

MIRACLE Academy

قال تعالى (يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ)

تفريغ المناة
زميلتكم لين قطيشات



لجان الرفعات

Antigen Antibody Reaction

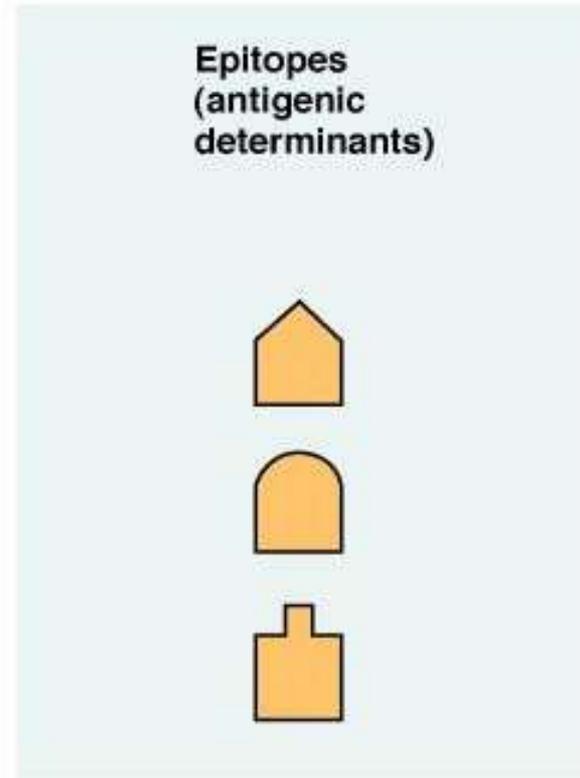
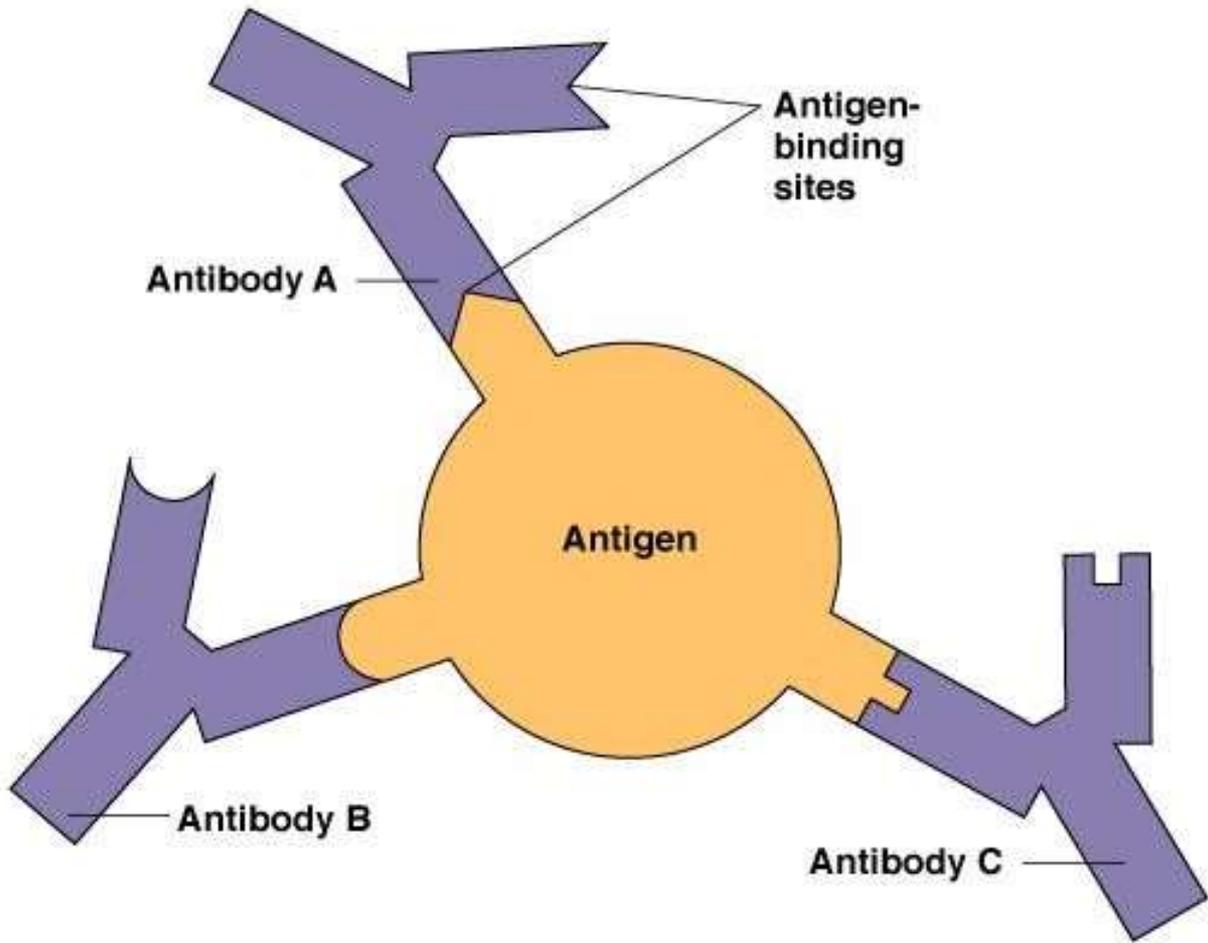
Objectives

