Lab 6
Lab Techniques

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1. Flow Cytometry

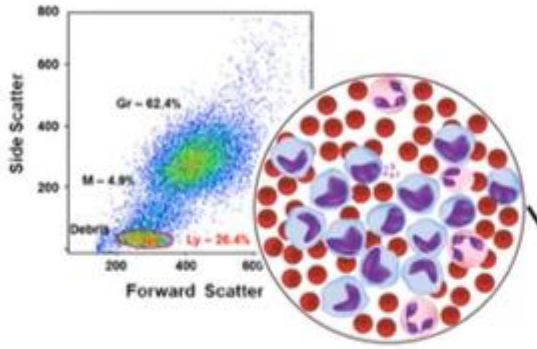
- The term '**cytometry**' is defined as a measurement of the physico-chemical properties of cells. 'Flow cytometry' is the measurement of cells and other particles in a flow.

➢ Can analyze the cell surface or intracellular expression of different molecules.

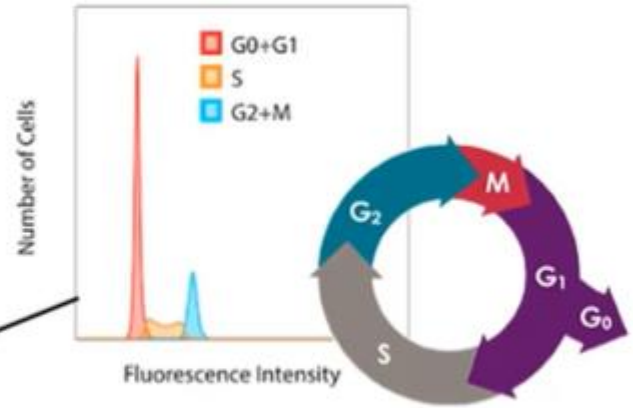
➢ The maturation stage, cytokine secretion (inside cells and bound) or cell cycle, apoptotic cell and numerating B, T, and other leukocyte cells.

- The flow cytometer is a specialized instrument that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule.

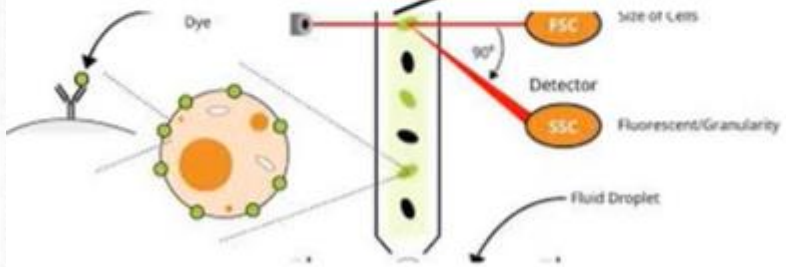
Flow cytometry



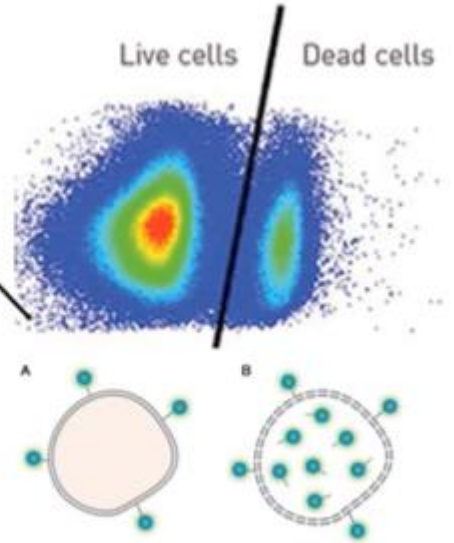
Cell sorting by size and complexity



Cell cycle analysis



Sorting cell based on fluorescence



Cell viability analysis

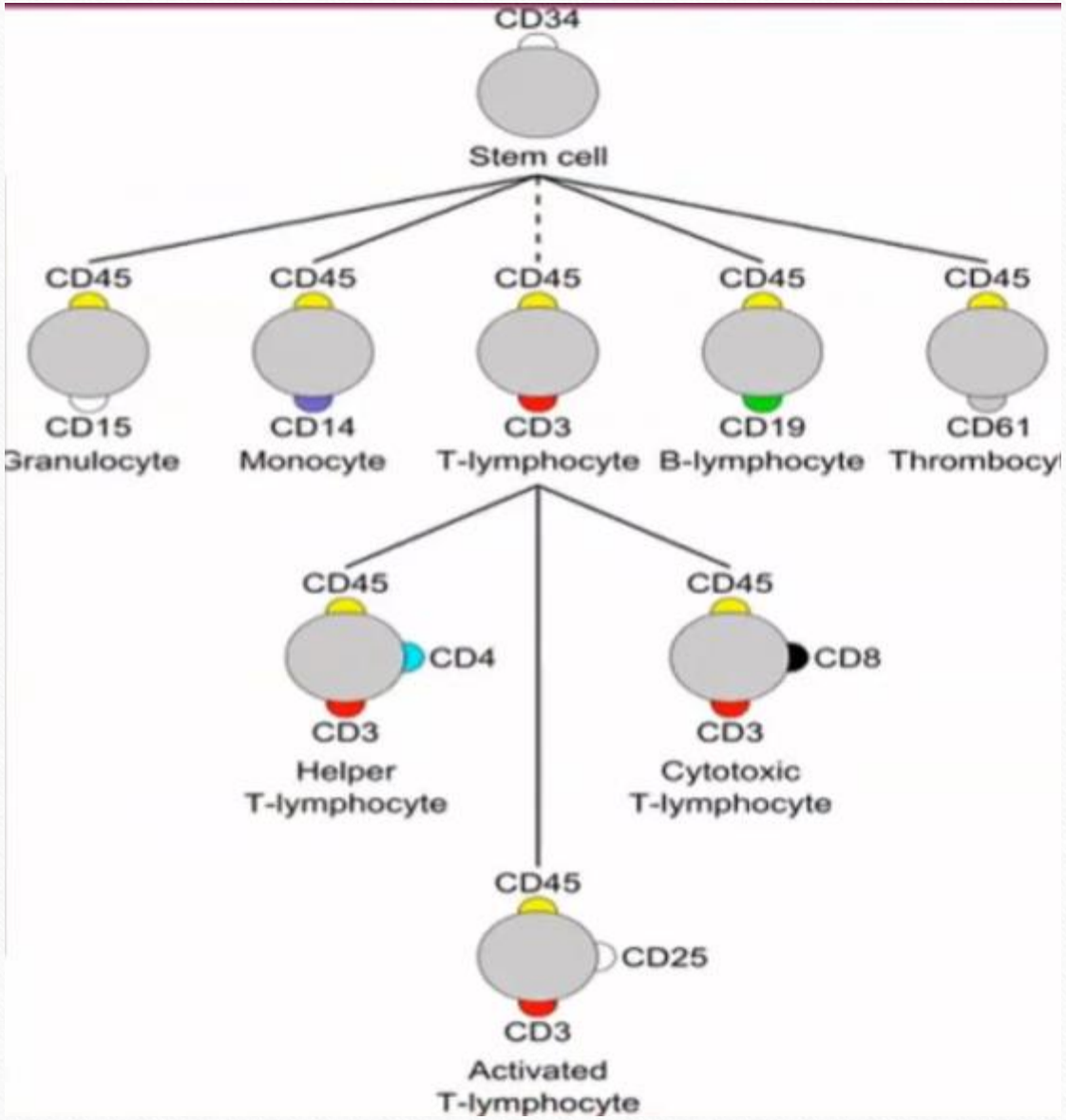
- Cytoplasmic molecules can be stained by temporarily permeabilizing cells and permitting the labeled antibodies to enter through the plasma membrane.
- lipophilic Fluorescent dyes can be used to study the proliferation of T and B cells in vivo. One commonly used dye of this type is **CarboxyFluoroscein Succinimidyl Ester** (CFSE).

