Antigen Antibody Reaction

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الدكتورة صابتشرح عل نفس سلايدات التمز
عشان هيك إلي مش موجود عملت له screen shot
وكتبت عليه، وبالآخر حطيت الشرح من تفريغ صيركل
لأنه مش ملحقته اكمله 😐

لا تنسوا زميلنا إلهم الله يرحمه
من دعائكم

Objectives

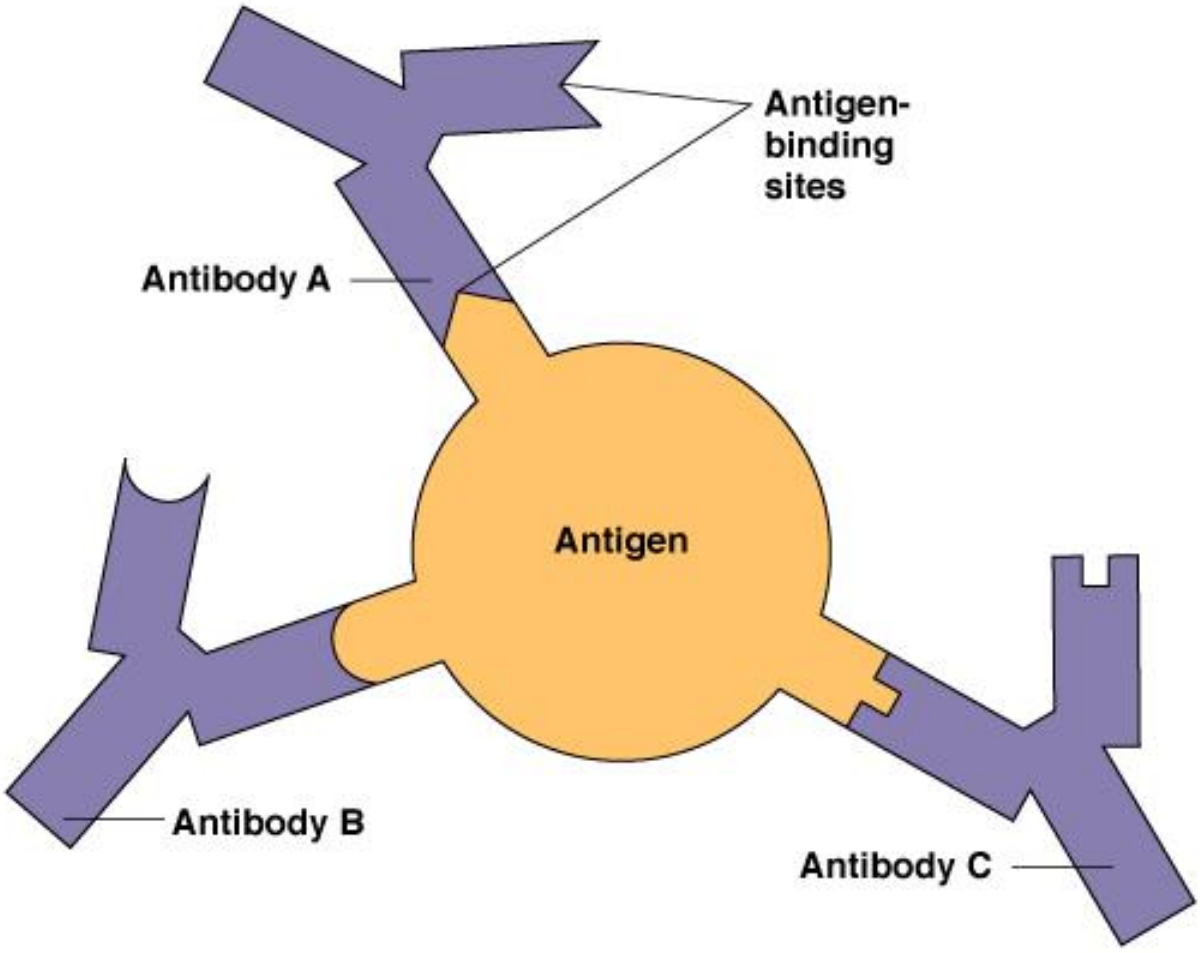
- Discussion of general principles of antigen-antibody interactions
- Definition and importance of affinity, avidity, and cross reactivity
- Laboratory methods used for visualizing antigen-Antibody Reactions

كل إشي بهذا السلايد مراجعه

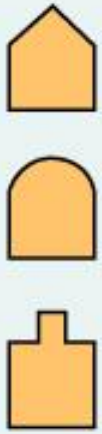
Definitions

حكينا نه الantigen اي chemical قادرة على انها تعمل reactمع الجسم وتعمل immun respond

- Antigen: Any chemical that creates immune response, most are proteins or large polysaccharides
  - Microbes: Capsules, cell walls, toxins, viral capsids
  - Non microbes: Pollen, egg white
- Antibodies: Immunoglobulines that recognize and bind to a particular antigen with high specificity and is made in response to exposure to the antigen
- Epitope: Small part of an antigen that interacts with an antibody (10-12 amino acids). Any given antigen may have several epitopes. Each epitope is recognized by a different antibody



Epitopes
(antigenic
determinants)



Antibody-Antigen Interaction

- The interaction of the antibody with an antigen causes a change in shape of the antibody
- May cause the exposure of another site which then is responsible for the various reactions elicited by the antibody to destroy the foreign substance.
- The interaction of antibodies and antigens may produce a network type complex

Antibody-Antigen Interaction

- The interaction of the antibody with an antigen causes a change in shape of the antibody. This is known as conformational change or induced fit.

- **The Binding Site (Paratope):** Antibodies have a specific binding site for an antigen called the paratope. This site is located at the tips of the Fab (fragment antigen-binding) regions of the antibody.

- **Lock-and-Key vs. Induced Fit**: The initial concept of antibody-antigen binding was based on the "lock-and-key" model, where the antibody's binding site was pre-formed and perfectly complementary to the antigen, like a key fitting into a lock. However, it's now understood that antibodies often undergo conformational changes upon antigen binding.

- **Induced Fit**: In the induced fit model, the antibody's binding site is not perfectly complementary to the antigen before binding. Instead, when the antibody encounters its antigen, the interaction induces a change in the shape of the antibody, allowing the binding site to "mold" itself around the antigen.

لما يصير في ارتباط بين الantigen and antibody ممكن يصير تغير في شكل الantibody(conformational chang) او induce fit
هسا الbinding site الي بنسميه paratope(زي الموجود بالmonomer والpentamore غيرهم) فال paratope هي المكان الي رح يوتبط في الantibodyبالantigen هسا ممكن يرتبطو ببعض بكون زي الlock and key فاذا كانو متناسبين مارح يصير في تغير وفي كلمه ركزت عليها الدكتوره الي هي pre-form
والinduce fit مش زي الkey-lock(يعني مش pre-form) يعني رح يصير في تغير بشكل الreceptor لما يصير ارتباط الantibody والantigen

طيب لما يكون في conformational chang شو رح تكون النتيجة؟بالاول رح تزيد الaffinity (strenth of interaction) عن طريق انها تعمل tighter and more stable binding وبرضه في allosteric effect يعني انه هذا التغير الي صار يمتد من الantibody molecule ويروح لother molecul زي الFC region(للتذكير هي بتكون responsible للbiological activity) وهذا الاشي بأثر على بقدرة الantibody بالتفاعل مع ال other immun componant
واخر نتيجة انه الhiddin site بالreceptor للantibody بصير الها exposure واشهر مثال ال C1q الموجود بالIgG المهم بعملية الcomplemant activation

Antibody-Antigen Interaction

- **Consequences** of Conformational Change: This shape change has several important consequences:

- **Increased Affinity**: The conformational change often results in a tighter and more stable binding between the antibody and the antigen, increasing the affinity of the interaction.

- **Allosteric Effects**: The shape change can propagate through the antibody molecule, affecting other parts of the antibody, such as the Fc region. This can influence the ability of the antibody to interact with other immune components, like complement proteins or Fc receptors on immune cells.

