

Radioimmunoassay (RIA)

Radioimmunoassay (RIA) is very sensitive (can detect at a concentration of $<0.01 \mu\text{g/mL}$) and a specific technique that is used for the quantitative detection of antigens or haptens.

RIA is an extremely important tool in biomedical research and clinical practice. It is commonly used to assay microbial antigens, proteins, vitamins, hormones, or drugs in serum. A specialized RIA called radioallergosorbent test (RAST) is used to measure the amount of serum IgE antibody that reacts with a known allergen (antigen).

PRINCIPLE AND PROCEDURE OF RIA

Radioimmunoassay uses radioisotope-labeled purified known antigen which competes with unlabeled (unknown) antigen for binding sites on a known amount of antibody.

When the sample contains a high amount of antigen, much of the antigen-binding sites of the antibodies are occupied by **un-labelled antigen**. So the bound complex will show little radioactivity. The radioactivity generated, in fact, is inversely proportional to the amount of antigen present in the sample.

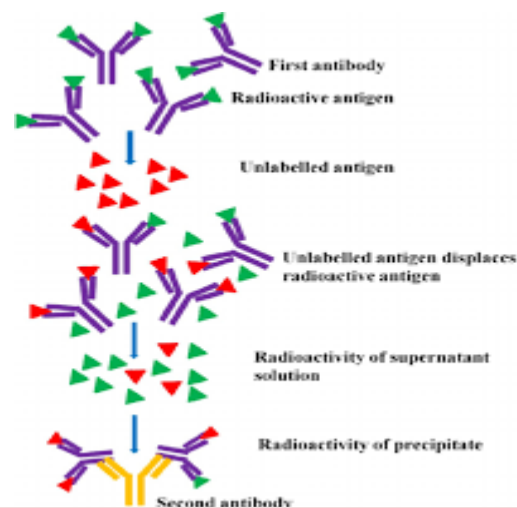
Then the concentration of the unknown (unlabeled) antigen or hapten present in a sample is determined by plotting the value of radioactivity generated in a standard chart generated using different concentrations of same antigens against the radioactivity of the bound complex.

Advantages

1-Highly sensitive so it can be used for the detection of small quantities of analyte or antigens.

2-quantification of antigens or analytes is possible with RIA.

Limitations: The handling of radioisotopes requires specific safety measures. Because of this limitation, RIA has been replaced by ELISA in clinical laboratories.



Latex agglutination

Agglutination (is the clumping of particles) or the visible expression of the aggregation of antigens and antibodies.

There are 3 types of agglutination depends on the carrier that carry the antigens.

1. Haem agglutination
2. Bacterial agglutination
3. Latex agglutination

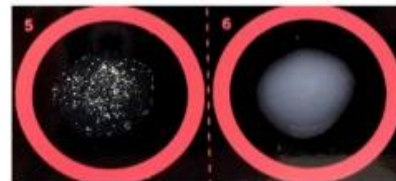
Latex Agglutination: a type of agglutination reactions has been used to detect of microbial and viral infections, autoimmune diseases, hormones, drugs and serum proteins have been developed in the worldwide.

Latex is inert **Polystyrene latex particles** (0.8 – 1 μm in diameter) are widely employed to adsorb several types of antigens. This test is convenient, rapid and specific Used for detection of hepatitis B antigen, ASO, CRP, RF factor, HCG and many other antigens.

Rheumatoid Factor Test

is the first example based on latex agglutination. Sometimes autoantibodies are produced by the human body against self antigens, the presence of these autoantibodies serves as a marker of some disease. In rheumatoid arthritis (RA) IgG produced by lymphocytes in the **synovial joint react** with IgM antibodies (RF, rheumatoid factor) to generate immune complexes that activate the complement and cause the destruction. Test principle The RF

latex agglutination is slide agglutination method depends on mixing of specimen which containing of RF with anti-RF coating with latex reagent and allowed them to react; if RF is present the **visible agglutination** is observed but when RF is absent the **no agglutination** is observed. Reagents Uniform suspension polystyrene latex particles coated with IgG reagent and controls (positive and negative). **Specimen:** Only **serum** should be used.



Test procedure

1. Pipetted one drop of onto dry and clean black slide.
2. Add one drop of latex RF reagent beside the serum drop.
3. Using mixing stick to mix the serum with RF latex reagent onto reaction circle.
4. Immediately observed of agglutination macroscopically after two min.

Neutralization Reaction

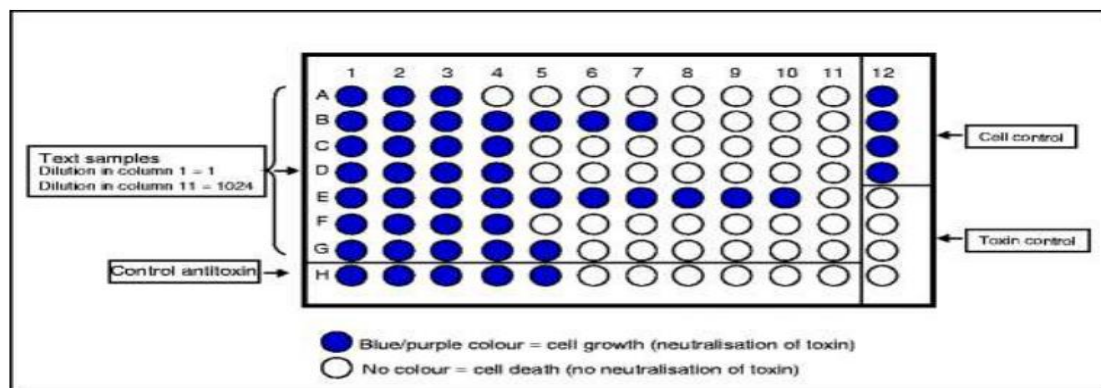
Neutralization is an antigen–antibody reaction in which the biological effects of viruses and toxins are neutralized by homologous antibodies known as neutralizing antibodies. These tests are broadly of two types: (a) **virus neutralization tests** and (b) **toxin neutralization tests**.