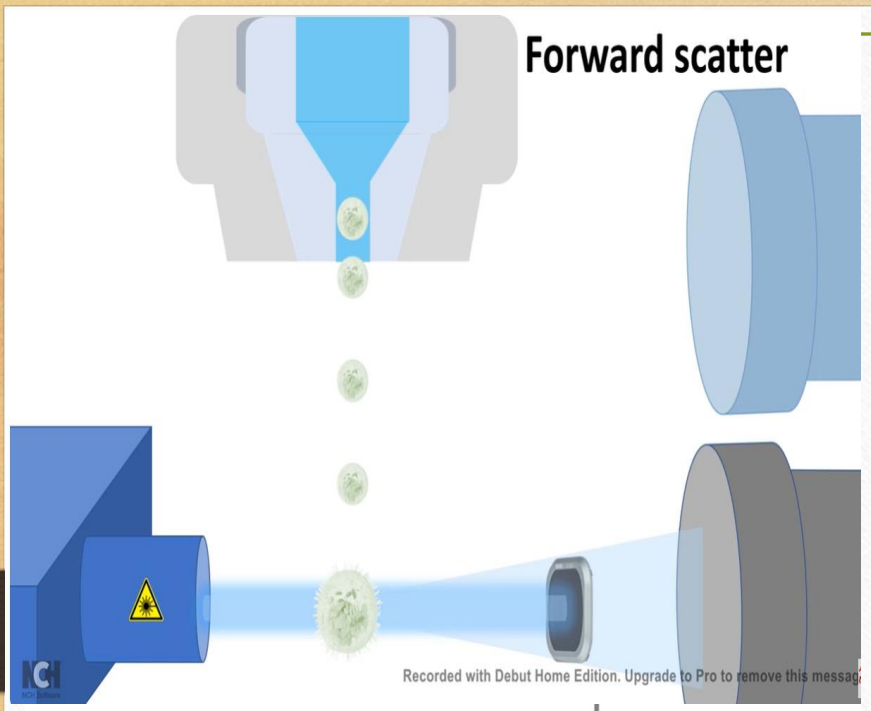
- Flow cytometers also identify and separate the cells depending on the cell **size** and **internal complexity** by forward (size)and side light-scattering (complexity) properties of cells.
- This information is often used to distinguish different cell types. For example, neutrophils and monocytes.