- **Exposure of Hidden Sites**: As mentioned earlier, the conformational change can expose previously hidden sites on the antibody (e.g., the C1q binding site on IgG, which is necessary for complement activation).

Antibody-Antigen Interaction

- May cause the exposure of another site which then is responsible for the various reactions elicited by the antibody to destroy the foreign substance such as complement fixation.

- The interaction of antibodies and antigens may produce a network-type complex known as an immune complex. More specifically, the process of forming these networks is called **immune complex formation** or **cross-linking**, and when it becomes visible it's often referred to as agglutination or precipitation, depending on the nature of the antigen.

ارتباط الantigen مع الantibody بكون complex بنسميه immun
complex والعمليه كلها بنسميها immun complex formation
تو cross linking وبتكرن visible

Nature of Antigen Antibody Reaction

شرحته فوق

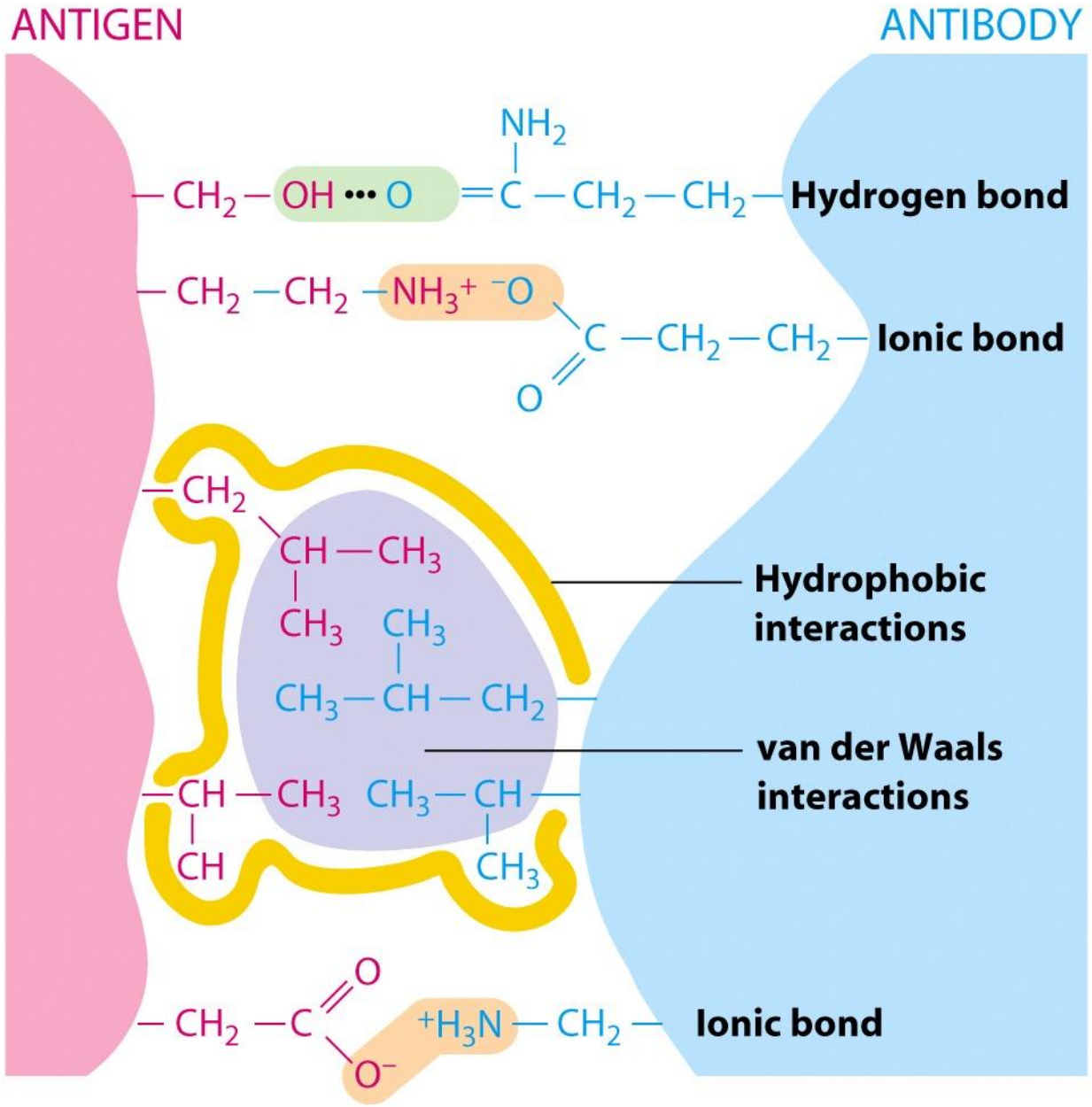
- Lock and Key Concept: The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains

مابدي الbond الي بين الantigen والantibody تكون covalent
لانه مابدي الارتباط يكون ابدي

- Non-covalent Bonds: The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds.
- Reversibility: Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible

مطلوب

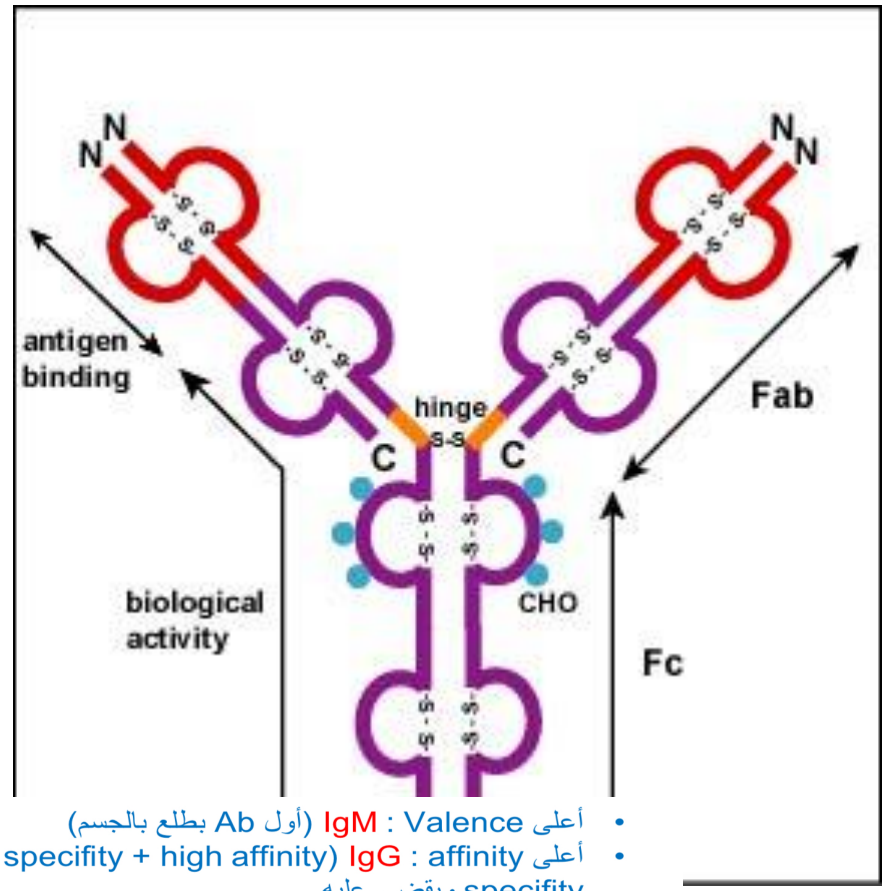
The Ag-Ab interaction is due to lots of non-covalent interactions- lock and key!