- 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

Definitions

مراجعة

- 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 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



- بس يرتبط الـ Ab بالـ antigen رح يصير في تغيير بالـ structure تبع الـ Ab منسميه **Conformational change** بالـ Ab وبصير هاد التغيير حتى يساعد على ارتباط الـ antigen بالـ Ab
- كل monomer يرتبط بـ 2 antigen
- كل Ab يختلف عن الثاني بالـ Fc region لأنه كل واحد الـ Function مختلف عن الثاني
- **Lock and key vs. Induced Fit**
 - * بالـ **Lock and Key** يكون عنا already الـ Ab pre-formed مجهز وبروح يرتبط بالـ antigen
 - أما بالـ **Induced Fit** يكون لسا الـ Ab مش perfectly complement للـ antigen لكن بس يتفاعل الـ Ab مع الـ antigen بصير عنا conformational change بتغير الـ Ab فبصير Fit exactly مع الـ antigen .

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.

Antibody-Antigen Interaction

بترتب عليه انه يزيد ال affinity لل antigen-Ab reaction

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

Strength of binding

- **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.

هنا ال conformational change يمتد لأجزاء ثانية من ال Ab فبخلي ال Ab عنده قدرة يروح يرتبط مع other immune component زي ال complement protein

- **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.

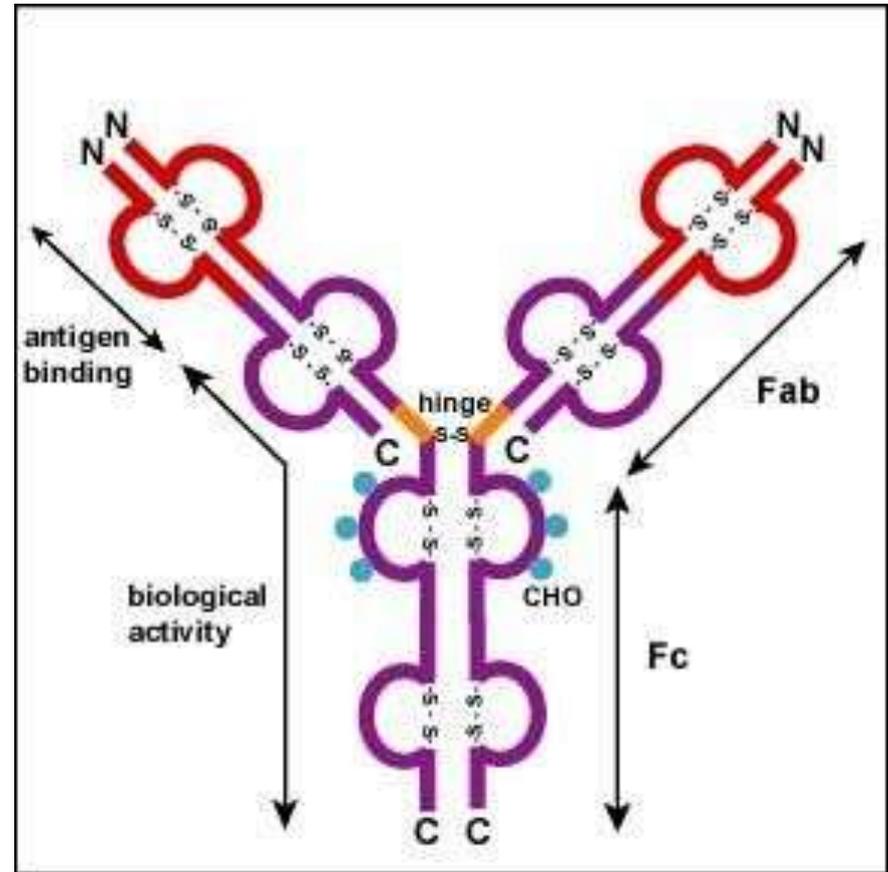
Nature of Antigen Antibody Reaction

- **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
- **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**

الارتباط بين الantigen والAb يكون reversable وأخذنا انو الbond الـ بتكون reversable الي هي
الnon-covalent bond (زي hydrogen bonds , electrostatic bonds , Van der Waals forces ,
(hydrophobic bonds,

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 = carbohydrate.)



