17ABBZPD - Fundamentals of Pathology, Hygiene and Epidemiology

Fundamentals of Laboratory Techniques in Hematology

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Introduction

- what is hematology
- the basis of hematopoiesis
- the basis of hemostatis
- the basic laboratory methods in hematology

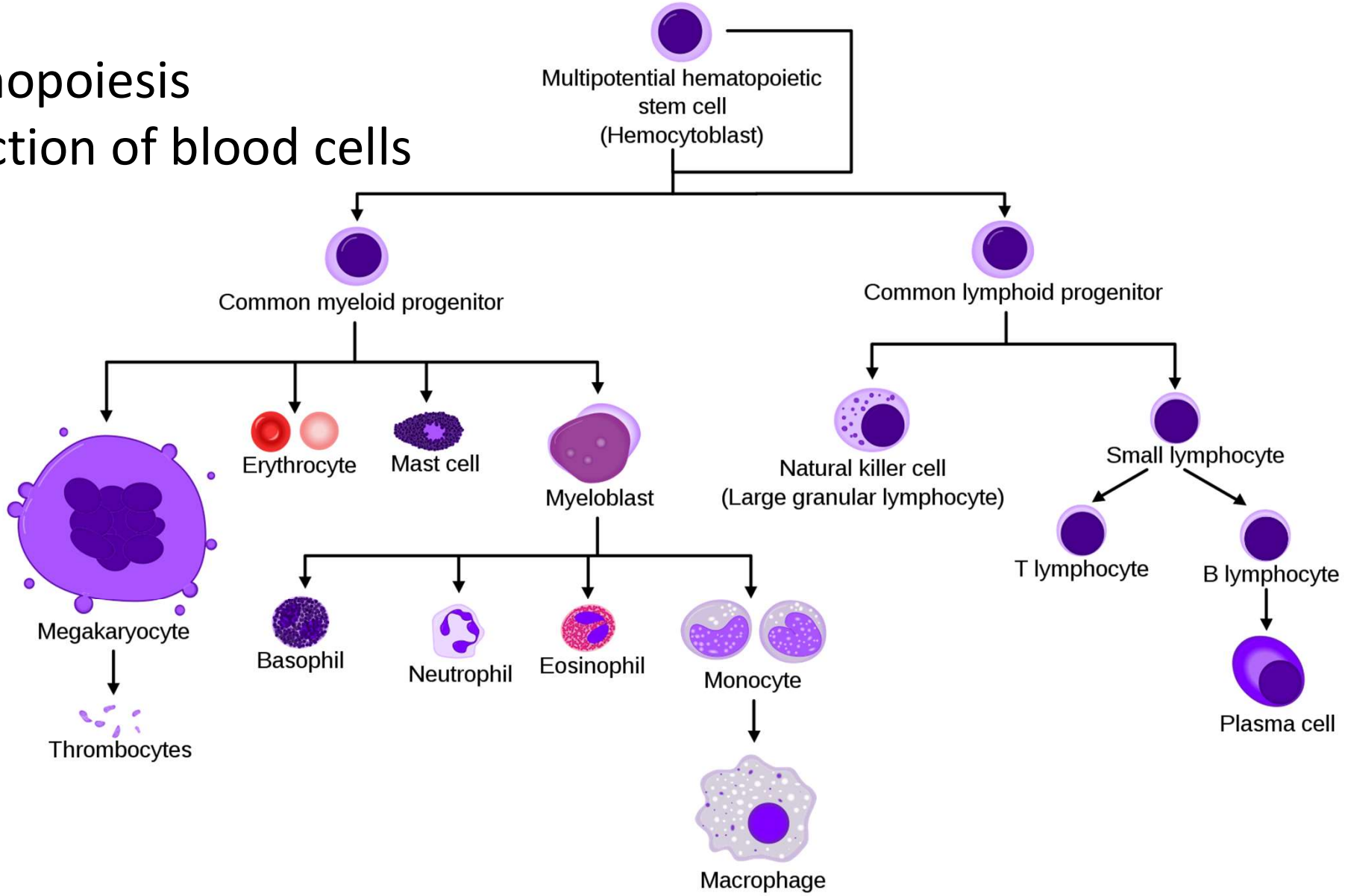
Hematology

Hematology – haima = blood, logos = word

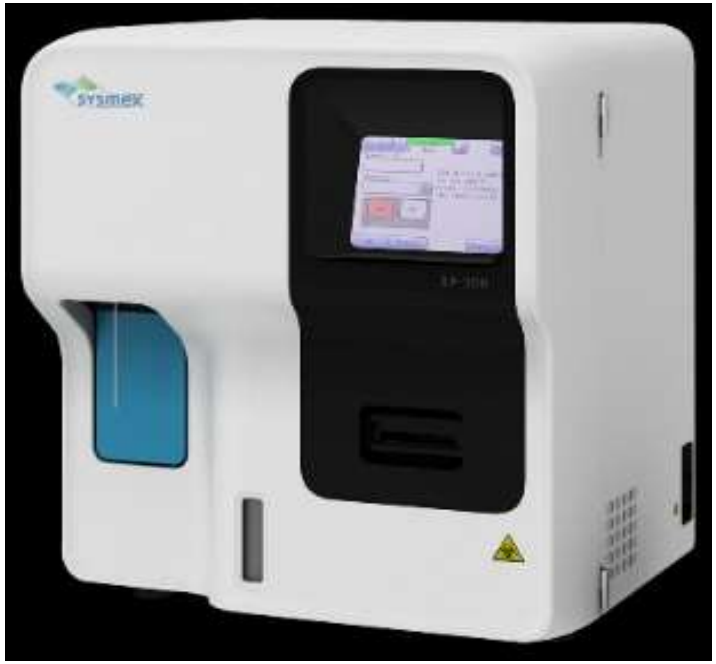
clinically oriented discipline

includes - the basic knowledge of pathology,
- laboratory techniques and oncology,
- the sofisticated techniques of imunology
- genetic ingeneering plays the main role in diagnosis of inheritted diseases

The hemopoiesis
= production of blood cells



Hematological analysers



based on – change of impedance
- optical analysis

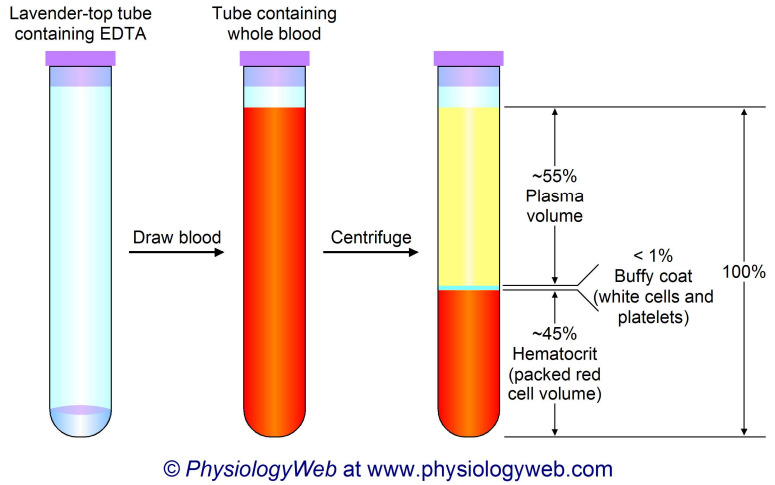
assessment: CBC (complete blood count)

samples – blood with EDTA (*ethylenediaminetetraacetic acid*)) or sodium citrate

- **WBC** 4.0-10.0 x 10^9/L
- **Platelets PLT** 150-450 x 10^9/L
- **RBC** 4.5-5.9 x 10^{12}/L

