Antigen – Antibody Reactions

The antigen-antibody reactions are reactions between specific antigens and matching antibodies that occur in a laboratory under controlled conditions (*in vitro*). These reactions form the basis of immunological tests or **immunoassays** used for determining the presence of a variety of antigens or antibodies in samples. Since antibodies can bind with high specificity many different antigens, such as structural molecules of microbes and their secreted products or products of immune and other cells.

In order to detect an antigen of interest in a given sample, an antibody specific for that antigen should be used. However, if the goal is to detect antibodies in the sample, in addition to a known antigen, another antibody must be added as a reagent in the reaction. This antibody is specific for the epitopes of Fc fragment (constant region of heavy immunoglobulin chain) of an antibody of a particular isotype (class), such as γ-chain in the case of IgG detection, and, therefore, it is called anti-immunoglobulin antibody or anti-antibody.

Anti-immunoglobulin antibodies are sometimes also referred to as the secondary antibodies, considering the fact that the antibodies that bind directly to an antigen are, so called, primary antibodies.These anti-immunoglobulin antibodies are usually obtained from animals that are immunized with immunoglobulins of another species (typically human) and they not only enable detection of specific antibodies in a sample, but also allow determining their class, which can help in diagnosis of many diseases.

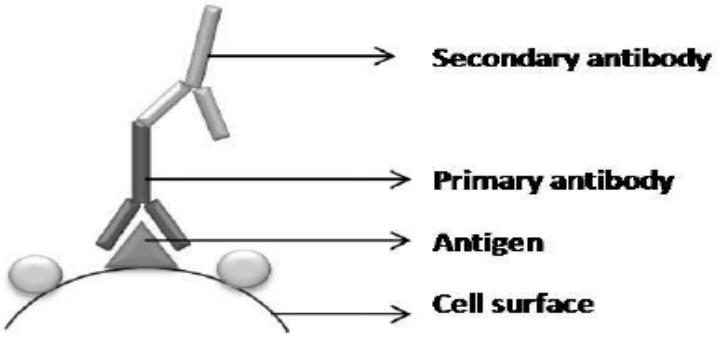
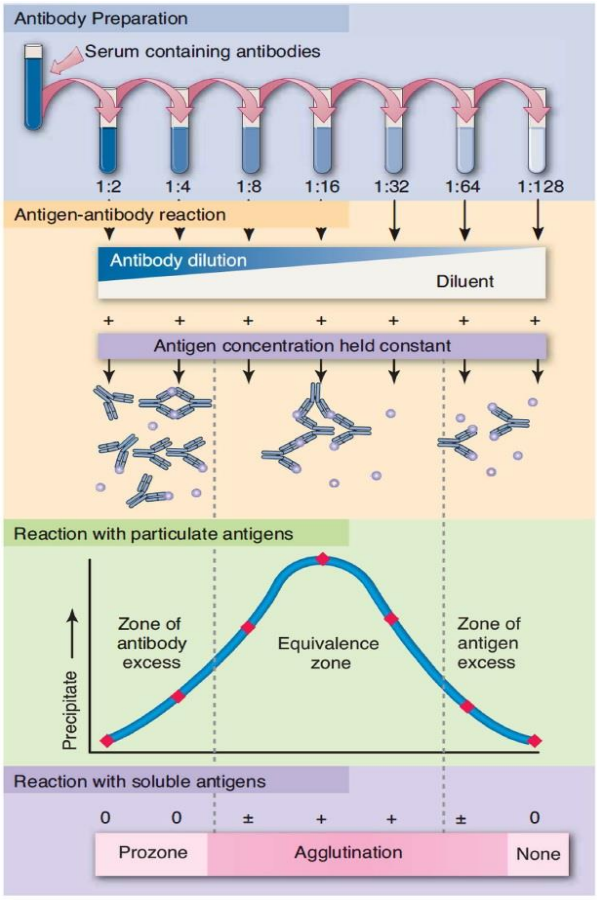
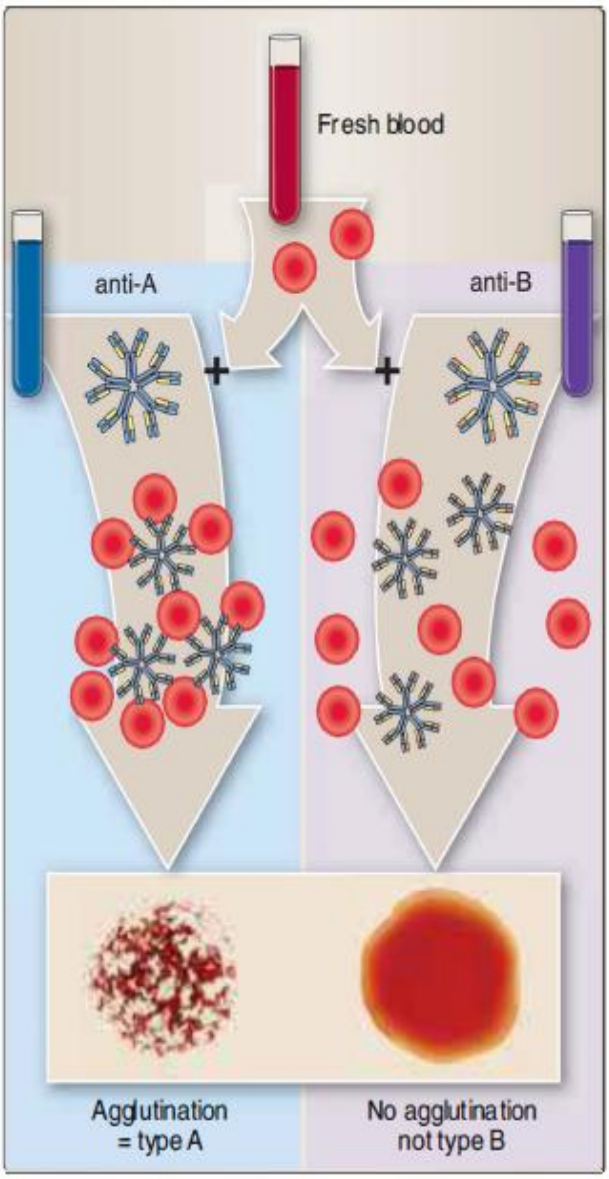