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

Antibody affinity

الbinding يكون non-covalent اذن هو مجموع ال net forces اشي يكون attractive واشي repulsive فالمحصلة النهائية هي بتحددلي ال affinity فإذا كانت more attractive than repulsive معناته ال affinity بتكون أعلى .

- **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. Most antibodies have a high affinity for their antigens.
- A higher affinity means a stronger interaction.

Antibody Avidity and Valence

- **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.

كل ما كان الantigen كبير ومعد أكثر كان يحتوي على epitope أكثر فبالتالي الAb التي بتكوّن
بكون عنده valence أكثر.
والAb التي له affinity أعلى و valence أعلى بكون الAvidity له عالية .

Antibody Specificity

- **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

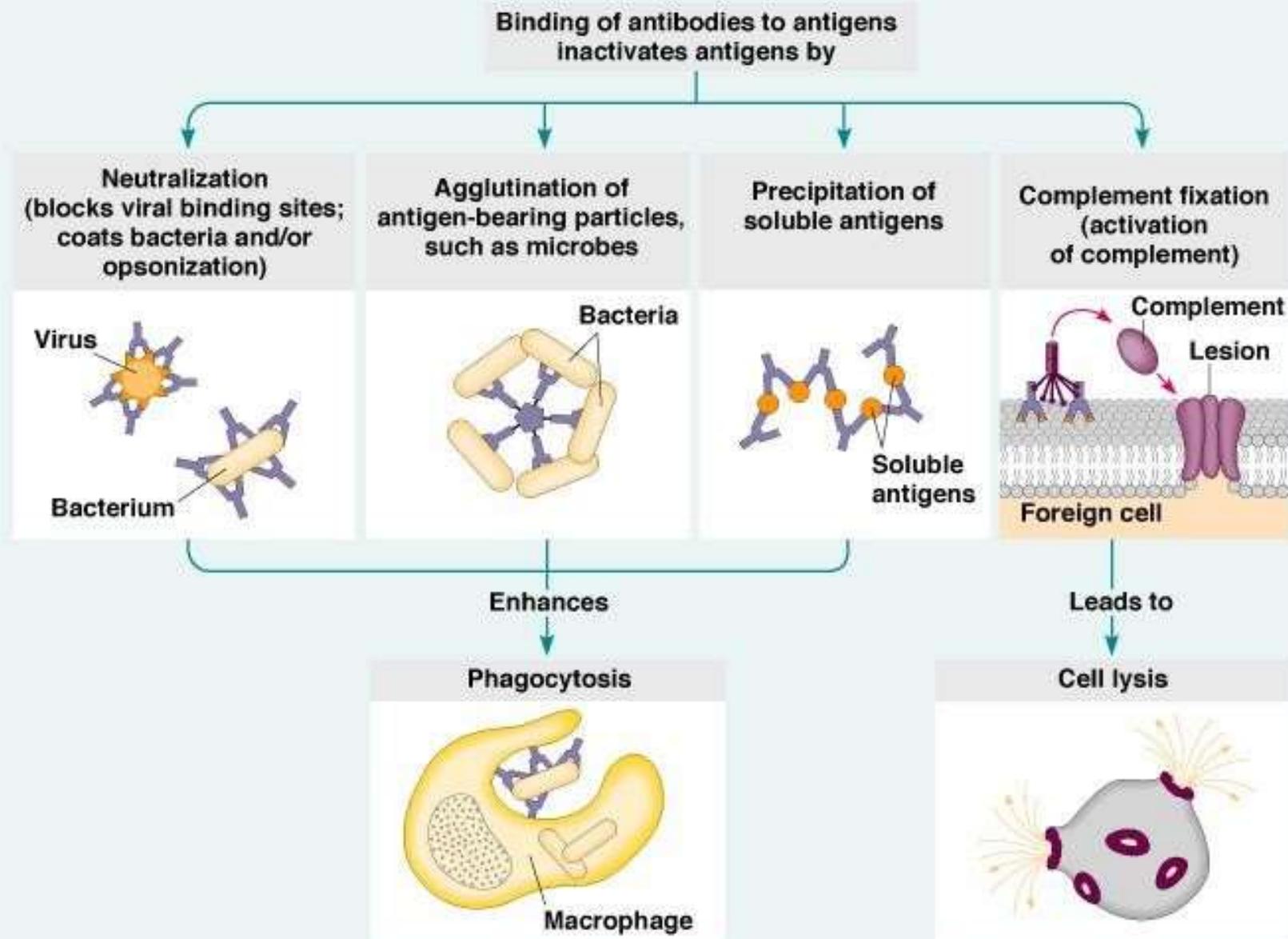
Cross Reactivity

يعني الـ Ab المفروض يروح يتفاعل مع antigen معين لكن بييجي antigen ثاني كثير بشبهه بروح الـ Ab بتفاعل معه وزى ما حكينا بالشابتر الي قبل انه هاد الاشي ايجابي