Hematological analysers

Hemoglobin and red cells indices



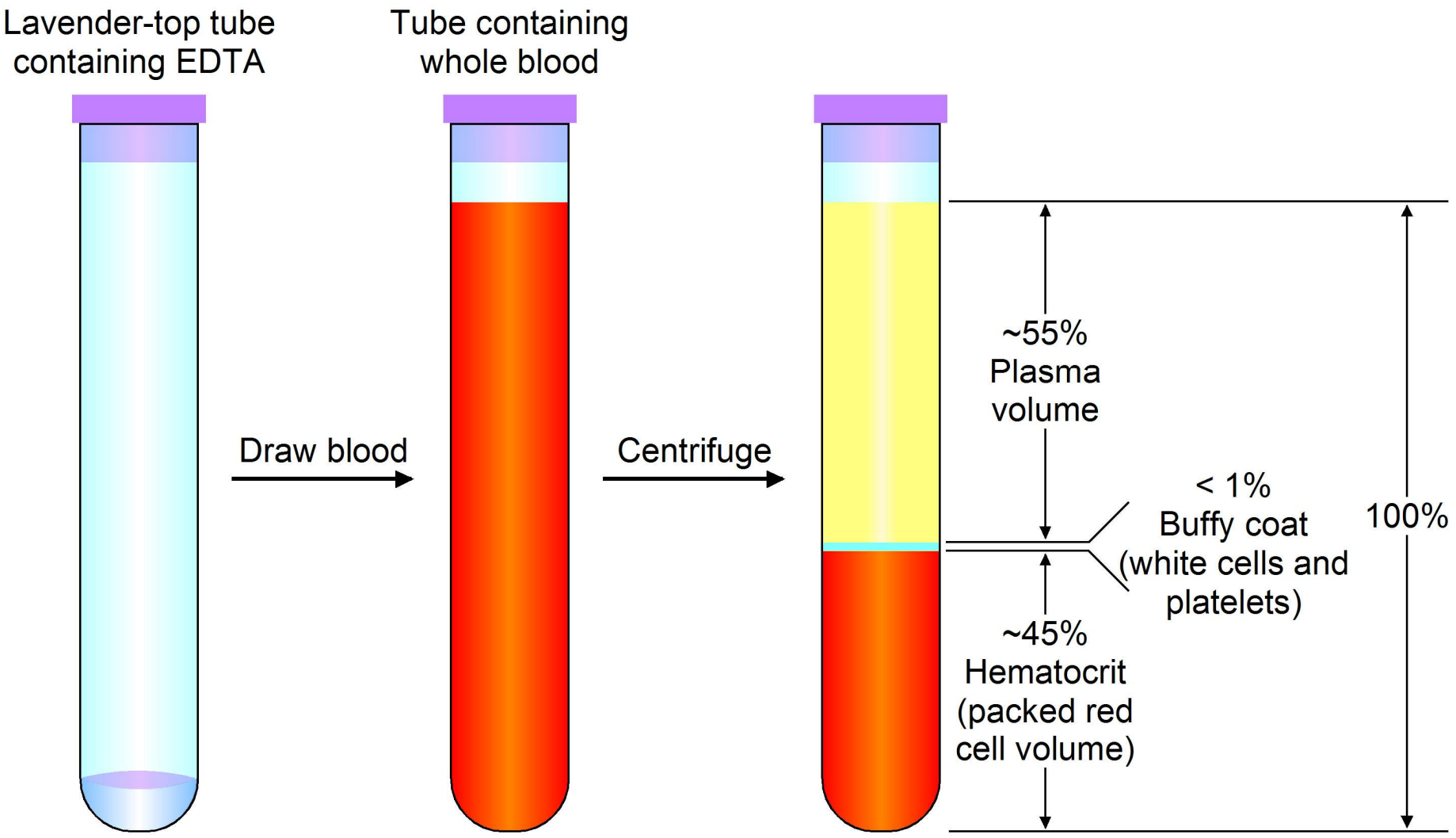
Hematocrit

Mean cell (corpuscular) *volume* or MCV 78 -100 *femtoliters*

Mean cell *hemoglobin concentration* or MCHC Normal: 31-37 g/dL (*of erythrocytes*)

Mean cell *hemoglobin content* or MCH 26 - 32 pg per red cell

Hemoglobin 120 – 140 g/L female, 140-160 g/l male



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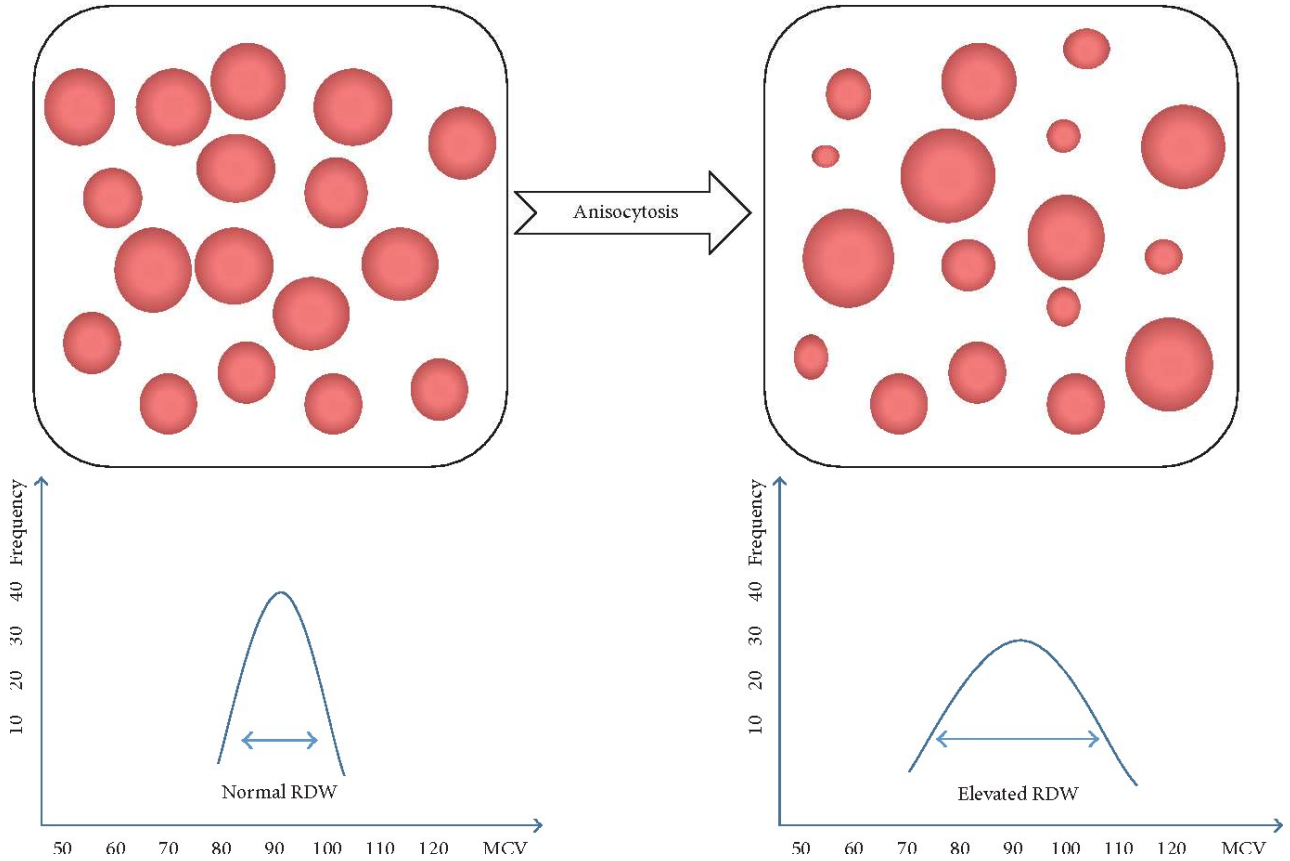
Distribution width

