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The history of the ELISA

The format of the ELISA was developed in the 1960s independently at the same time by two research groups; Peter Perlmann and Eva Engvall at Stockholm University, and the Dutch research group of Anton Schuurs and Bauke van Weemen. The assay was based on the underlying principles of conventional radioimmunoassay, with the key difference that the antibodies are labelled with an enzyme, rather than radioisotopes.

Typical Steps of an ELISA

The success of an ELISA assay is dependent upon the underlying level of immunoreactivity of the capture and detection antibodies to the target analyte. Box 1. shows one format of the assay, which uses two separate antibodies: the first to recognise and bind the target analyte, the second to detect the bound target. In Step 1, one of the antibodies is applied to the well of a microtitre plate: this is known as the capture antibody. The capture antibody binds to the plate via passive adsorption and this step is often performg

subsequent incubation steps. Excess blocking agen

If the test sample contains the target analyte, this is bound by the capture antibody that is anchored to the plate (Step 2). After the incubation step with the test sample, the plate is washed prior to the addition of the enzyme-linked detection antibody (Step 3). The detection antibody is conjugated with an enzyme which is commonly either horseradish peroxidise, alkaline phosphatase or -*D*-galactosidase. These enzymes are proteins that catalyse the hydrolysis of a chromogenic substrate, such as 3,3',5,5'-tetramethylbenzidine or 2,6-dichlorophenolindophenol, which undergoes a colorimetric change that is measurable using a spectrophotometric plate reader at specified wavelengths (Step 4). With the catalysis of the traditional chromogenic substrates the reaction is terminated by the addition of a stop solution prior to measuring the absorbance of each of the wells of the microtitre plate.

Indirect detection

In addition to the direct detection method, as illustrated in Box 1, ELISAs may utilise an indirect detection approach. In this instance, as illustrated in Figure 1, the secondary antibody used will recognise and bind to the appropriate species-specific sub-class of the antibodies (or immunoglobulins). It is the secondary detect1t410. 510.9209 Tm(sub)TjTm(10.02 0 0 10.02 23.920a522.38084 Tm(i)Tj9.02 0 0