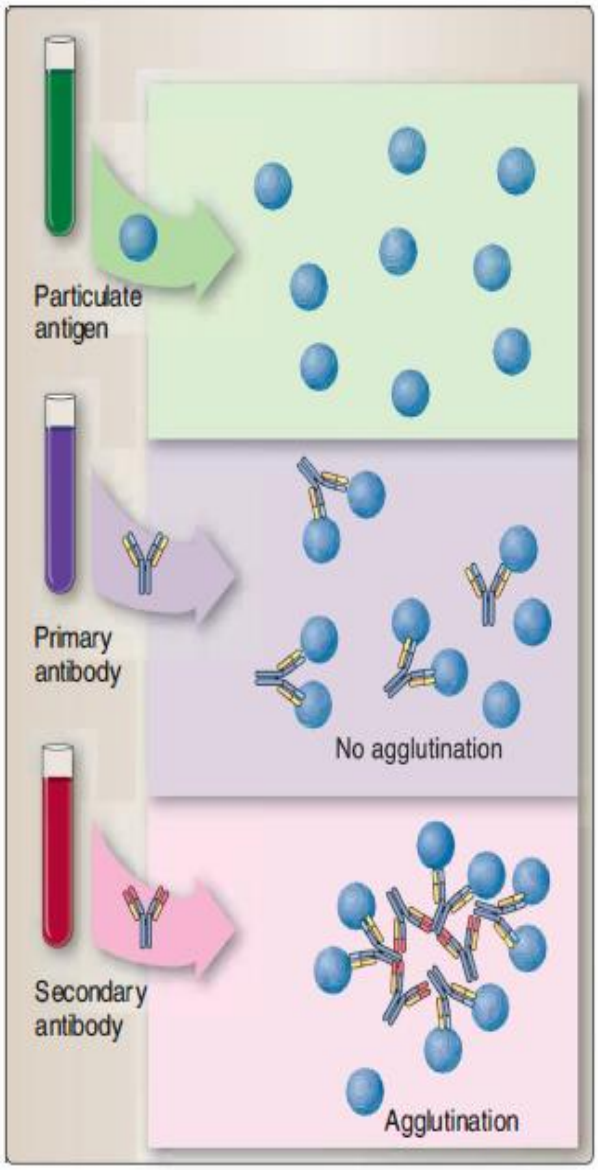
Figure 1. Primary and secondary antibody