is a measure of the range of variation of red blood cell (RBC) volume



Hematological analysers

Hemoglobin

basic method – with KCN and ferrikyanid

principle

$HbFe^{2+} + Fe^{3+}(CN)_6^{3-} \rightarrow HbFe^{3+} + Fe^{2+}(CN)_6^{4-}$

$HbFe^{3+} + CN^{-} \rightarrow HbFe^{3+}CN$

measured by photometer at 540nm, result g/l

recently the toxic kyanid is replaced by nontoxic laurylsulfate (SLS)
laurylsulfate derivatives absorb at 535 nm

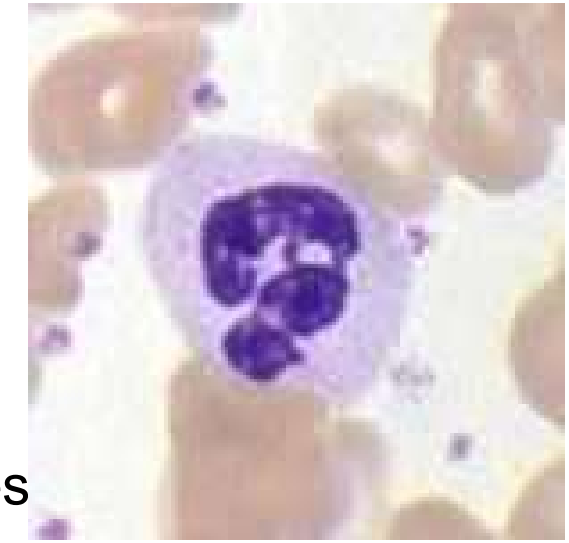
Microscopical assessment

Stained (Giemsa-Romanovski) peripheral blood smear is examined carefully using 40 x to 100 x objective