ومفيد في حالة لما ناخذ المطعوم باخذ مناعة مش بس من المرض الي أخذت المطعوم عشانه برضو أعطاني مناعة للأمراض تانية وممكن يكون ضار إذا هاد الـ Ab راح على antigen طبيعي موجود بجسمي هون بصير autoimmune disease

- **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 (multi-specificity).

Consequences of Antibody Binding

تلخيص



Visualizing Antigen-Antibody Reactions

• Agglutination → Clumping and aggregation

• Precipitation ترسب

• Complement fixation →

انه يكون عندي antigen-Ab complex بروح
يعمل stimulation لل precursor protein تبع
ال complement system وبصيرلها
activation وبتروح ترتبط بال Fc portion تبعت
ال Ab

• Fluorescent antibody tests

• ELISA and RIA

• Western Blot

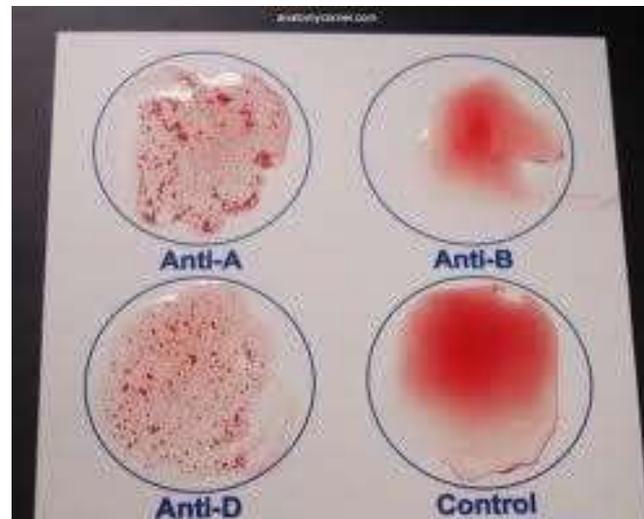
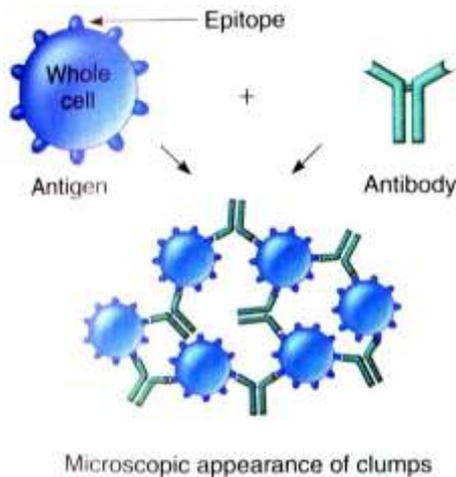
بستخلص البروتين من عينة الدم

ال Ab بربطه ب flourescent peptide فإذا
كان في antigen بعرضه ل Ab الي يكون معاه
المشع إذا ارتبط بال antigen وأخذت image
فأكيد رح يطلع إشعاع بما انه صار ارتباط بينهم
فهاد الاشئ منستدل من خلاله انه عنا
antigen-Ab reaction