شرحناه قبل

Antigen-antibody binding site

- The Fab portion of the antibody has the complementarity-determining regions (red) providing specificity for binding an epitope of an antigen.
- The Fc portion (purple) directs the biological activity of the antibody.
- (S-S = disulfide bond; N = amino terminal of glycoprotein; C = carboxy terminal of glycoprotein; CHO = carbohydra



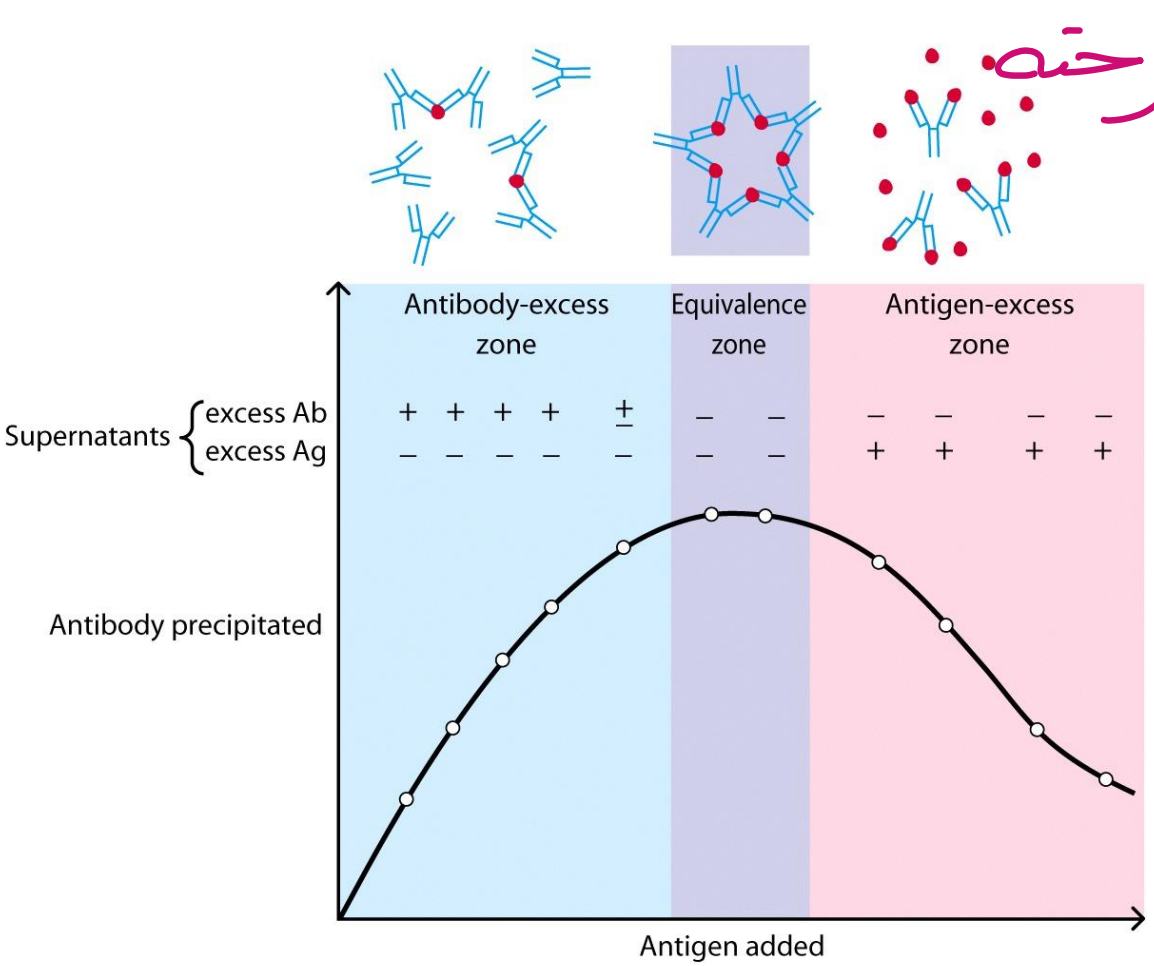
- أعلى Valence : IgM (أول Ab بطلع بالجسم)
- أعلى affinity : IgG (high specifity + high affinity) برتبط بال antigen more specifity وبقضي عليه

ما شرحته

Antibody Binding Variations

- The various genes the cell splices together determine the order of amino acids of the Fab portion of both the light and heavy chain; the amino acid sequence determines the final 3-dimensional shape.
- Therefore, different antibody molecules produced by different B-lymphocytes will have different orders of amino acids at the tips of the Fab to give them unique shapes for binding epitope.
- The antigen-binding site is large enough to hold an epitope of about 5-7 amino acids or 3-4 sugar residues.

Antigen Antibody Binding Equilibrium



Affinity

- Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody
- Affinity is the equilibrium constant that describes the Ag-Ab reaction as illustrated. Most antibodies have a high affinity for their antigens.

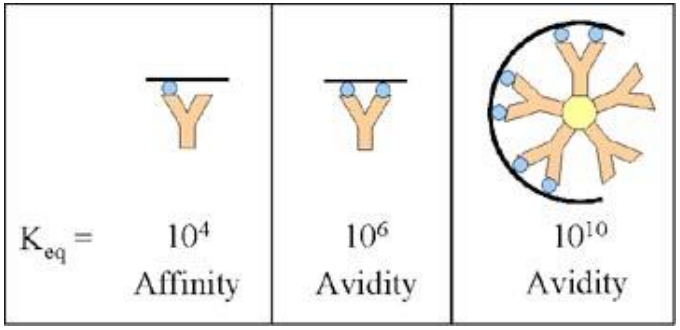
↑ affinity → ↑ Interaction

Antibody Avidity and Valance

- **Affinity** refers to the **strength of binding** between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the **overall strength of binding** between multivalent antigens and antibodies.
- **Valence**: This refers to the **number** of binding sites.
  - **Antibody Valence**: IgG, for example, is divalent (valence of 2) because it has two Fab regions, each capable of binding an antigen. IgM is pentameric (valence of 10, though sterically hindered), meaning it has five antibody monomers joined together, providing ten antigen-binding sites.
  - **Antigen Valence**: An antigen's valence refers to the number of repeating epitopes it presents. A large, complex antigen (e.g., a bacterial cell with numerous identical surface proteins) will have high valence.
- **Avidity**: This is the overall strength of the interaction between an antibody and an antigen. It takes into account BOTH the affinity of the individual binding sites AND the number of binding sites involved (valence).
- **Avidity** is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities.

الantigen valence عدد الrepeted epitop بالantigen فكل ما كان
الantigen اكبر وcomplex اكثر كان الantigen valenc اعلى

Avidity



- Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies.
- Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.
- Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. This is illustrated in the on the next page.

Specificity

قدره ال combining site الي1بالantibody ليتفاعل مع ال antigenic determinant

- Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen.
- In general, there is a high degree of specificity in Ag-Ab reactions. Antibodies can distinguish differences in
  - the primary structure of an antigen
  - isomeric forms of an antigen
  - secondary and tertiary structure of an antigen

