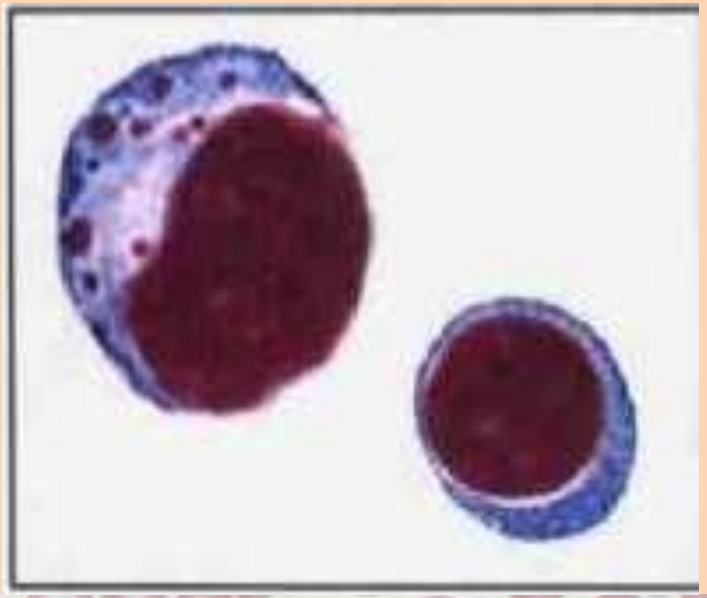
- **Cell diameter :** 12-15 µm
- **Nucleus : Bilobed, clumped chormatin.**
- **Cytoplasm : Large, coarse purplish granules obscuring the nucleus.**
- **Normal % : 0-1.**
- **Absolute count per µl : 10-100.**

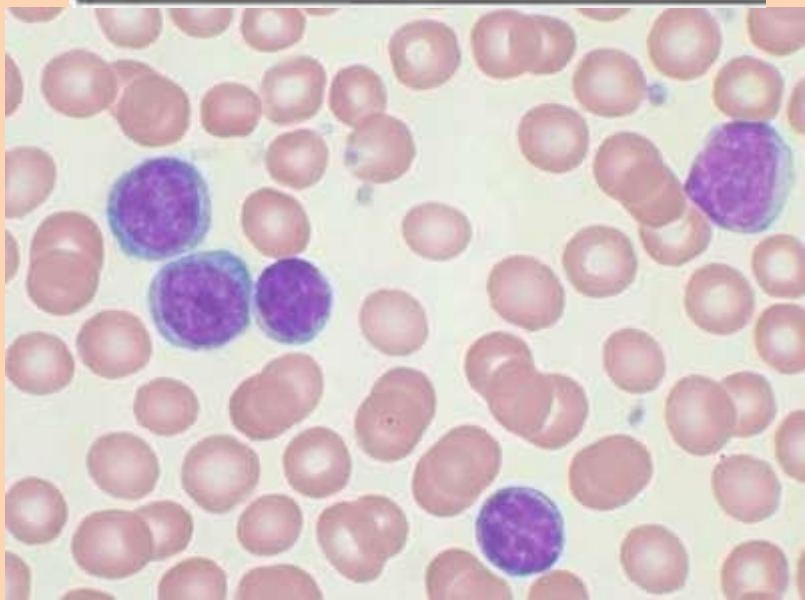




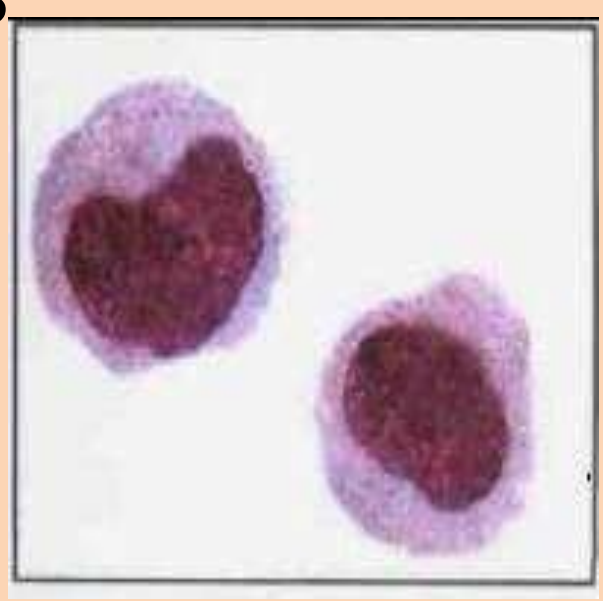
Agranulocytes
Lymphocytes

- **Cell diameter :**
  - **Small Lymphocyte: 9-12 µm**
  - **Large Lymphocyte : 12-16 µm**
- **Nucleus : Large nucleus round to indented fills the cell, clumped with chromatin**
- **Cytoplasm : Peripheral rim of basophilic cytoplasm, no granules**
- **Normal % : 20-40**
- **Absolute count per µl : 1500-4000**

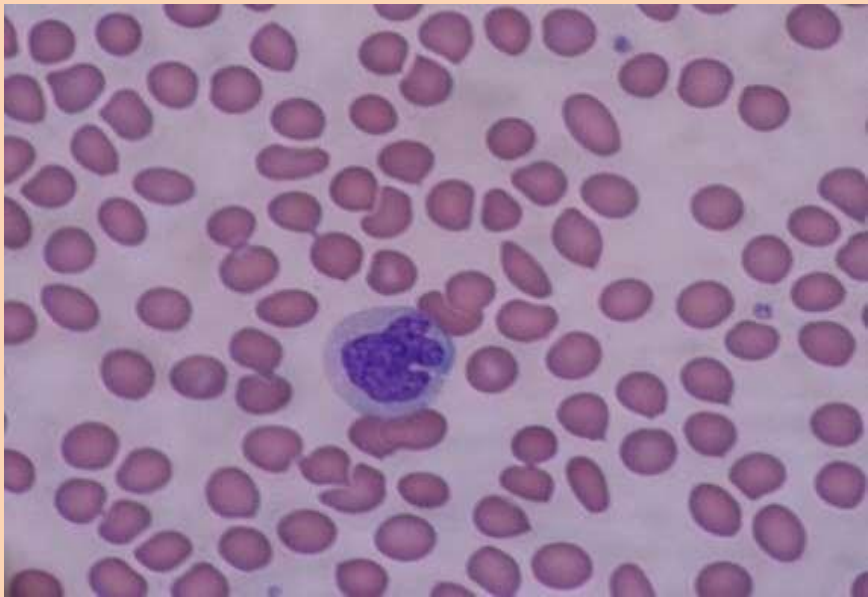




Agranulocytes
Monocytes



- **Cell diameter :** 12-20 µm
- **Nucleus : Large lobulated, indented, with fine chormatin**
- **Cytoplasm : Light basophilic, may contain fine granules or vacuoles.**
- **Normal % : 2-10**
- **Absolute count per µl : 200-800**



Learning Objectives

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- Differential Counting of WBCs
- Pathologic variations in DLC

Blood can be collected from 3 different sources:

- Capillary blood.
- Venous blood.
- Arterial blood.

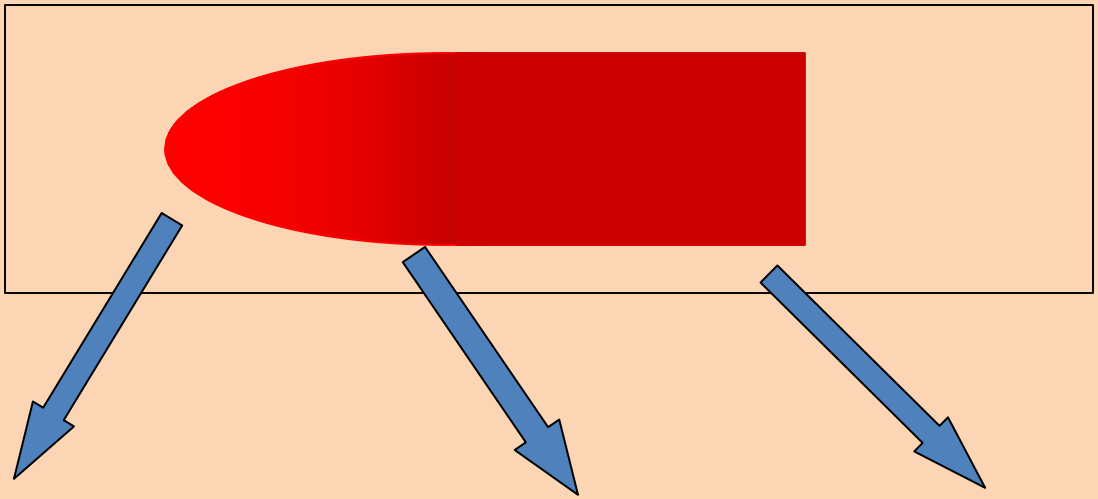
Tube cap color	Additive	Function of Additive	Common laboratory tests
Light-blue 	3.2% Sodium citrate	Prevents blood from clotting by binding calcium	Coagulation
Red or gold (mottled or "tiger" top used with some tubes is not shown) 	Serum tube with or without clot activator or gel	Clot activator promotes blood clotting with glass or silica particles. Gel separates serum from cells.	Chemistry, serology, immunology
Green 	Sodium or lithium heparin with or without gel	Prevents clotting by inhibiting thrombin and thromboplastin	Stat and routine chemistry
Lavender or pink 	Potassium EDTA	Prevents clotting by binding calcium	Hematology and blood bank
Gray 	Sodium fluoride, and sodium or potassium oxalate	Fluoride inhibits glycolysis, and oxalate prevents clotting by precipitating calcium.	Glucose (especially when testing will be delayed), blood alcohol, lactic acid