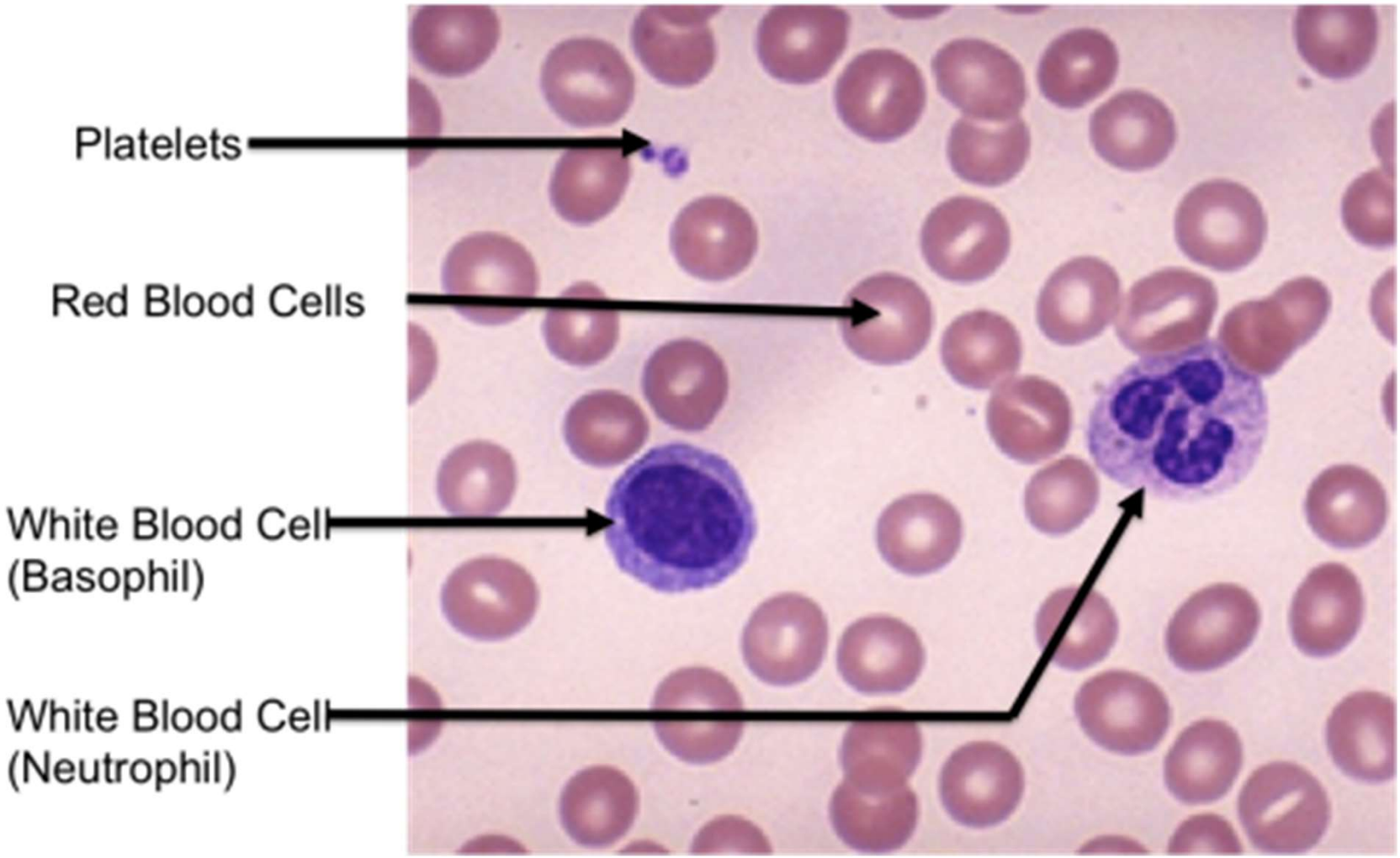
100 white blood cells are counted

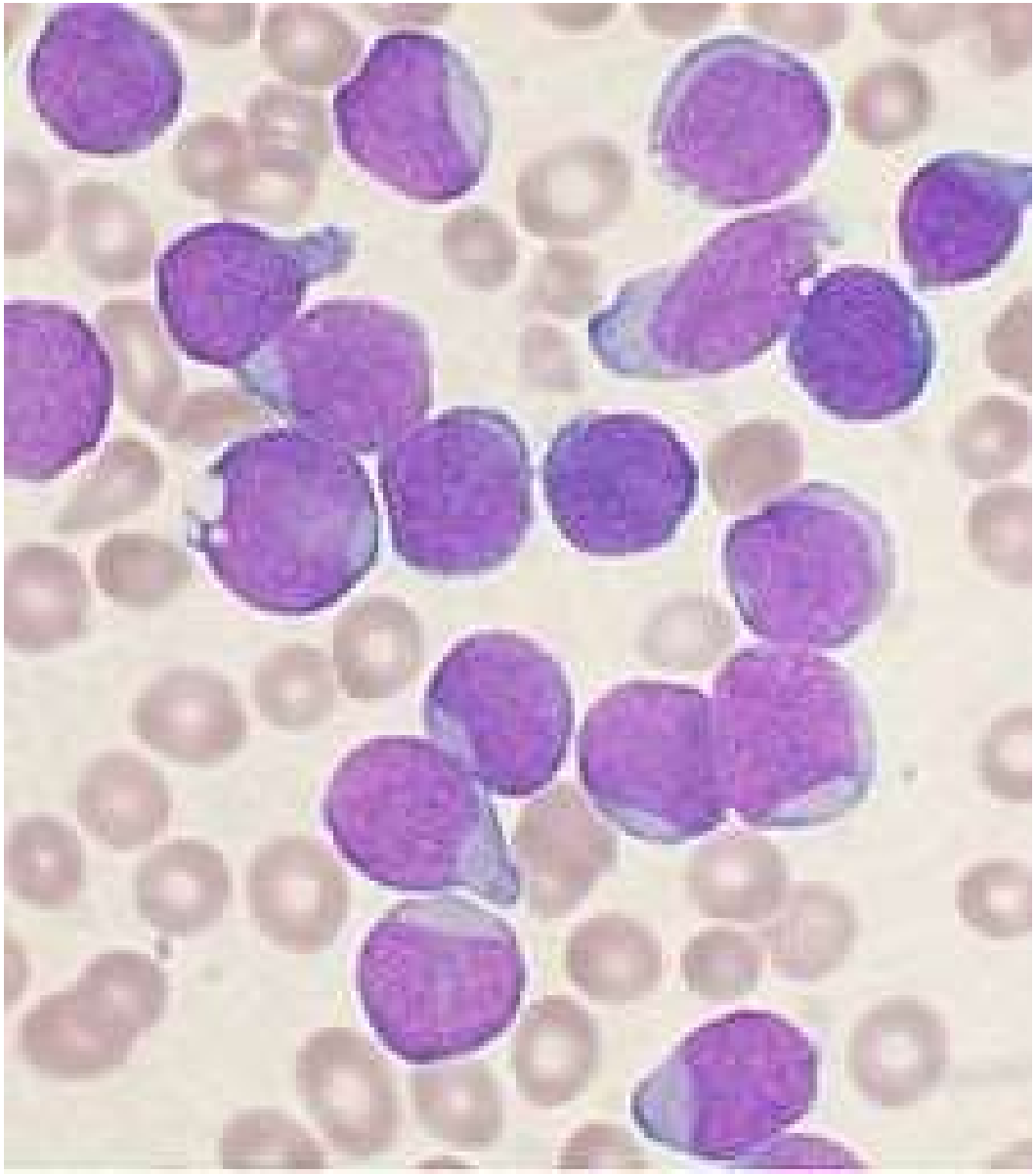
Cells are classified by morphology→

Neutrophils
Bands
Lymphocytes
Monocytes
Eosinophils
Basophils
Metamyelocytes
Myelocytes
Promyelocytes
Blasts



Human Blood Slide





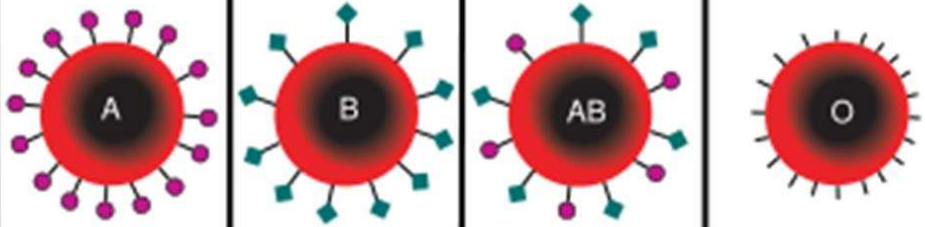
How to diferentiate blood groups AB0 system

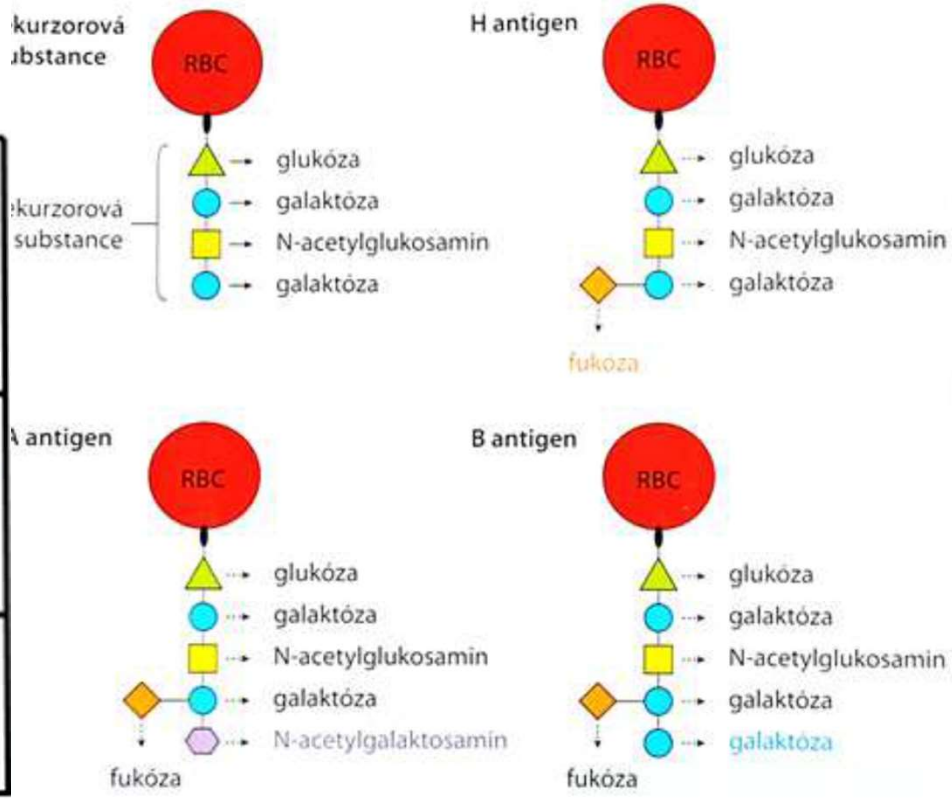
Basic blood groups – A, B, AB, 0

on RBC – aglutinogens - erytrocytes A – have aglutinogen A
erytrocytes B – have aglutinogen B
erytrocytes AB – have aglutinogen A and B
erytrocytes 0 – neither A nor B

in plasma – there are present aglutinins – A anti b
B anti a
AB – no
0 ani A and anri b