1. Agglutination Testing → بعمله لما اجي أفحص فصيلة دمي وهو كثير 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

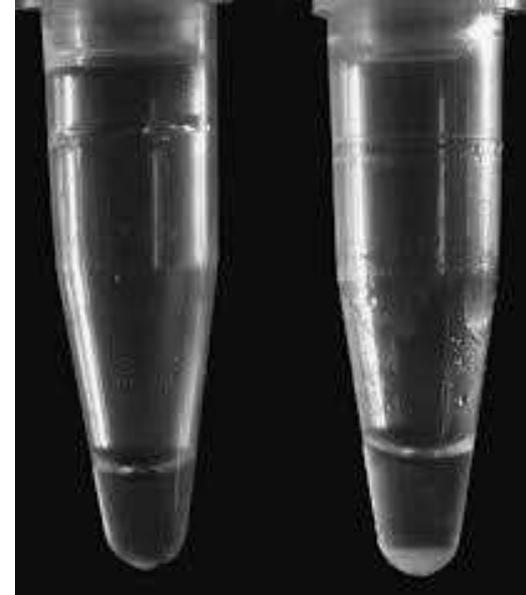
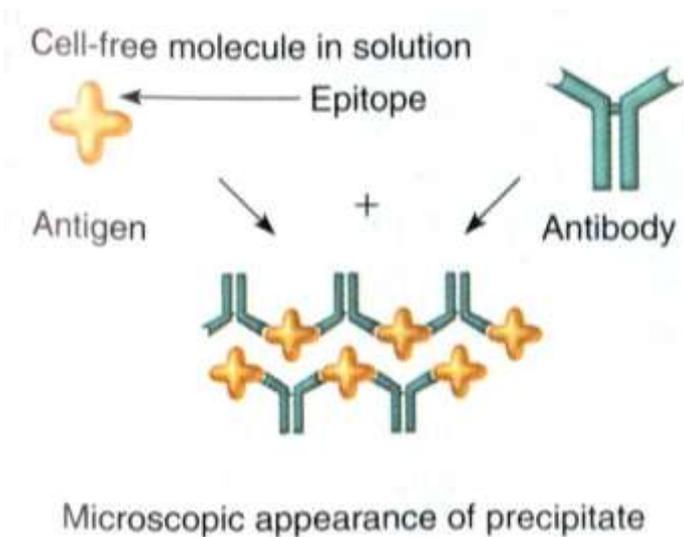


2. Precipitation Tests

هدول التتين إذا تفاعلوا بترسبوا

- Precipitation is the interaction of a **soluble Ag** with a **soluble Ab** to form 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 (Venereal Disease Research Lab) test**.

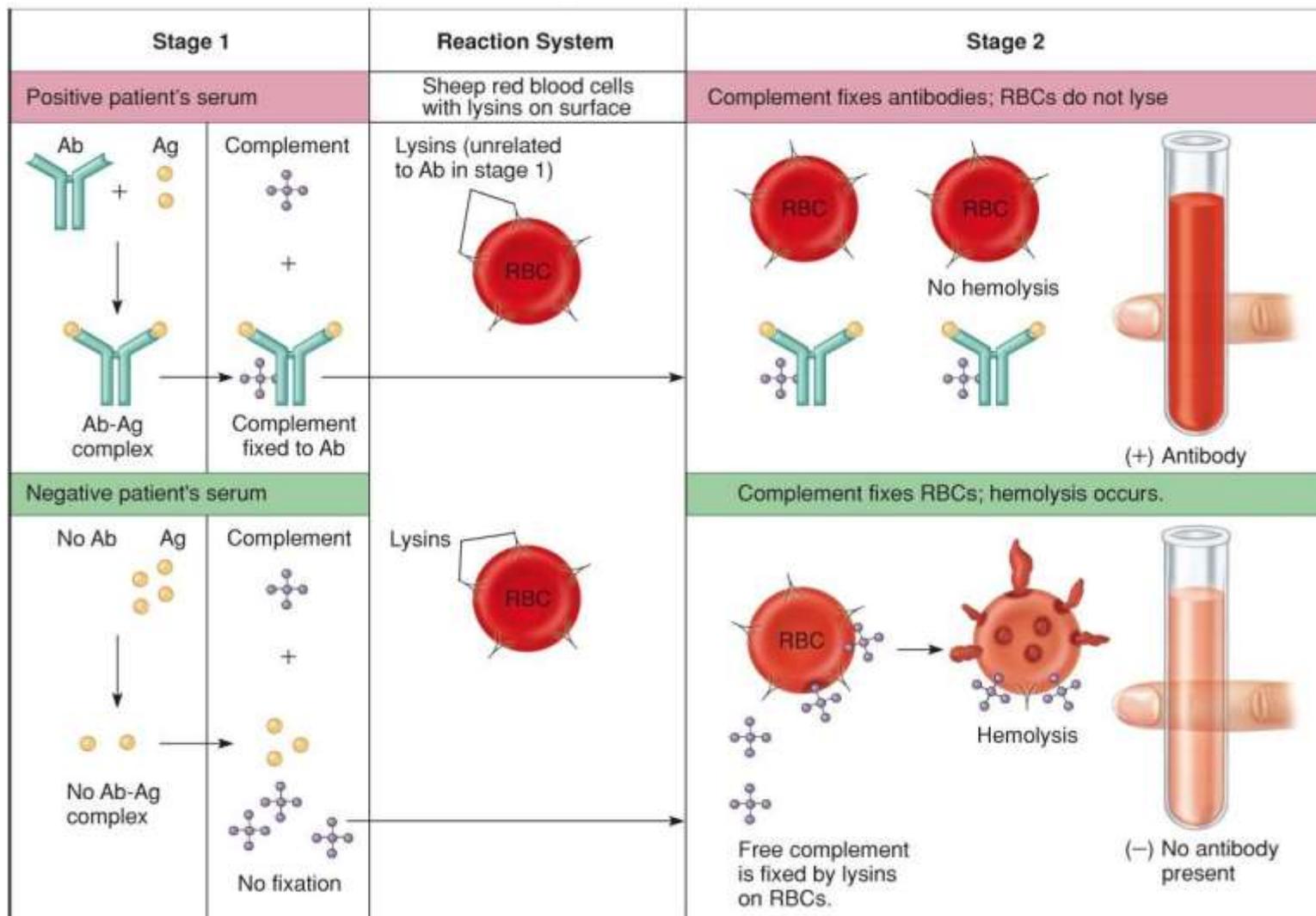
إذا المريض عملته فحص ال Syphilis (مرض ينتقل جنسيًا) وطلعت النتيجة positive يكون مصاب لأنه في Ab و antigen تفاعلوا مع بعض وانتقلوا من ال soluble لل insoluble