Myeloblast

Promyelocyte

Myelocyte





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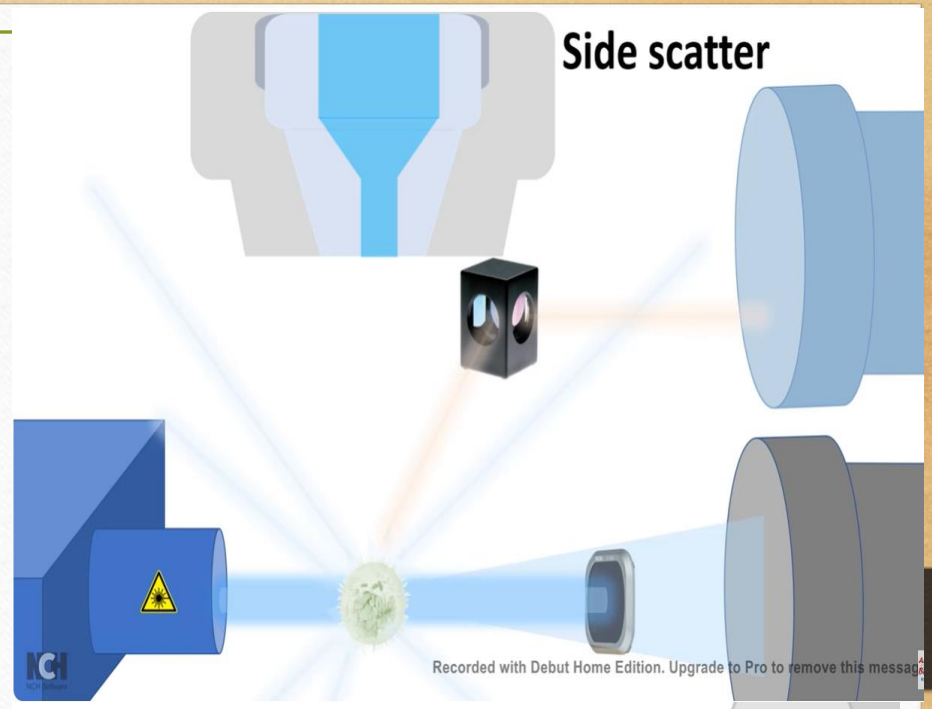
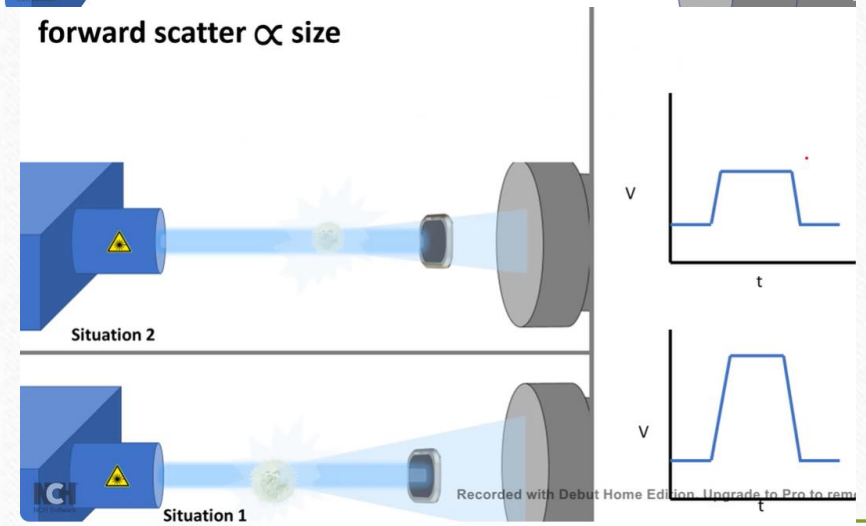
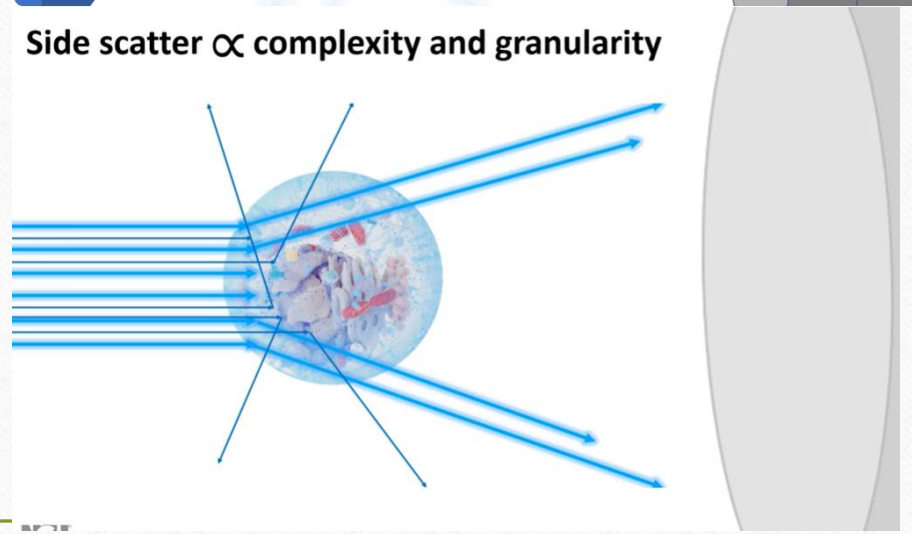
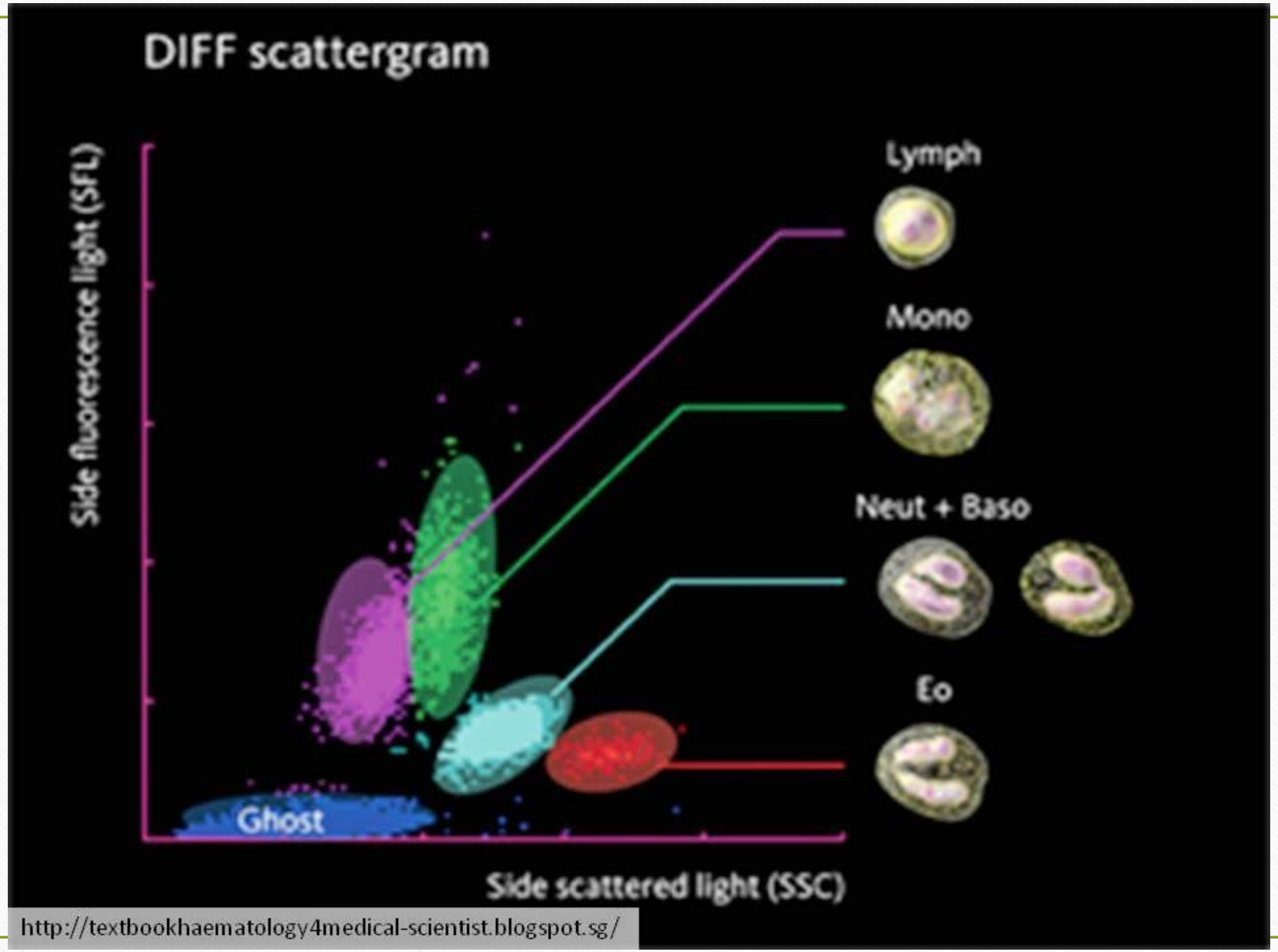








