Immunology Lab Techniques

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Immunology,

- **Serology** is the scientific study of serum and other fluids. In practice, the term usually refers to the diagnostic identification of antibodies in the serum.
  - Infection
  - Blood typing
  - Autoimmune diseases
  - Immune deficiency as X- linked agammaglobulineamia

- An **immunoassay** is a method of target (detection/quantitation) antigen (or antibody) capture in samples using a specific antibody (or antigen),
- 1.Precipitation; Soluble antigen + specific antibody = Insoluble Precipitate of Ag-Ab complex (IGG)
- 2. Agglutination; if the antigen is insoluble or cell/ tissue bound and Ab is soluble (IGM, IGA)
- 3.Complement fixation (CFT); if the antibody in patient serum unites specific antigen, the added complements will be fixed, the indicator sheep RBC will not be lysed(positive)
- 4. radio-immunoassays (color)

- 5. ELISA; enzyme-linked immunosorbent assay is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones using an antibody linked to enzyme react with a substrate give color.
- 6. Western blot . In this technique a mixture of proteins is separated based on molecular weight through electricity (gel electrophoresis). These results are then transferred to a membrane producing a band for each protein, the specific protein is identified by binding specific radiolabeled or enzyme linked antibody.

- Visualizing an antibody-antigen interaction can be accomplished in a number of ways.
  - an antibody is conjugated to an enzyme, such as peroxidase, that work on a substrate and produce color(ELISA and western staining).
  - Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein (immunofluorescence).

    -Immunofluorescence

    -Flowcytometer
  - Indicator RBC lysis in CFT
  - Clotting in bottom in agglutination
  - Line of precipitation

Immuno-cyto/histo-chemistry :

- Purpose: to detect the presence and localization of antigens in tissues (histo-) or in cells (cyto-) grown in culture using radiolabeled antibody and Immunofluorescent microscope as in identification of anti-nuclear antibody and autoantibodies in autoimmune diseases
- Widely used in cancers
- Visualizing an antibody-antigen interaction can be accomplished in a number of ways, mainly either of the following:
  - Chromogenic immunohistochemistry (CIH), wherein an antibody is conjugated to an enzyme, such as peroxidase (the combination being termed immunoperoxidase), that can catalyse a colour-producing reaction.
  - Immunofluorescence, where the antibody is tagged to a fluorophore, such as fluorescein.

Precipitation reaction

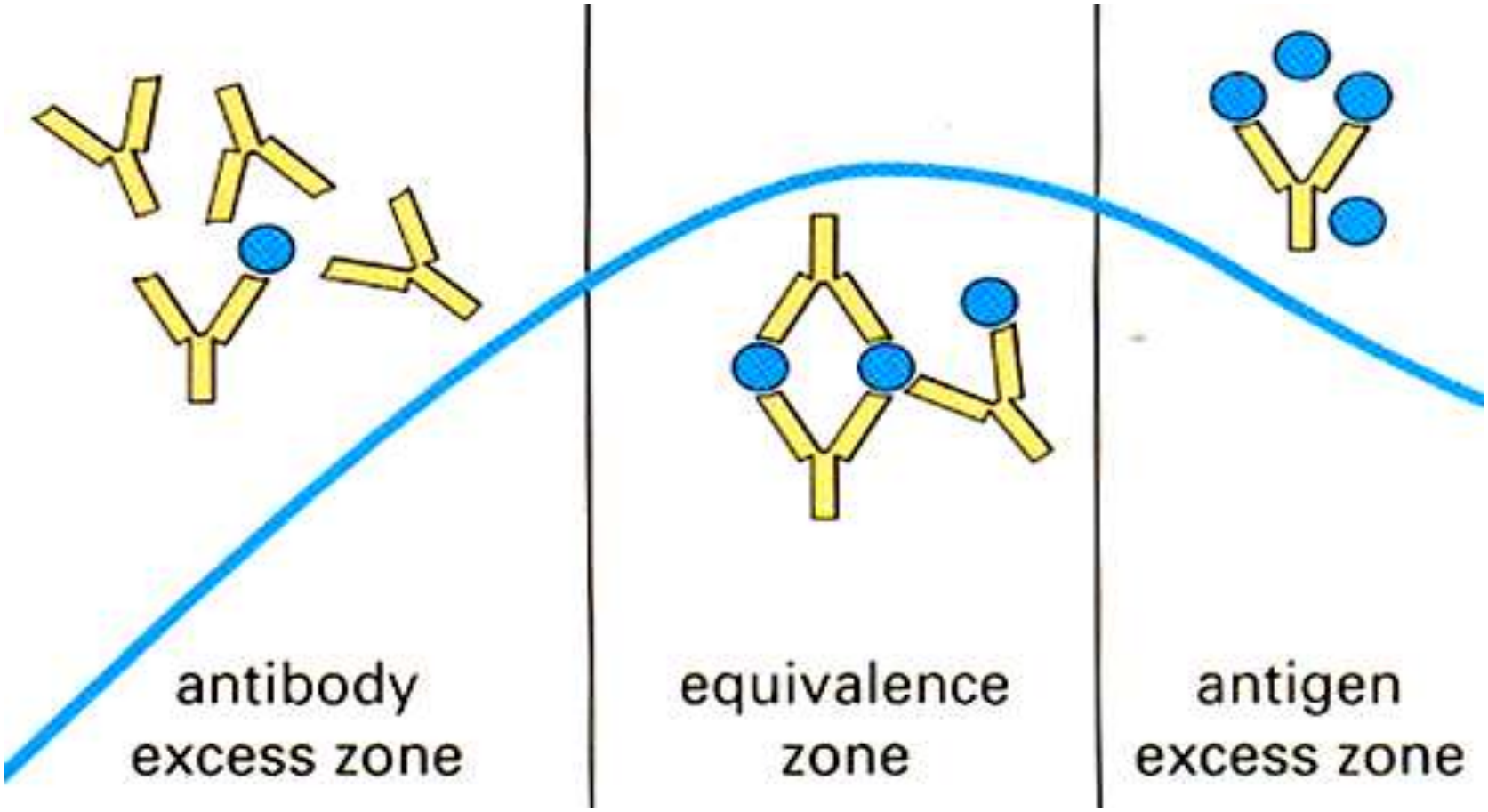
- Amount of precipitate Influenced by - Relative proportions of Ag & Ab
- Maximum precipitation occurs when

  Ags & Abs at optimal or equivalent proportions
- Precipitation occur when a lattice (line or lattice) formed

Precipitation Reactions

- ***prozone phenomenon:***antibody excess, no lattice network is formed.
- ***postzone phenomenon:*** antigen excess. no lattice network is formed.
- ***for precipitation reactions to be detectable, they must be run in the zone of equivalence.***

Lattice formation or precipitation curve



1-Single radial diffusion On a slide

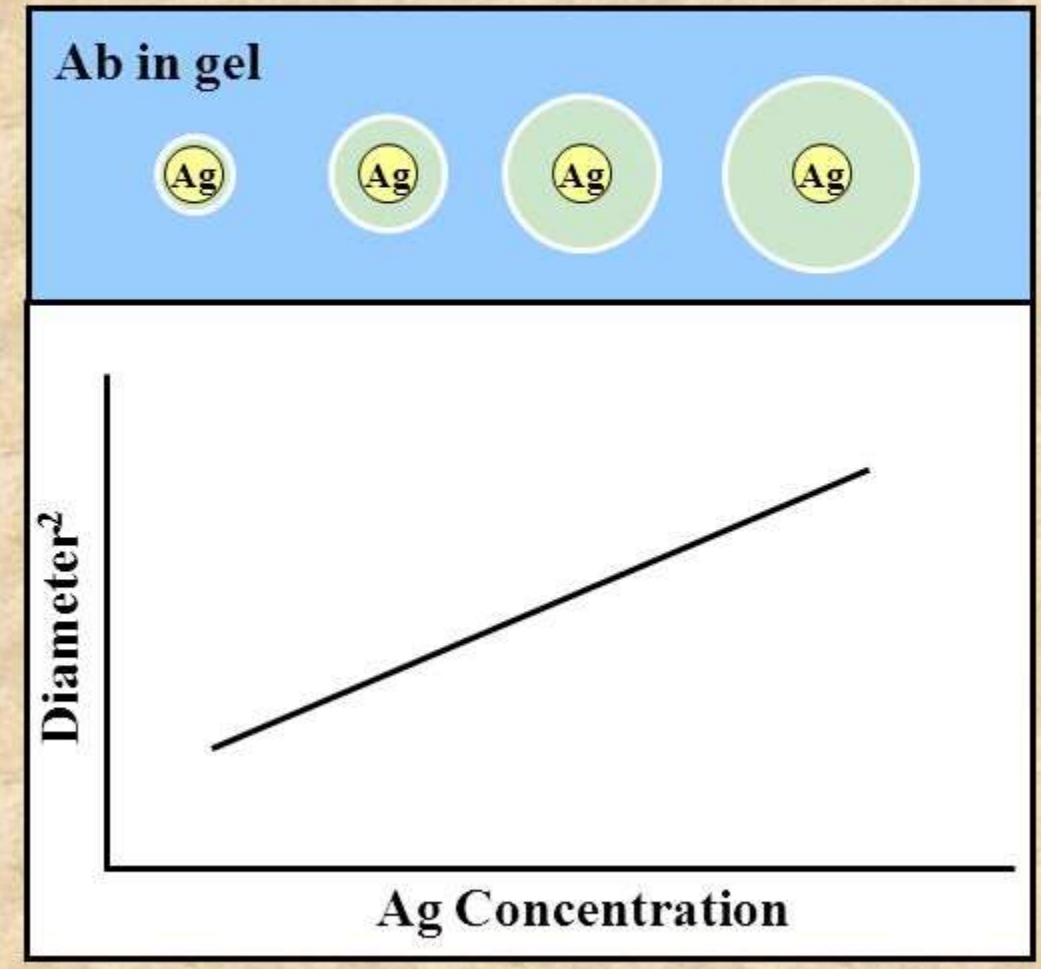
- Single diffusion in two dimensions (Radial immunodiffusion))
  - Ab incorporated in agar gel
  - Ag. added to wells in agar.
  - Ag. diffuses radially from the well
  - Forms precipitation ring around antigen
  - Diameter of halo – estimate of conc. of Ag.
  - Used for estimation of

    Igs levels in serum,

    screening Abs against viruses (Influenza) or bacteria

Radial Immunodiffusion (Mancini)

- Method
  - Ab in gel
  - Ag in a well
- Interpretation
  - Diameter of ring is proportional to the concentration
- Quantitative
  - Ig levels



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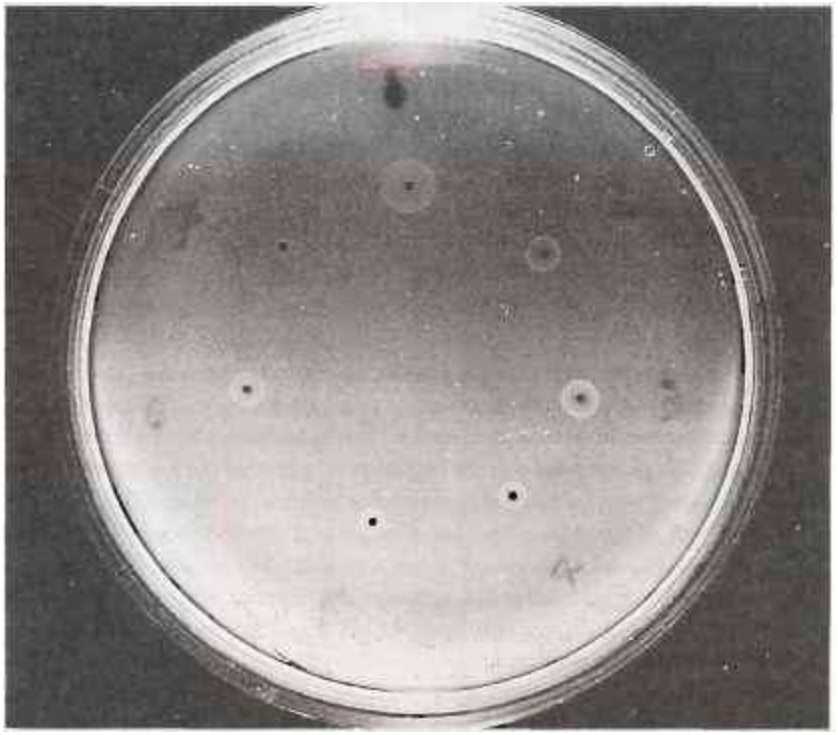


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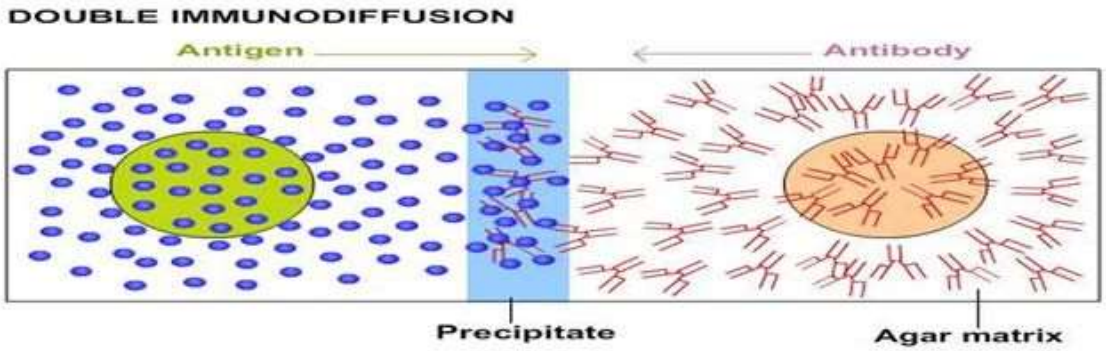
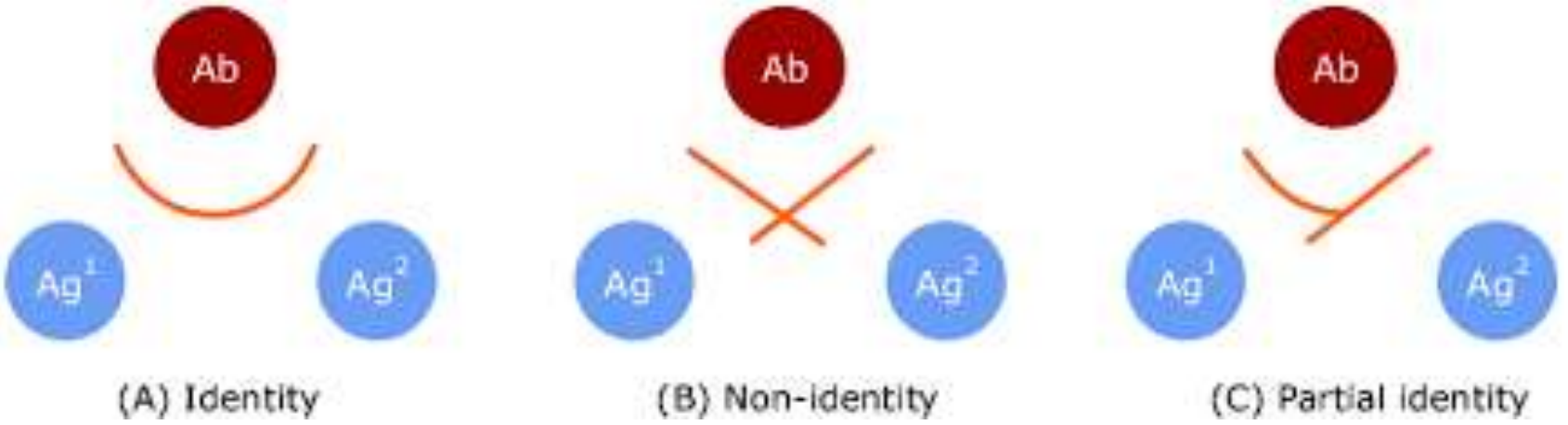
2-

Double Immunodiffusion

- Diffusion of antibody and antigen towards each other in an Agarose gel.
- A line of precipitate will form if the antibody binds to antigen.
- Used to determine if an antigen or antibody is present



Ouchterlony results for the detection of antigens and antibodies and determination of homologies between antigen



Types of Agglutination

- Direct Agglutination
- Indirect or Passive Agglutination
- Reverse Passive Agglutination
- Agglutination Inhibition
- Coagglutination

II.Passive (indirect) agglutination

What is a difference between passive and active agglutination?