3. Complement Fixation

شرحها بالاسلايد الجاي

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



Stage 1 (Positive patient's serum):

بكون الشخص مصاب بكون بالدم عنده Ab و antigen الاثنين بتفاعلوا مع بعض وبتكون ال complex بعدين بيجي معهم ال complement protein برتبط بال Fc region فبصير عنا complement fixation فال complex بصير مكون من Ab و antigen و complement 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 ال antigen-Ab complex ما ارتبطوا مع بعض اذن ما عندي complement protein مرتبط فهو بكون حر فبروح برتبط بال lysin الي عال RBC فبعمل rupture لل RBC فعينة الدم بصير لونها على صفار .

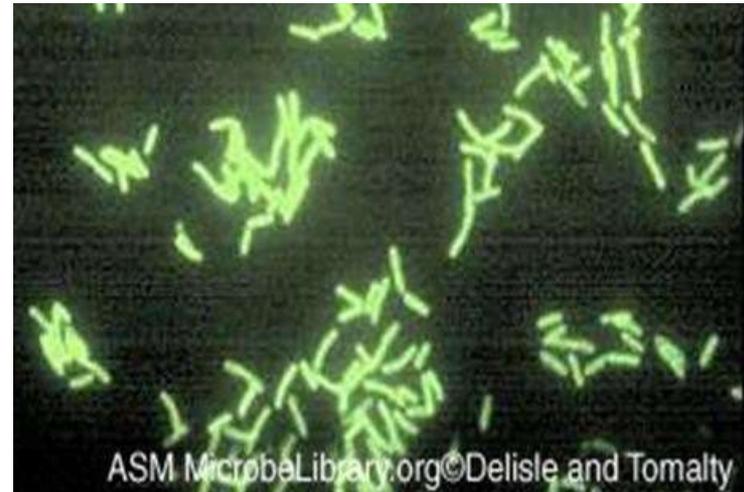
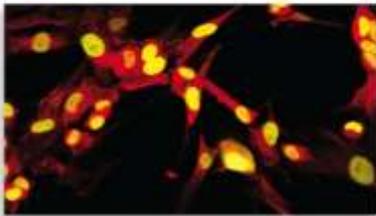
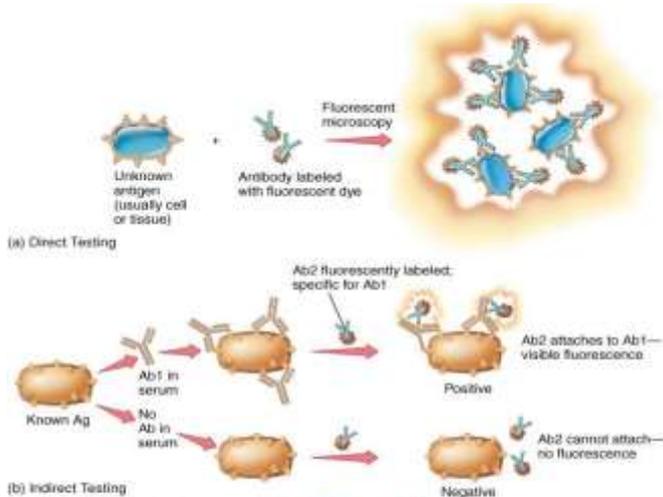
ال* lysin بحتاج complement protein حتى يشتغل .