http://textbookhaematology4medical-scientist.blogspot.sg/

- Once a cell/object comes into the path of a ray of light (Laser), the light changes its orientation. This phenomenon is called light scatter and occurs under all angles between 0 and 360°.

- Detection of this scattered light provides information on the size and the quality of the object.

Properties of CMF:

✓ Accurate
✓ Fast measurements
✓ Qualitative & Quantitative measurements.
✓ Measures cells or particle with size (0.5-150 μm)

2. A. Ag-Ab binding affinity

- A method, more commonly used today, to measure the kinetics of antigen-antibody interactions depends on **Surface Plasmon Resonance**.
- In this method, an antibody passed over an antigen that is fixed over a metal film. A light source is focused on this film before passing antibody through a prism at a specific angle (resonance), and the reflected light provides a surface plasmon resonance readout. Adsorption of an antibody to the antigen alters the surface plasmon resonance readout, and this alteration makes an angular shift and as it increase means increase antibody affinity.

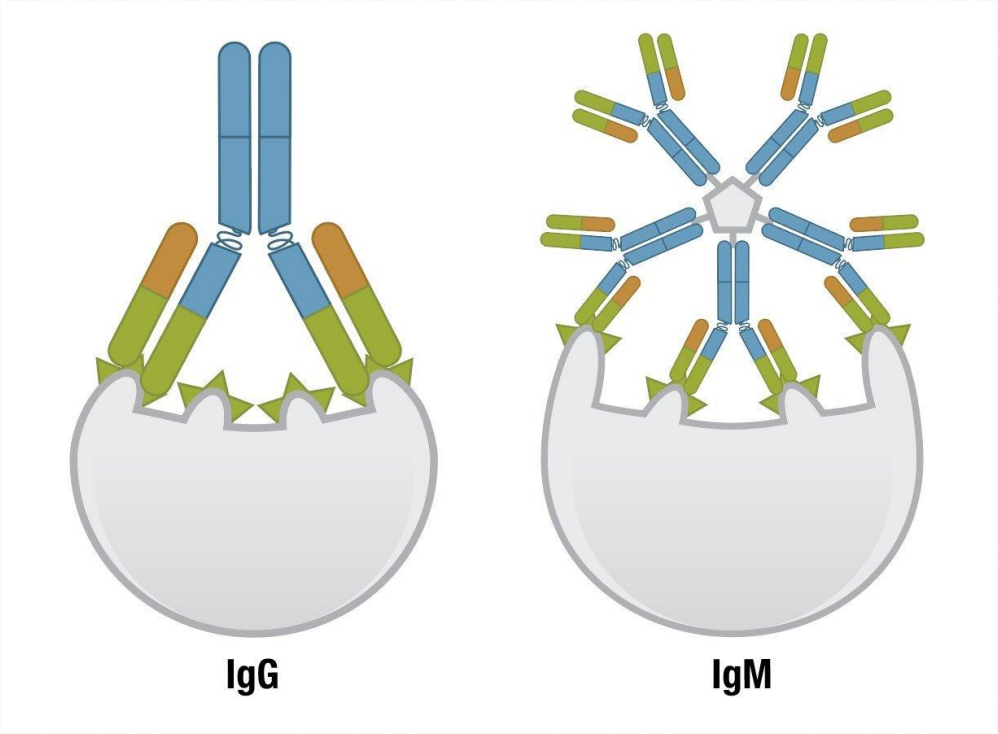
Affinity and Avidity

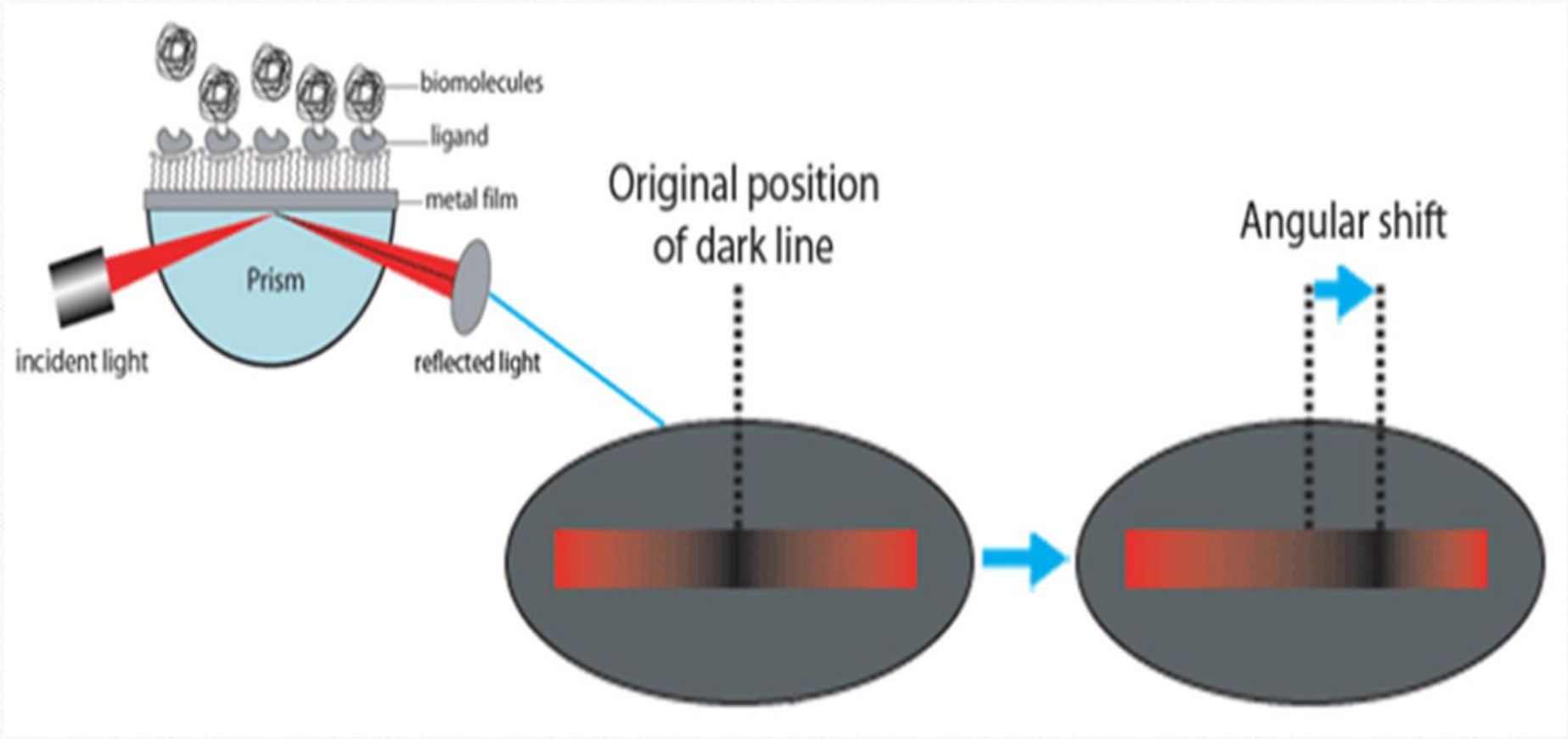
Are two terms used in immunology and microbiology to describe binding strength between an antibody and antigen.