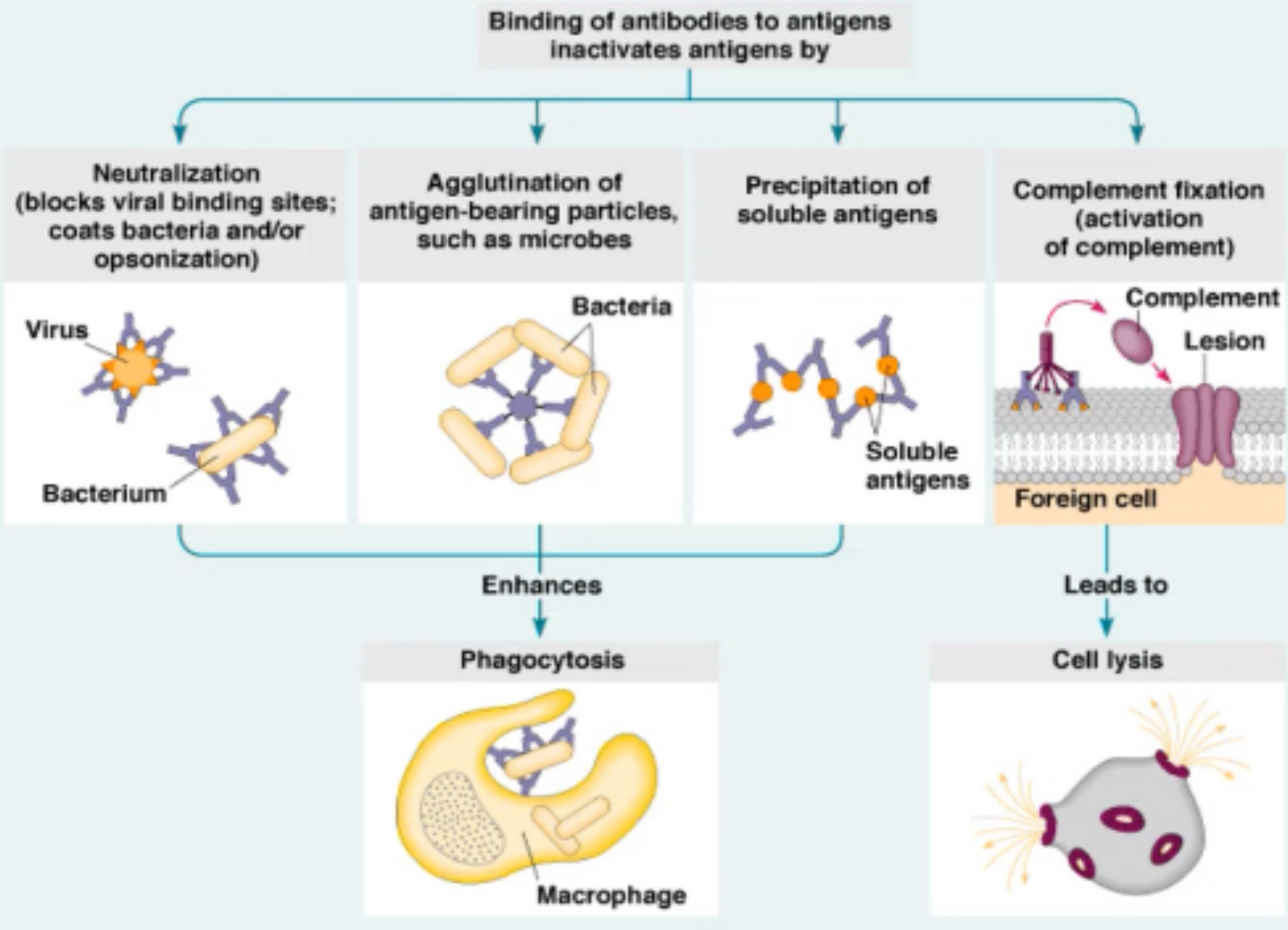
Cross Reactivity

- Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.
- Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

Visualizing Antigen-Antibody Reactions

- Agglutination
- Precipitation
- Complement fixation
- Fluorescent antibody tests
- ELISA and RIA
- Western Blot

Consequences of Antibody Binding

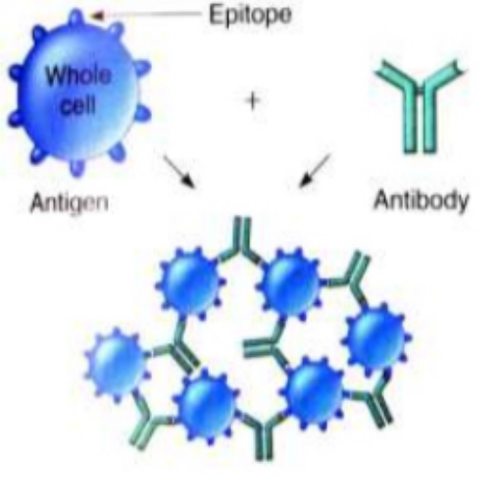


1. Agglutination Testing

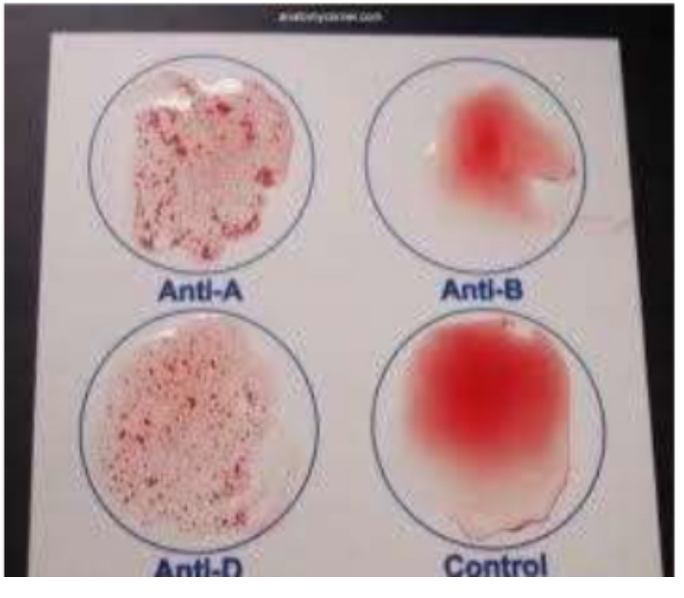
بعمله لما اجي أفحص فصيلة دمي وهو كتير عملي

- Agglutination means the clumping or aggregation of particle.
- Antibodies cross-link the antigens to form visible clumps
- Performed routinely to determine ABO and Rh blood types
- Widal test: tube agglutination test for diagnosing salmonella and undulant fever.
- Latex agglutination tests are used for the rapid detection and identification of various antigens and antibodies in biological samples. It is tiny latex beads with antigens affixed.

بفصيلة الدم بكون على سطح كريات الدم الحمراء الantigen وبالserum بكون الAb



Microscopic appearance of clumps

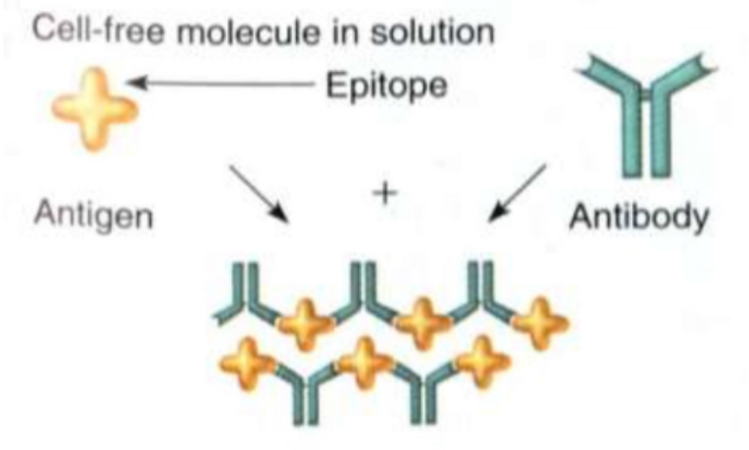


2. Precipitation Tests

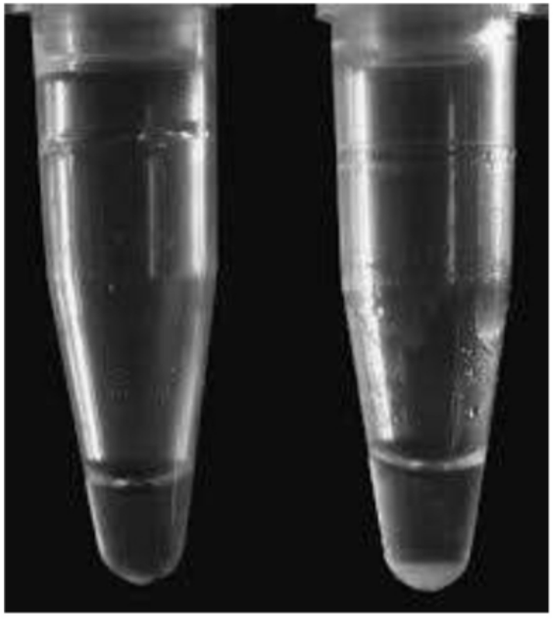
هدول التنين إذا تفاعلوا بترسبوا

- Precipitation is the interaction of a soluble Ag with a soluble Ab to for an **insoluble complex**.
- The complex formed is an aggregate of Ag and Ab
- Reaction is observable as a cloudy or opaque zone at the point of contact
- Example: VDRL (Veneral Disease Research Lab) test.