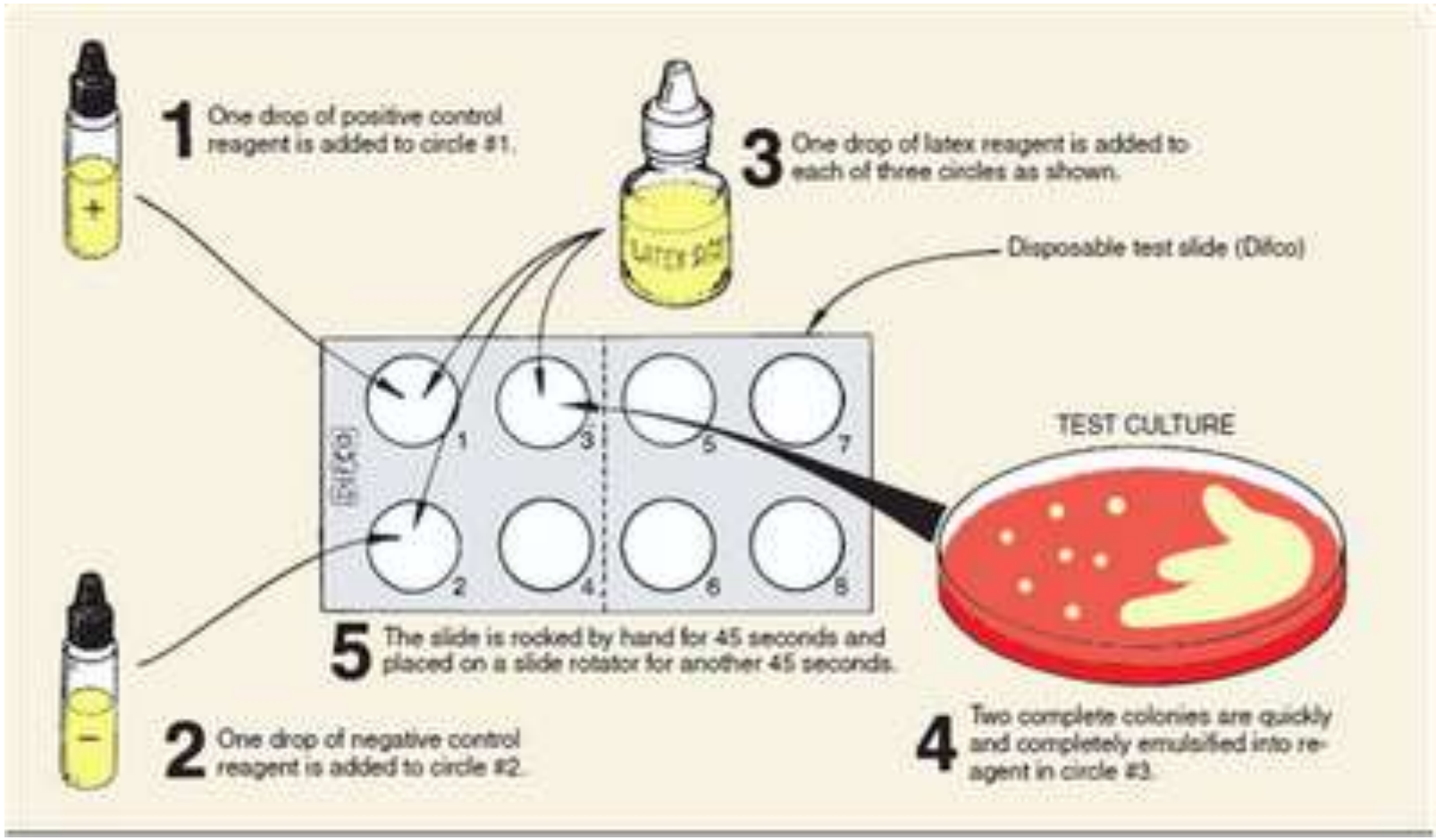
- In active agglutination you have a particulate Ag + Ab, since the Ag is particulate, large, when a complex is formed it is visible.
- In passive agglutination the Ag is soluble so it must first be attached to something like latex beads, red blood cells so when agglutination occurs it can be seen with the naked eye.

Application on direct

1. If antigen on red blood cells, then it is called hemagglutination.

- Uses of heamagglutination are Blood grouping & Cross matching,
- Antisera of the IgM type can be used to in blood grouping
- Smooth suspension of blood on 3 slides + drop of antibody (anti-a, anti B and anti RH on each slide. Clumping of blood means it has that antibody specific antigen

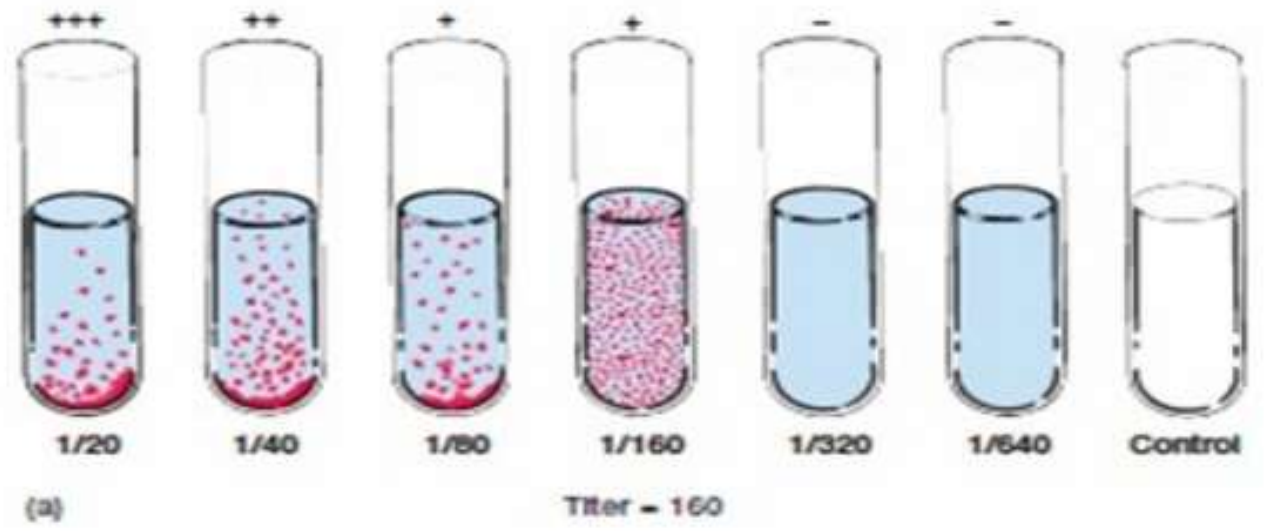
2. Used in identification and typing of micro-organisms as pneumococci


1 One drop of positive control reagent is added to circle #1.
3 One drop of latex reagent is added to each of three circles as shown.
Disposable test slide (Difco)
TEST CULTURE
5 The slide is rocked by hand for 45 seconds and placed on a slide rotator for another 45 seconds.
2 One drop of negative control reagent is added to circle #2.
4 Two complete colonies are quickly and completely emulsified into reagent in circle #3.

- **2-direct agglutination test (on tube) (Titer) (quantitative)**
  In this test, serial dilutions are made of a antibody sample (patient serum) and then a fixed number of particulate antigen is added. Then the last dilution that gives agglutination is determined and called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.

2) Tube agglutination test :

- Standard quantitative method for determination of antibodies.
- Serum diluted serially by doubling dilution in test tubes.
- Equal vol of particulate antigen is added to all tubes.
- Highest dilution of serum at which agglutination occurs is antibody titre



Agglutination

- Widal test: a rapid screening test to help determine the possibility of typhoid fever. The antigens used in this procedure include Salmonella O (somatic) and H (flagellar) antigens.
- Brucella agglutination test

Titer

- Or level of antibody in serum is expressed as the highest dilution of antibodies that gives a positive reaction with antigen. It can be diagnostic or prognostic

3- Coombs test

- The key component of agglutination in coombs test is antibody to human globulin that is made in animals or by means of hybridoma techniques (Coombs reagent).
- The ***direct antiglobulin test (coombs test)*** is used to demonstrate in vivo attachment of antibody to an individual's red blood cells.
- Patient RBC sample is mixed with coombs reagent, if agglutination occurs this mean that the RBCs have antibody on their surfaces (sensitized)
- This test serves as an indicator of :

✓ autoimmune hemolytic anemia

✓ hemolytic disease of the newborn

✓ sensitization of red blood cells caused by the presence of drugs, or a transfusion reaction.

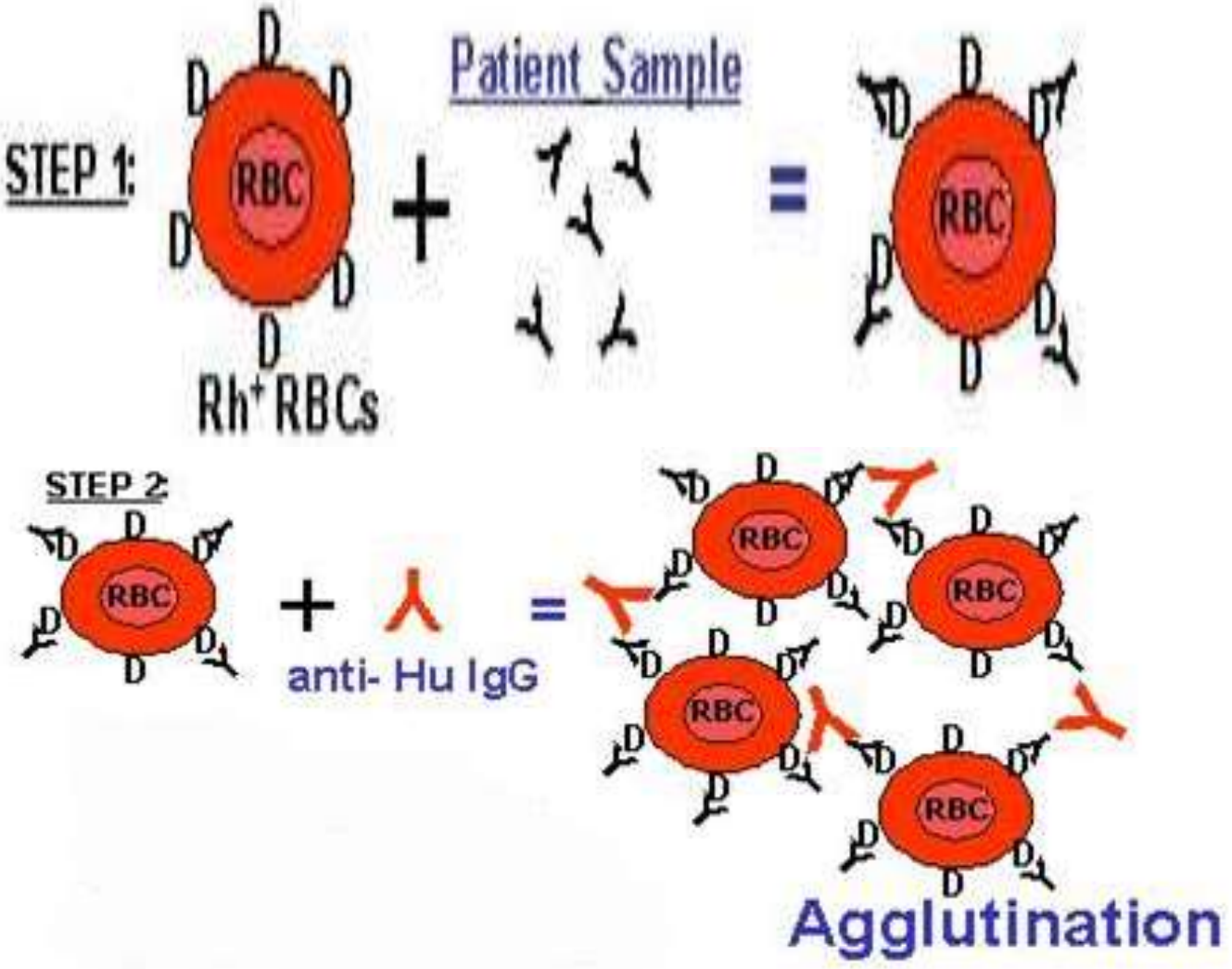
5-Indirect coombs test

- The ***indirect antiglobulin test (indirect coombs test)*** is used to determine the presence of a particular antibody in a patient.
- This is a two-step process.
- Patient serum is mixed with RBC have the RH antigen, and the cells are then carefully washed again to remove any unbound antibody.
- coombs reagent is added, a visible reaction occurs if the patient is positive for the antibody
- is used In antenatal care, to screen pregnant women for antibodies that may cause hemolytic disease of the newborn , Pregnant serum + RH+ RBC
- and to screen for antibodies in the preparation of blood for blood transfusion (Cross match). Recipient serum + donor RBC

cross match

- To perform a cross match, a small amount of the recipient's serum is mixed with a small amount of the donor RBCs. The mixture is then examined coombs reagent. If the proposed transfusion is incompatible, the donor RBCs are agglutinated by antibodies in the recipient's serum.