Affinity is the interaction between a single antigen binding site at the antibody and an antigen epitope (net force), whereas **Avidity** is the (total strength) of interaction between a multimeric antigen and a multivalent antibody.

Antibodies IgG, IgE, and IgD binds the epitopes with high affinity than IgM. But IgM binds the epitopes with higher avidity.

So, IgG consider the most common type of Ab used in cell biology methods



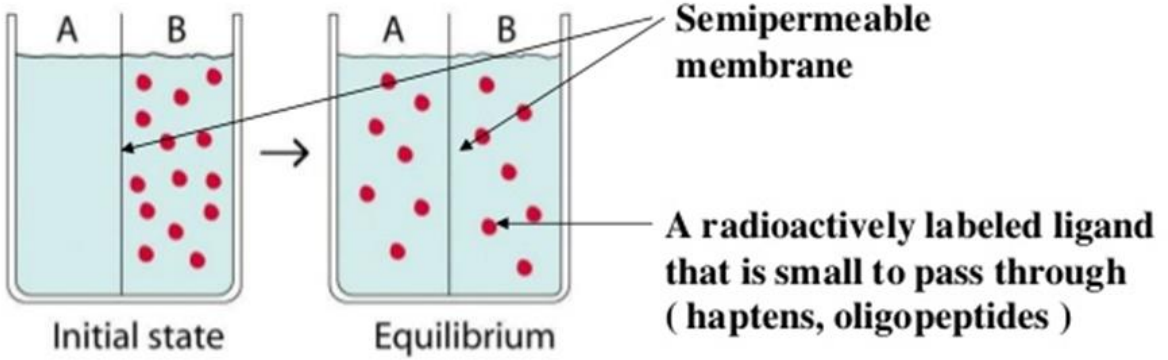

biomolecules
ligand
metal film
Prism
incident light
reflected light
Original position
of dark line
Angular shift

B. Equilibrium dialysis

- Antibody affinities for antigen can be measured directly for small antigens (e.g., haptens) by a method called **equilibrium dialysis**.
- In this method, the antigen with radioactive material in the bathing solution enters until the concentration of antigen within the two membrane-side (semi-permeable for antigen) compartments becomes exactly the same (the same radioactivity).
- A solution of antibody is confined in one compartment. when antibody is present in one compartment , the net amount of antigen inside the antibody compartment increases and the radioactivity increases by the quantity that is depending on antibody affinity.