إذا المريض عملتله فحص الSyphilis (مرض بنتقل جنسيًا) وطلعت النتيجة positive بكون مصاب لأنه في Ab و antigen تفاعلوا مع بعض وانتقلوا من الsoluble للinsoluble

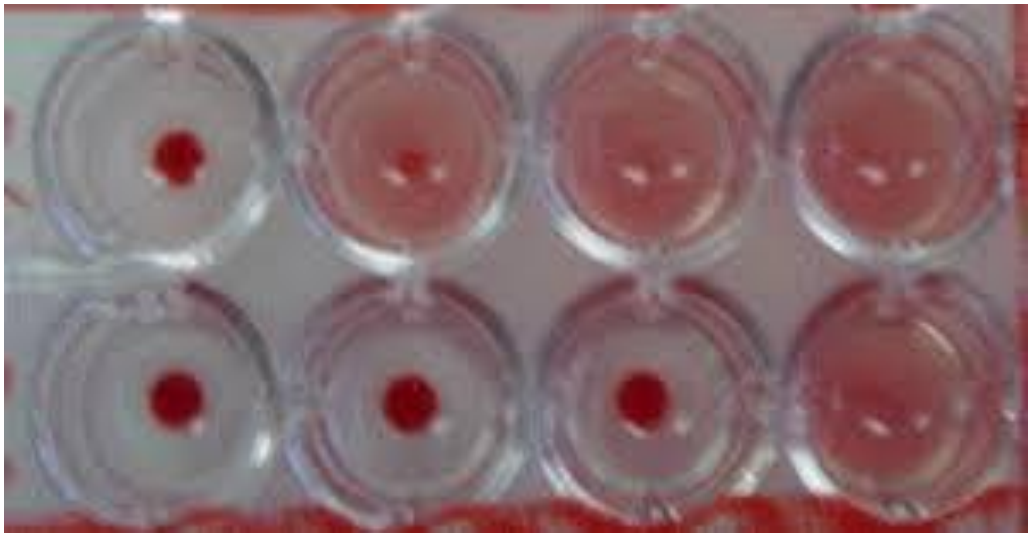


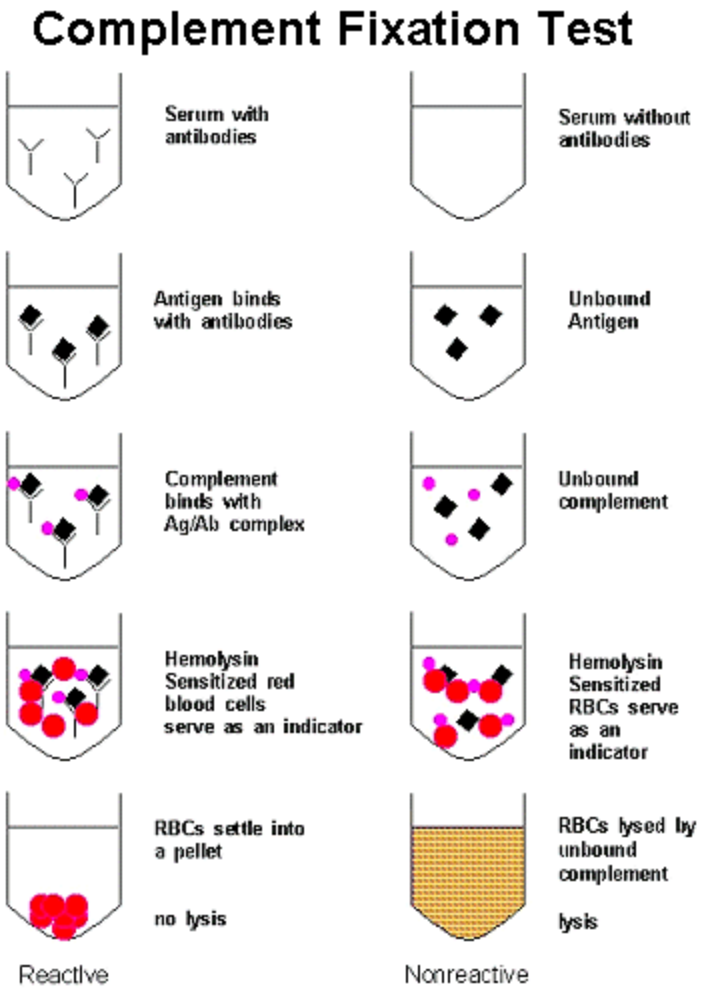
Microscopic appearance of precipitate

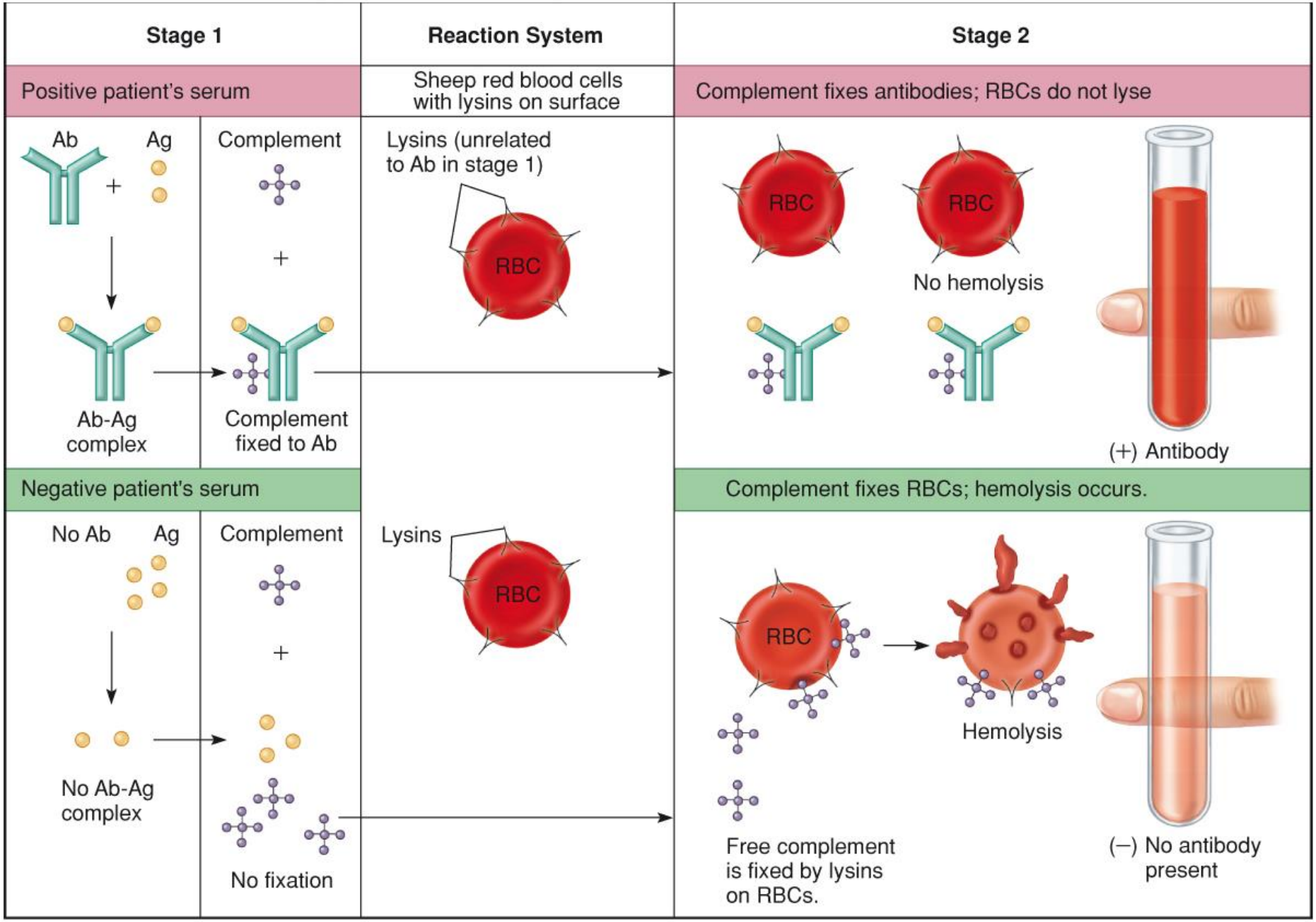


3. Complement Fixation

- Lysin or cytolysin: an antibody that requires complement to complete the lysis of its antigenic target cell