How to diferentiate blood groups AB0 system

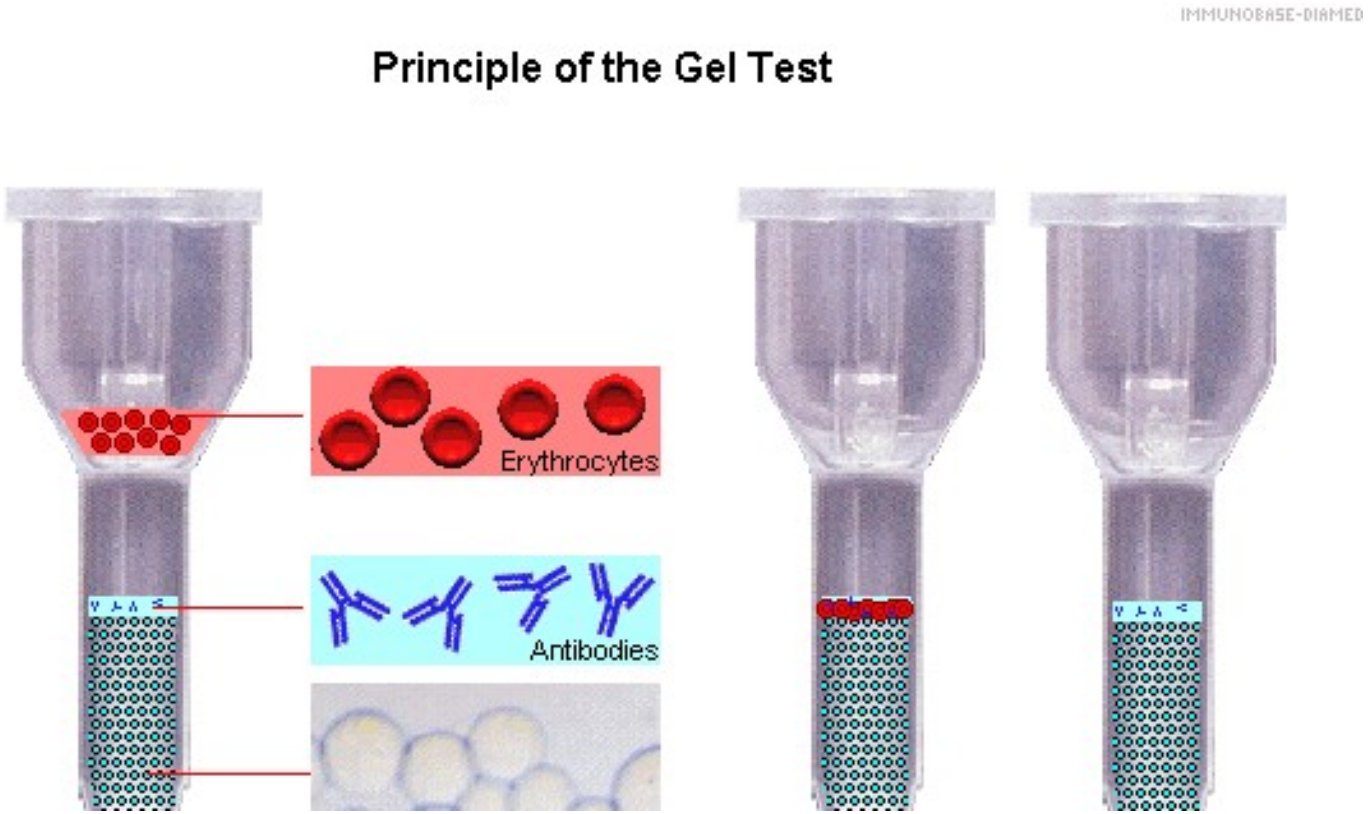
	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	 A antigen	 B antigen	 A and B antigens	No antigens



Principle of gel technology

Sepadex matrix serves as a sieve

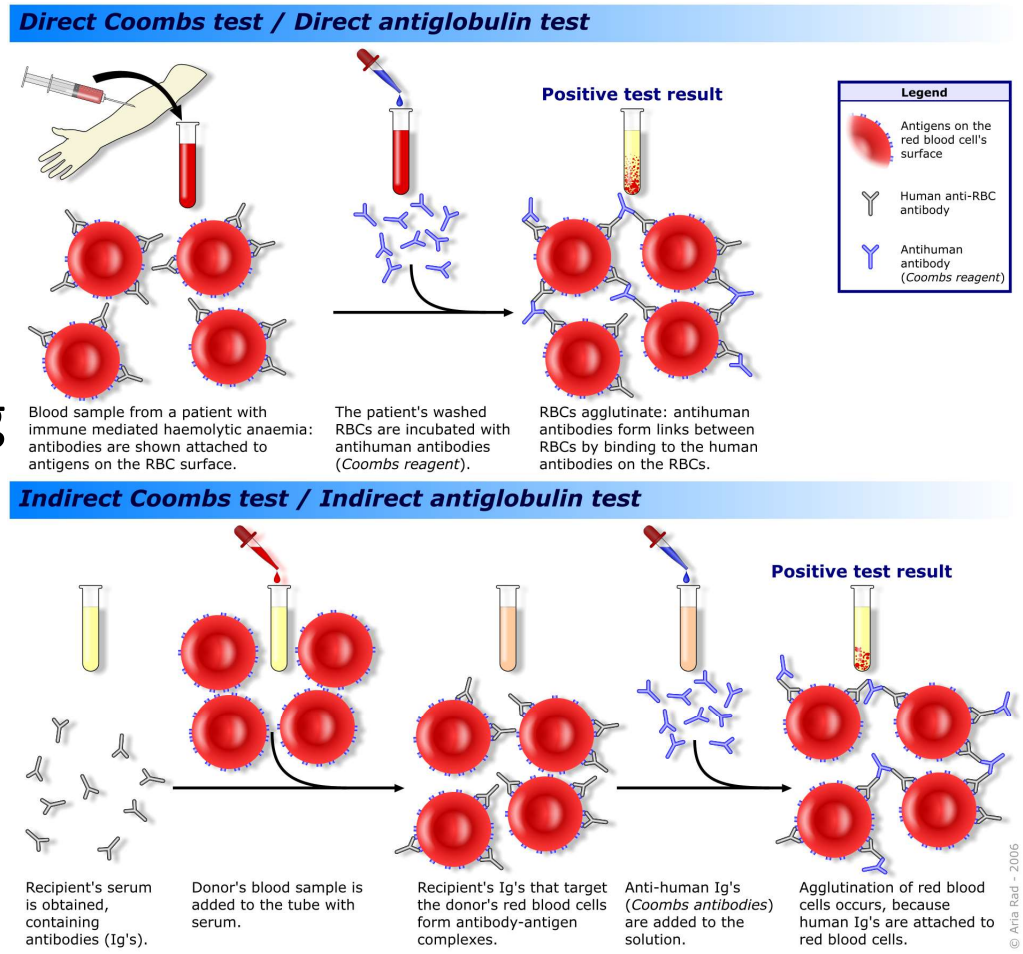
- large aglutinates remains on or near the top of gel interface
- unaglutinated ERY sink to the bottom of the gel cartridge



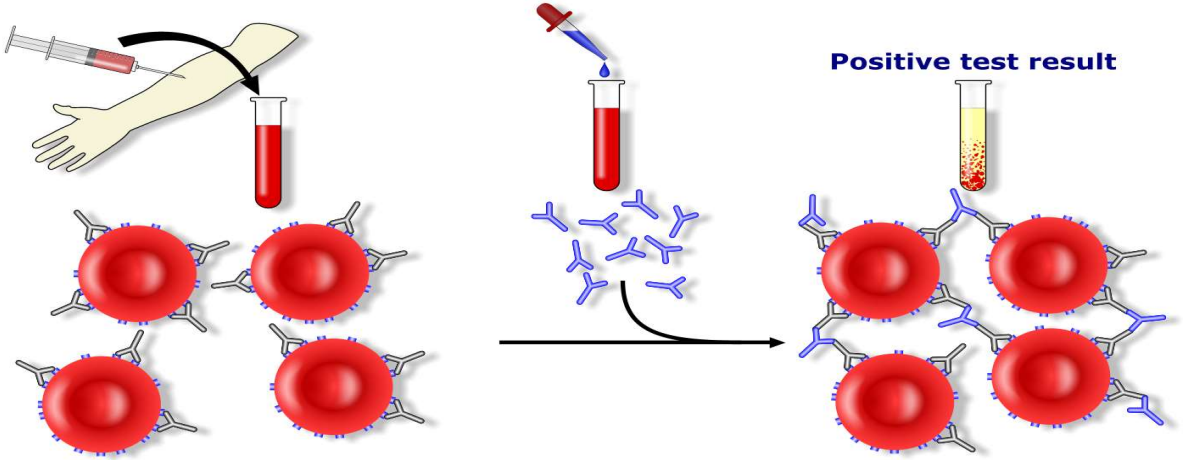
Coombs test

direct – searches for antibodies covering antigens of RBC
(detection of autoantibodies directed against RBC surface)