In Preparation for blood transfusion; recipient serum mixed with donor's blood



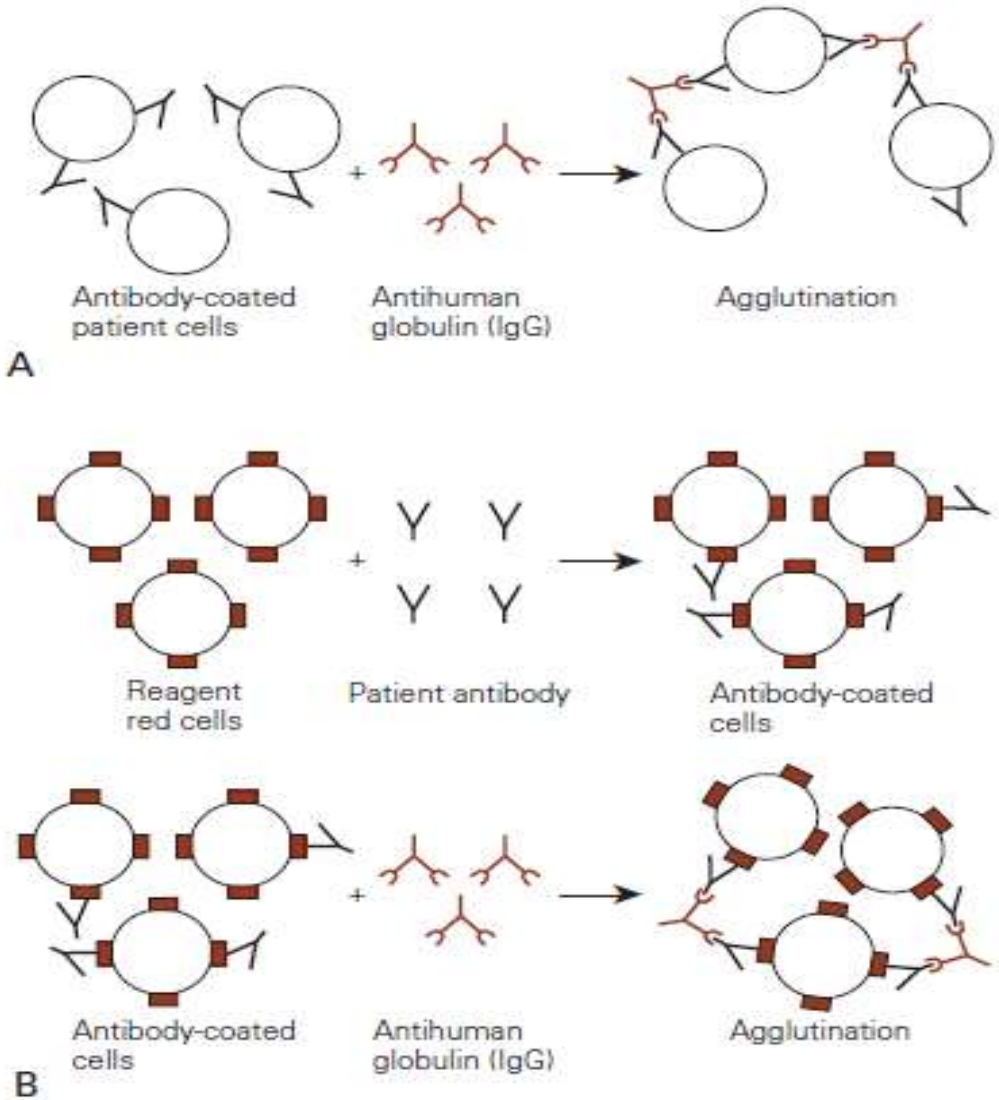


FIGURE 9–6. Direct and indirect antiglobulin tests. *(A)* Direct antiglobulin test (DAT). Antihuman globulin is combined with patient cells that have become coated with antibody in vivo. *(B)* Indirect antiglobulin test (IAT). Reagent cells are reacted with patient antibody. These are washed, and then antihuman globulin is added to enhance agglutination.

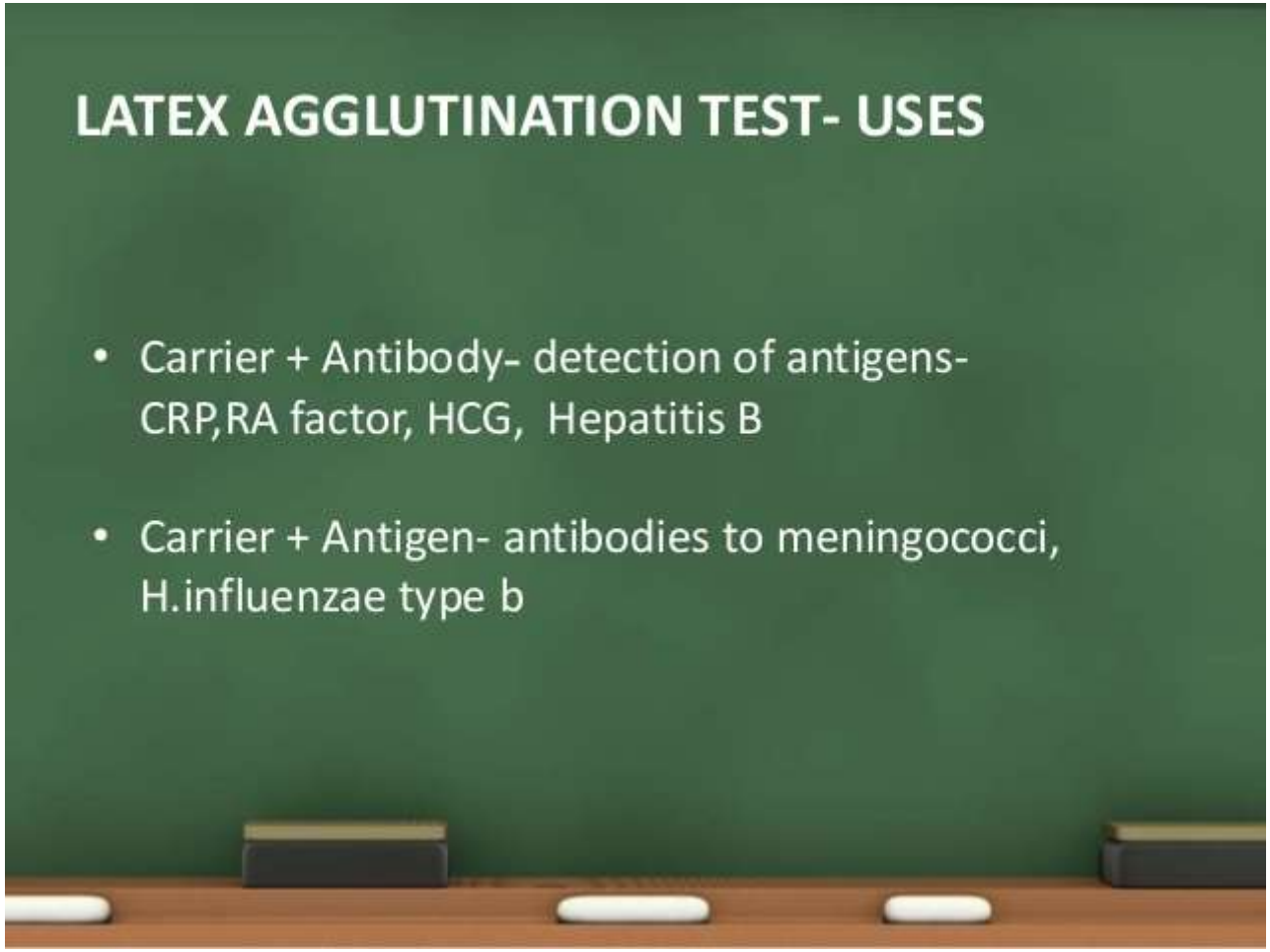
Indirect agglutination test

Passive Agglutination Test

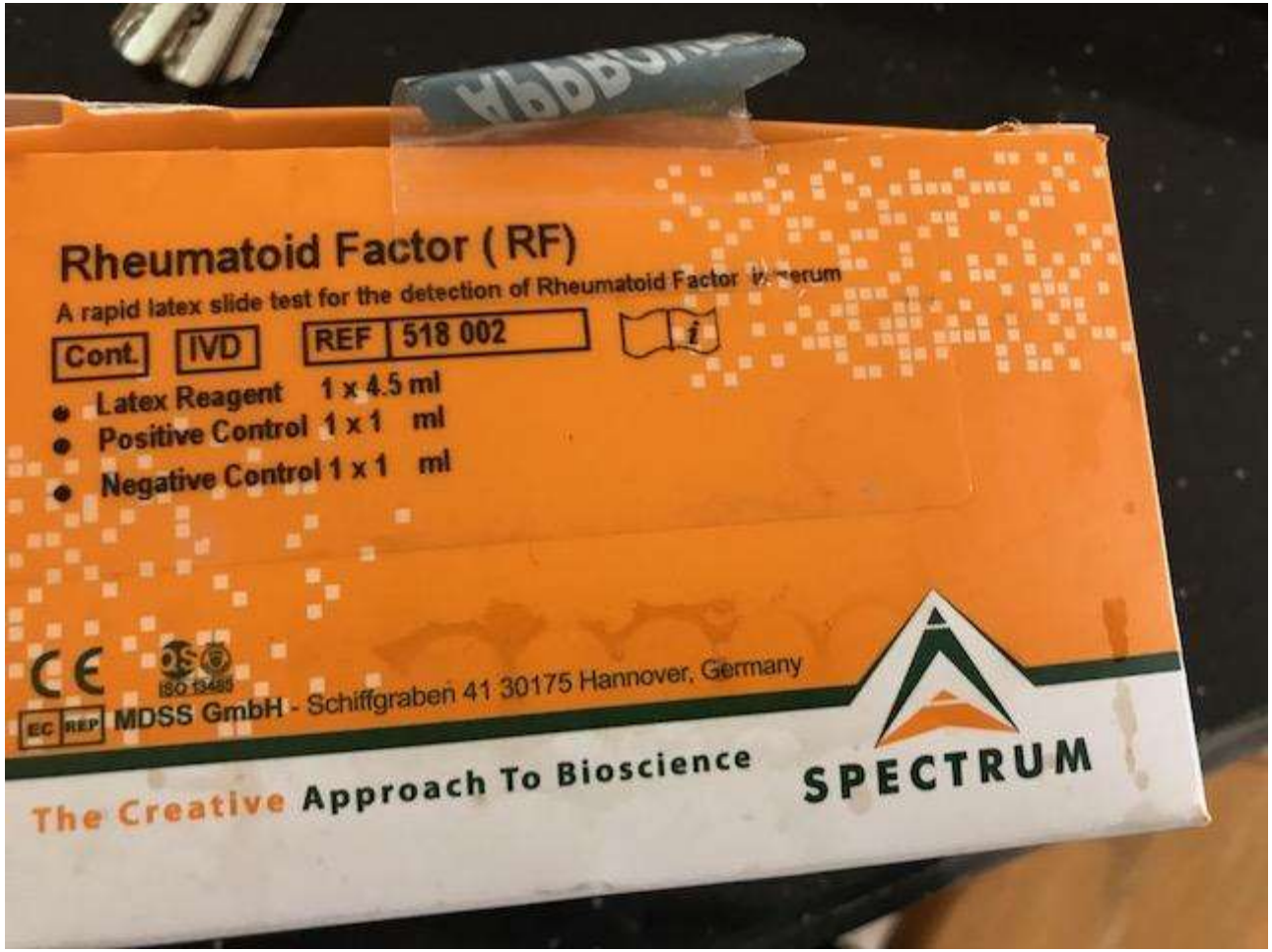
- Converting a precipitating test to an agglutinating test
- Chemically link soluble antigen to inert particles such as LATEX or RBC
- Addition of specific antibody will cause the particles to agglutinate
- Reverse PAT: antibody linked to LATEX

e.g. Lancefield grouping in Streptococci.

1-Latex agglutination test



Latex agglutination test


Rheumatoid Factor (RF)
A rapid latex slide test for the detection of Rheumatoid Factor in serum
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Latex Reagent 1 x 4.5 ml
Positive Control 1 x 1 ml
Negative Control 1 x 1 ml
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(0.5 ML)
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LATEX AGGLUTINATION

TEST	SPECIMEN	TYPE OF REACTION SENSITIVITY	ANTIGEN Ag	ANTIBODY Ab	PRINCIPLE	CAUSATIVE AGENT	DISEASE CASE
HCG qualitative	urine / serum	passive agglutination	HCG	anti-HCG on latex	HCG +anti-HCGon latex = agglu. Within 2 min.		pregnancy
CRP qualitative& semiquantitative	serum	passive agglutination	CRP- C reactive protien	anti-CRP on latex	CRP+anti-CRPon latex = agglu. Within 2 min.	many bacteria & viruses	acute stage of inflammation diseases
RF qualitative& semiquantitative	serum	passive agglutination	human IgG on latex	IgM or IgG in serum(RF)	RF+human IgG on latex = agglu. Within 2 min.	UNKNOWN	Rheumatoid arthritis
ASO qualitative& semiquantitative	serum	passive agglutination	StreptolysinO on latex	anti-SterptolysinO in serum	StreptolysinO on latex + anti-StreptolsinO in serum= agglu. Within 2 min.	Streptococci pyogenes group A	streptococcal infections rheumatic fever

Passive Agglutination

2. Coagglutination is the name given to systems using bacteria as the carrier particles to which antibody is attached.

- *Staphylococcus aureus* is most frequently used, because it has a protein on its outer surface, called protein A, which naturally adsorbs the (FC) portion of antibody molecules.

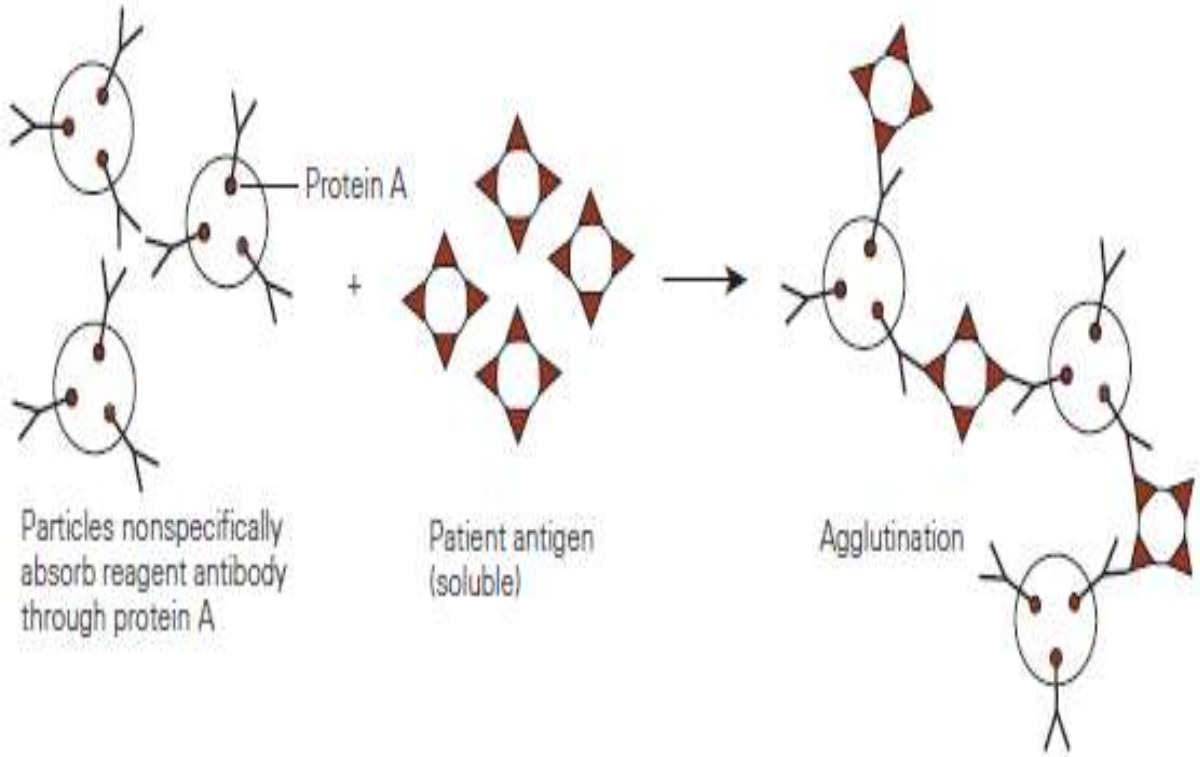


FIGURE 9–5. Coagglutination with *Staphylococcus aureus*. *Staphylococcus aureus* particles nonspecifically bind the Fc portion of immunoglobulin molecules. When reagent antibody is used, combination with patient antigen produces a visible agglutination reaction.