Stage 1
Reaction System
Stage 2
Positive patient's serum
Sheep red blood cells with lysins on surface
Complement fixes antibodies; RBCs do not lyse
Ab
Ag
Complement
Lysins (unrelated to Ab in stage 1)
RBC
RBC
RBC
No hemolysis
Ab-Ag complex
Complement fixed to Ab
(+) Antibody
Negative patient's serum
Complement fixes RBCs; hemolysis occurs.
No Ab
Ag
Complement
Lysins
RBC
RBC
Hemolysis
No Ab-Ag complex
No fixation
Free complement is fixed by lysins on RBCs.
(–) No antibody present

Stage 1 (Positive patient's serum):

بكون الشخص مصاب بكون بالدم عنده Ab وantigen الاثنين بتفاعلوا مع بعض وبتكون الcomplex
بعدين بيجي معهم الcomplement protein برتبط بالFc region فبصير عنا complement
fixation فالcomplex بصير مكون من Ab وantigen وcomplement protein .

Stage 2 (complement fixes antibodies: RBCs do not lyse)

هون العينة بتكون حمراء ما تغيرت .

Stage 2 (complement fixes RBCs: hemolysis occurs)

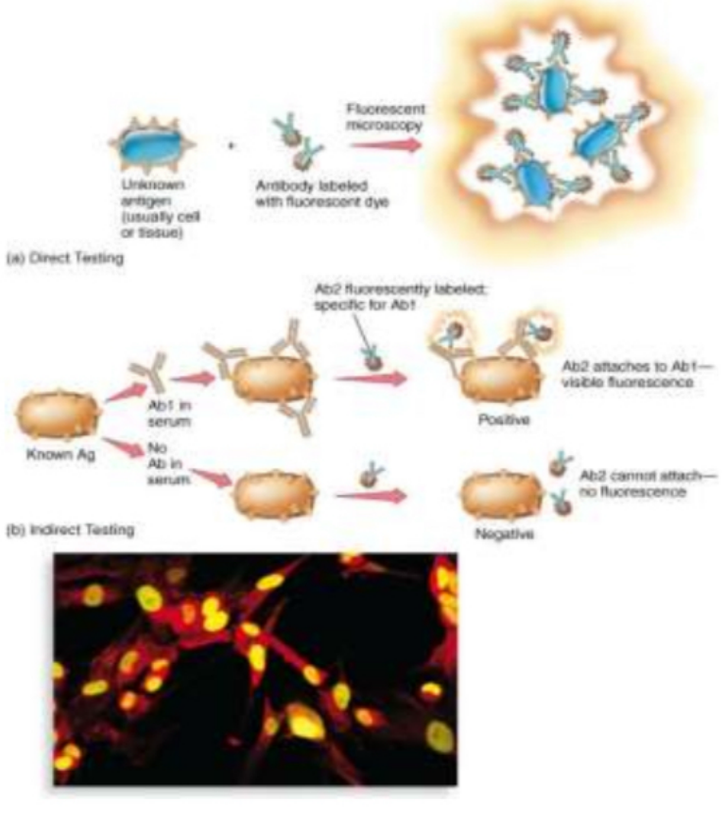
هون مش مصاب المريض في عندي الantigen-Ab complex ما ارتبطوا مع بعض اذن ما عندي
complement protein مرتبط فهو بكون حر فبروح برتبط بالlysin الي عالRBC فبعمل rupture
للRBC فعينة الدم بصير لونها على صفار .
*الlysin بحتاج complement protein حتى يشتغل .

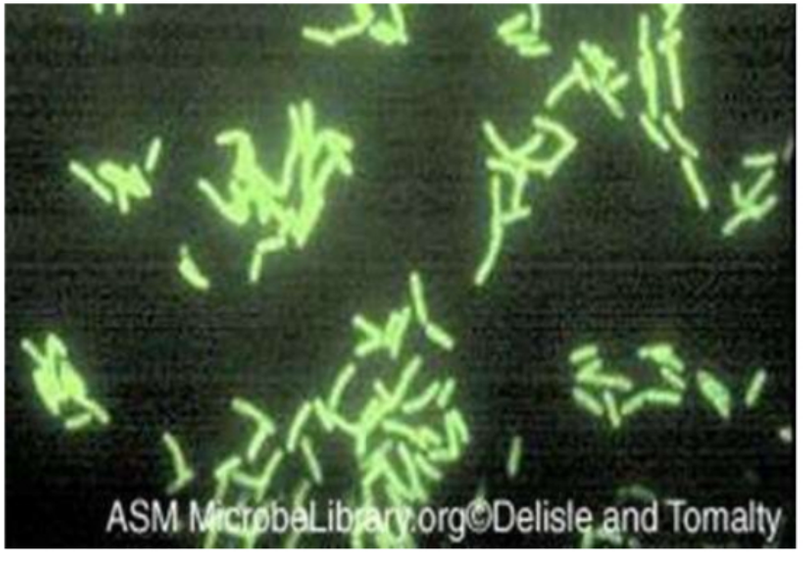
4. Fluorescent Antibodies and Immunofluorescence Testing

هون انا بكون بدي اعرف هل في antigen او لا فبروح بجيب الantigen بثبته على slide وبجيب الflourescent Ab solution وبخلي الضوء خافت بالغرفة لانه الflourescent Ab اذا حطيته على ضوء الغرفة بتكسر بعدين منقط أكم نقطة حسب الprocedure منخليه شوي بعدين منشوف إذا اعطاني إشعاع اذن في detection (الإشعاع ما بصير الا اذا ارتبط الAb بالantigen)

- **Direct testing**: an unknown test specimen or antigen is fixed to a slide and exposed to a fluorescent antibody solution of known composition
- **Indirect testing**: the fluorescent antibodies are antibodies made to react with the Fc region of another antibody

هون الAb الي عليه fluorescent dye ما رح يرتبط بالantigen وانما رح يرتبط بالAb الموجود بالFc region المربوط بالantigen .