1.Immunoagglutination (often simply called agglutination) is a laboratory diagnostic test based on the reaction between a particular antigen and the matching specific antibody. qualitative tests. specificity and sensitivity and they are inexpensive and easy to perform.

1.Direct agglutination **2.Indirect**

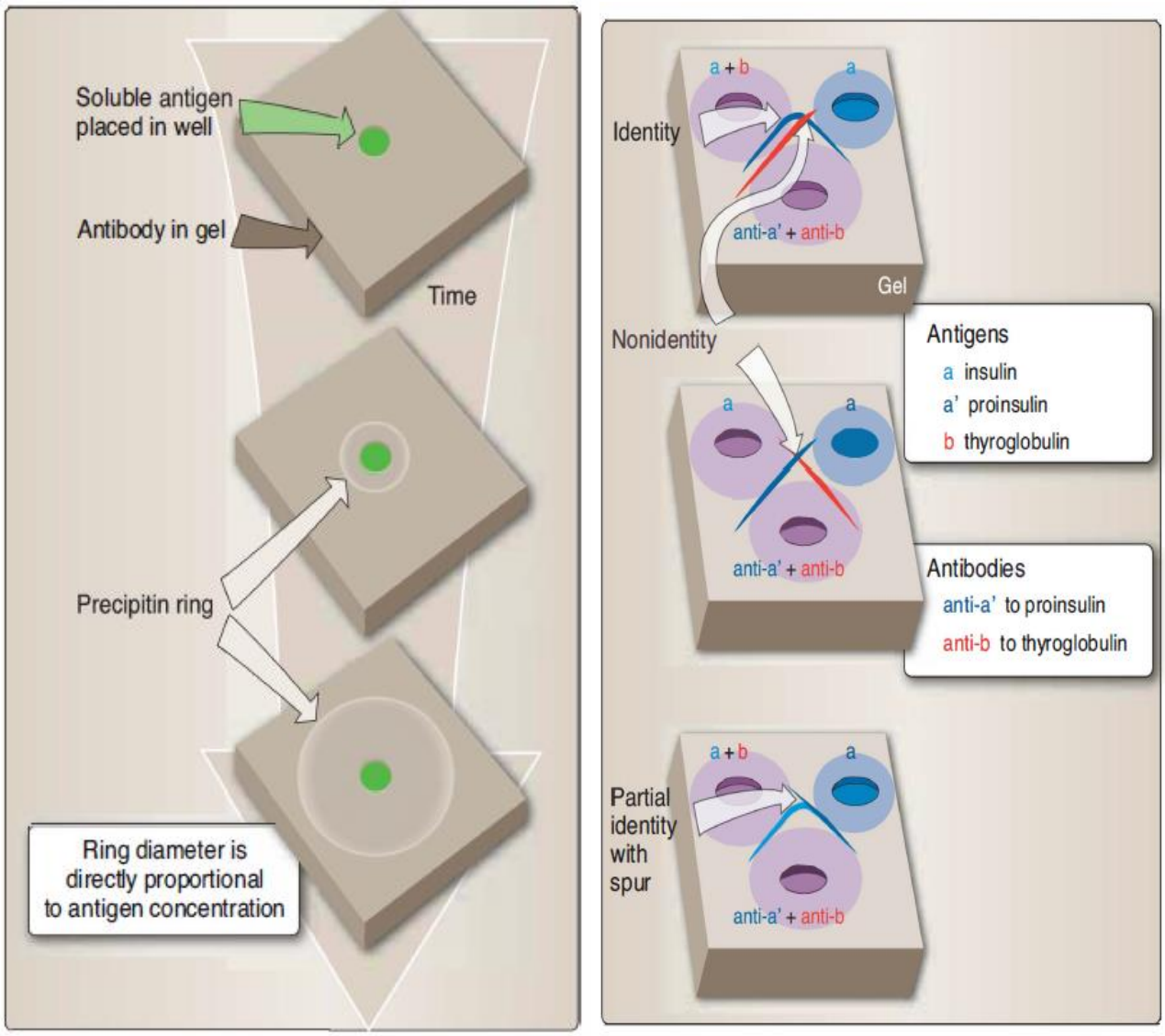




2.A Coombs test (or antiglobulin test) is a special form of immunoagglutination used for the detection of anti-erythrocyte antibodies that bind to red blood cells but do not lead to their agglutination.

(e.g. the presence of anti-Rh antibodies in a serum of Rh negative mothers).

3.**The radial immunodiffusion** (RID test) is also quantitative method, which is based on a diffusion of the soluble antigen through a gel that contains incorporated uniformly distributed antibody (given that only the antigen diffuses, this technique is sometimes called single radial immunodiffusion assay). e.g. IgG, IgM, IgA, C3, C4.

