Clinical tests: Enzyme assays, radioactive immunoassays, ELISA

Radioactive immunoassays (RIA)

A low cost and commonly used technique that can be used to measure low levels of antigens (e.g hormone levels in blood) by use of antibodies. A known quantity of an antigen is radioactively labelled and mixed with a known quantity of the antibody, and the two specifically bind together. A sample from a patient containing an unknown quantity of the same antigen is added. This causes the unlabelled antigen from the patient to compete with the labelled antigen to form the antigen-antibody complex. Bound antigens are separated from unbound antigens, and the radioactivity of the bound antigen remaining is measured by a gamma counter. These assays are very **sensitive** and **specific**.

However, human antibodies may **interfere** with an RIA and react either positively or negatively with the immunoassay reagents to give a false result.

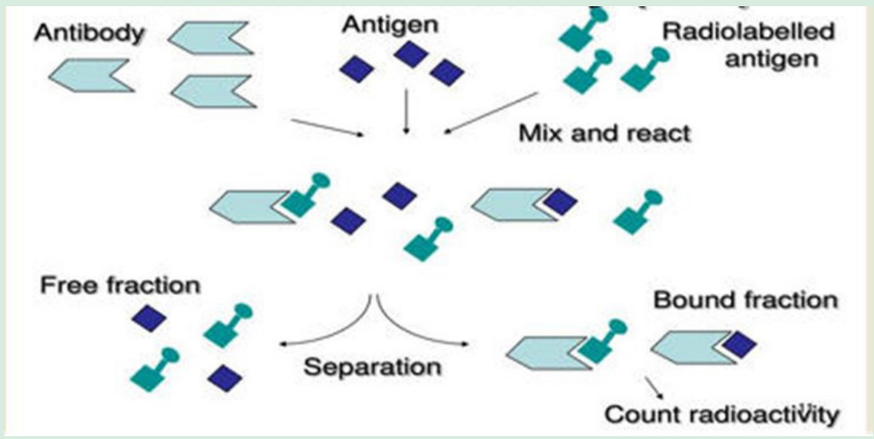

Diagram to show principle of RIA

Enzyme assays

These measure enzyme activity either by the consumption of the substrate or formation of a product over time. Enzyme assays can be split into two groups. The split is created according to their sampling method. The groups are **continuous assays** (where the assay gives a continuous reading of activity), and **discontinuous assays** (where samples are taken, the reaction stopped and then the concentration of substrates/products determined).

ELISA

The enzyme-linked immunosorbent assay (ELISA) is a technique used to detect antibodies or infectious agents, such as antigens, in a sample.

Antibodies are made in response to infection. Therefore, antibody ELISA testing can indicate if the patient has been in contact with a certain virus. An antigen ELISA can tell whether the patient is infected with a virus by detecting it directly.

For an antibody ELISA, antigens are stuck onto a plastic surface, a sample is added and any antibodies for the disease being tested for will bind to the antigens. Next, a second antibody with a marker is added and a positive reaction is detected by the marker changing colour when an appropriate substrate is added. If there are no antibodies in the sample, the second antibody will not be able to stick and there will be no colour change.

For an antigen ELISA, antibodies are bound to a plastic surface, a sample is added and if antigens from the virus being tested for are present, they will stick to the antibodies. This test then proceeds in the same way as the antibody ELISA.

ELISA testing is used to diagnose HIV infection, pregnancy, and blood typing, among many other things.

The **sensitivity** and **specificity** of ELISA tests currently in use are generally reported to be greater than 90% and 95%, respectively. Immunoassay interference is typically caused by interactions between antibodies within the assay itself and endogenous antibodies present in a patient's sample. Any type of interference can cause an assay to report a false result.