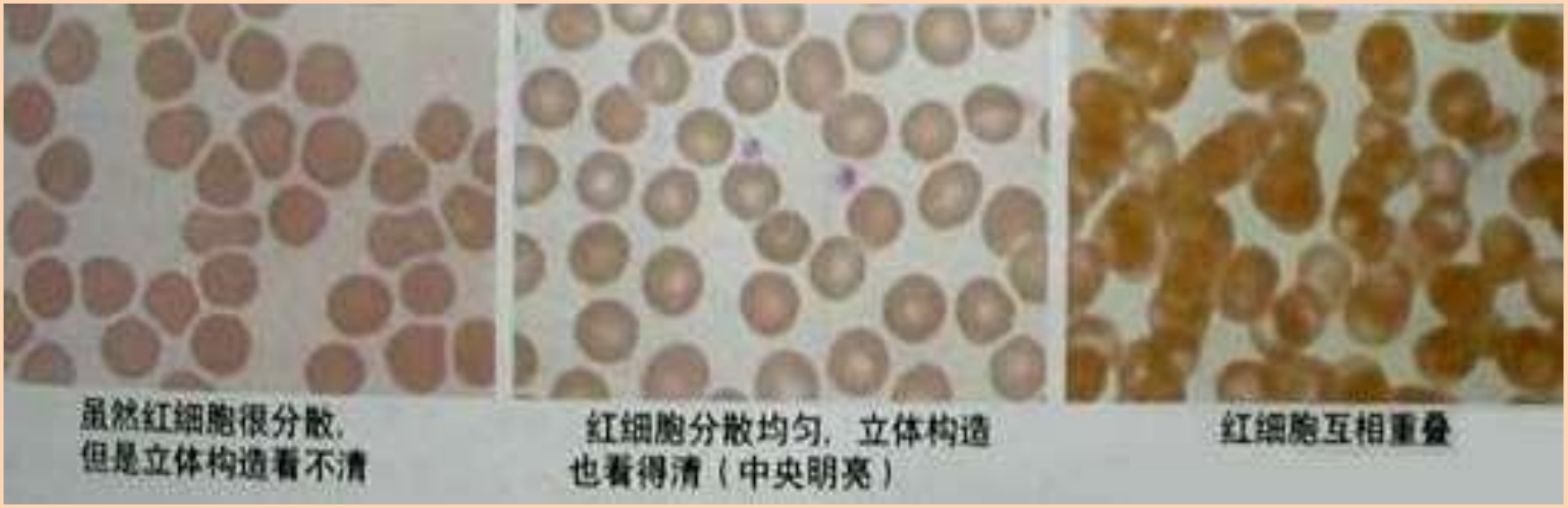
FOCUSING

- 4X to see the general formation of slide.
- 10X for WBC counting
- For a differential WBC count, an oil-immersion objective with around 100x magnification (1.4NA) is used.

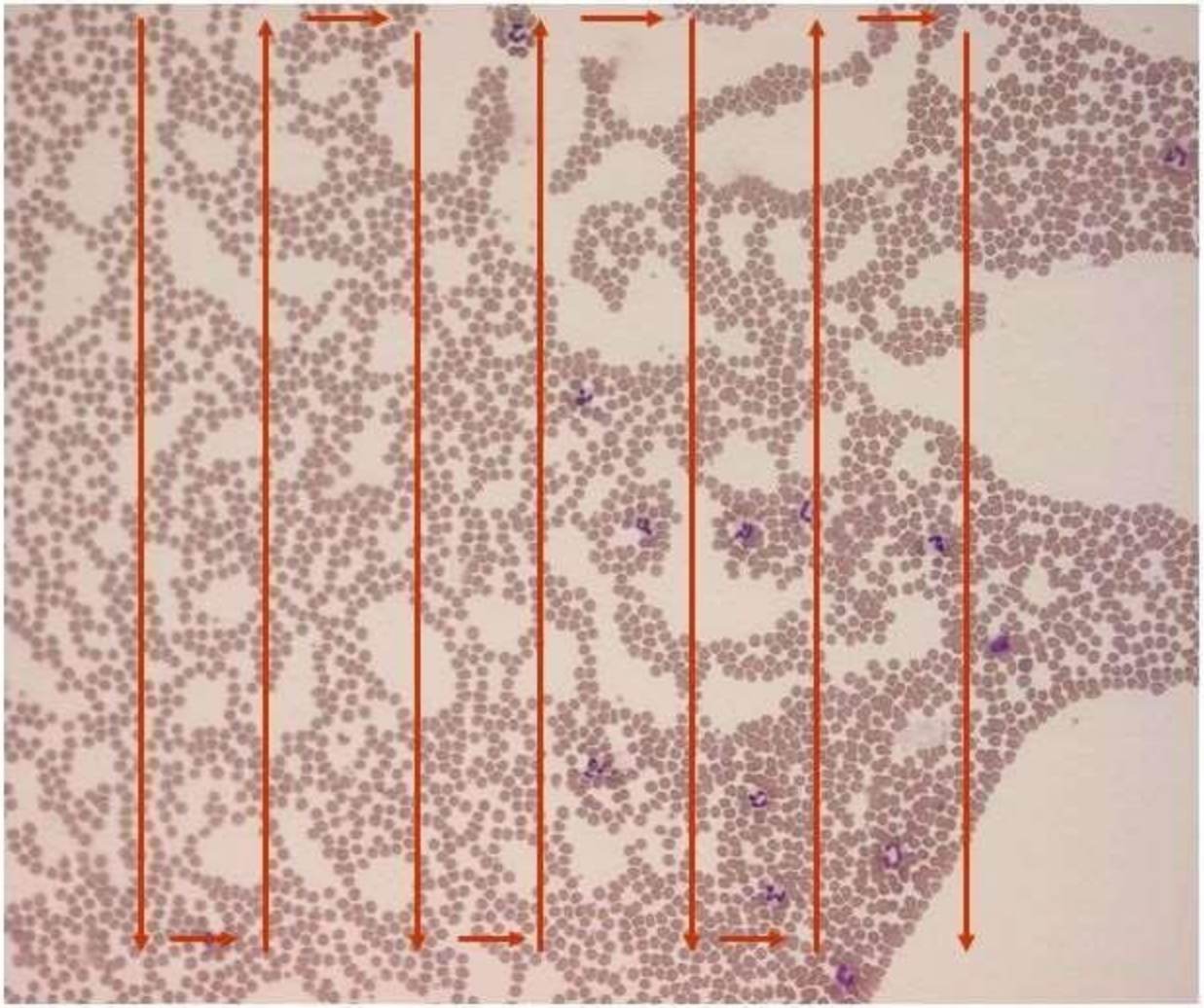
Choose an area near the junction of body with the tail of the smear

tail body head





DIFFERENTIAL COUNT IN A STAINED BLOOD SMEAR: BATTLESHIP METHOD



Start counting

L	P	P	L	P	L	P	P	L	P
P	L	P	P	L	P	P	L	P	P
P	P	P	L	P	P	L	P	P	L
P	P	P	L	P	P	P	L	P	P
L	P	L	P	M	L	L	P	M	P
P	B	E	P	P	L	P	E	P	P
P	M	P	L	L	P	L	P	L	L
L	P	E	P	P	P	L	L	P	P
P	P	P	L	P	L	P	P	L	P
P	L	L	P	P	M	P	L	P	L

Result of DLC

P	L	M	E	B
60%	32%	4%	1%	3%

Normal Reference Range

- White blood cell count 4.0–11.0 x 10^9 /l
- Differential white cell count
  - Neutrophils 2.0–7.0 x 10^9 /l (40–80%)
  - Lymphocytes 1.0–3.0 x 10^9 /l (20–40%)
  - Monocytes 0.2–1.0 x 10^9 /l (2–10%)
  - Eosinophils 0.02–0.5 x 10^9 /l (1–6%)
  - Basophils 0.02–0.1 x 10^9 /l (<1–2%)

AUTOMATED COUNTING

- It is done by electronic counting method.

- Coulter – Automated haemanalyser.

- There are 3 types of electronic methods—
- by cell size analysis,
- by flow cytometry
- high resolution pattern recognition.

- Automated DLC counters have a differential counting capacity of counting either
- 3-part DLC (granulocytes, lymphocytes and monocytes)
- 5-part DLC (P, L, M, E, B).