4. Fluorescent Antibodies and Immunofluorescence Testing

هون انا بكون بدني اعرف هل في antigen او لا فيروح بجيب ال antigen بثبته على slide وبجيب ال fluorescent Ab solution وبخلي الضوء خافت بالغرفة لانه ال fluorescent Ab اذا حطيته على ضوء الغرفة بتكسر بعدين منقط اكم نقطة حسب ال procedure منخله شوي بعدين منشوف إذا اعطاني إشعاع اذن في detection (الإشعاع ما بصير الا اذا ارتبط ال Ab بال antigen)

- **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 ال Ab الي عليه fluorescent dye ما رح يرتبط بال antigen . وانما رح يرتبط بال Ab الموجود بال Fc region المربوط بال antigen .

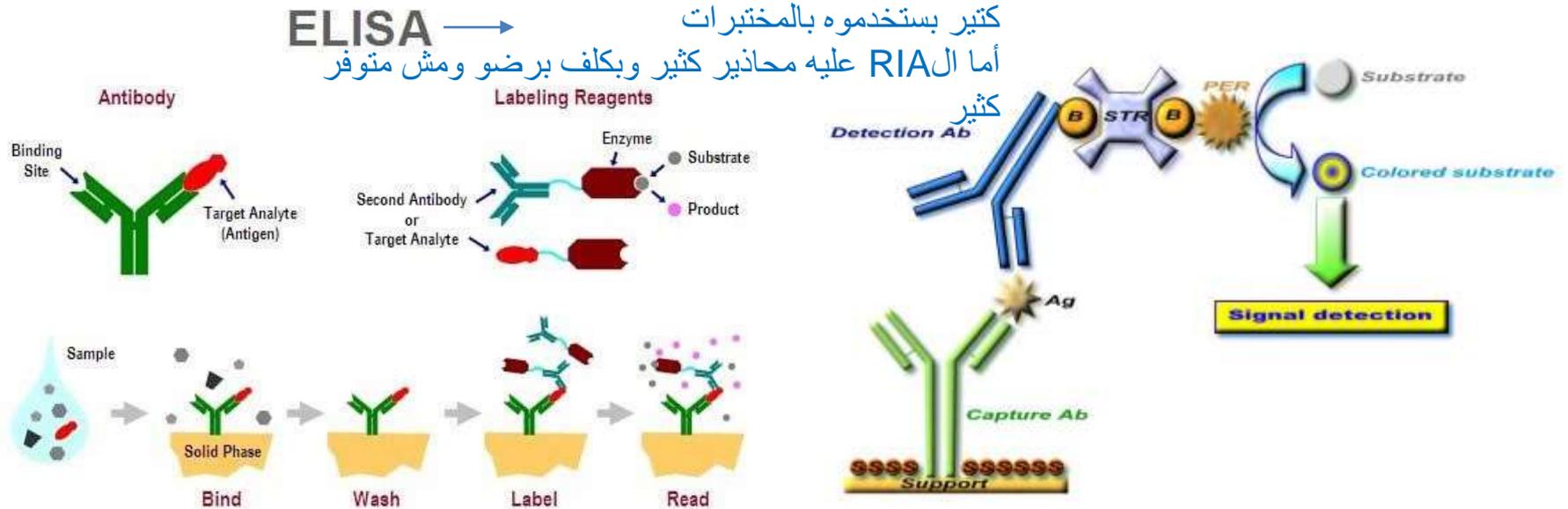


5. Radioimmunoassay (RIA)

Enzyme-Linked Immunosorbent Assay (ELISA)