3. Passive heamagglutination

- , when known antigen is coated on RBC and then mixed with serum from patient, to diagnose
- TAPH test, treponema pallidum heamagglutination test for syphilis

Agglutination inhibition test

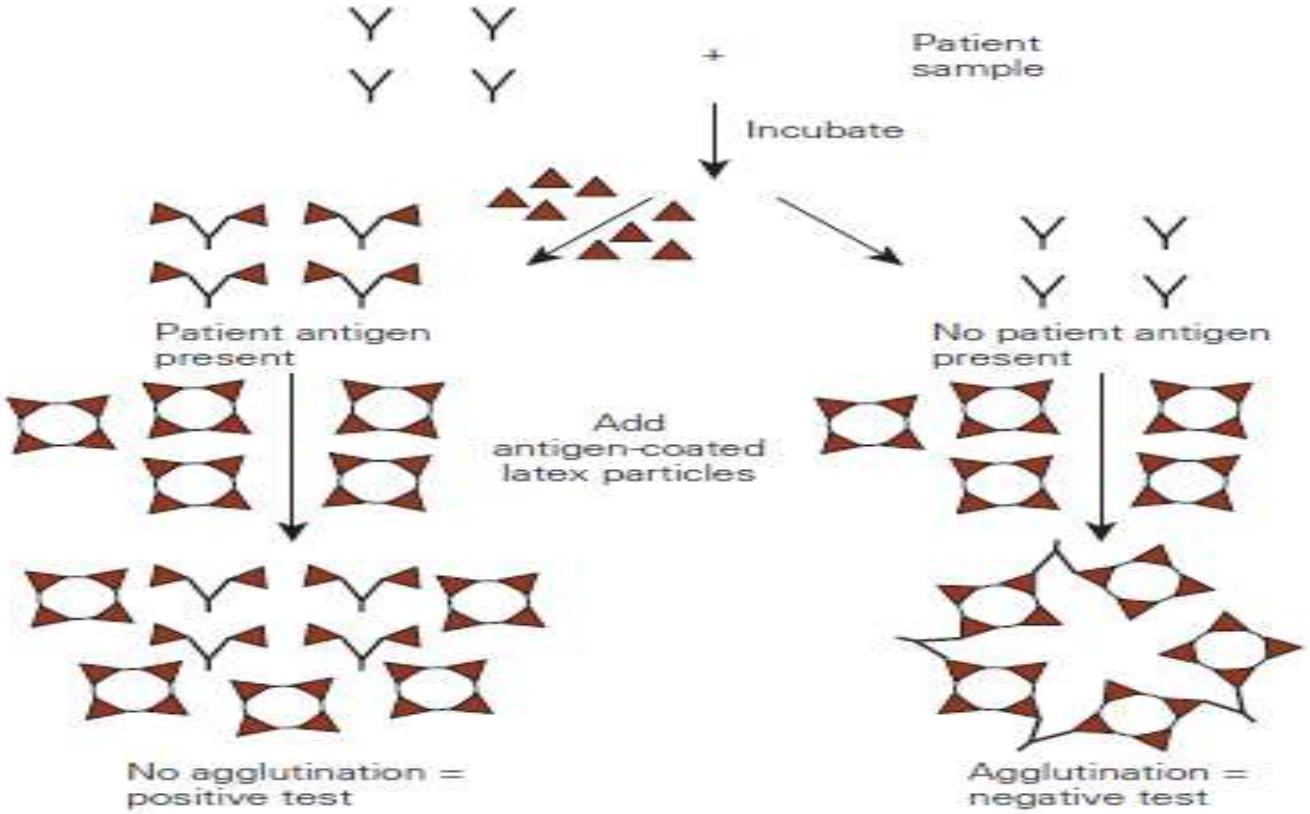


FIGURE 9–4. Agglutination inhibition. Reagent antibody is added to the patient sample. If patient antigen is present, antigen–antibody combination results. When antigen–coated latex particles are added, no agglutination occurs, which is a positive test. If no patient antigen is there, the reagent antibody combines with latex particles, and agglutination results, which is a negative test.

Complement fixation test

Principle

- Complement takes part in many of the immunological reactions. It gets absorbed during the combination of antigens and antibody.
- This property of antigen–antibody complex to fix the complement is used in complement fixation test for the identification of specific antibodies.
- The haemolytic system containing sheep erythrocytes (RBC) and its corresponding antibody (amboceptor) is used as an indicator which shows the utilization or availability of the complement.
- If the complement is fixed then there will be **no lysis** of sheep erythrocytes, thus denoting a **positive test**.
- If the complement is available then there will be **haemolysis** which is a property of complement, denoting a **negative test**.

Complement fixation test(CFT)

Serum(? Abs)+ known Ag+C ⟶Incubate for 1 hour, then add indicator system(Sheep RBCs+Ani-sheep RBCs).⟶Incubate for 1 hour

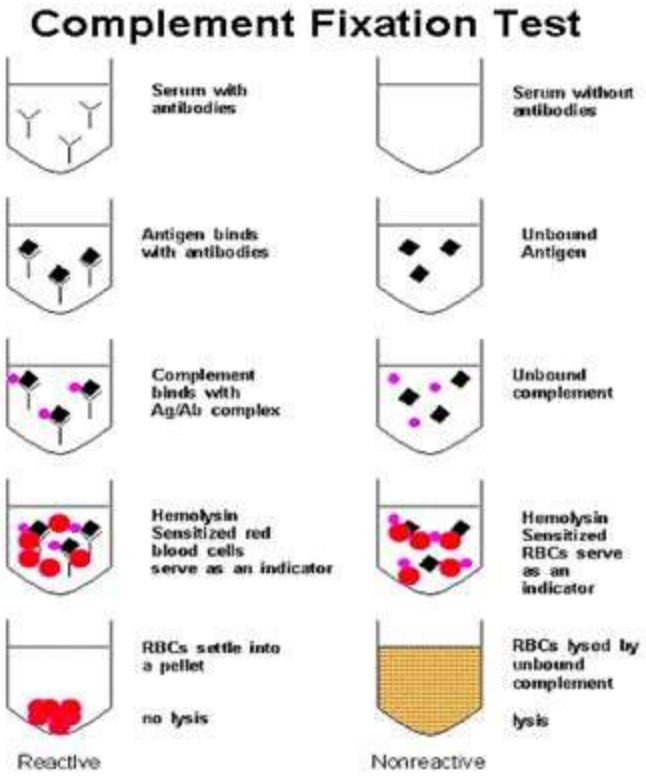
+ve = No lysis

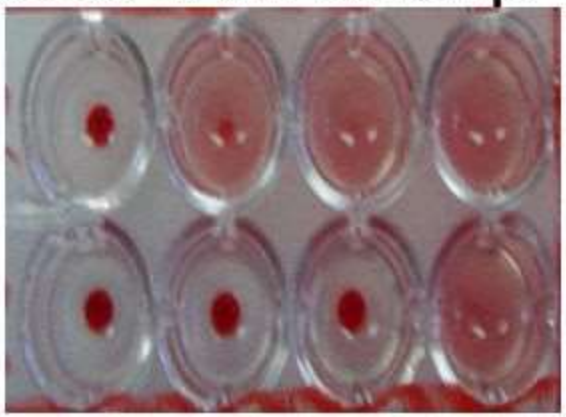
-ve = lysis

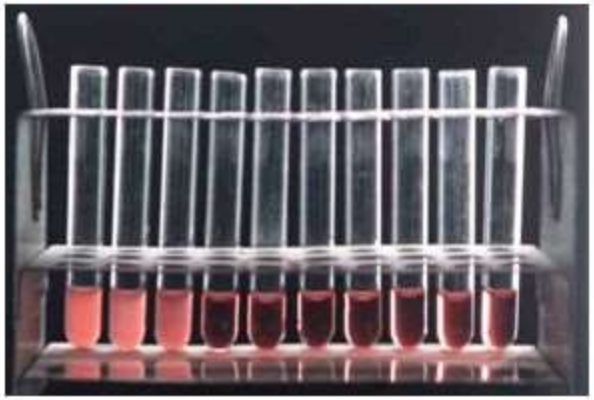
Uses:

Diagnosis of:

Syphilis, Viral Infections, Gonorrhoea,....



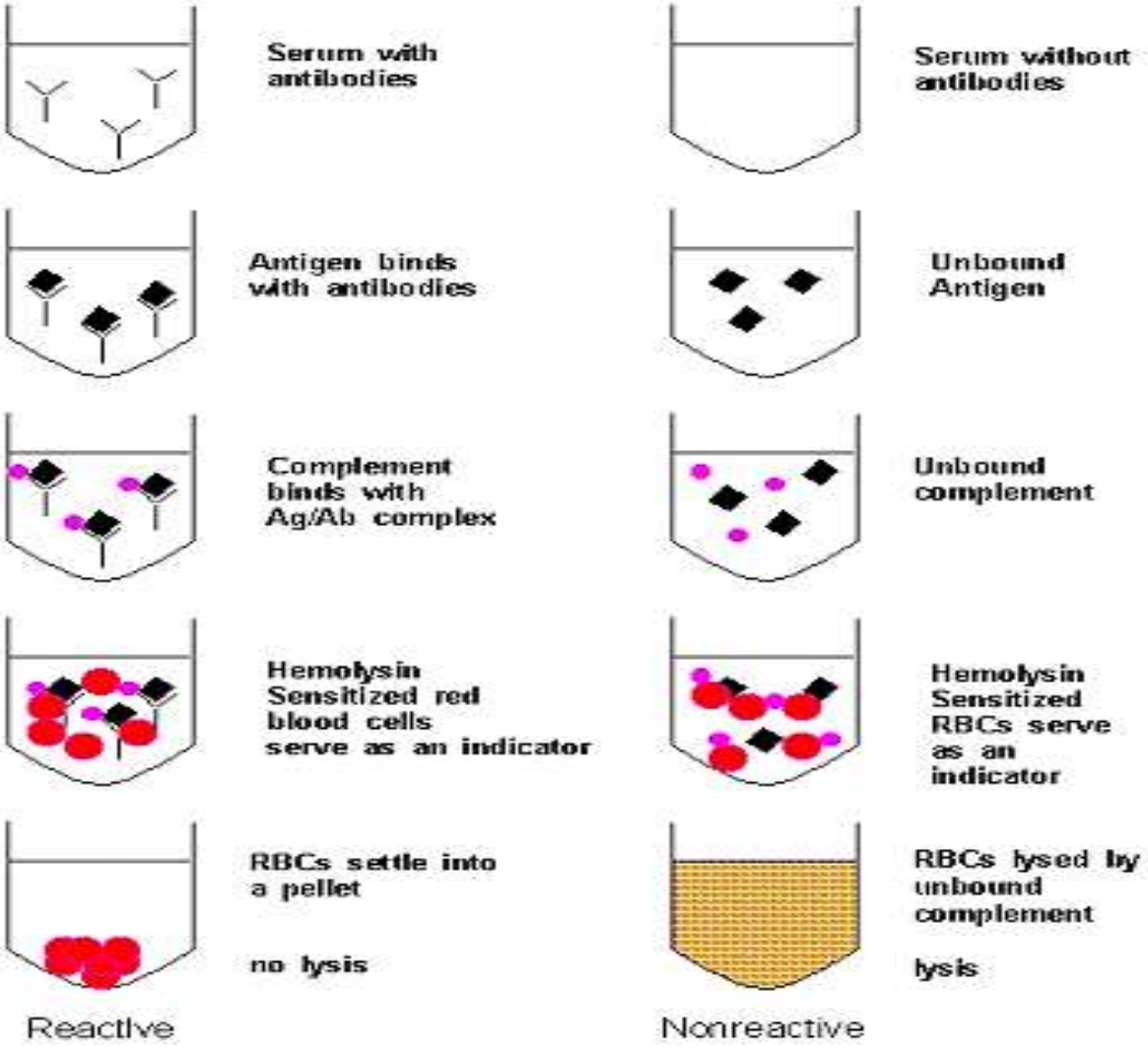




Uses of CFT

- To diagnose
  - syphilis (Wassermann reaction)
  - Gonorrhea
  - Reckettsial infection
  - Viral infection