AUTOMATED COUNTING

- Coulter – Automated haemanalyser

Advantages

- ✓ Easy and rapid method.
- ✓ Time saving.
- ✓ Provide additional information on cell size, shape, nuclear size and density.
- ✓ Very large number of cells are counted rapidly
- ✓ High level of precision

Disadvantages

- ✓ Costly
- ✓ Calibration error
- ✓ Nucleated RBCs/normoblasts are counted as lymphocytes
- ✓ Platelet clumps counted as leucocytes

18/07/16 14:24 M_ ABX

RunNo: 0030
IdNo: 2571
Id:
Type: standard
Birth:
Ph.:
Dep:
Com:

R: 1

Age:

m: s: p:
Sex: Loc:

Date:

V 2.0.0

Pathologies

Leuko

Erythro

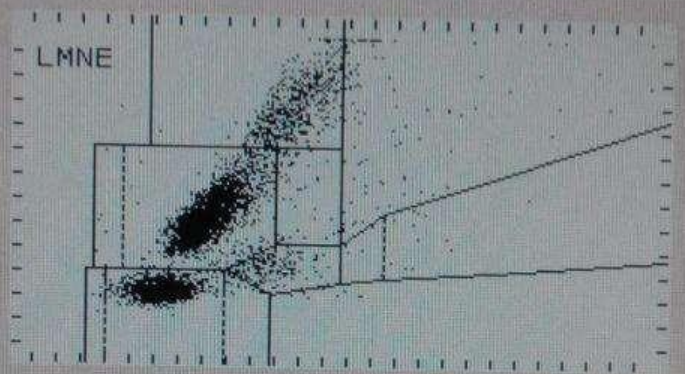
Thrombo

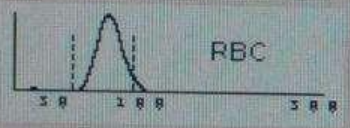
----------Alarms----------
WBC: Baso+ Rn

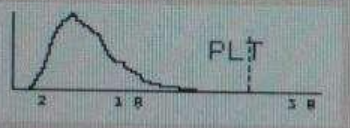
RBC: PLT:

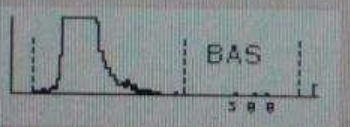
WBC	12.1 !		$10^3/mm^3$
RBC	4.19		$10^6/mm^3$
HGB	12.4		g/dl
HCT	36.7	l	%
MCV	88		μm^3
MCH	29.6		pg
MCHC	33.8		g/dl
RDW	14.5		%
PLT	307		$10^3/mm^3$
MPV	8.8		μm^3
PCT	0.272		%
PDW	15.0		%

LYM%	25.7	LYM#	3.10!
MON%	3.9	MON#	0.47!
NEU%	56.5!	NEU#	6.82!
EOS%	13.1	EOS#	1.58!
BAS%	0.8!	BAS#	0.10!
ALY%	0.5	ALY#	0.06!
LIC%	3.2!	LIC#	0.37!
IML%	0.2!	IML#	0.02!
IMM%	0.4!	IMM#	0.05!
IMG%	2.6!	IMG#	0.30!









0031 DIF

MENU

F1 F2 F3 F4 F5
F6 F7 F8 F9 F10

Learning Objectives

- Introduction
- Identification of WBCs
- Differential Counting of WBCs
- Pathologic variations in DLC

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

1. Acute infections (By bacteria, fungi,parasites and some viruses)

i. Pneumonia
ii. Acute appendicitis
iii. Acute cholecystitis
iv. Salpingitis
v. Peritonitis
vi. Abscess and physical agents
vii. Acute tonsillitis
viii. Actinomycosis
ix. Poliomyelitis
x. Furuncle
xi. Carbuncle

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

2. Intoxication

i. Uraemia
ii. Diabetic ketosis
iii. Poisoning by chemicals anaemia
iv. Eclampsia

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

3. Inflammation from tissue damage

i. Burns
ii. Ischaemic necrosis
iii. Gout
iv. Hypersensitivity reaction

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

4. ***Acute haemorrhage***

i. *Acute haemolysis*

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

5. ***Neoplastic conditions***

i. *Myeloid leukaemia (CML)*
ii. *Polycythaemia vera*
iii. *Myelofibrosis*
iv. *Disseminated cancers*

Neutrophilia

Increase in neutrophil count above 7,500/µl.

Causes

6. Miscellaneous conditions

i. Administration of corticosteroids

ii. Idiopathic neutrophilia

Neutropenia

Fall in neutrophil count below 2,000/µl

Causes –

1. **Infections**

i. Typhoid
ii. Brucellosis
iii. Measles
iv. Malaria
v. Kala azar

Neutropenia

Fall in neutrophil count below 2,000/µl

Causes –

2. Drugs and chemicals and physical agents

i. Antimetabolites
ii. Benzene
iii. Nitrogen mustard
iv. Irradiation

Neutropenia

Fall in neutrophil count below 2,000/µl

Causes –

3. Haematological and other diseases

i. Aplastic anaemia
ii. Pernicious anaemia
iii. SLE
iv. Gaucher's disease
v. Cachexia
vi. Anaphylactic shock

Lymphocytosis

Increase in absolute lymphocyte count to more than 4,000/µl

Causes –

1. Acute Infections

i. Pertussis
ii. Infectious mononucleosis
iii. Viral hepatitis

2. Chronic Infections

i. Tuberculosis
ii. Brucellosis
iii. Secondary syphilis

3. Haematopoietic Disorders

i. CLL
ii. NHL

Lymphopenia

absolute lymphocyte count below 1,500/µl

Causes –

i. Aplastic anaemia
ii. High dose of steroid administration
iii. AIDS
iv. Hodgkin's disease
v. Irradiation

Monocytosis

Rise in absolute monocyte count above 800/µl

Causes –

1. Bacterial infections

i. Tuberculosis
ii. SABE
iii. Syphilis

2. Protozoal infections

i. Malaria
ii Kala azar
iii. Trypanosomiasis

Monocytosis

Rise in absolute monocyte count above 800/µl

Causes –

3. Haematopoietic disorders

i. Monocytic leukaemia
ii. Hodgkin's disease
iii. Multiple myeloma
iv. Myeloproliferative disorders

4. Miscellaneous conditions

i. Sarcoidosis
ii. Cancer of ovary, breast, stomach

Eosinophilia

Increase in the absolute esosinophil count above 400/µl

Causes –

1. **Allergic disorders**

i. Bronchial Asthma
ii. Urticaria
iii. Hay fever
iv. Drug hypersensitivity

2. **Parasitic infestations**

i. Round worm
ii. Hookworm
iii. Tape worm
iv. Echinococcosis

Eosinophilia

Increase in the absolute esosinophil count above 400/µl

Causes –

3. Skin diseases

i. Pemphigus
ii. Dermatitis herpetiformis
iii. Erythema multiforme

4. Pulmonary diseases

i. Loeffler's syndrome
ii. Tropical eosinophilia

Eosinophilia

Increase in the absolute esosinophil count above 400/µl

Causes –

5. Haematopoietic diseases

i. Chronic myeloid leukaemia
ii. Polycythaemia vera
iii. Hodgkin's disease
iv. Pernicious anaemia

6. Miscellaneous conditions

i. Rheumatoid arthritis
ii. Polyarteritis nodosa
iii. Sarcoidosis
iv. Irradiation

Eosinopenia

Fall in the absolute eosinophil count below 40/µl

Causes –

Steroid administration

Basophilia

Increase in the absolute basophil count above 100/µl

Causes –

i. Chronic myeloid leukemia
ii. Polycythaemia vera
iii. Myxoedema
iv. Ulcerative colitis
v. Hodgkin's disease
vi Urticaria pigmentosa

Thank you

RETICULOCYTE COUNT

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INTRODUCTION:

Reticulocytes are :-

→ Immature Red blood cells.

→Larger than RBC's .

→Non-nucleated.

→Cytoplasm contains ribosomal RNA

→ Contain other cytoplasmic organelles, such as mitocondria, remnants of the Golgi appartus, Centrioles, Ferritin molecules etc.

→They are stainable with basic dyes like Brilliant cresyl blue and new methylene blue.

Gilmer and Koepke defined reticulocyte in 1976:

DEFINATION:

'A reticulocyte is a non-nucleated red blood cell, which consists of at least two or more particles ('dots') of blue-stained basophilic polyribosomal material in the cytoplasm after staining with new methylene blue. The dots should be at a clear distance from the cell wall to avoid being mistaken for Heinz bodies. Cells with clear, blue cytoplasmic granulae, which can be seen without fine focussing, are to be regarded as reticulocytes of maturation stage IV'.

STAGES OF MATURATION

Identified by their morphological features:-

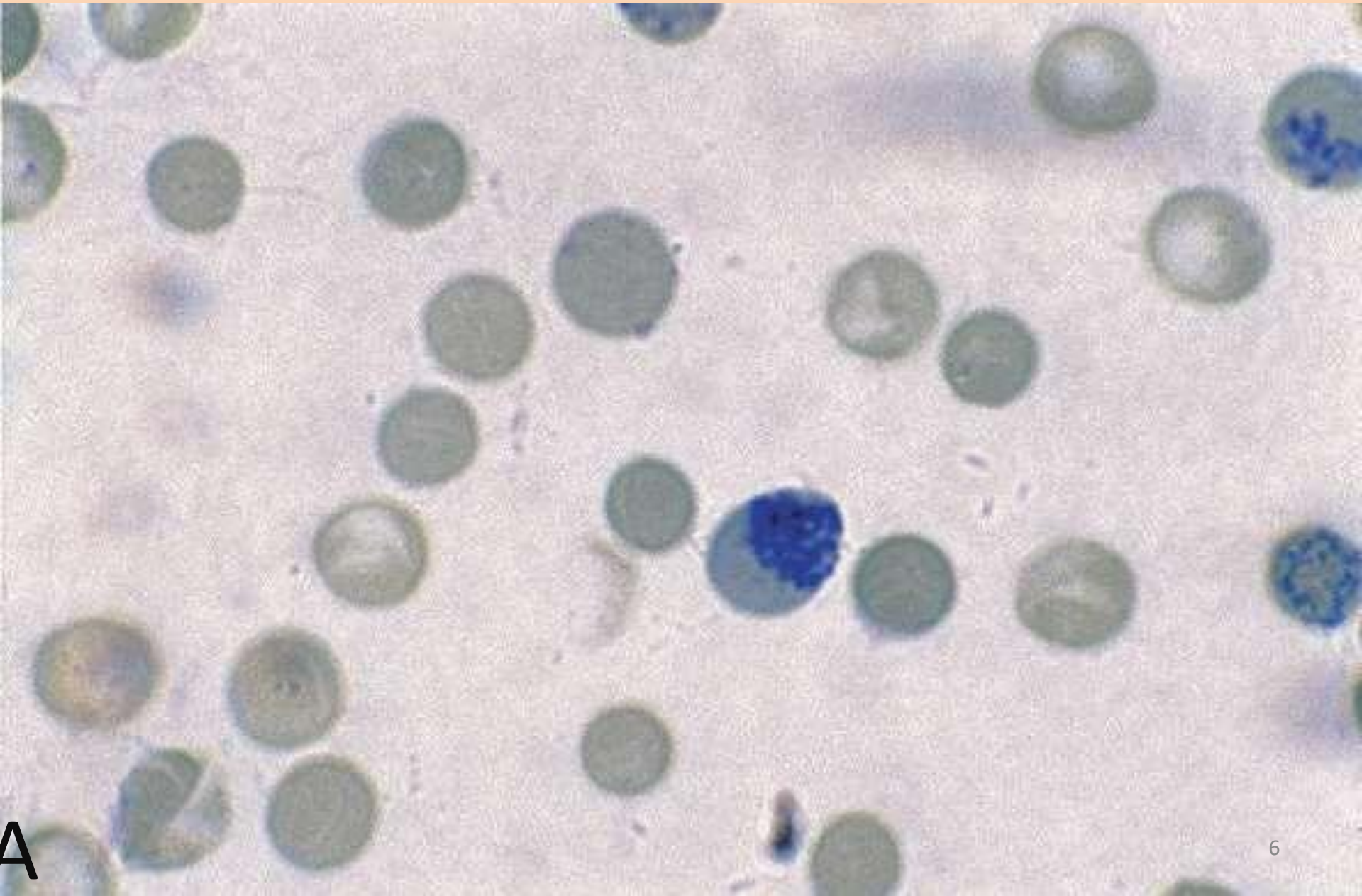
1.Most immature Reticulocytes → Large clumps of reticulin.