indirect – searches for antibodies diluted in serum



Direct Coombs test / Direct antiglobulin test

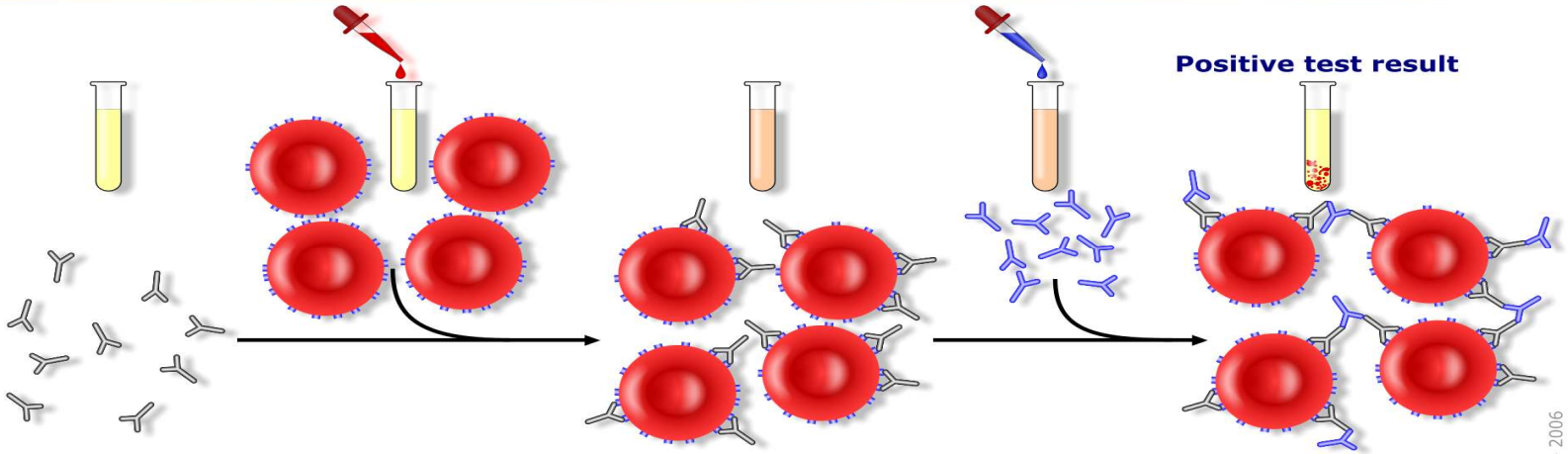


Blood sample from a patient with immune mediated haemolytic anaemia: antibodies are shown attached to antigens on the RBC surface.

The patient's washed RBCs are incubated with antihuman antibodies (*Coombs reagent*).

RBCs agglutinate: antihuman antibodies form links between RBCs by binding to the human antibodies on the RBCs.

Indirect Coombs test / Indirect antiglobulin test



Recipient's serum is obtained, containing antibodies (Ig's).

Donor's blood sample is added to the tube with serum.

Recipient's Ig's that target the donor's red blood cells form antibody-antigen complexes.

Anti-human Ig's (*Coombs antibodies*) are added to the solution.

Agglutination of red blood cells occurs, because human Ig's are attached to red blood cells.

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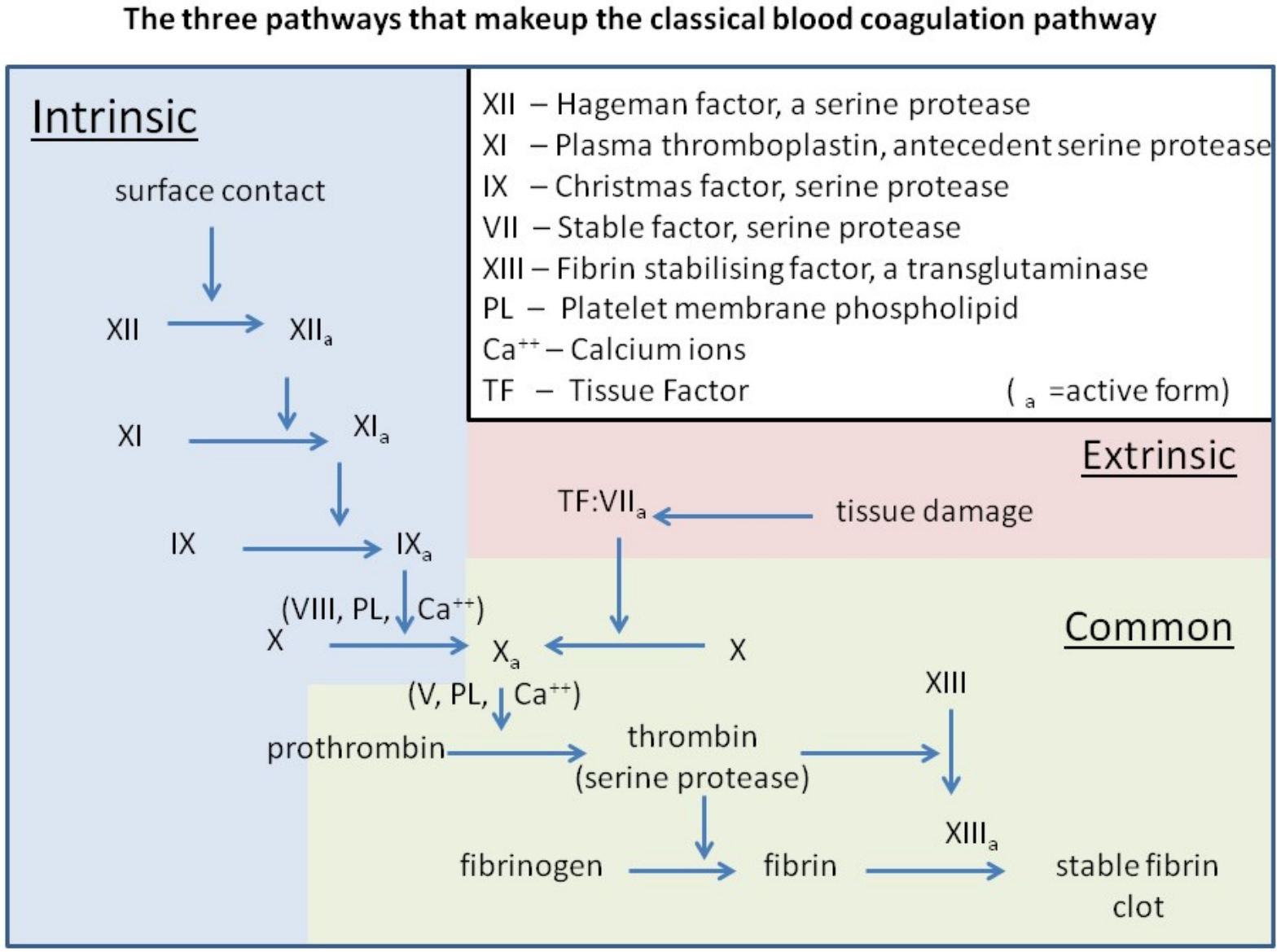
Hemostasis