Complement Fixation Test

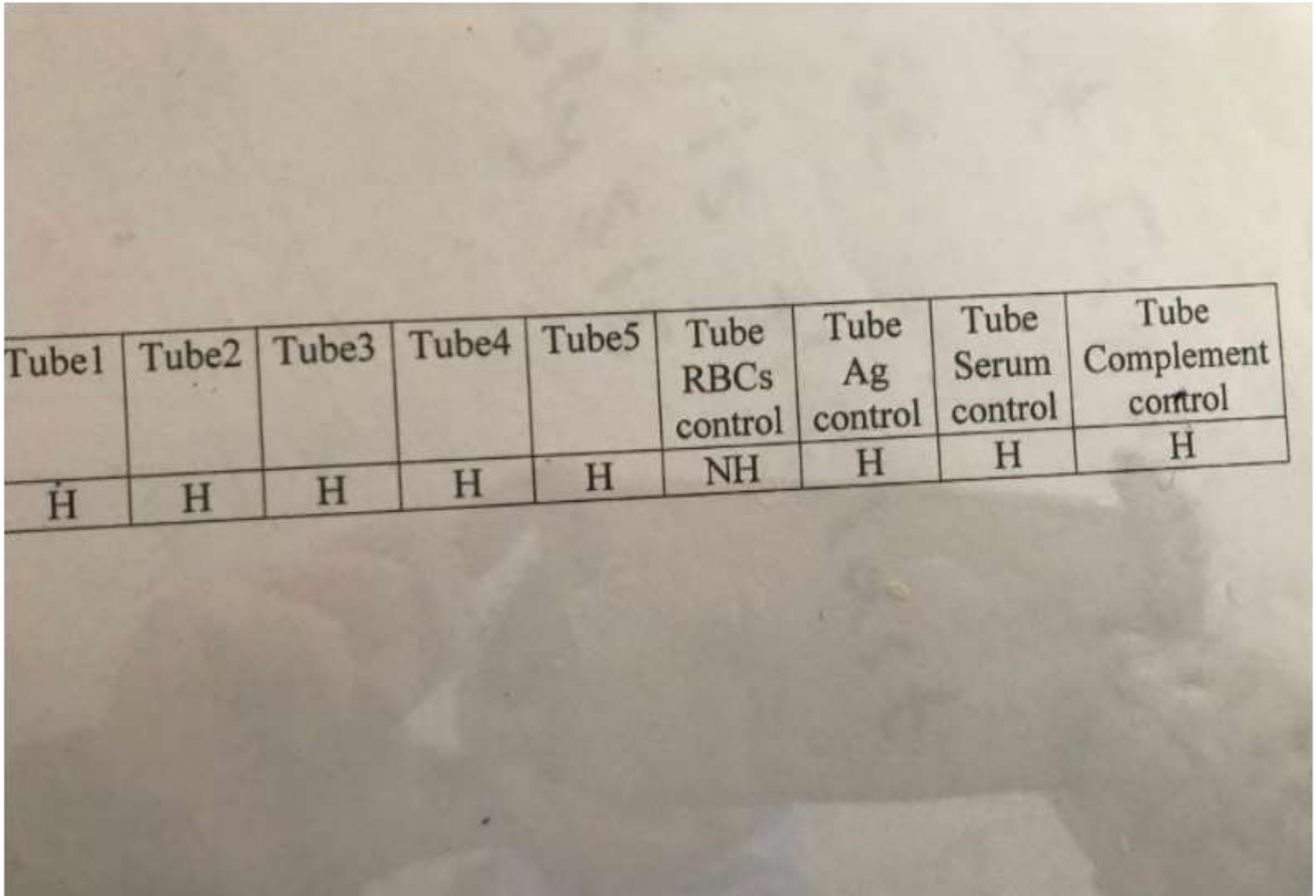


Positive

Antibody present

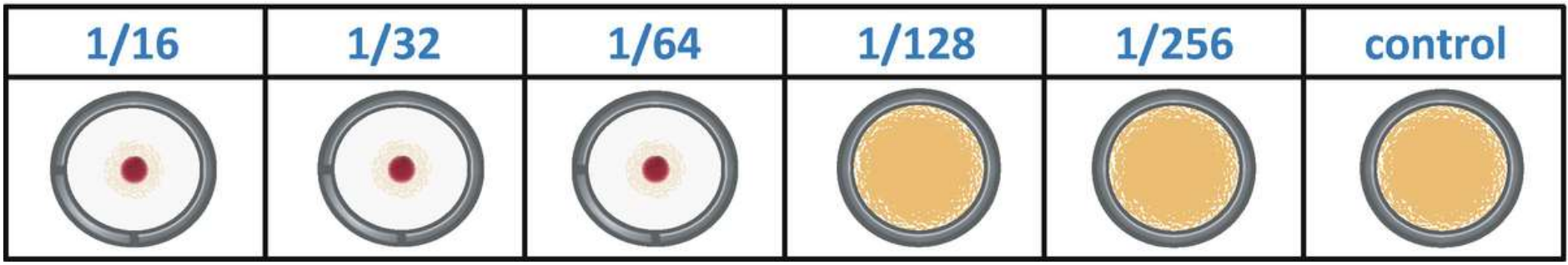
negative (No AB)

Negative case



Tube1	Tube2	Tube3	Tube4	Tube5	Tube RBCs control	Tube Ag control	Tube Serum control	Tube Complement control
H	H	H	H	H	NH	H	H	H

Positive case with titer 64



No Heamolysis

Heamolysis

COMPLEMENT FIXATION TEST

Anti-complementary reaction: NO haemolysis in all test tubes + NO Haemolysis in the serum, RBCs control tube.

The value of serum control tube is to test:

-The inability of serum alone to bind complement.

-The anti-complementary action of the serum, which is due to one of the followings:

a-presence of immune complexes in serum that binds to complement and prevent its haemolytic activity

b-Heparin therapy which inactivates complements due to consumption of Ca, Mg.

c-Old or contaminated serum has destructive action on complement.

Tube1	Tube2	Tube3	Tube4	Tube5	Tube RBCs control	Tube Ag control	Tube Serum control	Tube Complement control
NH	NH	NH	NH	NH	NH	H	NH	H

radio- immune assay (RIA) and ELISA

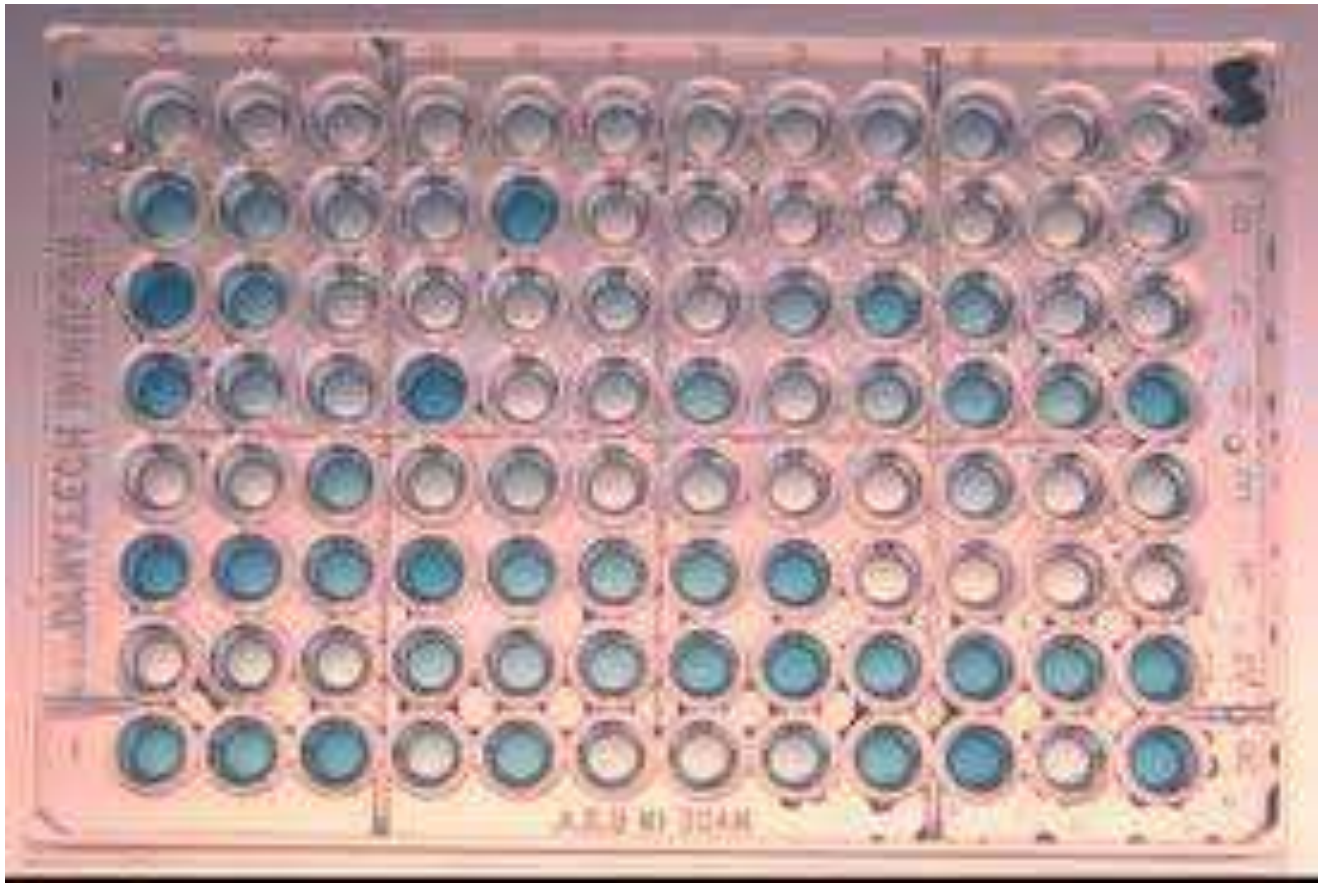
- When the antigen or antibody is labeled with a radioisotope, it may be quantified by instruments that detect radioactive decay events; the assay is called a radioimmunoassay (RIA).
- When the antigen or antibody is covalently coupled to an enzyme, the rate at which the enzyme converts a clear substrate to a colored product may be quantified by determining the resulting color wavelength with a spectrophotometer and then blotting the wavelength (absorbance) with a corresponding concentration of target antigen on a slop (different pattern for each antigen) ; the assay is called an enzyme-linked immunosorbent assay (ELISA).

- Measures amount quantities of antigens or antibody (protein) using radiolabelled sign:
  - Hormones
  - Drugs
  - Tumour markers
  - Abs
  -Viral and bacterial antigens

Quantitation of Antigen by Immunoassays ELISA

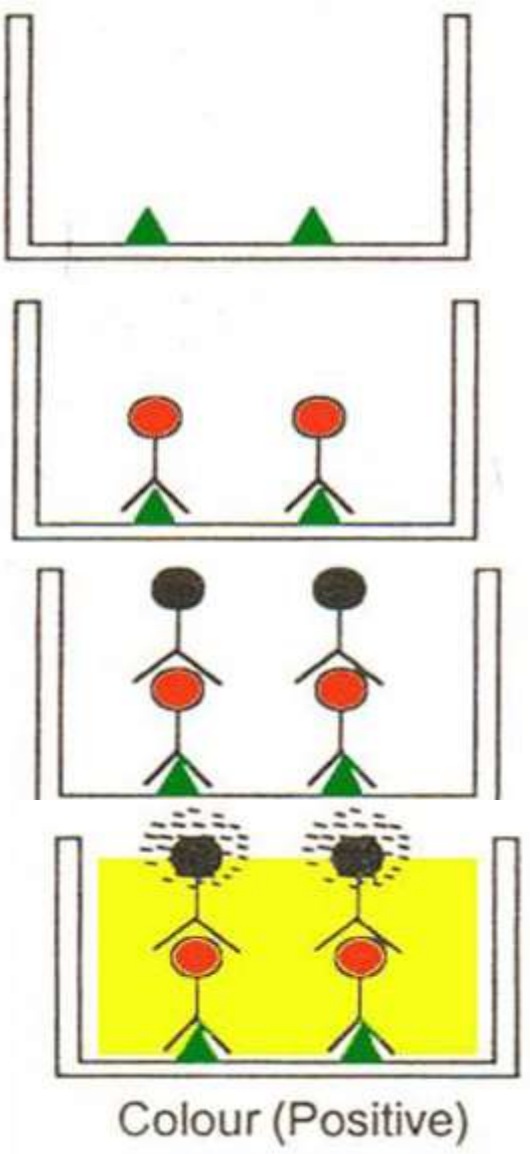
- Enzyme linked immunosorbent assay(1971)
- the antibody is enzyme linked,
- Done in 96 well microtitre plates
- To detect the presence of a protein and its quantity
- Ag or Ab coated on wells

ELISA plate



Indirect ELISA

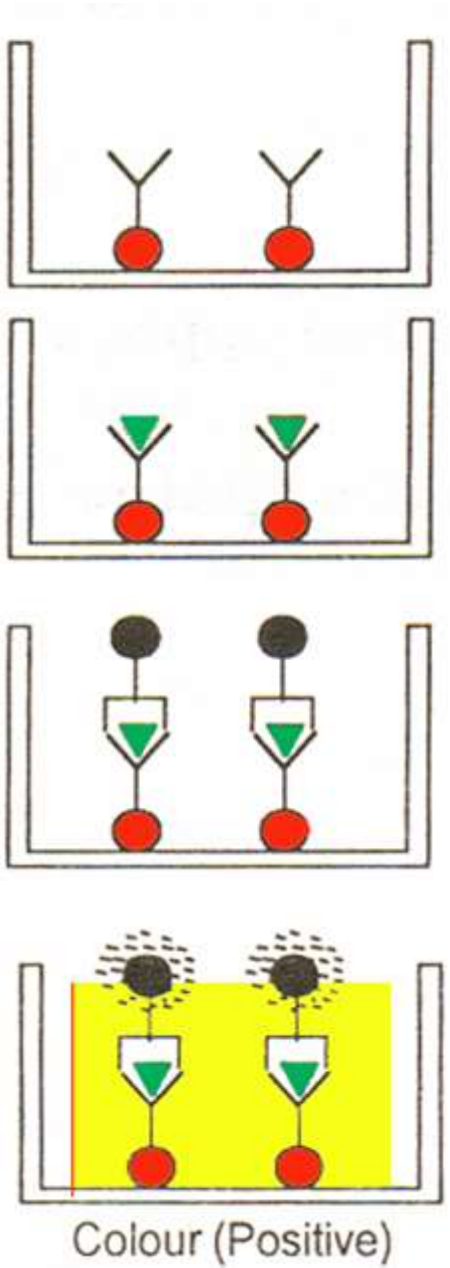
For Ab detection,
Ag coated wells used
Add patient serum then wash
(If Ab present, it binds to Ag)
Add goat anti-human
Ig antibody enzyme linked and
substrate
If there is color means positive



Sandwich ELISA (the most common)

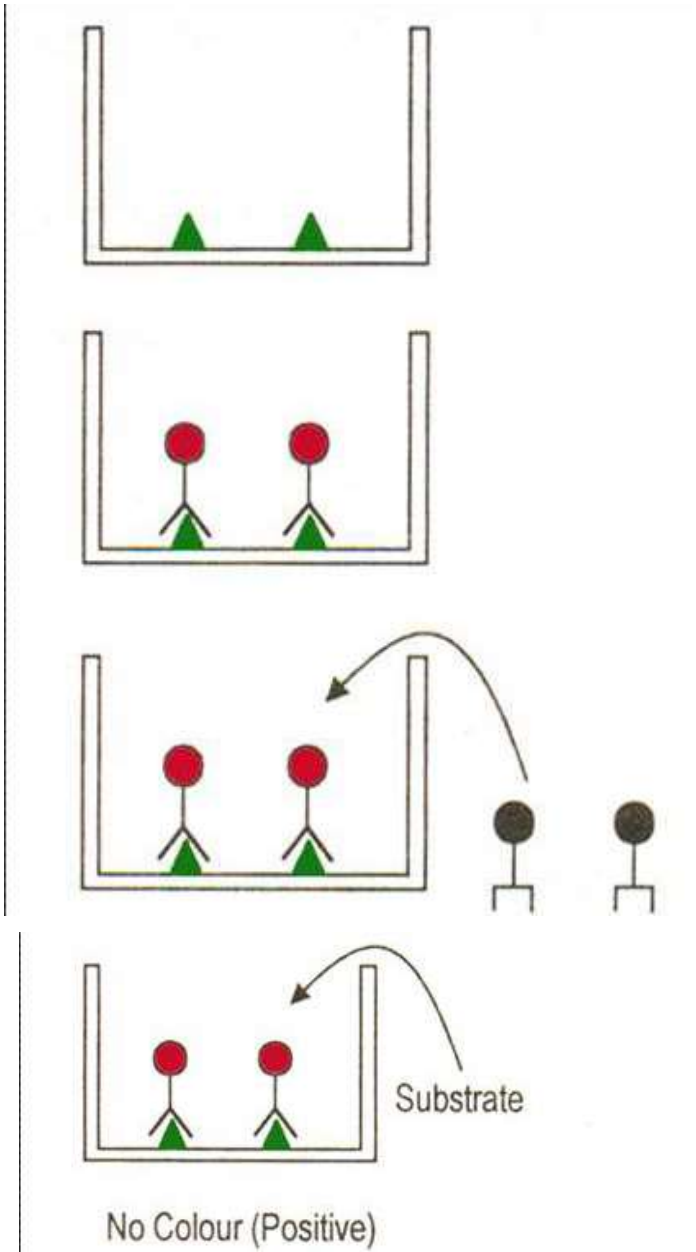
- For Ag detection, wells coated with Sp. Ab.
- add patient specimen then wash Ag in specimen binds to coated Ab.
- Add antibody enzyme linked Ig antibody enzyme linked and substrate