2.Most mature Reticulocyte → Few granules of reticulin.

Classification of maturation stages by *Heilmeyer-1932*

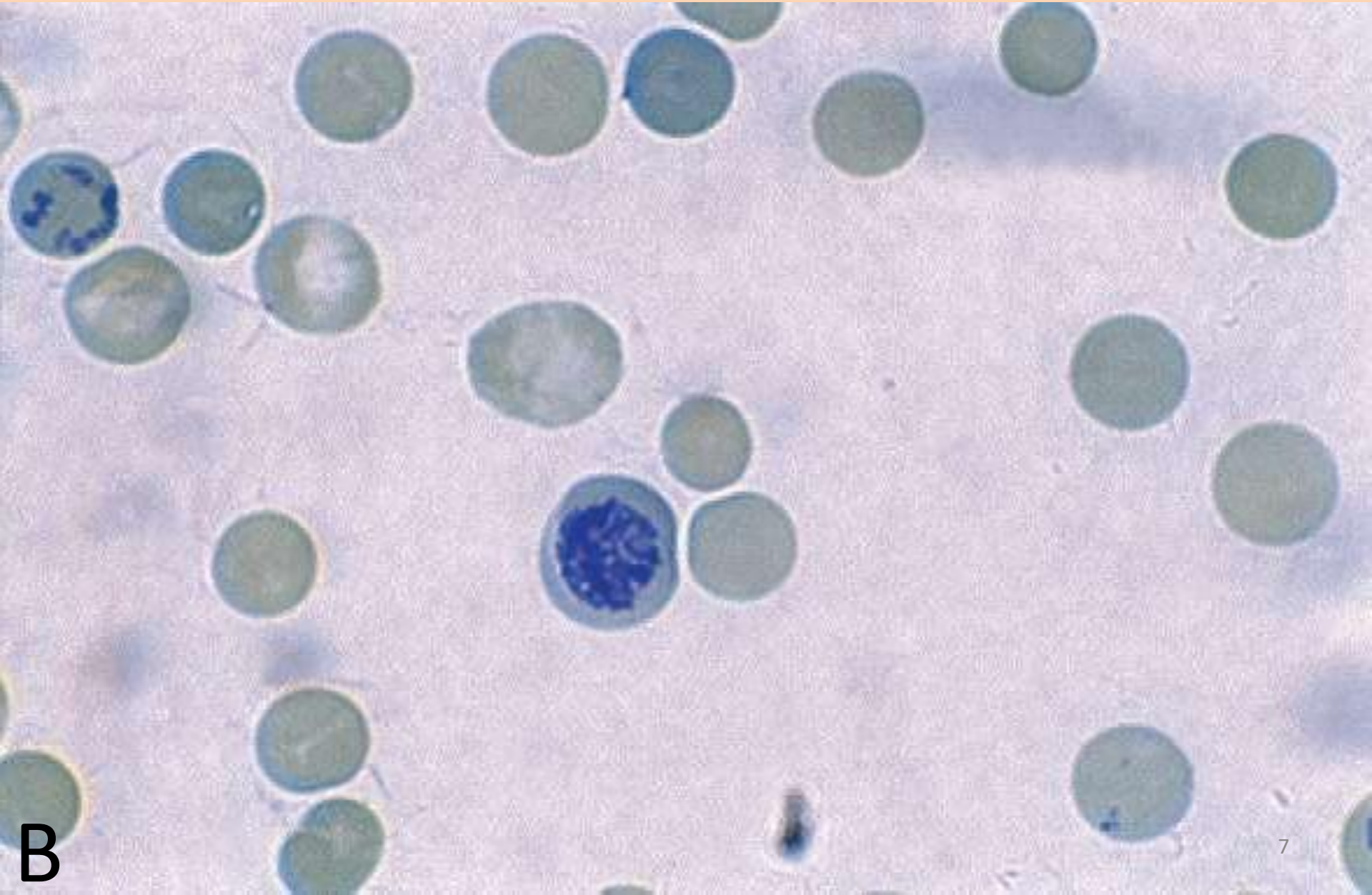
Maturation Stage	Morphological Description
Stage I	Reticulum consists of dense clots
Stage II	Loosely arranged reticulum
Stage III	Diffusely arranged reticulum
Stage IV	Some scattered granulae

Stage I(most immature reticulocyte)



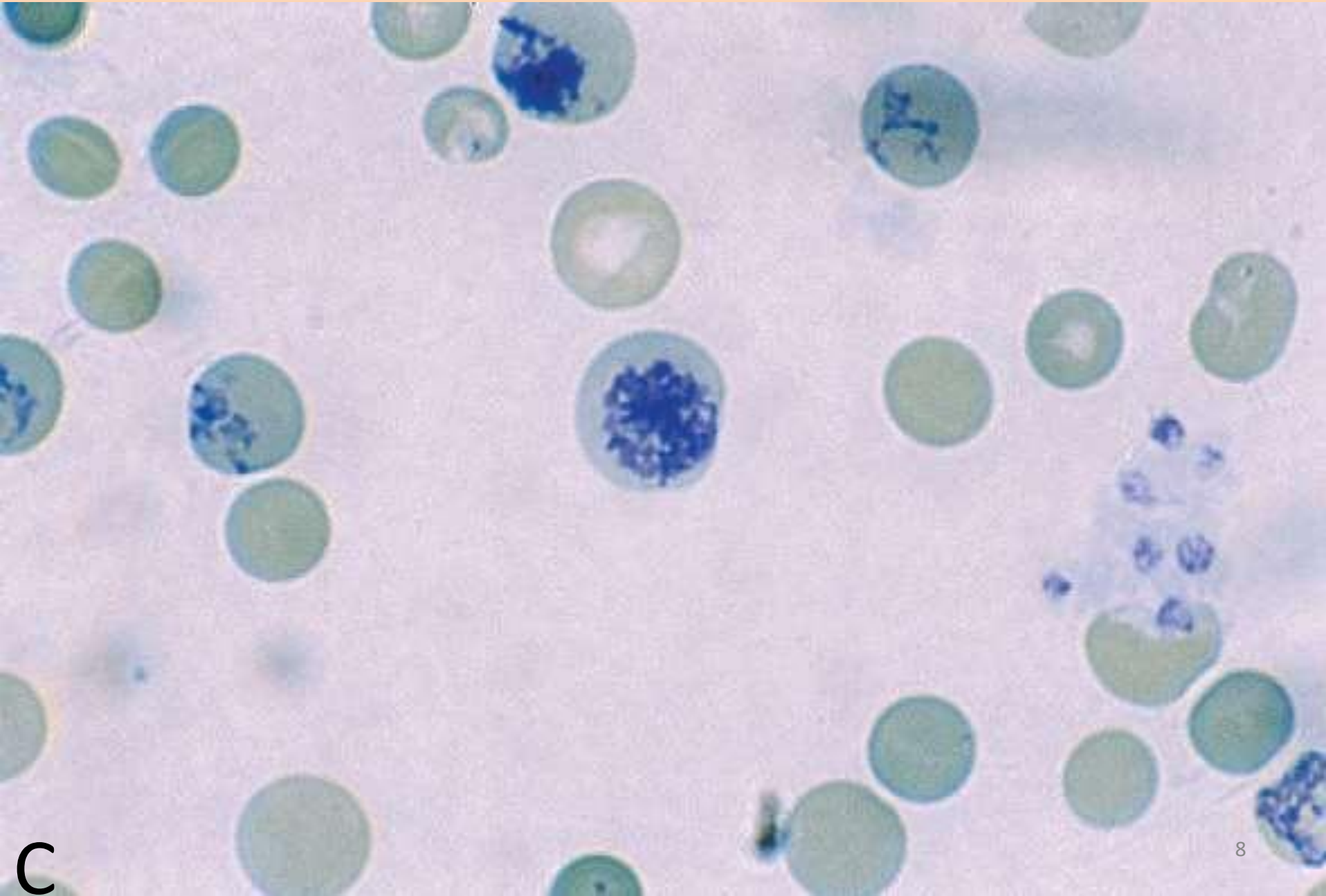
A

Stage I(most immature reticulocyte)