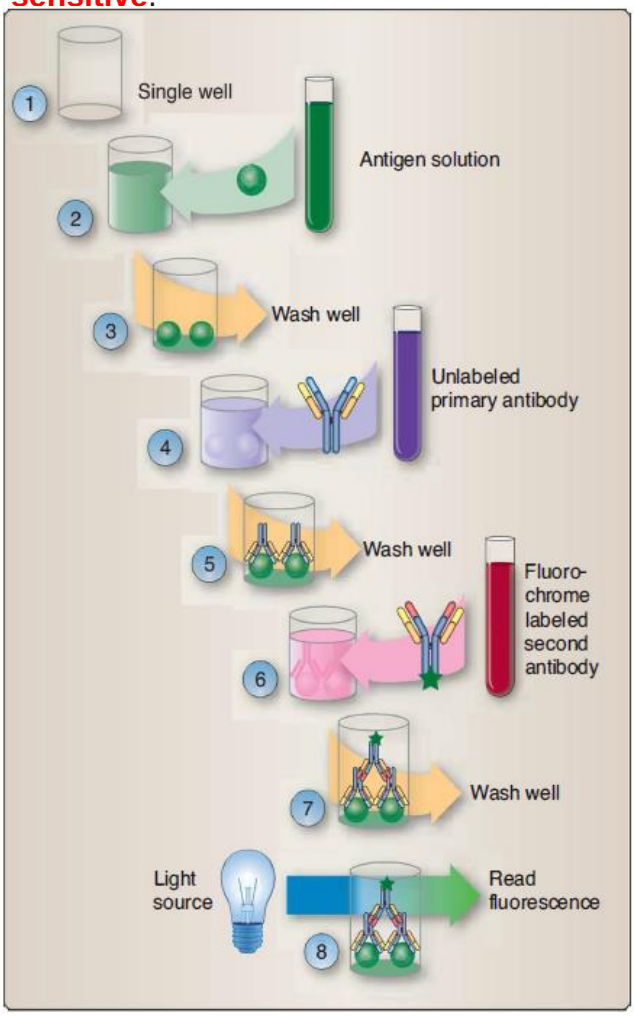


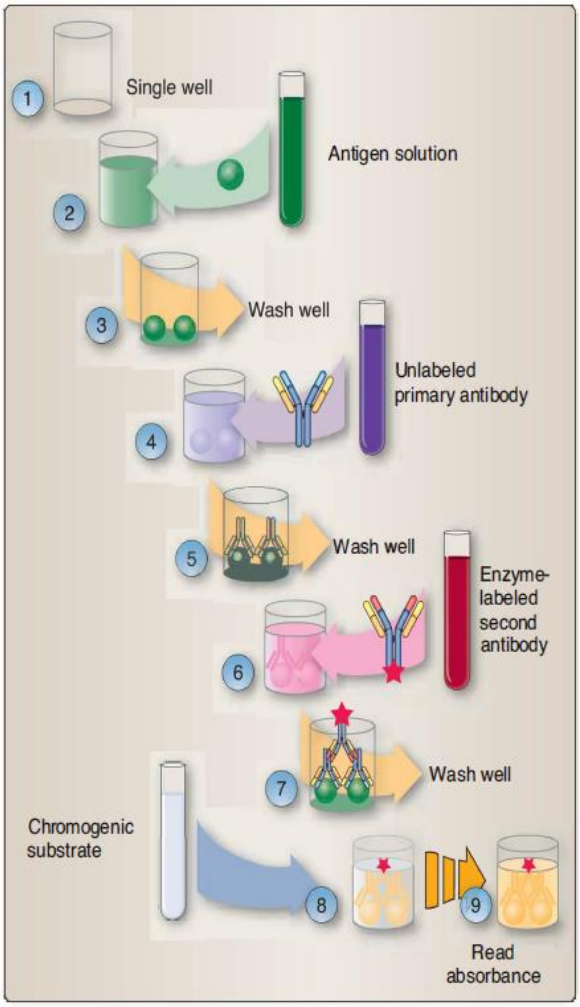
4.Double gel immunodiffusion is often used for qualitative detection of an antigen or specific antibody in a tested sample, this assay is a qualitative.

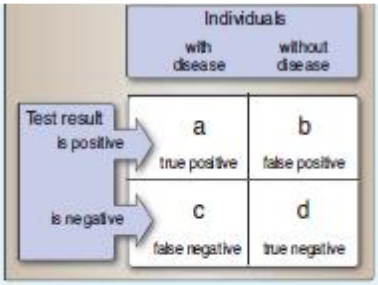
e.g. Rheumatic diseases.

5.**Enzyme-linked immunosorbent assay** {ELISA, also called enzyme immunoassay EIA) has replaced RIA in several tests. ELISA offers the advantage of safety and speed . Because there is no radioactive decay, the reagents that are used are relatively stable. Its sensitivity is often equal to or greater than that of RIA or fluorescent immunosorbent assay because an enzyme-labeled reactant is used to turn a chromogenic substrate from colorless to a color . Color change of the substrate indicates that an enzyme-labeled reactant has bound. Increasing substrate incubation time al lows low-concentration enzyme to convert more substrate to enhance test sensitivity . ELISAs are both specific and quantitative.

6.**Fluorescent immunosorbent assay (FIA).** The FIA design is similar to the ELISA design . 1 . The assay may be performed in proteinadsorbing, 96-well polystyrene plates (a single well is shown here) . 2. Soluble antigen is added and noncovalently binds to the plastic. 3. Unbound antigen is washed from the well . 4. Unlabeled (often sera to be tested) primary antibodies are added to the well and allowed to bind. 5. Unbound primary antibodies are washed from the well . 6. Fluorochromelabeled anti-immunoglobulin antibodies are added to the well and allowed to bind. 7. Unbound-labeled antibodies are washed from the well . 8. Fluorescence indicates t h e presence of epitopes. F I A is specific and relatively **sensitive**.







$$Sensitivity = \frac{a}{(a + c)}$$

$$Specificity = \frac{d}{(b + d)}$$