If there is color means positive



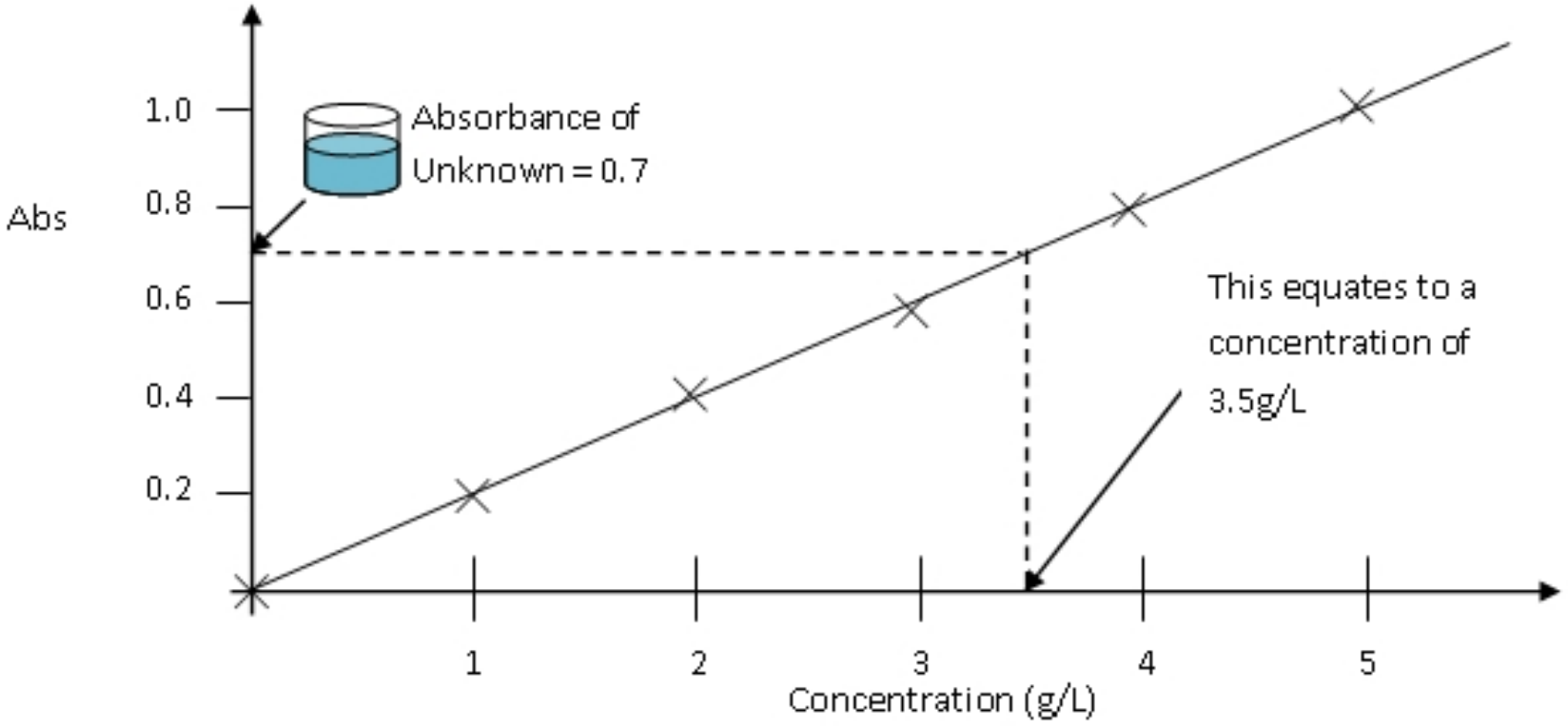
Competitive ELISA

- Ag coated wells
- Two specific Abs,
(enzyme linked Ig antibody enzyme linked and substrate& patient serum) added simultaneously then wash
- More specific tested Abs attach to Ag
- Positive test- no colour because the enzyme linked antibody go with wash



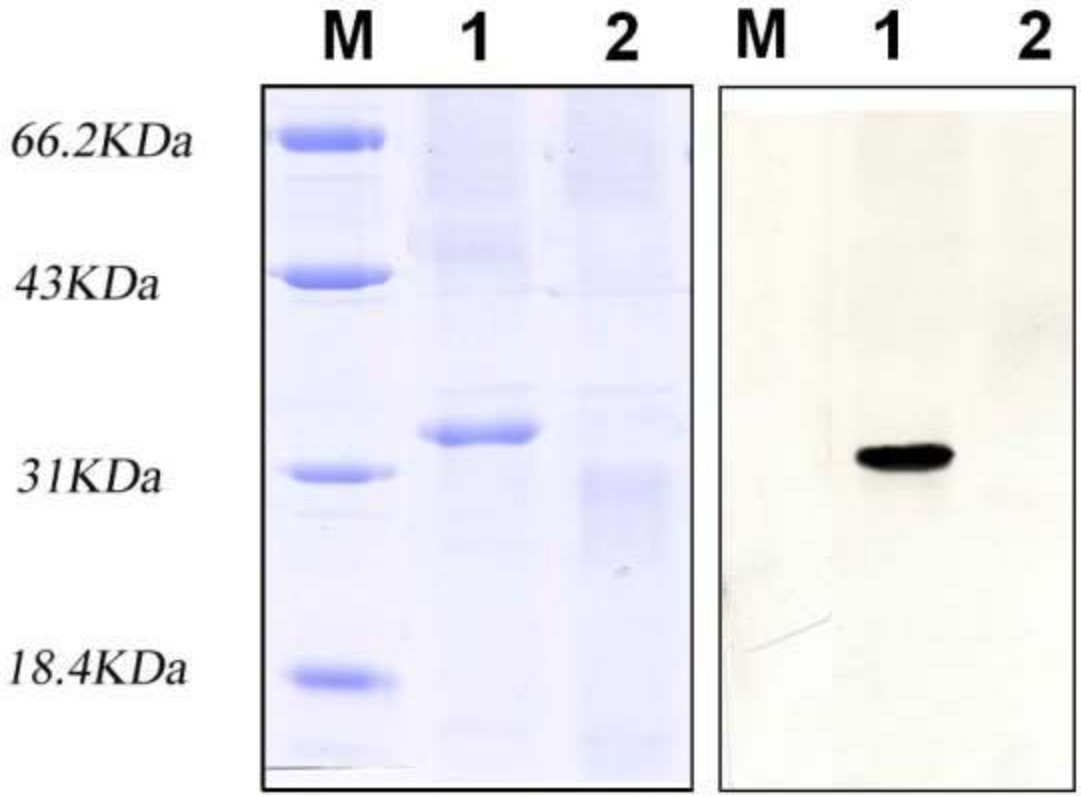
Spectrophotometer for reading the color

Spectrophotometry absorbance and antigen concentration slop



Western blot

- In this technique a mixture of proteins is separated based on molecular weight through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein, the specific protein is identified by binding specific radiolabeled or enzyme linked antibody. Identification of HIV antibodies
- When the proteins transferred from gel to membrane## and The position of the protein antigen on the membrane is depending on molecular weight,
- then it can be detected by an antibody that may be conjugated to an enzyme such as horseradish peroxidase, that generate signals and leave images on photographic film. Or by radiolabeled antibody


M
1
2
M
1
2
66.2KDa
43KDa
31KDa
18.4KDa

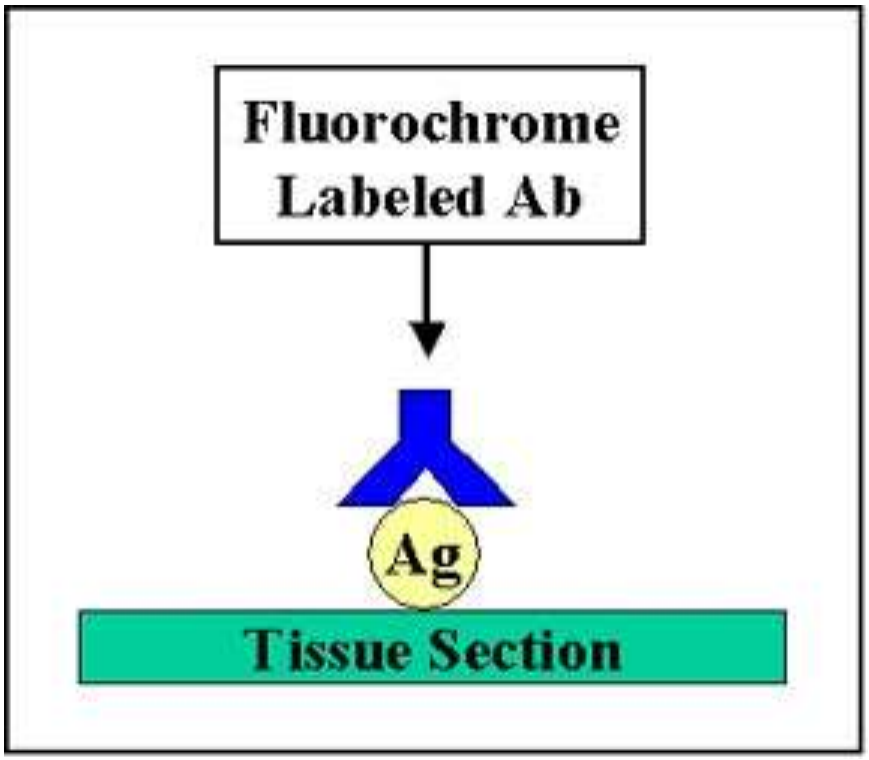
Southern blot, a technique for DNA detection (transfer from gel to a membrane) developed earlier by Edwin Southern. Detection of RNA is termed northern blot

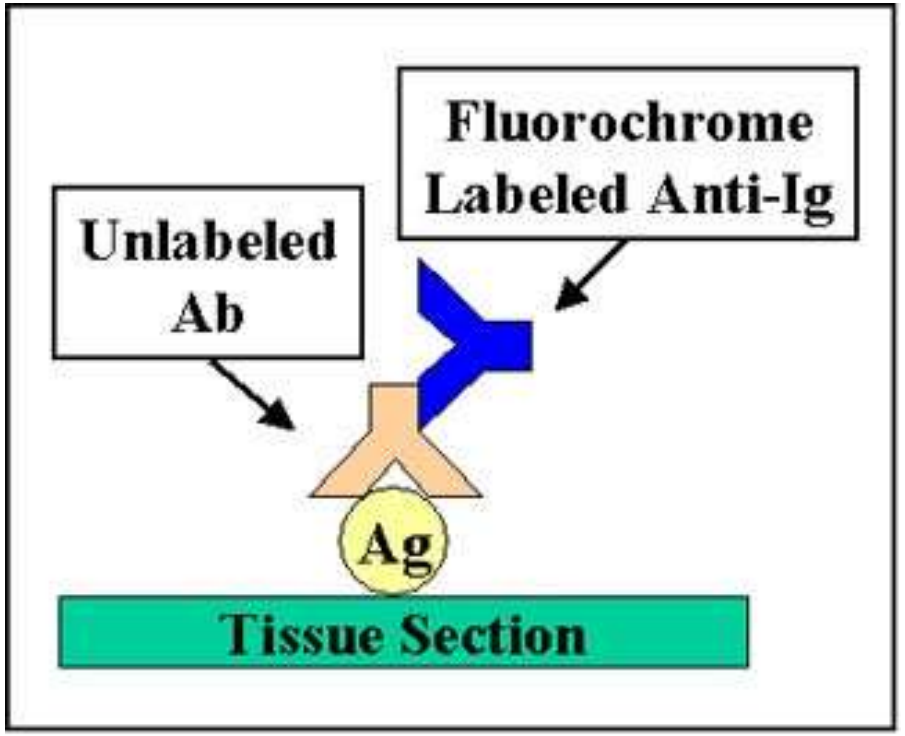
Tests for Cell/ tissue Associated Antigens.Immuno-cyto/histo-chemistry

Detection of cell surface or intracellular antigens

1. **Immunofluorescence**
   Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein) Determine the anatomic distribution of antigen in tissues using the fluorescence emitted by the bound antibody. Uses in tumor antigen detection, ANA in SLE and Rheumatoid arthritis and in autoimmune diseases.
   - **Direct Immunofluorescence**
     attaching fluorescein to a specific mouse antibody directed against the antigen of interest,
   - **Indirect Immunofluorescence**
     In indirect immunofluorescence, fluorescein can be attached to a second anti-antibody (e.g., rabbit anti-mouse Ig antibody) that is used to bind to the first unlabeled antibody. Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