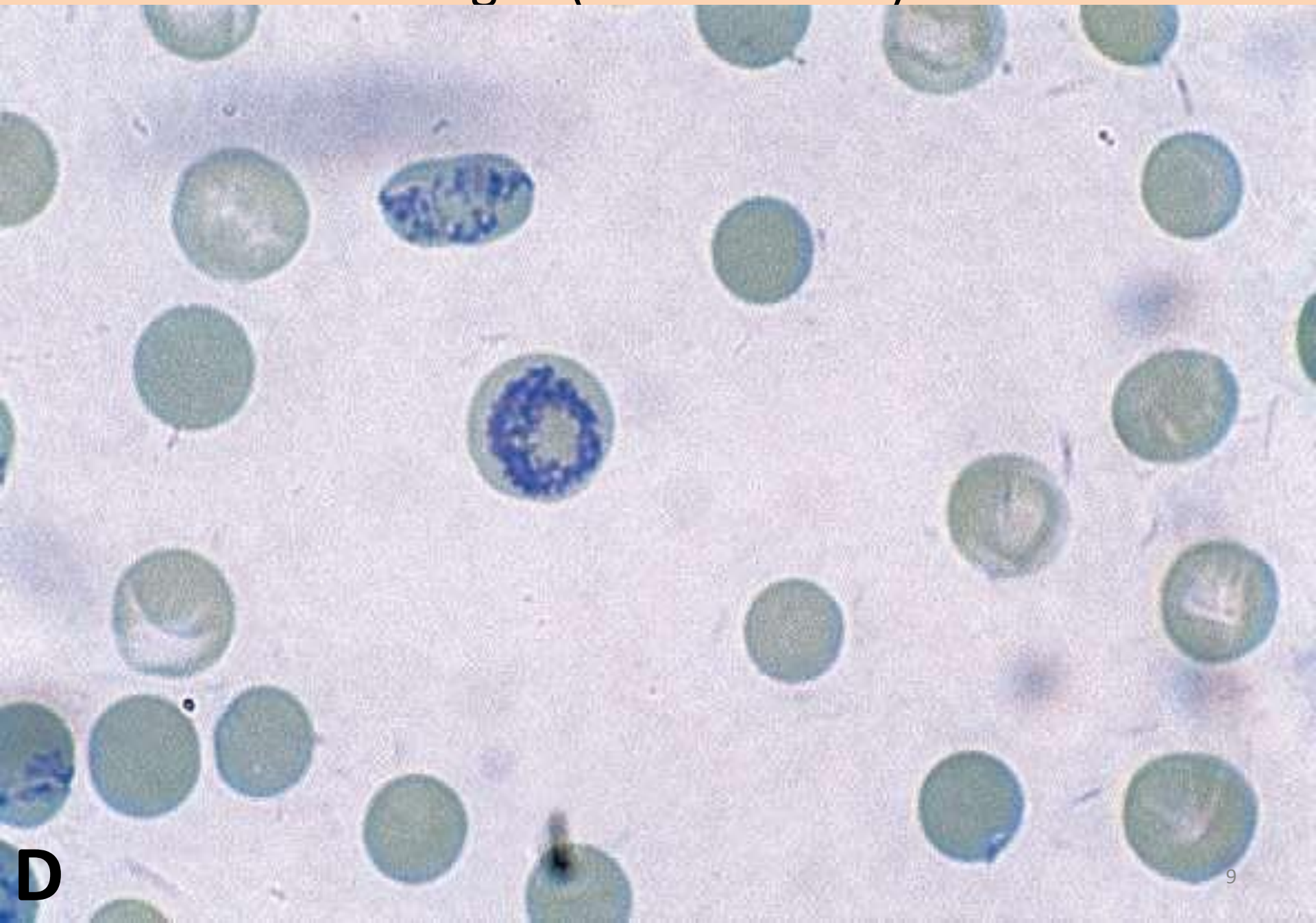
B

Stage II(intermediate)



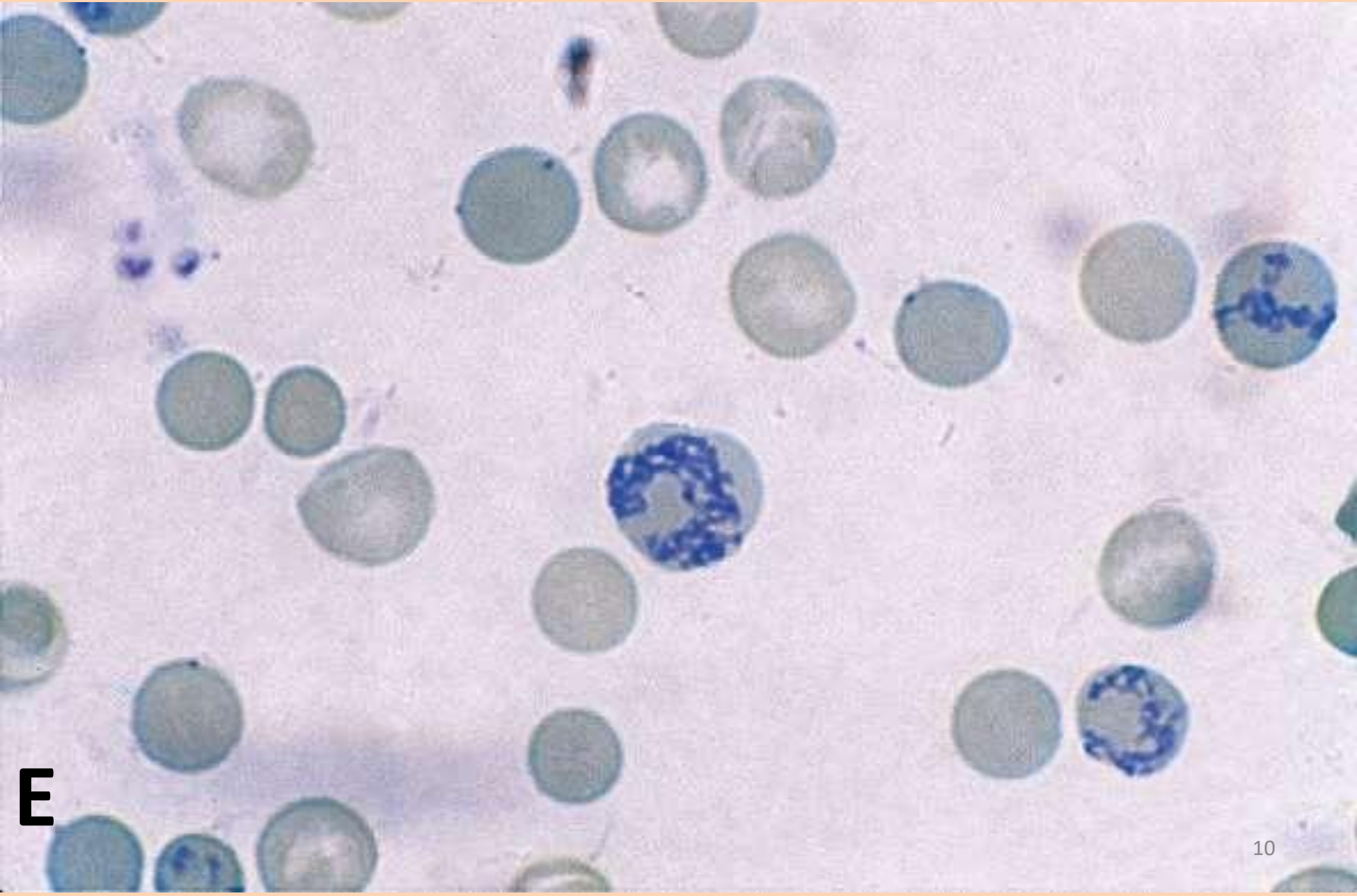
C

Stage II(intermediate)