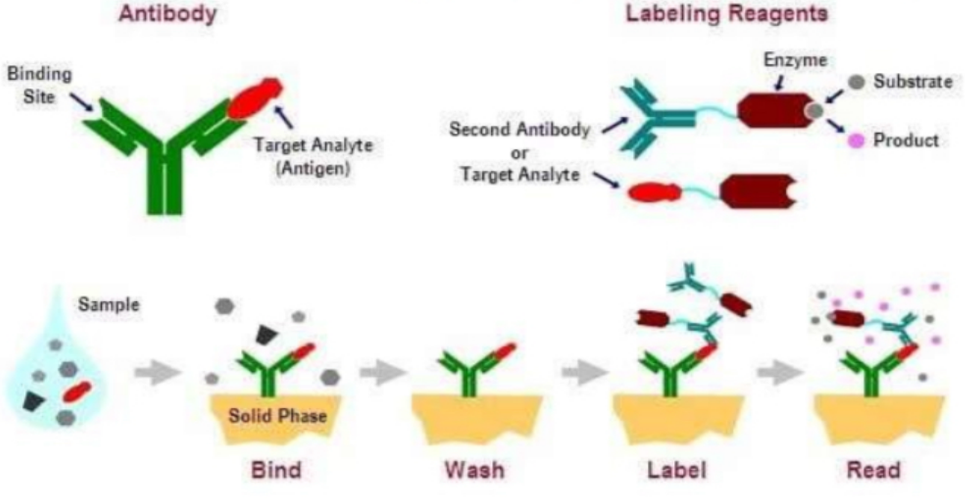


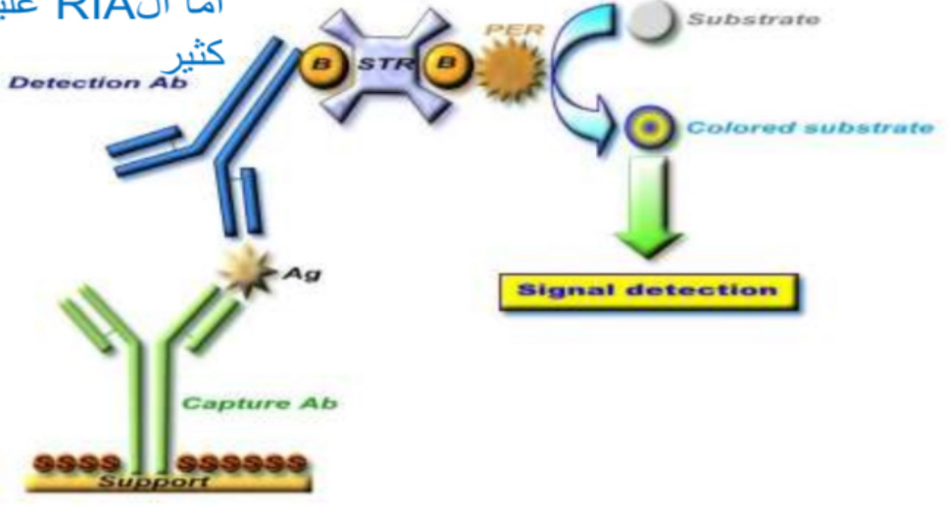
5. Radioimmunoassay (RIA)
Enzyme-Linked Immunosorbent Assay (ELISA)

زي لما يجوا يفحصوا السرطان الAb بربطوه بisotopes حتى نعرف وين الlocalization للسرطان
أو مثلا لما يفحصوا نشاط الغدة الدرقية بشربوا الشخص سائل بتحرك بالجسم بعدين بالاشعة بشوفوا الisotopes اذا كانت functional ولا non-funtional

- Antibodies or antigens labeled with a radioactive isotope (RIA) or Enzyme (ELISA) used to pinpoint minute amounts of a corresponding antigen or antibody
- Compare the amount of radioactivity present in a sample before and after incubation with a known, labeled antigen or antibody.

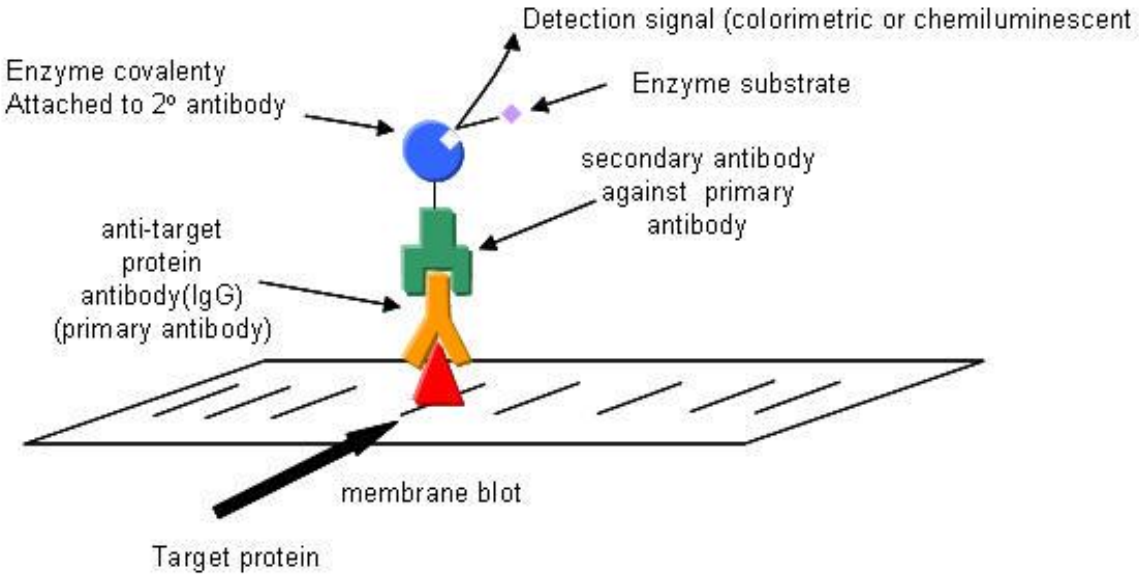
ELISA ← كتير بستخدموه بالمختبرات
أما الRIA عليه محاذير كثير وبكلف برضو ومش متوفر كثير