1. Strength of Ag-Ab Interactions

Control: No antibody present
(ligand equilibrates on both sides equally)



Experimental: Antibody in A
(at equilibrium more ligand in A due to Ab binding)

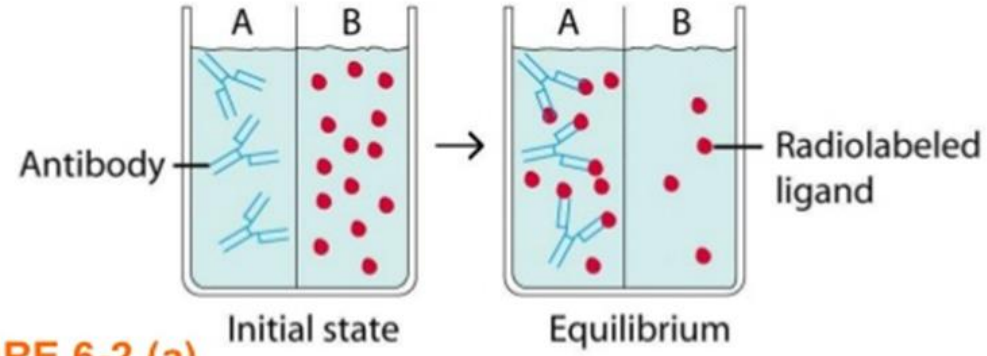


FIGURE 6-2 (a)

Determination of Ab affinity (Ka) by equilibrium dialysis.

Immunohematology

- *The term **immunohematology** refers to the serologic, genetic, biochemical, and molecular study of antigens associated with membrane structures on the blood cells, as well as the immunologic properties and reactions of blood components and constituents.*

- Transfusion medicine is a multidisciplinary specialty encompassing all aspects of:

✓blood donation.

✓blood component preparation.

✓blood cell serology.

✓blood transfusion therapy.

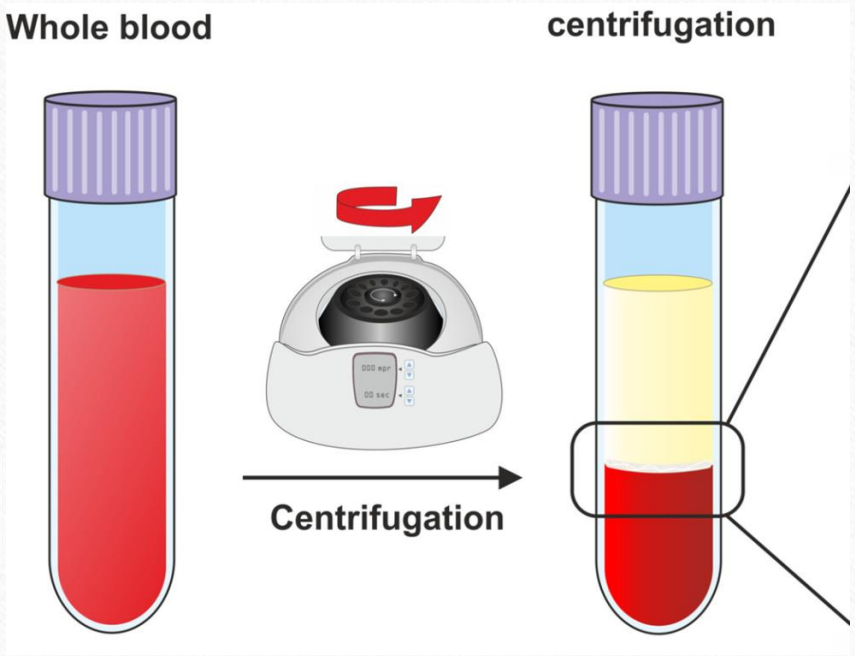
- *Operationally, transfusion medicine is divided between blood centers and transfusion services.*

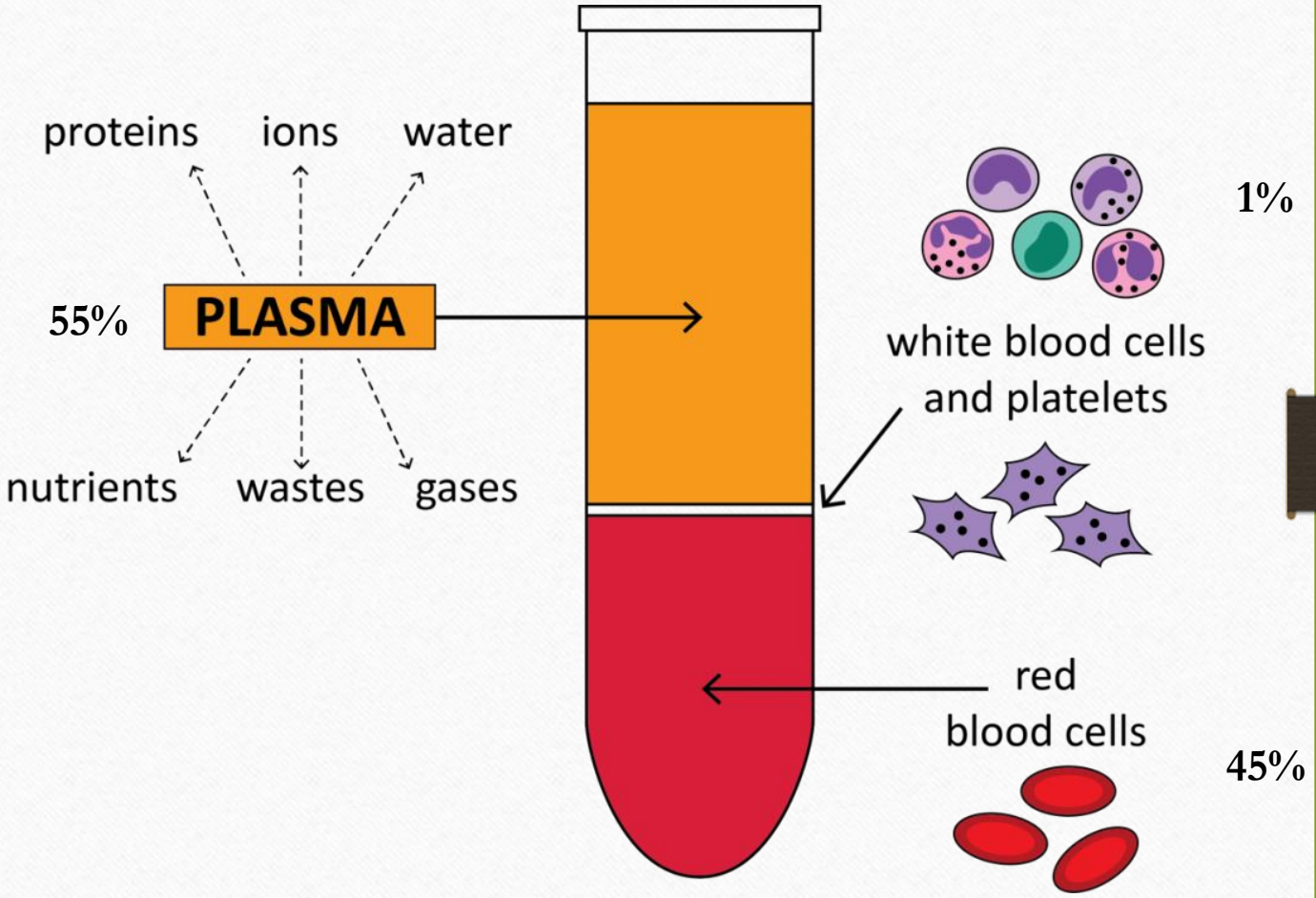
- *Blood centers* recruit and collect blood from donors and manufacture and distribute blood components.
- *Transfusion services* perform pretransfusion compatibility testing (blood grouping and cross match), select and issue blood components for patients, and provide medical support for blood transfusion.