The three pathways that makeup the classical blood coagulation pathway
Intrinsic
surface contact
XII
XIIa
XI
XIa
IX
IXa
(VIII, PL, Ca++)
X
Xa
(V, PL, Ca++)
prothrombin
thrombin
(serine protease)
fibrinogen
fibrin
XIIIa
stable fibrin clot
XII – Hageman factor, a serine protease
XI – Plasma thromboplastin, antecedent serine protease
IX – Christmas factor, serine protease
VII – Stable factor, serine protease
XIII – Fibrin stabilising factor, a transglutaminase
PL – Platelet membrane phospholipid
Ca++ – Calcium ions
TF – Tissue Factor
(a =active form)
Extrinsic
TF:VIIa
tissue damage
Common
XIII

Hemostasis

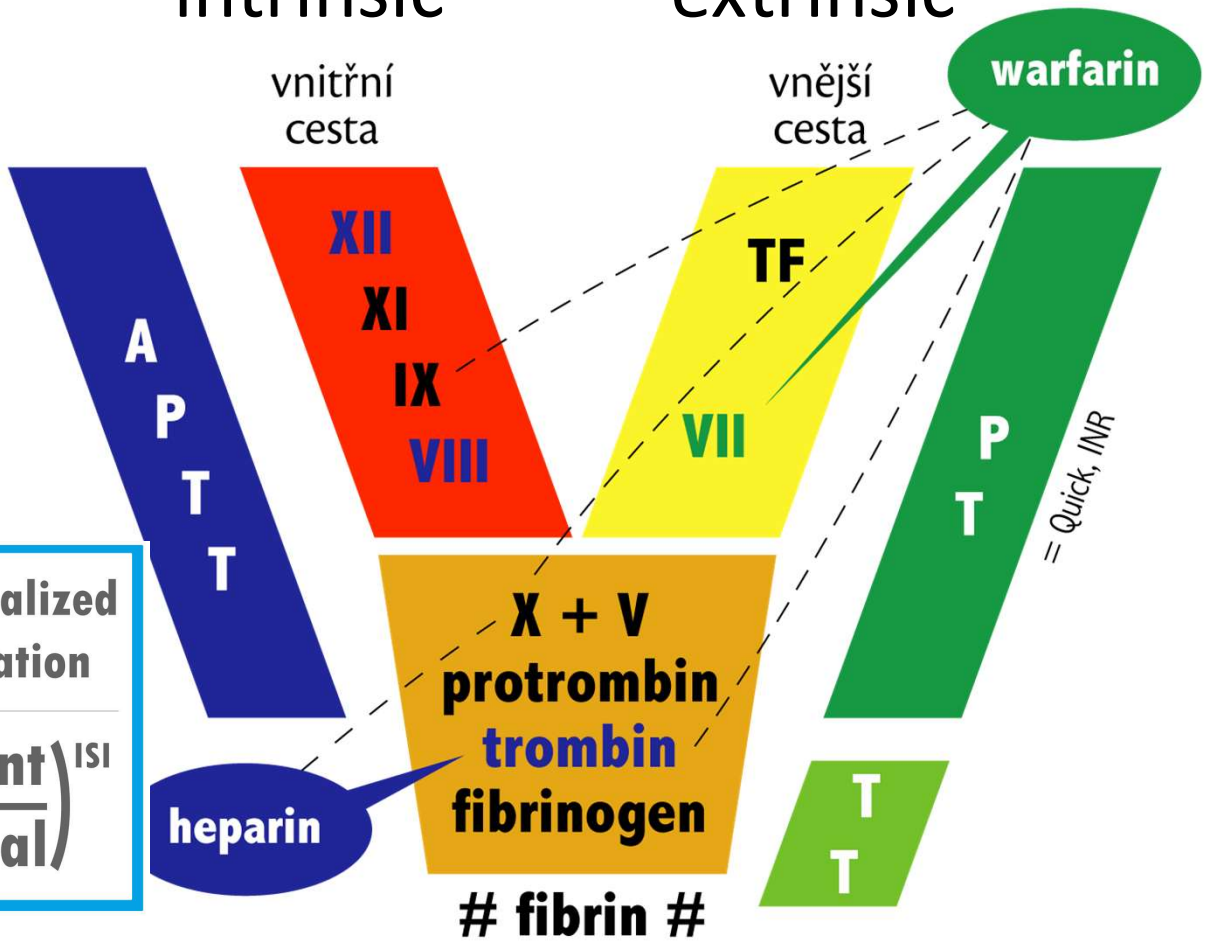
Prerequisite
– blood drawn to EDTA or citrate

Physiological values
PT
12–15 s
INR: 0,8–1,2 (80–120 %)