Direct and Indirect IF





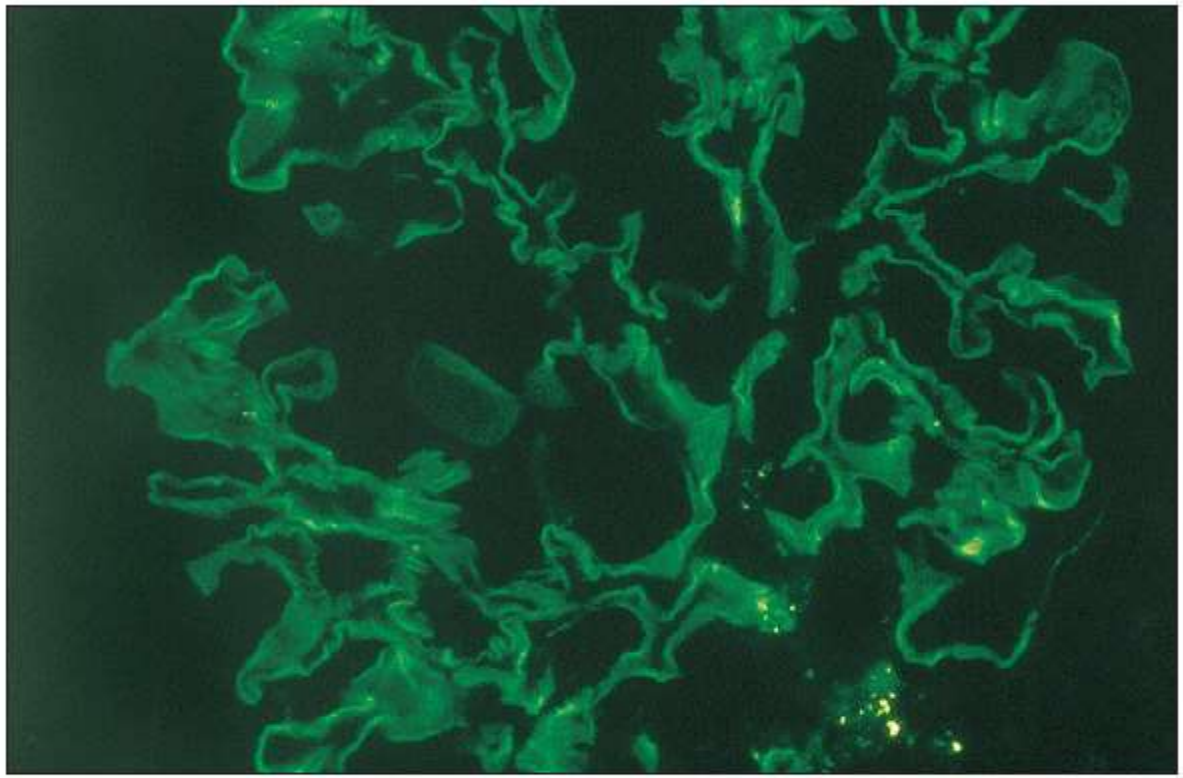
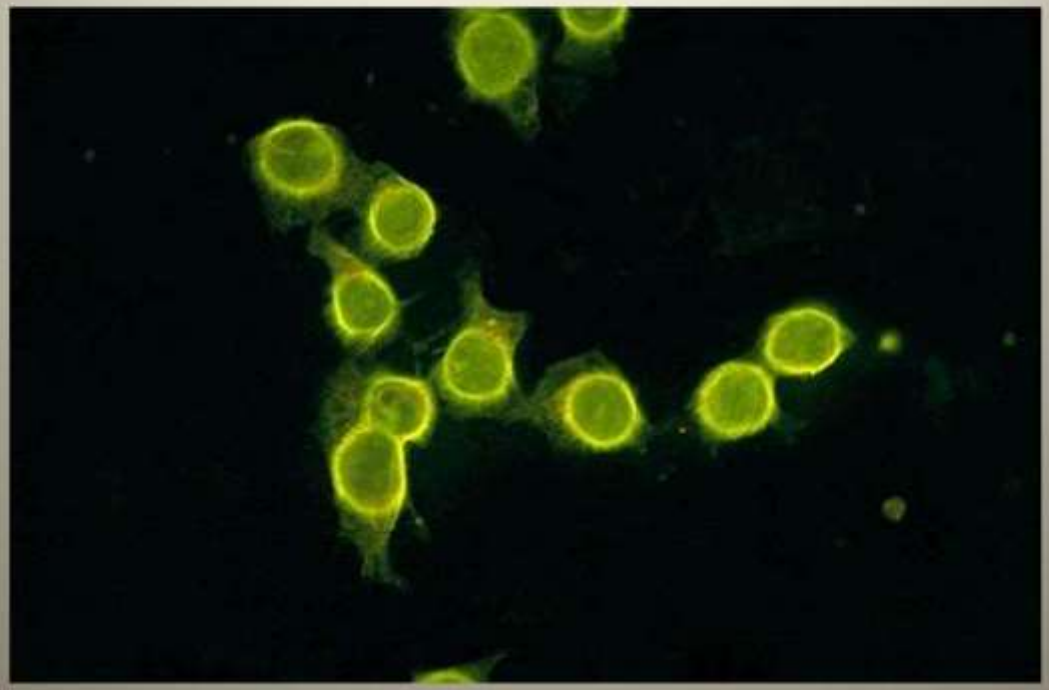


Figure 53-10 Direct immunofluorescence microscopy reveals global and linear glomerular basement membrane immunofluorescence in anti–glomerular basement membrane disease (×400).

Test ANA in autoimmune diseases

- Patient serum is added to a slide containing cells. If the patient has autoantibodies to the nuclei of the cells, they bind to the slide. After washing away any antibodies that don't bind, an antibody against human antibody is added. This antibody has radiolabeled molecules attached to it which, when viewed under Immunofluorescent microscope (light-up green).

ANA(Rim pattern)

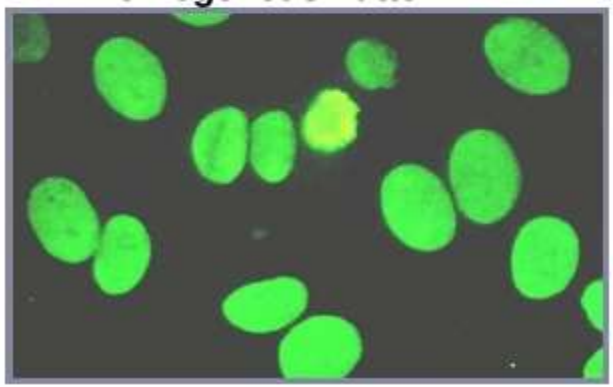


ANA follow

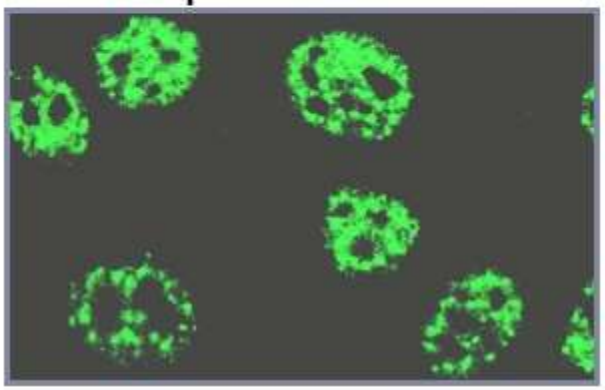
Screening Techniques

IIF (Indirect Immunofluorescence)

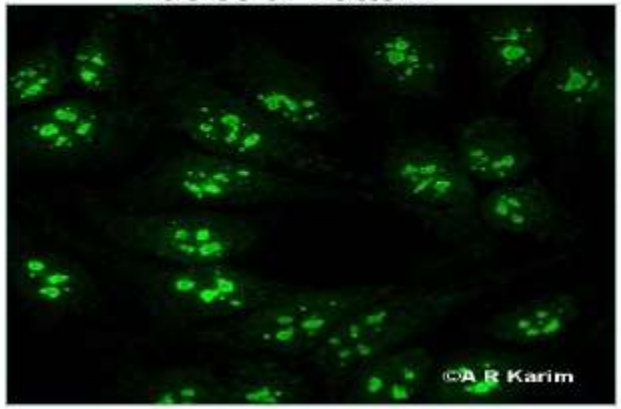
Homogeneus Pattern



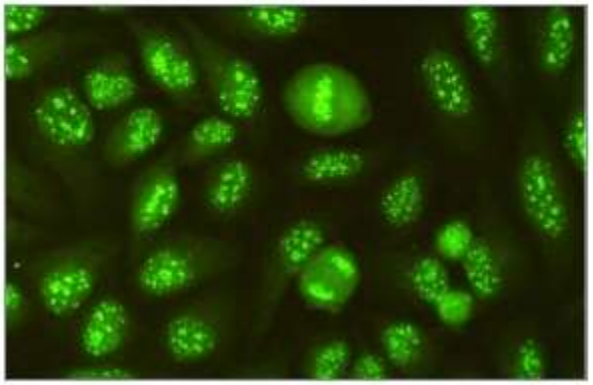
Speckled Pattern



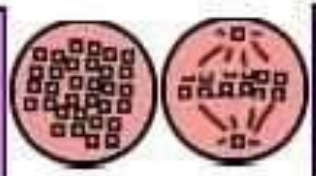
Nucleolar Pattern



Centromere-B Pattern



ANA Patterns

Peripheral (rim)		Anti-DNA (not seen on HEP-2)	SLE
Homogeneous (diffuse)		Anti-DNA Anti-histone Anti-DNP (nucleosomes)	RA & SLE Misc. Disorders (anti-ssDNA)
Speckled		Anti-Sm & RNP Anti-Ro & La Anti-jo-1 & Mi-2) Anti-Sci-70	SLE & SS PM/DM PSS (Systemic)
Centromere		Anti-centromere	PSS (CREST)
Nucleolar		Anti-nucleolar	SLE & PSS

2. Although sensitive, the fluorescence microscope is not an ideal tool to identify the detailed structure of the cell or tissue because of a low structural details. This problem has been overcome by new technologies including **confocal microscopy**,

3. Antibody can be coupled to an electron-dense probe such as colloidal gold, and the location of antibody can be determined subcellularly by means of an electron microscope,

Flow Cytometry

- 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.
- 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 proliferation of T and B cells in vivo. One commonly used dye of this type is (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.

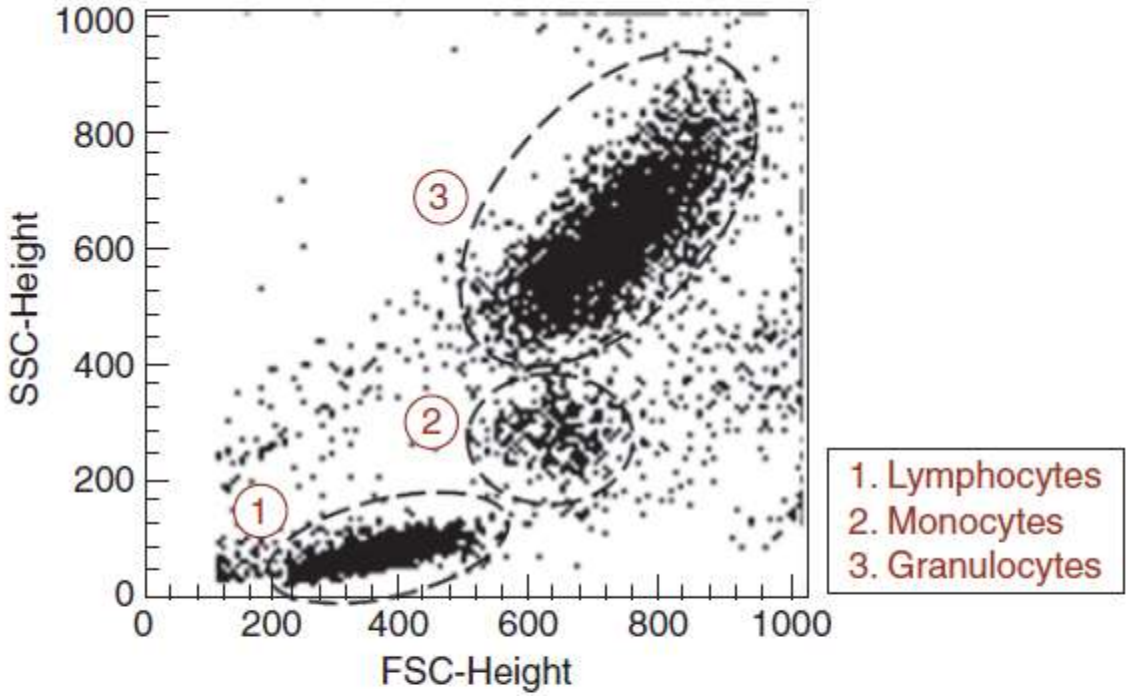
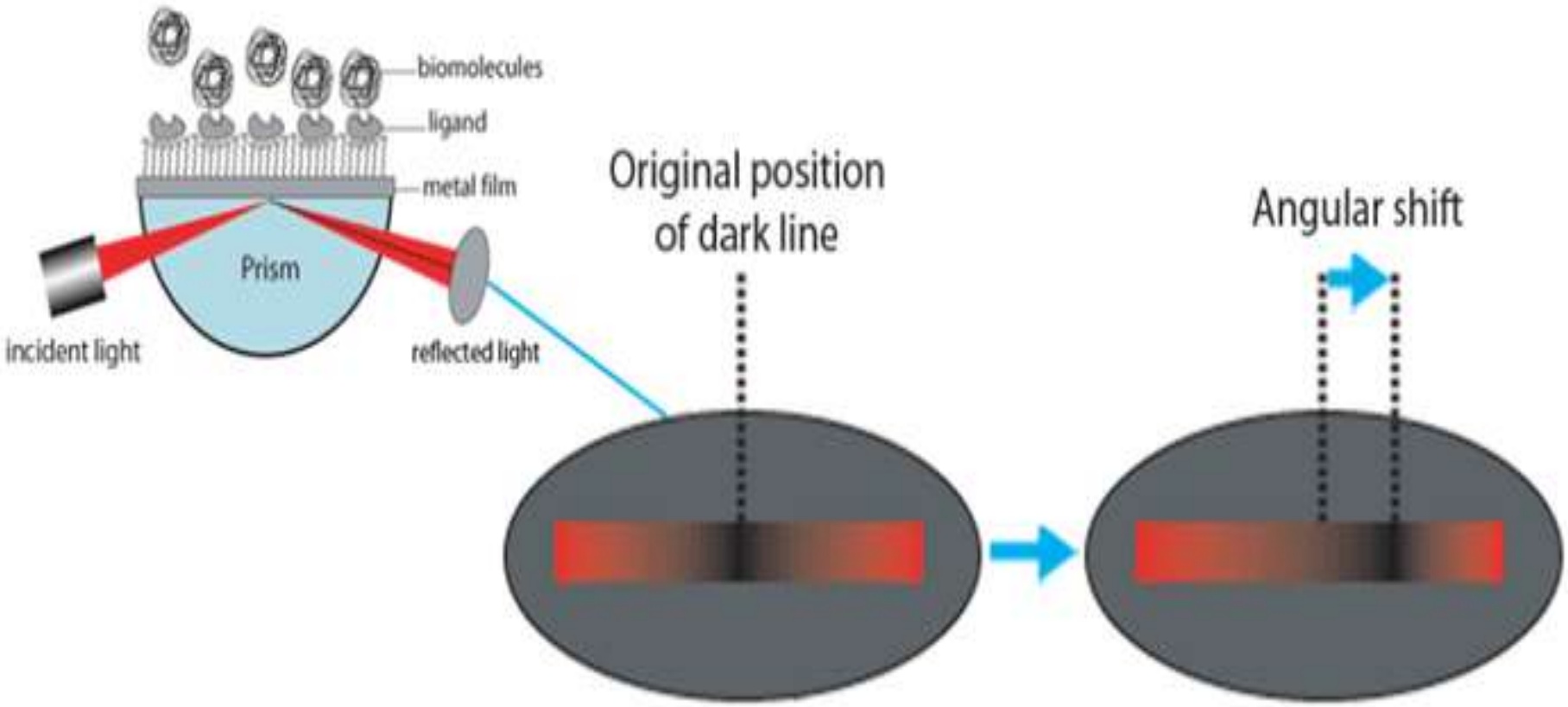


FIGURE 12–2. Peripheral blood leukocyte analysis by simultaneous evaluation of forward-angle light scatter (FCS) and 90-degree LS (SSC). Based on the intrinsic characteristics of size (FSC) and granularity (SSC) only, the three main populations of white cells (lymphocytes, monocytes, and granulocytes) can be discriminated into individual populations.