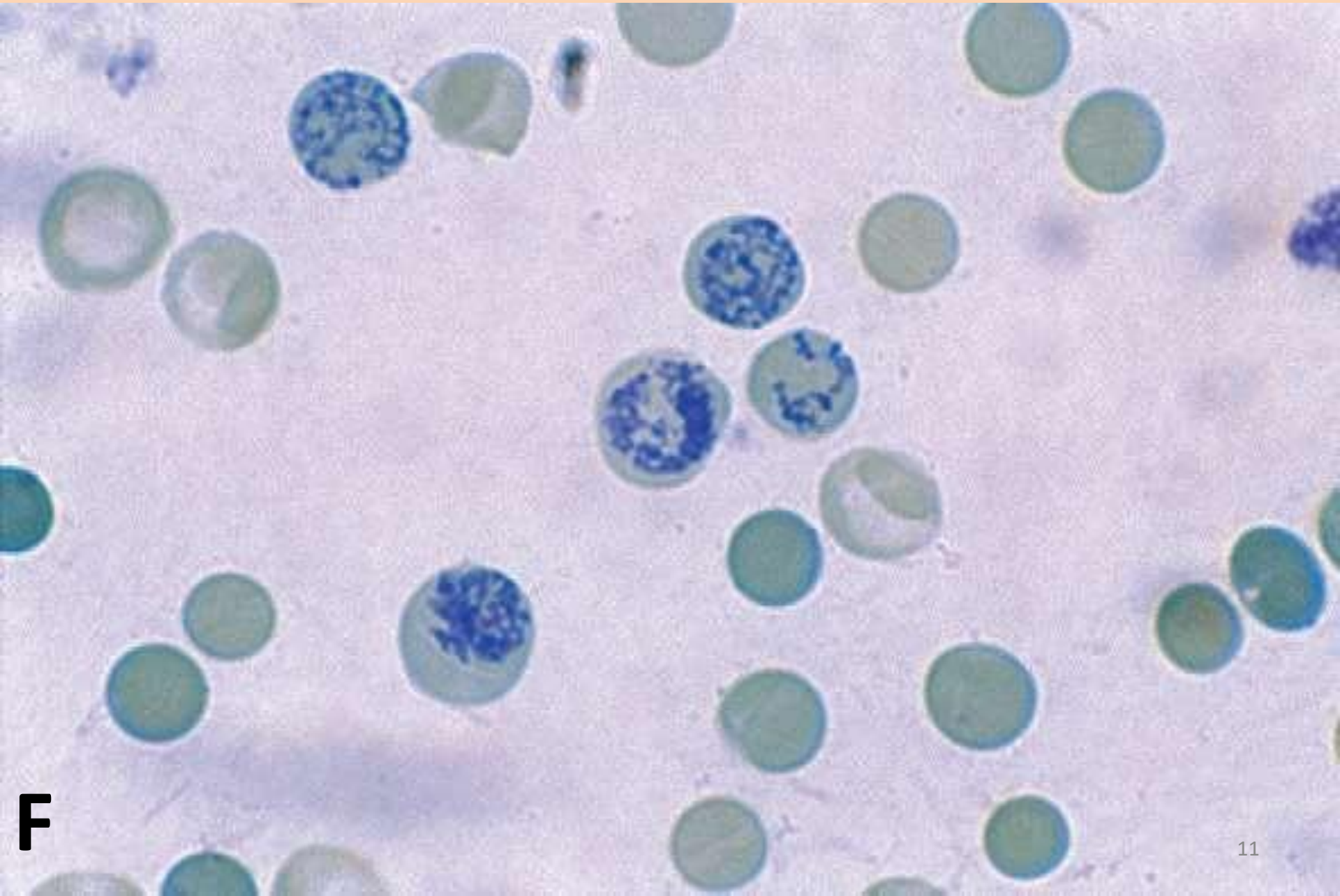


D

Stage III(later stage intermediate)

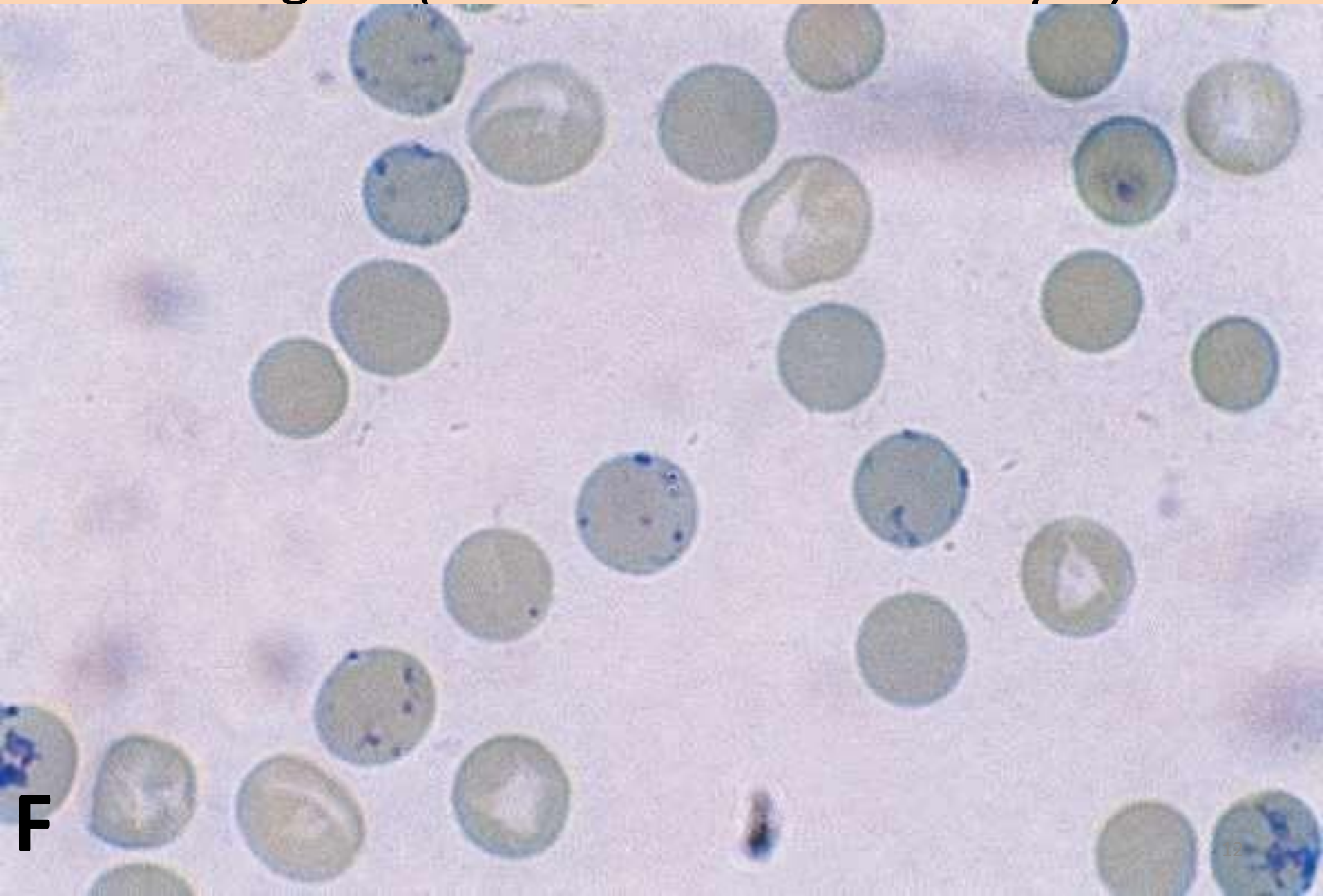


Stage III(later stage intermediate)

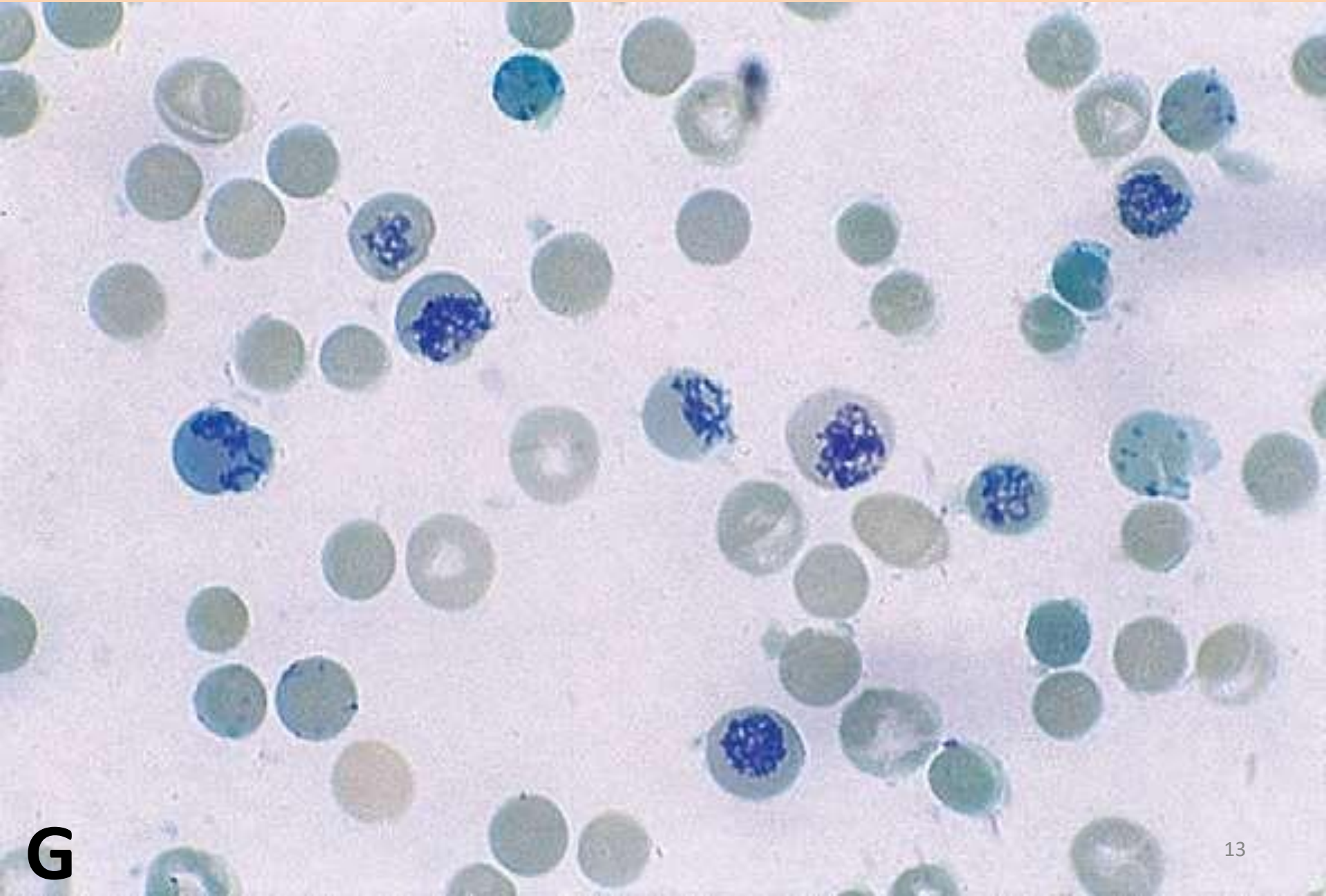


F

Stage IV(most mature reticulocyte)