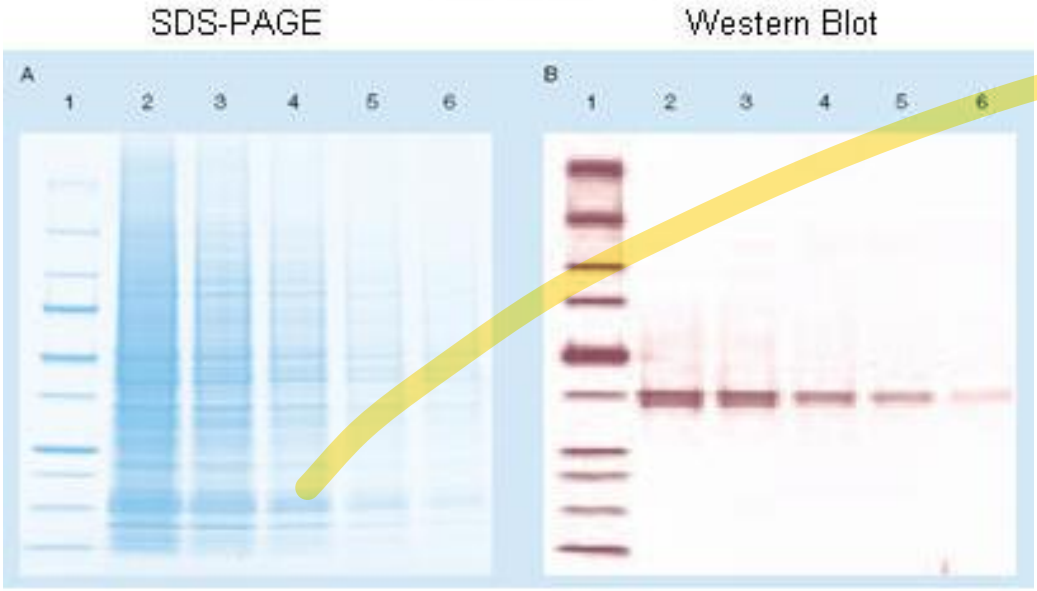


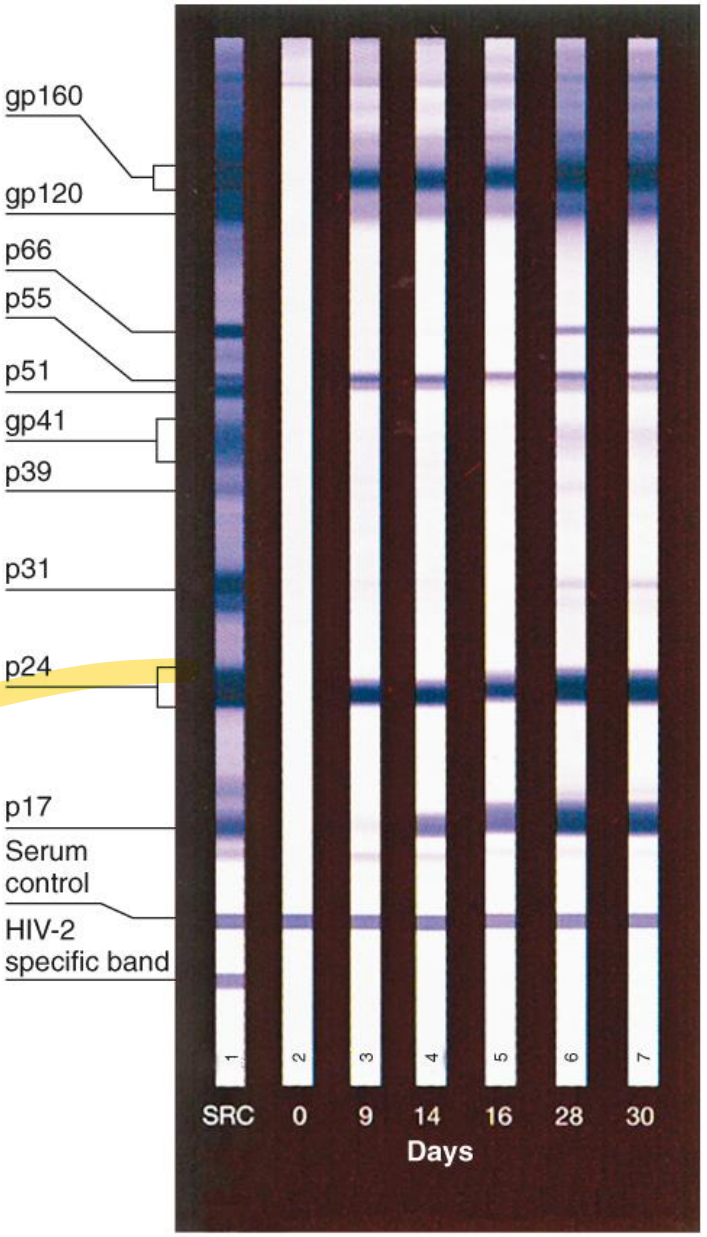


6. The Western Blot for Detecting Proteins

- Test material is electrophoresed in a gel to separate out particular bands
- Gel transferred to a special blotter that binds the reactants in place
- Blot developed by incubating it with a solution of antigen or antibody labeled with radioactive, fluorescent, or luminescent labels

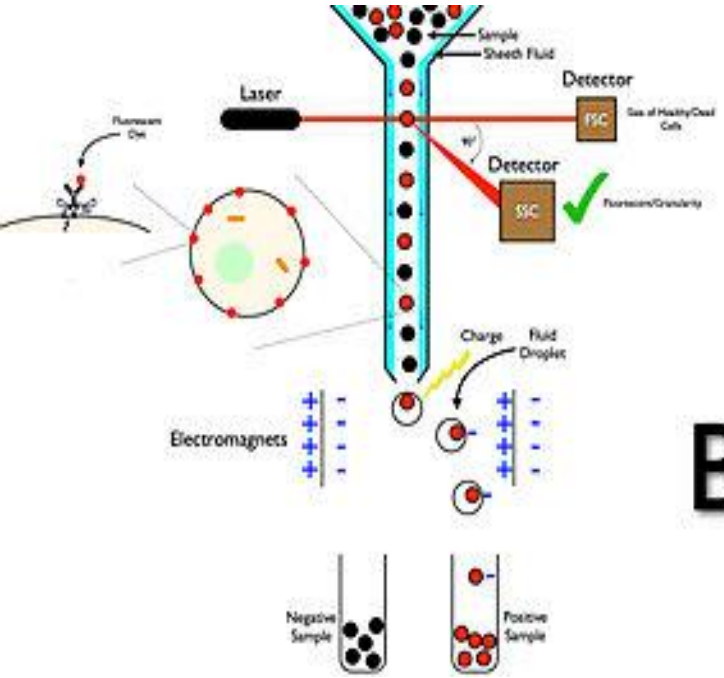




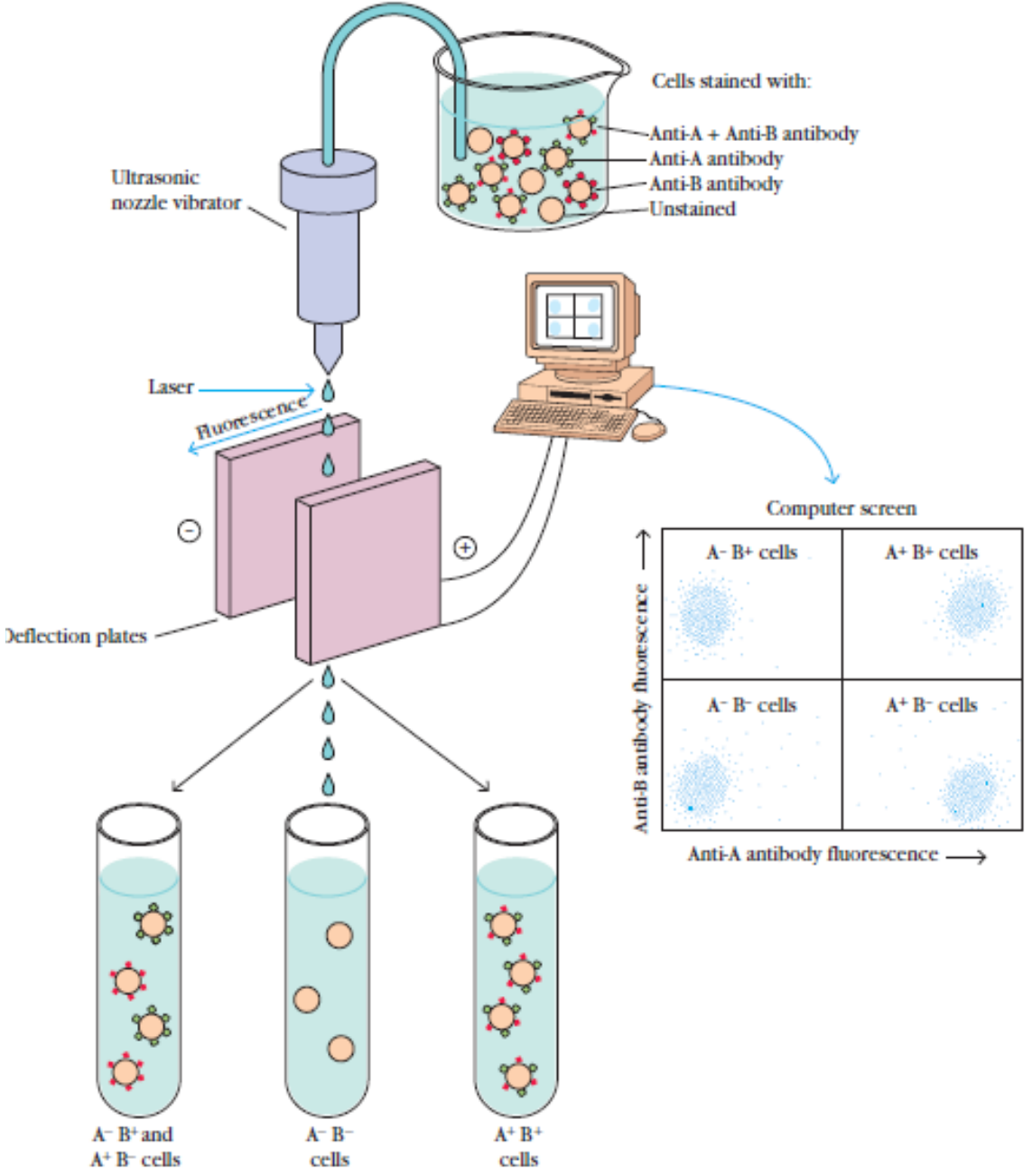


7. Flow Cytometry

- The flow cytometer was designed to automate the analysis and separation of cells stained with fluorescent antibody
- The flow cytometer uses a laser beam and light detector to count single intact cells in suspension
- Every time a cell passes the laser beam, light is deflected from the detector, and this interruption of the laser signal is recorded
- Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam
- It has large number of medical application for example in classification and treatment of leukemias


Sample
Sheath Fluid
Laser
Detector
FSC
Detector
SSC
Charge
Fluid Droplet
Electromagnets
Negative Sample
Positive Sample

B


Cells stained with:
Anti-A + Anti-B antibody
Anti-A antibody
Anti-B antibody
Unstained
Ultrasonic nozzle vibrator
Laser
Fluorescence
Deflection plates
Computer screen
A⁻ B⁺ cells
A⁺ B⁺ cells
A⁻ B⁻ cells
A⁺ B⁻ cells
Anti-B antibody fluorescence
Anti-A antibody fluorescence
A⁻ B⁺ and A⁺ B⁻ cells
A⁻ B⁻ cells
A⁺ B⁺ cells