Neutralizing antibodies block or distort the antigen sufficiently, so that pathogen fails to exert its biological activity. Neutralization reactions can occur in vitro or in vivo. Laboratory animals or tissue culture cells are used as “indicator systems” in neutralization tests. The toxin or virus to be assayed should have known effects on the indicator system which is neutralized by antibodies.

Toxin Neutralization Test: are based on the principle that biological action of toxin is neutralized on reacting with specific neutralizing antibodies called antitoxins. Examples of toxin neutralization tests include: **In vivo:** (a) Schick

test to demonstrate immunity against diphtheria and (b) *Clostridium welchii* toxin neutralization test in guinea pig or mice. **In vitro:** (a) antistreptolysin O test.

Neutralization of viruses by their specific antibodies are called virus neutralization tests. Inoculation of viruses in cell cultures, eggs, and animals results in the replication and growth of viruses. When virus specific neutralizing antibodies are injected into these systems, replication and growth of viruses is inhibited. This forms the basis of virus neutralization test. Neutralizing antibodies may interfere with virion binding to receptors, block uptake into cells, prevent uncoating of the genomes in endosomes, or cause aggregation of virus particles.



If antibodies to the virus are present then clinical disease, mortality in eggs or CPE will not be observed.

Hemagglutination

agglutination reactions that are performed on RBC's - RBC's mixed with antisera to the A or B blood-group antigens on a slide - if antigen is present on the cells, they agglutinate, forming a visible clump on slide.

Ex. ABO blood grouping.

The ABO Blood Group System

There are four major blood groups determined by the presence or absence of two antigens – A and B – on the surface of red blood cells:

Group A – has only the A antigen on red cells (and B antibody in the plasma)

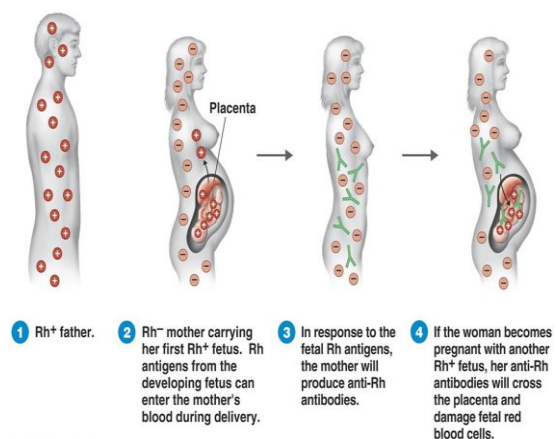
Group B – has only the B antigen on red cells (and A antibody in the plasma)

Group AB – has both A and B antigens on red cells (but neither A nor B antibody in the plasma)

Group O – has neither A nor B antigens on red cells (but both A and B antibody are in the plasma)

- **Rh System:** An individual either has, or does not have, the "*Rh factor*" on the surface of their [red blood cells](#). This term strictly refers only to the most immunogenic D antigen of the Rh blood group system, or the Rh– blood group system. The status is usually indicated by *Rh positive* (Rh+ does have the D antigen) or *Rh negative* (Rh– does not have the D antigen) suffix to the [ABO blood type](#). In contrast to the ABO blood group, immunization against Rh can generally only occur through [blood transfusion](#) or placental exposure during pregnancy in women. A mother who is Rh-negative may develop antibodies to an Rh-positive

baby. If a small amount of the baby's blood mixes with the mother's blood, which often happens in such situations, the mother's body may respond as if it were allergic to the baby. The mother's body may make antibodies to the Rh antigens in the baby's blood. This



means the mother has become sensitized and her antibodies may cross the placenta and attack the baby's blood. Such an attack breaks down the fetus's red blood cells, creating [anemia](#) (a low number of red blood cells). This condition is called **hemolytic disease or hemolytic anemia**. It can become severe enough to cause serious illness, brain damage, or even death in the fetus or newborn. Sensitization can occur any time the fetus's blood mixes with the mother's blood. It can occur if an Rh-negative woman has had a spontaneous or undetected miscarriage of a Rh positive fetus.

Haemagglutination Inhibition (HAI) Test

Some viruses can directly bind and agglutinate mammalian and avian red cells. This phenomenon is called as Haemagglutination. (Direct) Haemagglutination is not a serological test.

Haemagglutinating viruses include orthomyxoviruses, paramyxoviruses, alpha viruses, flaviviruses, and bunyaviruses as well as some adenoviruses, reoviruses, paravoviruses, and coronaaviruses.

Inhibition of viral haemagglutination by specific antibody can be used either as a **method for identifying a specific virus** or to measure antibody levels in the serum; this serological reaction is called as **HAI**

Dilutions of test sera are made, followed by the addition of an equal volume of virus suspension diluted to contain approximately 4 to 8 HA units.

The appropriate RBC suspension is then added and the plates are gently mixed and allowed to incubate for 1 – 2 hours

If the specific antibody is present in the test serum, agglutination of RBC will be inhibited and the RBC's will settle out in a welldefined "button".

♣ Agglutinated cells, in contrast, will settle out in a thin layer over the entire bottom of the test well.