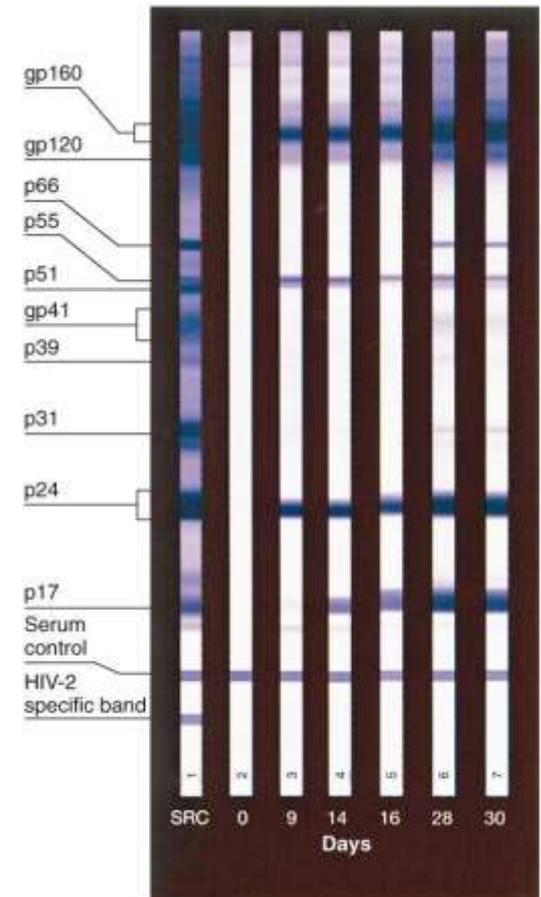
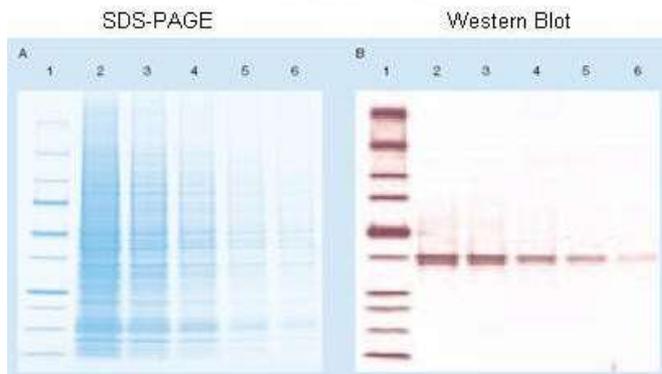
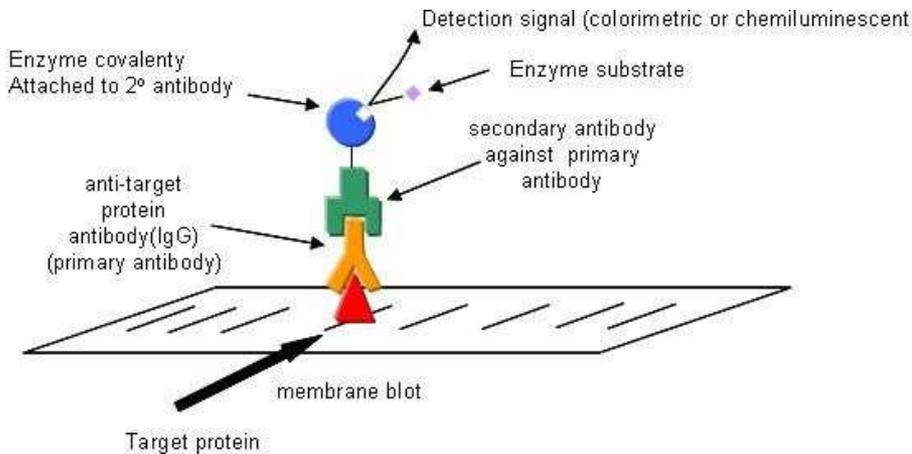
زي لما يجوا يفحصوا السرطان الـ Ab بربطوه بـ isotopes حتى نعرف وين الـ localization للسرطان
أو مثلا لما يفحصوا نشاط الغدة الدرقية بشربوا الشخص سائل بتحرك بالجسم بعدين بالاشعة بشوفوا الـ isotopes اذا كانت
functional ولا non-functional

- 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.



6. The Western Blot for Detecting Proteins M.Wt بعتمد عال

- 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

مش داخل اعرفوه فقط
كمسمى

- 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

لو هم أحدكم بإزالة جبل وهو واثق بالله لأزاله
دعواتكم ♡