Blood bank

- Units of WB and packed RBC are both kept refrigerated at 33.8 to 42.8 °F (1.0 to 6.0 °C), with maximum permitted storage periods of 35 and 42 days respectively.
- When keeping blood tube in standing position, a layer between the red cells and the plasma is formed and referred to as the **buffy coat** and is sometimes removed to make platelets and WBC for transfusion.
- Platelets have a shelf life of 5 to 7 days. Platelets are stored at room temperature (72 °F or 22 °C) and must be rocked/agitated

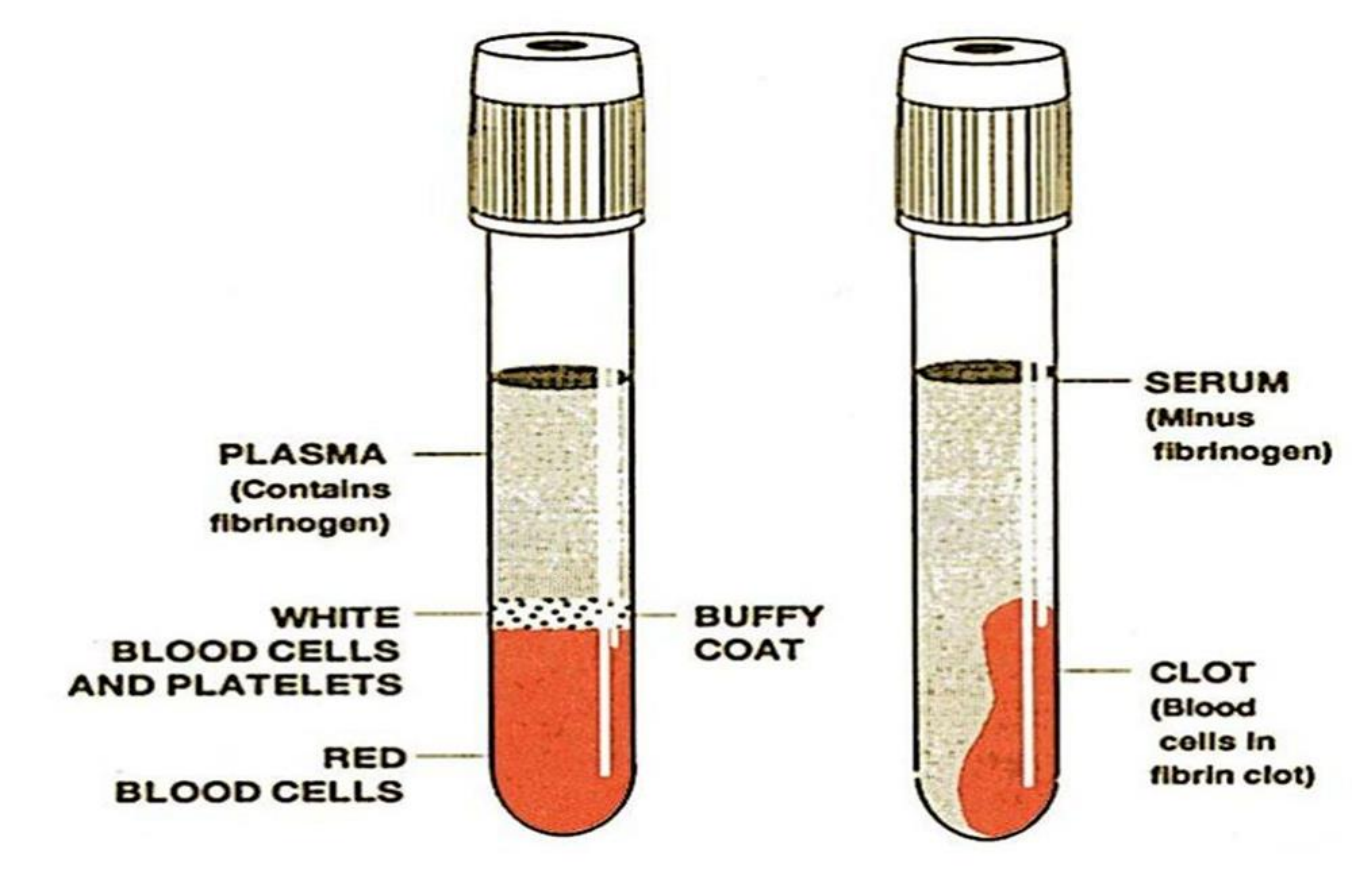
- If the plasma is frozen promptly and is intended for transfusion, it is typically labeled as fresh frozen plasma. If it is intended to be made into other products, it is typically labeled as recovered plasma or plasma for fractionation.