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


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

follow

- Another method, 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

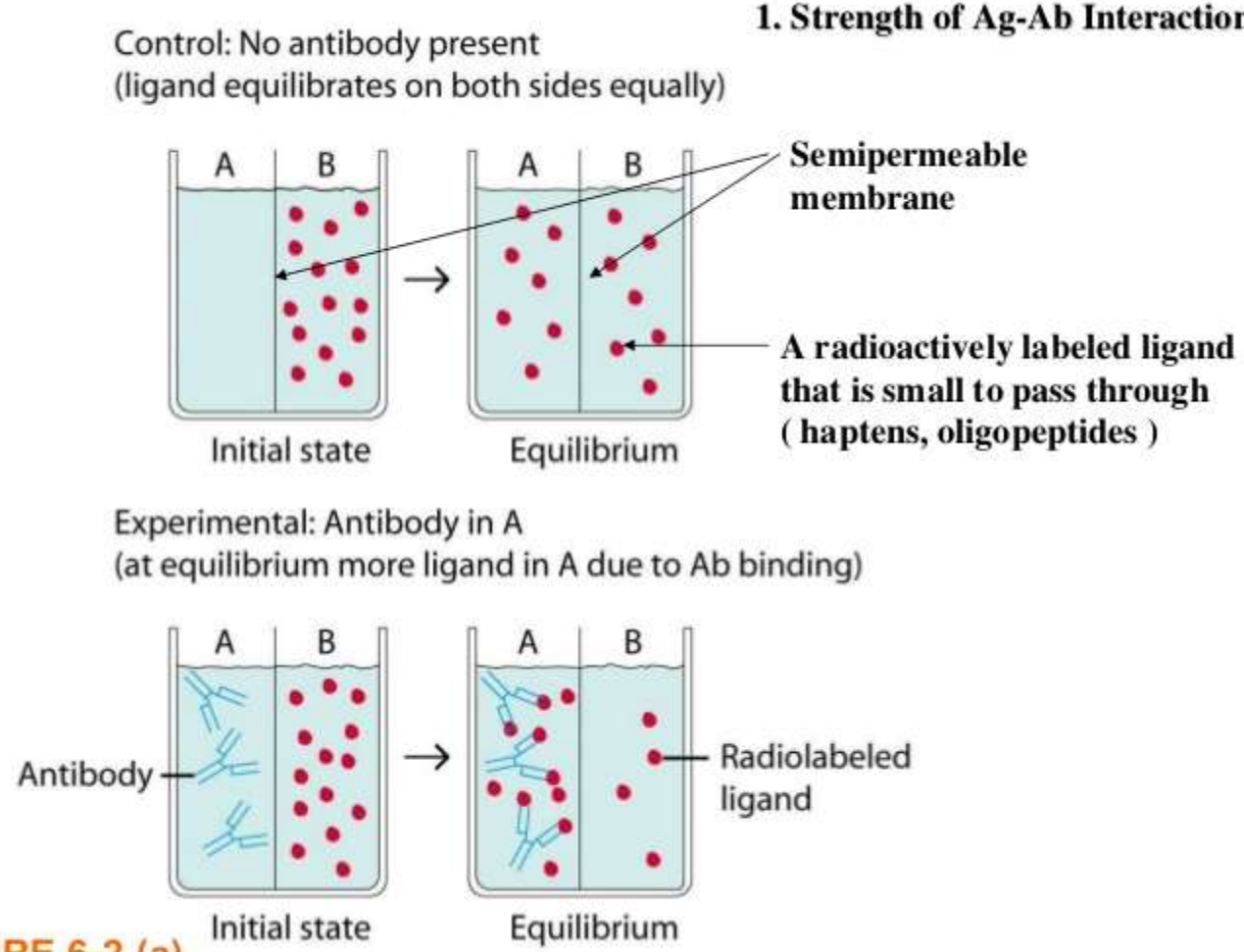


FIGURE 6-2 (a)

Determination of Ab affinity (Ka) by equilibrium dialysis.

Introduction

- *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.*

Introduction

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

✓ *blood donation.*

✓ *blood component preparation.*

✓ *blood cell serology.*

✓ *blood transfusion therapy.*

Introduction

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

Introduction

- ***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

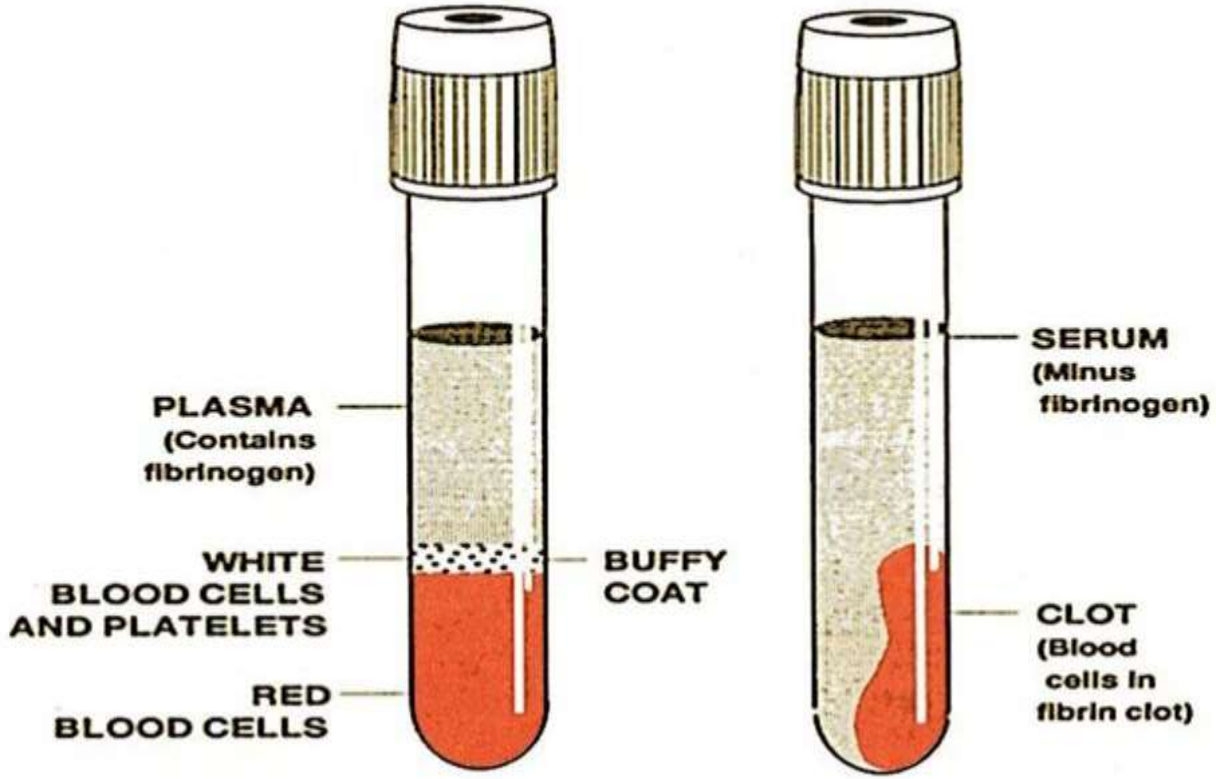
- Units of WB and packed RBC are both kept refrigerated at 33.8 to 42.8 °F (1.0 to 6.0 °C), with maximum permitted storage periods of 35 and 42 days respectively.
- When keeping blood tube in standing position,a layer between the red cells and the plasma is formed and referred to as the buffy coat and 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.

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

Plasma VS serum

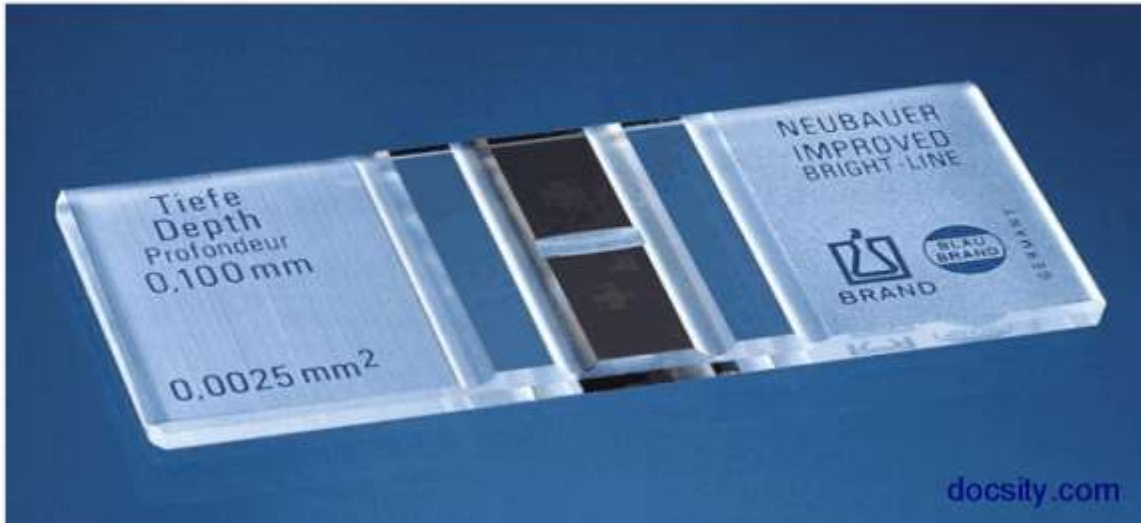
EDTA tube (purple) plain tube (Red)

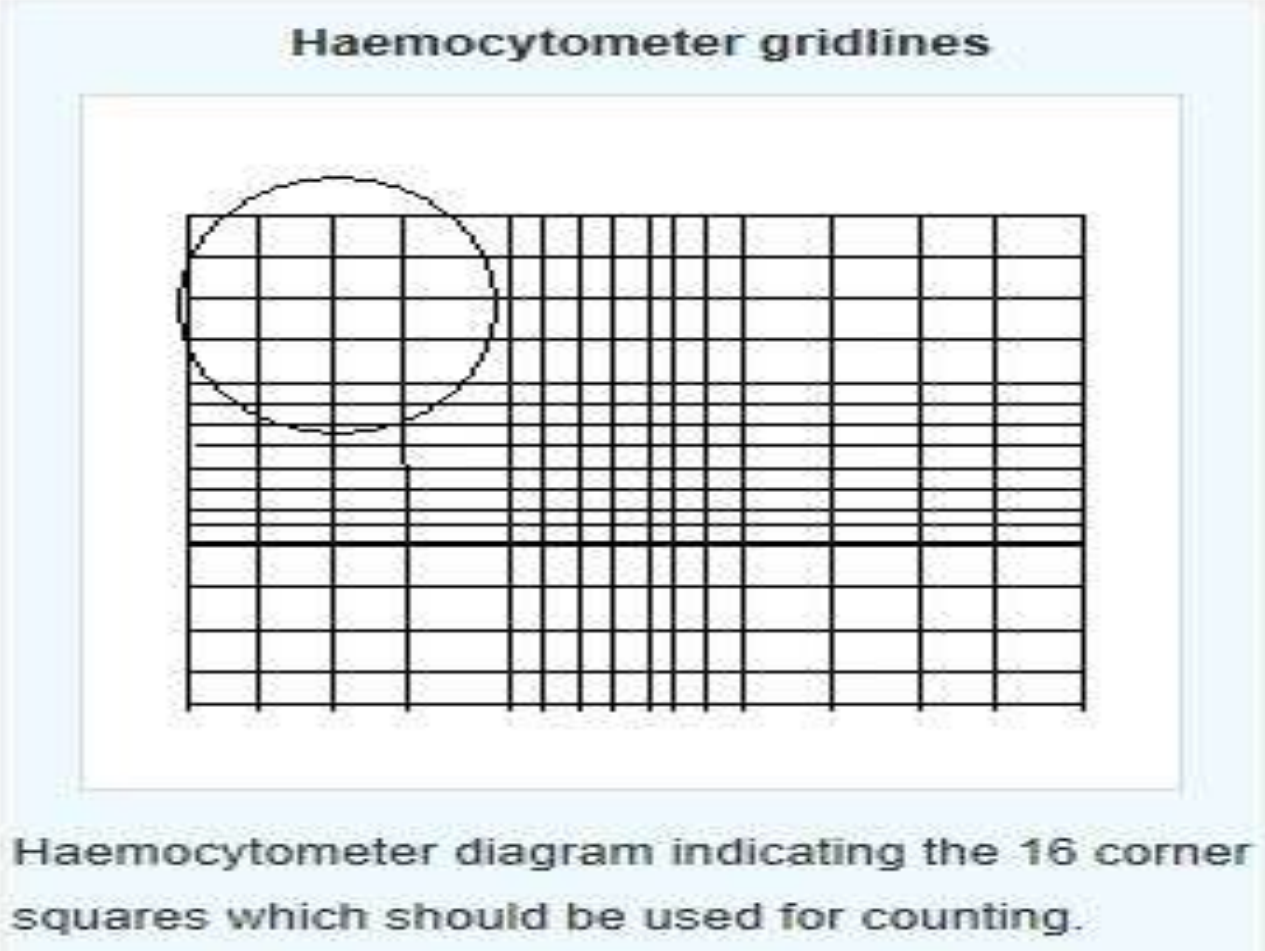


Purple tube for CBC, red tube for immunology

The Haemocytometer

The simplest, most convenient and cheapest mean of accurately determining the numbers of cells in a sample is to use a Haemocytometer and a microscope. A Haemocytometer is a specialised slide that has a counting chamber with a known volume of liquid.





Number of cells/cmm= counted cells in 16 corner sequare*10*dilution factor

White cell count by heamocytometer

The white cell count is the number of white cells in 1.00 cubic millimeter of undiluted blood. So we have 2 correction factors: dilution factor and volume factor.

Dilution factor: The blood has been diluted 1 in 20

Volume factor: the chamber has an area of 1 square millimeter and depth of 0.1

So the volume of the chamber"is = area X depth

=1 X 0.1 =0.1 cumm

Number of cells / cmm = number of cells in chamber X dilution factor X volume factor

= N X 20 X 10

...with higher total WBC and neutrophil counts in women of child-bearing age and during pregnancy. Counts also vary in different populations with lower total WBC and neutrophil counts being found in Africans

WBC reference range

(N.B. These are guideline figures which should be checked locally)

- Children at one year — 6.0-18.0 X 10^9 / L
- Children at 4-7 year — 5.0-15.0 X 10^9 / L
- Adults — 4.0-10.0 X 10^9 / L
- Pregnant women — up to 15.0 X 10^9 / L

Leukocytosis: the main causes of a raised WBC count are:

- Acute infections: e.g. pneumonia , meningitis , abscess tonsillitis , cholera. septicemia....etc Appendicitis, leukemia, meningitis, rheumatic fever, newborn, pregnancy, chickenpox,....
- Inflammation and tissue necrosis e.g. burns, ,trauma, arthritis , tumors,...etc
- Acute hemorrhage
- Stress, menstruation, exercise,

Leukopenia: the main causes of a reduced WBC count are:

- Viral, bacterial, parasitic infections, e.g. HIV, viral hepatitis, measles, rubella, influenza rickettsial infections, overwhelming bacterial infections such as military T.B, relapsing fever, typhoid fever , brucellosis, ,parasitic infections including leishmaniasis and malaria.
- Hypersplenism.
- Bone marrow infiltration.
- Ionizing radiation.

TABLE 41.2 Differential White Blood Cell Count

Cell Type	Normal Value (percent)	Elevated Levels May Indicate
Neutrophil	54–62	Bacterial infections, stress
Lymphocyte	25–33	Mononucleosis, whooping cough, viral infections
Monocyte	3–9	Malaria, tuberculosis, fungal infections
Eosinophil	1–3	Allergic reactions, autoimmune diseases, parasitic worms
Basophil	<1	Cancers, chicken pox, hypothyroidism

pipette


0.1
2

tips

eppendorf

labeled **BIOHAZARD** container and incinerated
°C) before final disposal or removal from the laborat
azard container must be labeled with the ward "BI
l biohazard symbol and must be leak-proof and pun

The international biohazard symbol

(2)

AWING AND PROCESSING OF VENOUS

tourniquet