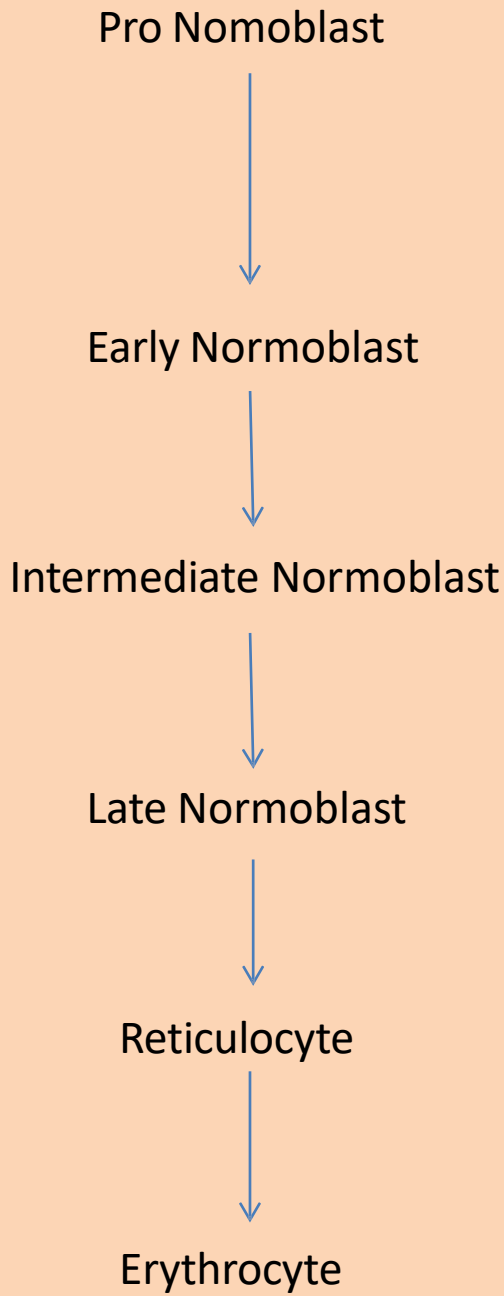


Stage IV(most mature reticulocyte)



G

RETICULOCYTES AND ERYTHROPOIESIS



Indication for counting reticulocytes:

- Basic diagnostic workup in all types of anaemias .
- Therapeutic monitoring during iron, vitamin B12 or folic acid replacement.
- Therapeutic monitoring under erythropoietin.
- Monitoring during stem cell transplantation.

Reticulocyte count

Methods:

a) Manual method

i) Using supravital stain

ii) Fluorescence method

iii) Miller ocular method

b) Automated method

a)MANUAL METHOD

PRINCIPLE:

Supravital stain is used for reticulocyte count. Blood is mixed with the stain and stain enters the cell in living condition .Reticulocyte contains ribosoms and RNA which stain with supravital stain and appears as blue filamentous or granular material.

STAINS:

I) Brilliant cresyl blue- An oxazine dye
II) New Methylene blue-An thiazine dye
III) Azure B

STAIN PREPARATION:

New Methylene blue or

Brilliant cresyl blue or = 1.0 g

Azure B

3% Sodium citrate solution = 20 ml

0.9% Sodium chloride = 80 ml

Dye→ stains the reticulofilamentous material.

Sodium citrate → an anticoagulant

Sodium chloride →provides iso-osmolality as that of blood

SPECIMEN: EDTA –anticoagulated blood .

PROCEDURE:

1) Take 2-3 drops of dye solution into a small test tube.
2) Add 2-3 drops of patients EDTA-blood and mix.
3) Incubate at $37^{o}C$ in a waterbath for 15-20 minutes .
4) Mix and prepare smear.
5) Air dry and observe under microscope.

COUNTING

- By using oil immersion objective choose an area of film where the cells are undistorted & staining is good.
- In the counting area of the film, cells should not overlap.
- Very large numbers of cells have to be surveyed if a reasonably precise count is to be obtained when only small numbers of reticulocytes are present.

- CALCULATIONS:

Count at least 1000 RBC's including reticulocytes which are easily identified with blue granular or reticular precipitate in the cytoplasm. Reticulocyte count is express as % of the red cells.

i) Retic count% =

Total no. of Reticulocytes/Total no. of RBC's X 100

ii) Absolute Retic count= Retic count% X Red cells

iii) Corrected Retic count =

Patient's Hb X retic count in% / Normal Hb of that age

(example, suppose retic count in a adult patient with 7.5 gm Hb is 2% ,

corrected reticulocyte count will be 7.5 X 2/15 =1%)

- NORMAL RANGE:

Adult :0.5-2%

Infants:2-6%

Cord blood: 1-2%

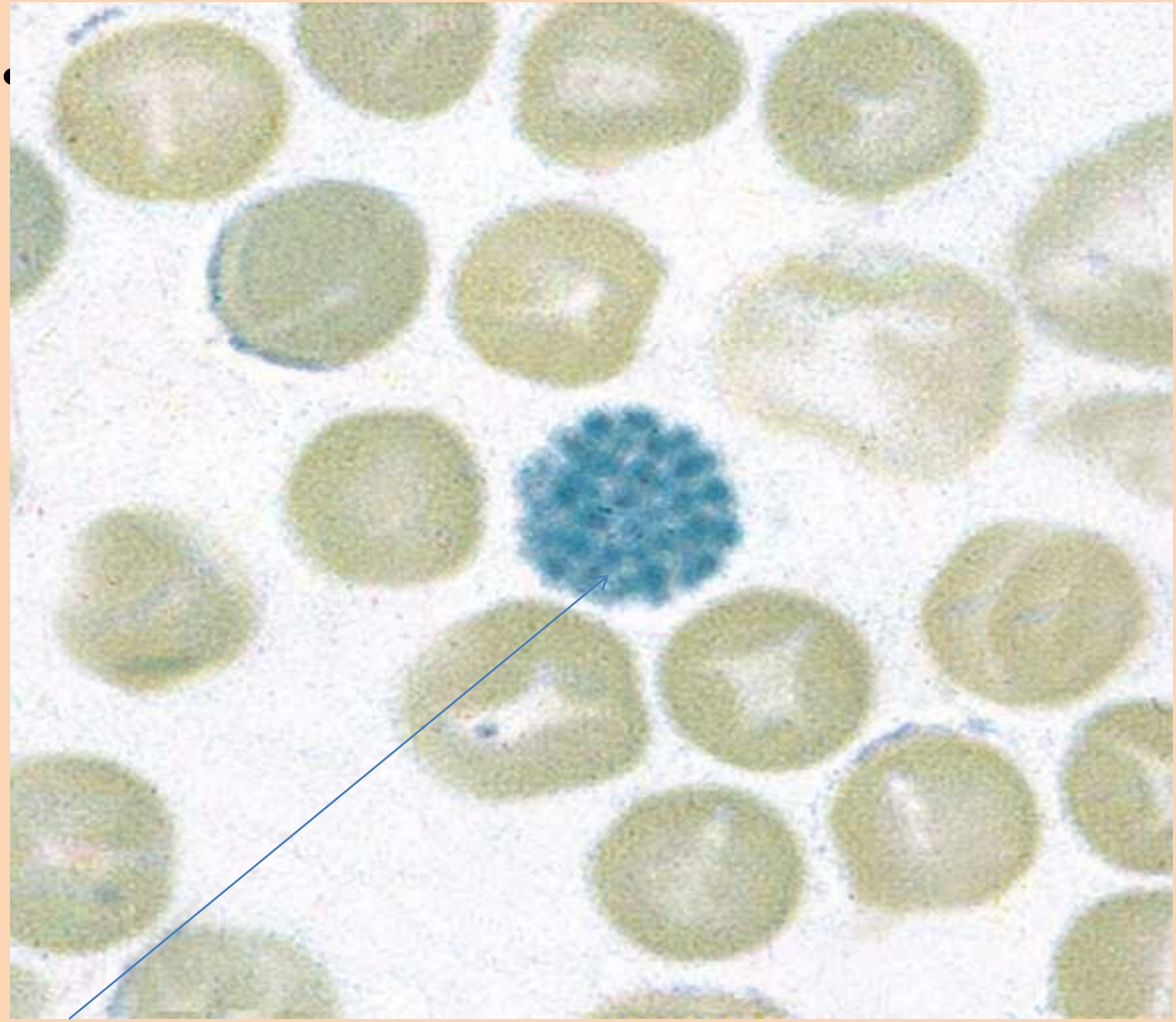
Other red cell inclusions can be seen in the brilliant cresyl blue/new methylene blue smears:

a) HbH bodies:

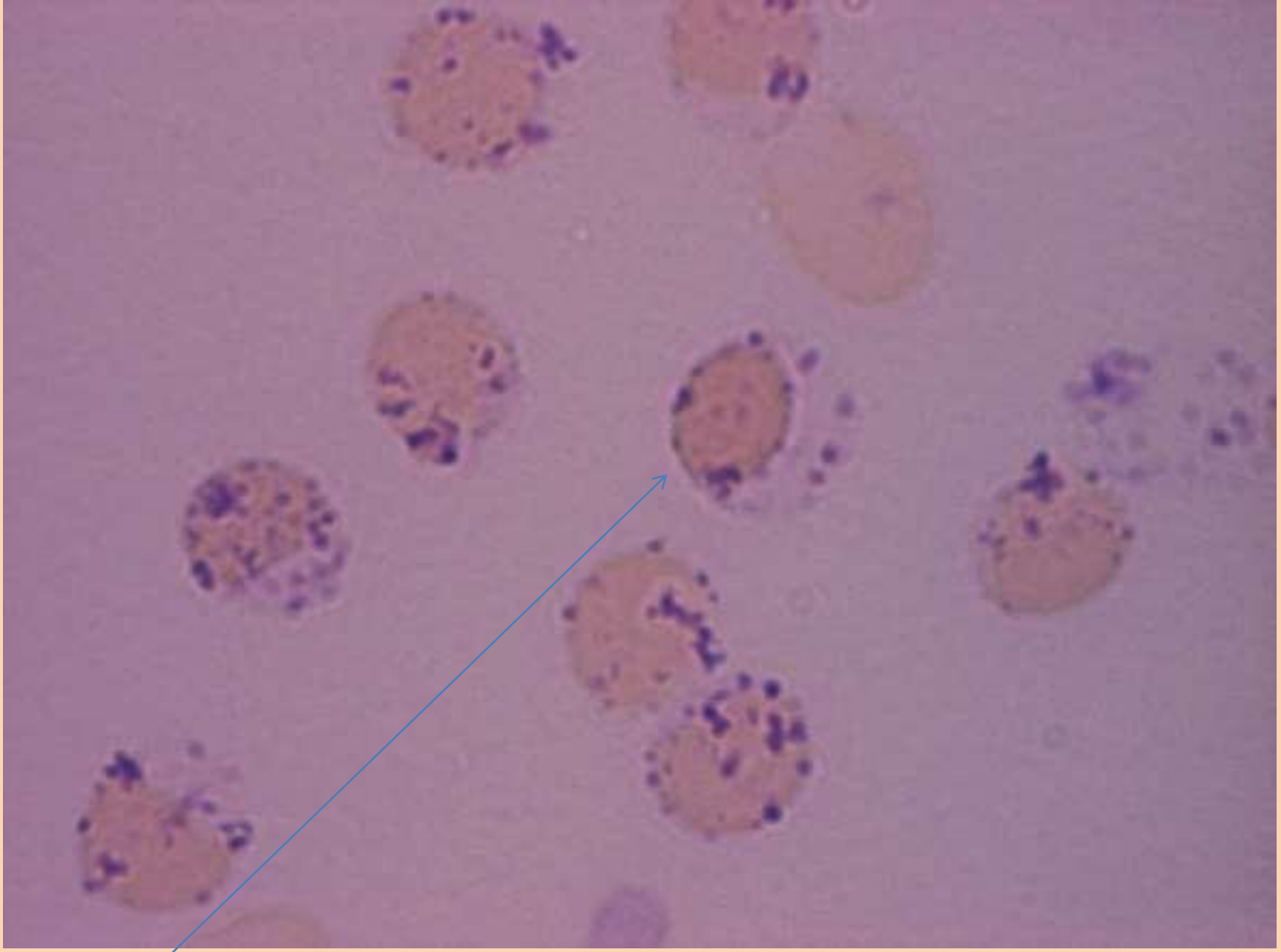
- Round inclusion bodies which stain greenish-blue.
- They are found in alpha thalassaemia or Haemoglobin H disease.

b) Heinz bodies:

- Seen as blue granules ,variable in size, lying to one side of the cell near the membrane.
- They are found in G6PD deficiency



HbH bodies



Heinz Bodies

FLOURESCENT METHOD

PROCEDURE:

1. Add 1 volume of Acridine orange solution with 1 volume of blood.
2. Mix for 2 minutes.
3. Make a film.

4.Dry and observe under Flourescent microscope.

Observation:

RNA gives an orange- red flourescence.

Other nuclear material(DNA)- Yellow flourescence.

AUTOMATION IN RETICULOCYTE COUNT

Example of automated instruments:

i) Sysmex XE-5000,

ii) XT-4000i,

iii) XE-2100,

iv) XT-2000i

Method of Detection:Flourescence(Forward light scatter & side fluorescent emission)

Reagent used:

Diluent:-Tricine buffer

Dye : Polymethine Dye

With Methanol in Ethylene glycol

- PRINCIPLE:

Nucleic acids remaining in immature erythrocytes are stained with a fluorescent dye RET Search (II), Reticulocytes are measured based on the principle of flow cytometry. The fluorescence-stained reticulocytes are divided into 3 fractions by the intensity of fluorescence:

- **Reticulocyte maturation**

HFR	MFR	LFR
High Fluorescence Fluorescence Reticulocytes	Medium Fluorescence Reticulocytes	Low Fluorescence Reticulocytes
Little RNA	More RNA	High level of RNA
Mature reticulocytes	Semi-mature reticulocytes	Immature reticulocytes
Reference range: 86.5 - 98.5%	Reference range: 1.5 - 11.3%	Reference range: 0 - 1.4%

Immature Reticulocyte Fraction (IRF)

IRF is the sum of MFR and HFR, i.e.

IRF = MFR + HFR

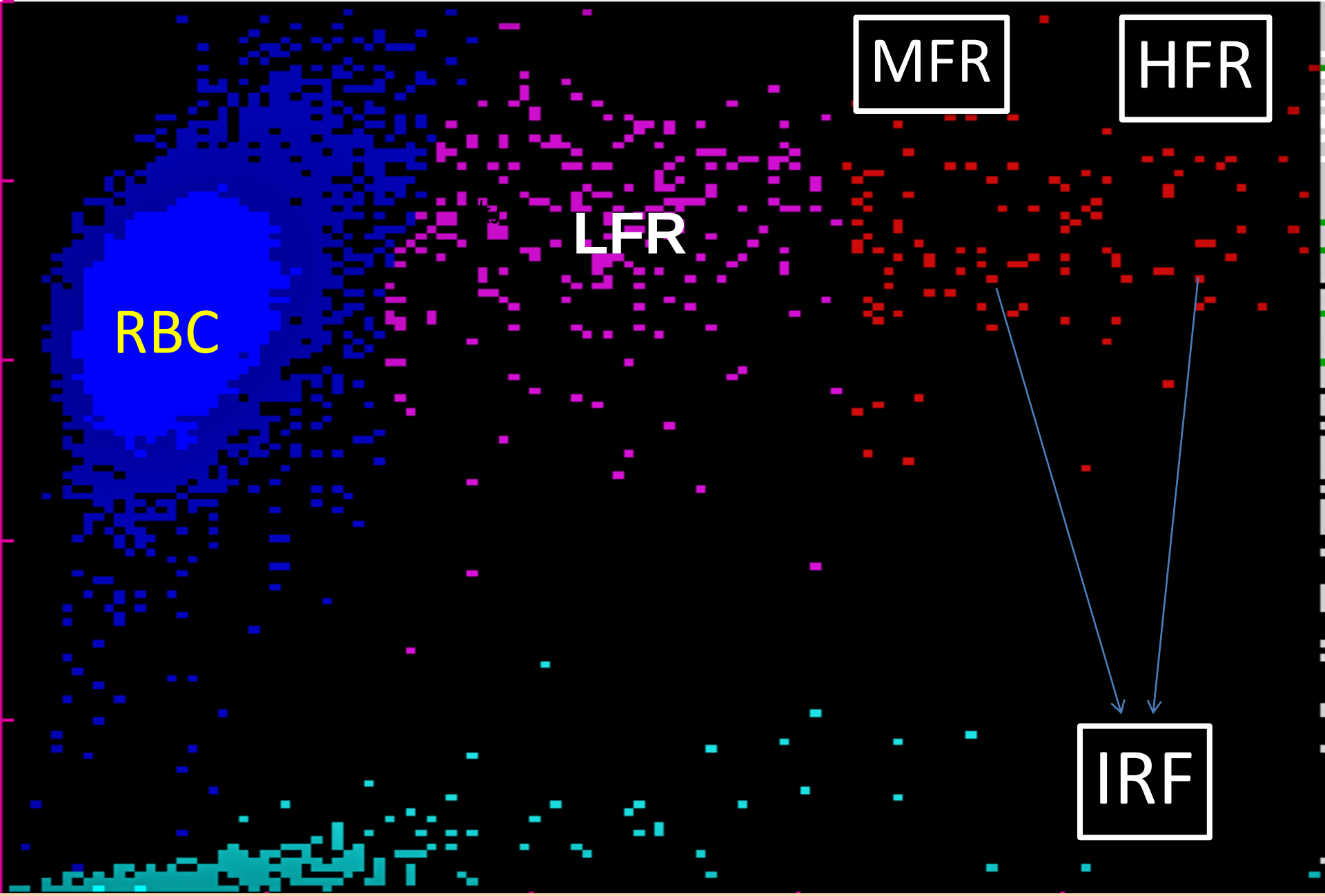
- **Reference range**

IRF: female 1.1 – 15.9 %

male 1.5 – 13.7 %

INDICATIONS OF IRF

- The IRF value is an early marker for evaluating the regeneration of erythropoiesis.
- The IRF percentage increases after only a few hours, the reticulocyte count increases after two to three days.
- If the IRF value does not increase during the treatment of deficiency anaemias with erythropoietin or vitamins, this indicates a lack of response to therapy.


MFR
HFR
LFR
RBC
IRF

Advantage of automated count:

- only 100µl sample required.
- More precise

Disadvantage:

- Costly
- Hawel jolley bodies, giant platelets are counted as reticulocytes.

Sources of errors:

- Fibrin microclots in sample.
- Presence of dust particles in the diluent.

CLINICAL SIGNIFICANCE

Abnormal findings:

INCREASED count in-

- Acute blood loss
- Hemolytic anaemia
- Therapy of iron deficiency
- Megaloblastic anaemia
- Response to specific therapy for megaloblastic anaemia.
- Sickle cell anaemia

DECREASED count in –

- Aplastic anaemia
- Anaemia of chronic disease
- Iron deficiency anaemia
- Deficient Red cell production
- Thalassaemia
- Sideroblastic anaemia
- Anaemia with chronic renal failure
- Acute leukaemia

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