Whole blood
centrifugation
Centrifugation


proteins
ions
water
55%
PLASMA
nutrients
wastes
gases
white blood cells
and platelets
1%
red
blood cells
45%

Plasma VS Serum

EDTA tube (purple) Plain tube (Red)

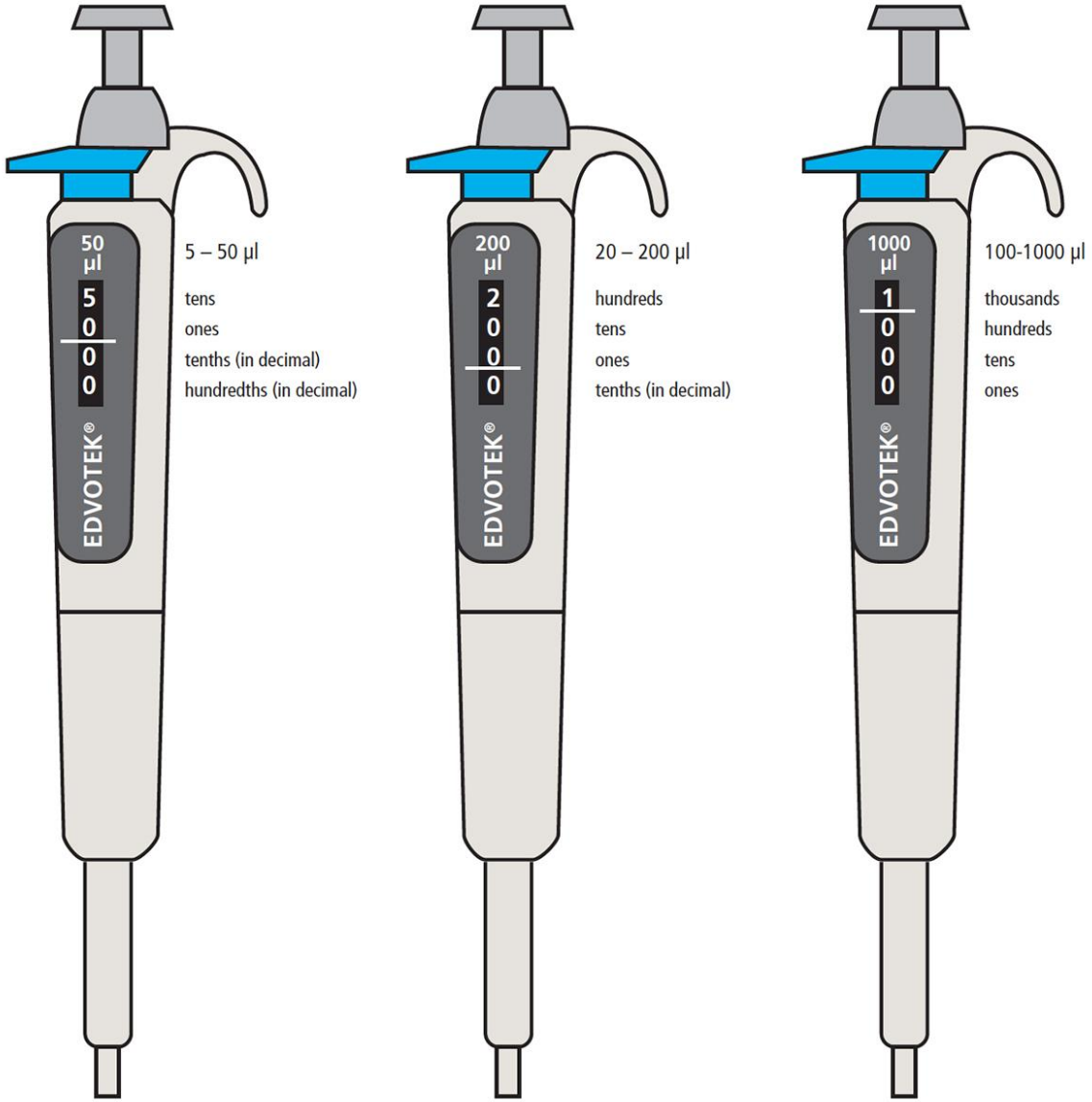


Purple tube for CBC

Red tube for immunology

Blood test tubes or Vacutainers

Order of Draw	Tube Stopper Color	Additive	Dept.	Tests	Liquid Part post - centrifugation
1	Yellow 	Sodium polyethanol sulfonate (SPS)	Microbiology	Blood Culture	Plasma
2	Light Blue 	Sodium Citrate	Coagulation	PT, PTT	Plasma
3	Red (plain) 	No additive	Tube Blood Bank	Type, RH, antibody screen, type & crossmatch	Serum
4	Red & Grey or Gold 	Clot Activator	Routine Chemistry	All STAT tests + Iron, folate	Serum
5	Green 	Heparin	STAT Chemistry	BMP, CMP, Glucose, K, Troponin, Bilirubin	Plasma
6	Lavender 	K2EDTA	Hematology	CBC, ESR	Plasma
7	Pink 	EDTA	Gel Blood Bank	Type, RH, antibody screen, type & crossmatch	Plasma
8	Gray 	Sodium Flouride (inhibits glycolysis)	Chemistry	Lactic Acid, Gluc (not run right away)	Plasma


50 µl
5 0 0 0
EDVOTEK®
5 – 50 µl
tens
ones
tenths (in decimal)
hundredths (in decimal)
200 µl
2 0 0 0
EDVOTEK®
20 – 200 µl
hundreds
tens
ones
tenths (in decimal)
1000 µl
1 0 0 0
EDVOTEK®
100-1000 µl
thousands
hundreds
tens
ones


MICROLIT RBO
1 0
1 - 10 µl
MICROLIT RBO
1 0 0
10 - 100 µl
MICROLIT RBO
1 0 0 0
100 - 1000 µl