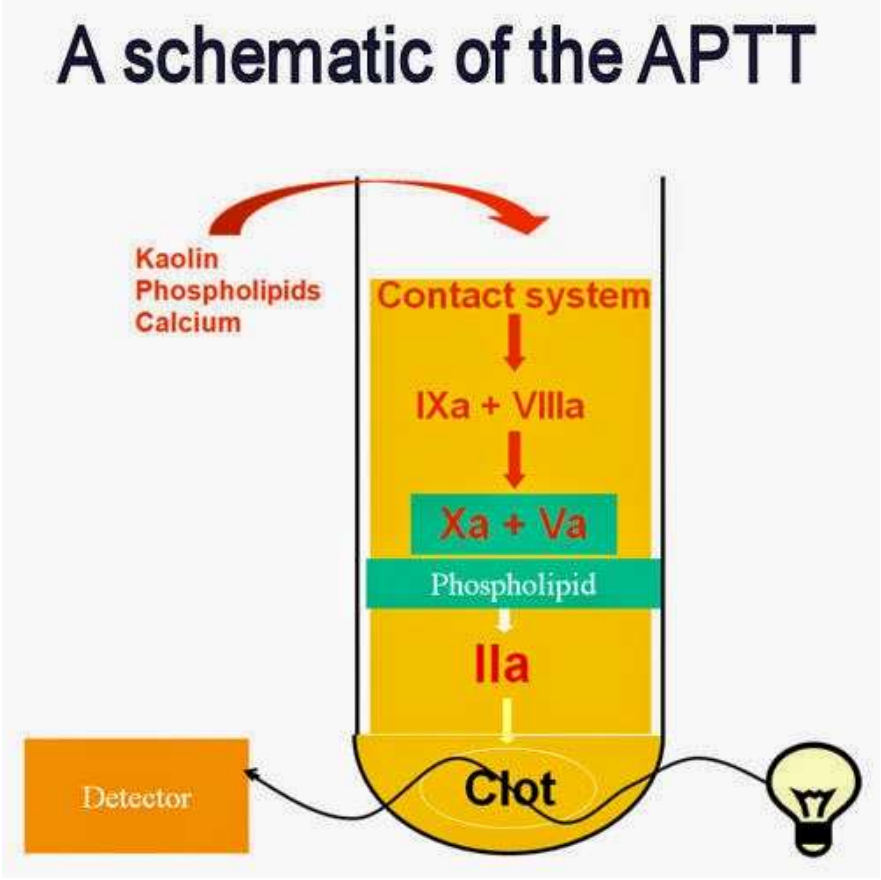
APTT
25,9–40 s

International Normalized Ratio (INR) Calculation

$$INR = \left(\frac{PT\ patient}{PT\ normal}\right)^{ISI}$$



A schematic of the APTT



Special methods in hematology

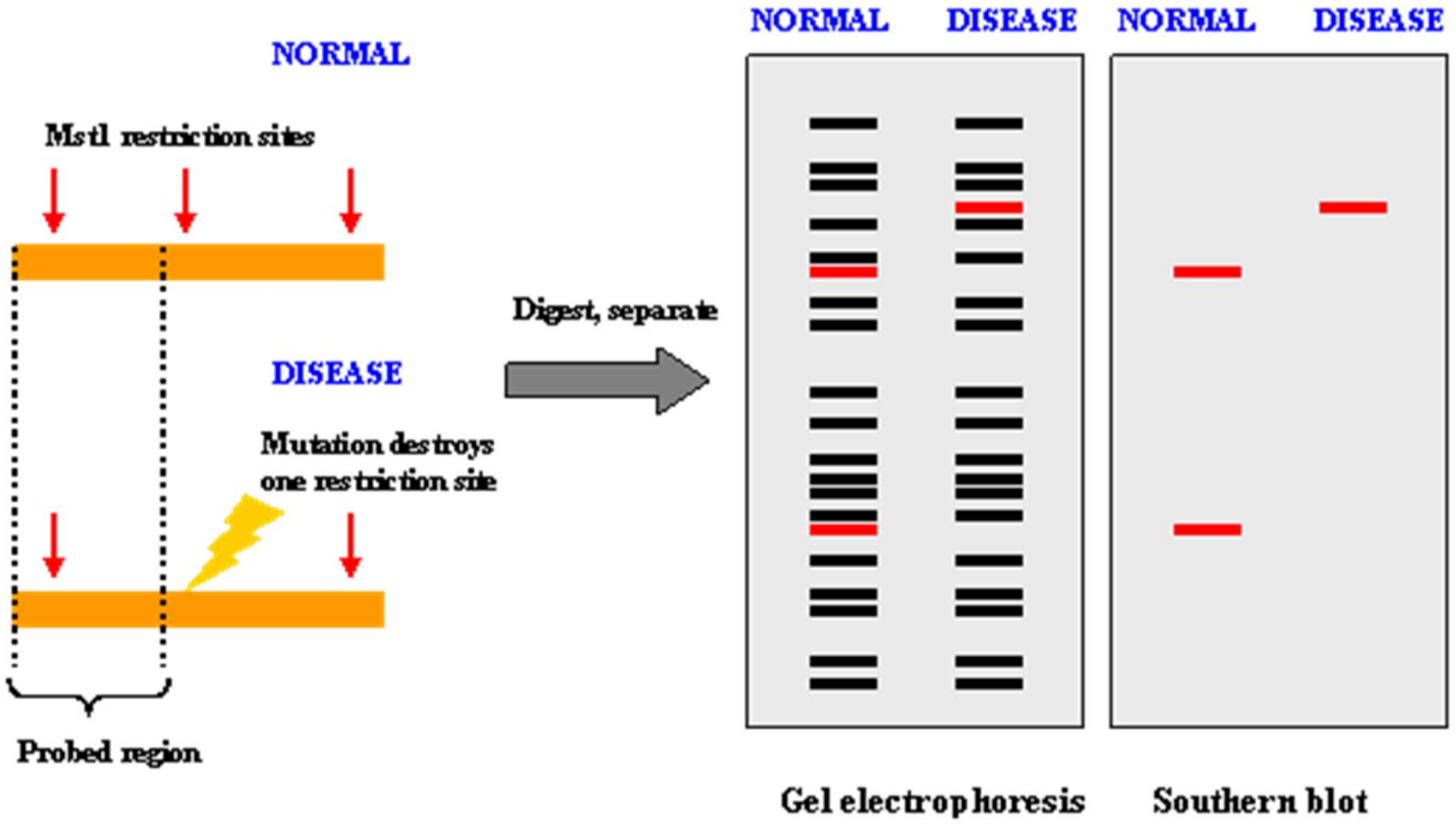
electropheretic methods

ELISA (Enzyme Linked Immunosorbent Assay)

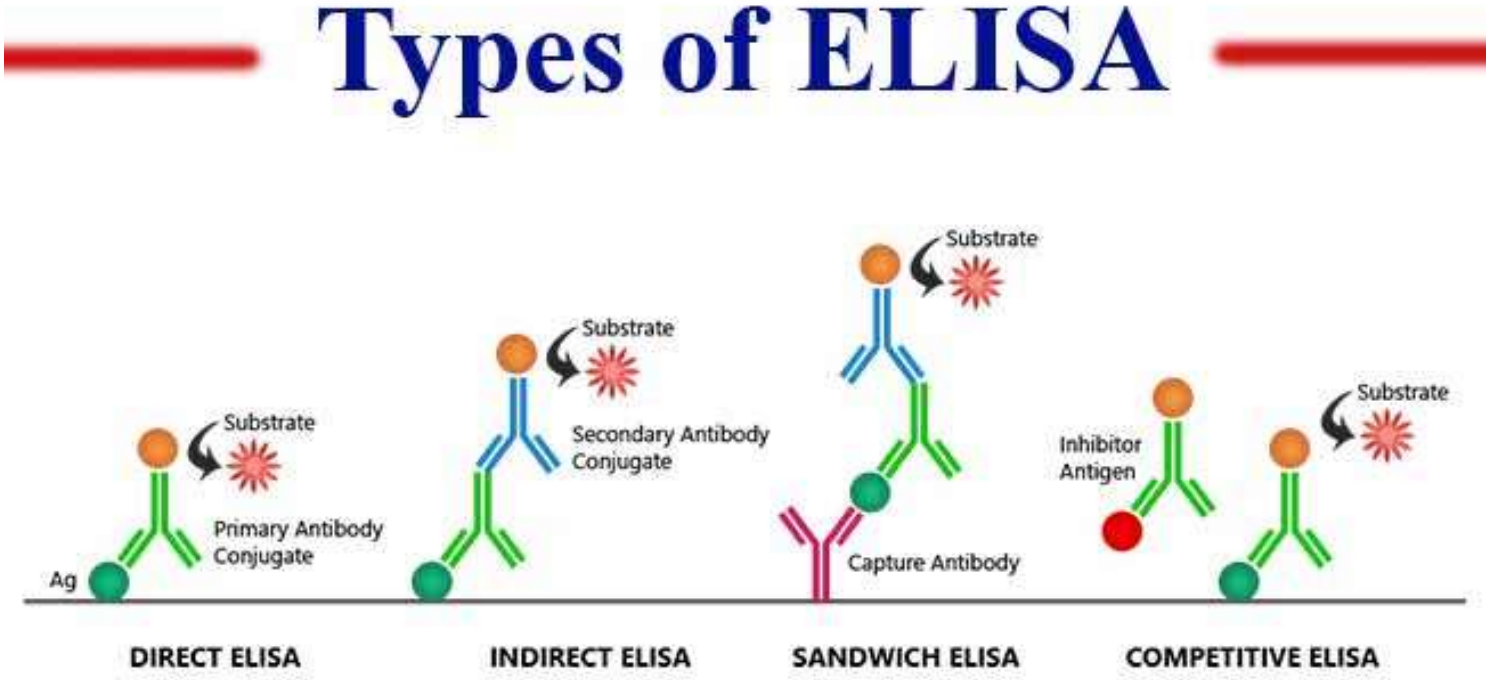
molecular genetics – RFLP, genes for thalasemias

Protein electrophoresis

RFLP
restriction fragment lenght polymorphism



ELISA
Enzyme Linked Immunosorbent Assay



Thank you for your attention