Principles and application of elisa

In this essay, I will discuss the uses of the biochemical technique ELISA (Enzyme Linked ImmunoSorbent Assay). ELISAs are the first and most basic test used to determine whether individuals are positive for certain biochemicals, such as hormones, drugs and immunoglobulins. (Glencross et al. 2010, pg 252)

Introduction

An ELISA involves immobilizing an antigen to a solid surface (e. g. a plastic multiwall plate) then blocking the sites that remain unbound to prevent false positive results. A primary antibody is then added and binds to the antigen before washing. A secondary antibody is added which is conjugated to an enzyme. A substrate is finally added which reacts with the enzyme to produce a coloured product, indicating a positive reaction (ELISA-Antibody, 2007)

The colour change is measured by a spectrophotometer which records the absorbance of the wells. The intensity of the colour is directly proportional to the quantity of antigen bound on the plate (Glencross et al. 2010 pg 398)

Various Principles of ELISA

The basis of ELISA involves immobilising antibodies onto a microtitre plate – the exact method used to prepare an ELISA plate can differ depending on the analyte being measured. Antibodies show specificity for an analyte, allowing qualification – a test which gives a positive or negative result like the Avian Influenza ELISA screening kit (Atlas Link Biotech, 2008) – or quantification –

an optical density interpolated into a standard curve through serial dilutions as in allergen detection (McSharry et al. 2006)

There are four main “ types” of ELISA – direct, indirect, sandwich and competitive.

Direct

A direct ELISA is the simplest ELISA and quickest to perform as it uses a single primary antibody that is linked with an enzyme this complex reacts directly with the antigen which is fixed to the plate. After washing, the substrate is added to give a colour change which can be measured. They can be used to test specific antibody-antigen reactions and help eliminate cross-reactivity between other antibodies. Direct ELISAs are also used in immunohistochemical staining (Thermo Fisher Scientific, 2011)a

Indirect

This format of ELISA is the most widely used in laboratories – again the antigen is fixed to the plate and there is a primary antibody added. However, a second antibody-enzyme complex is added which has a specificity for the primary antibody. The indirect ELISA is far more sensitive than direct as the primary antibodies’ “ signal” is amplified to give better readings. (Thermo Fisher Scientific, 2011)C: UserslawdunnettDesktopELISADirect ELISA. PNG

The indirect ELISA is used mostly for detecting the presence of antibodies in serum and is widely used for detecting HIV positive patients (Richalet-Sécordel and Regenmortel, 1991)

Sandwich

These ELISAs are name as such due to their method – the antibody is immobilized to the microtitre plate, not the antigen. This results in the antigen being “ sandwiched” between the primary antibodies and the patient’s antibodies (if they are present). There are two types of Sandwich ELISA – Double and Triple.

Double Antibody Sandwich

This ELISA is similar to the direct ELISA as there is no signal amplification. The microtitre plate is coated with a primary antibody then the sample is loaded into the plate. A secondary antibody/enzyme complex (the enzyme could be alkaline phosphatise or horseradish peroxidise) is added before finally adding a substrate to catalyse the colour change. The Double Antibody Sandwich ELISA is used to detect viruses in plants. (Edwards and Cooper, 1985)

Triple Antibody Sandwich

This type of ELISA can also be known as an indirect ELISA; again, the microtitre plate is coated with antibody, then antigen is added. The patient’s sample is then loaded before the antibody/enzyme complex is added.

This type of ELISA is used to identify the patient’s antibodies which may have resulted through infection or disease (e. g. Hepatitis B, Hepatits C, HIV). For Hepatitis B, the HBV coating antibody is bound to the wells of the plate and a synthetic Hepatitis B antigen is used; the live Hepatitis B virus is not used as the antigen due to the danger it presents to the laboratory. The secondary

antibody/enzyme complex is usually anti-human IgG (from mice/goats etc) to interact with the patient's human antibody. (Wilson and Walker, 2010) Again, a substrate is added to derive a colour change.

Competitive

ELISA Experiment

An indirect ELISA was carried out on two patient samples – these samples had previously tested positive for self-nuclear antigen (ANA) and were to be tested for antibodies to extractable nuclear antigens (ENA). The ELISA was qualitative assay – patient's were given a positive or negative result when compared to the negative control well.

The ELISA plates were coated with ENA (antigen), then anti-ENA antibodies were added. The plates were incubated then a polyclonal anti-human antibody tagged with an enzyme was put into the wells, incubated once more before adding a substrate to bring about a colour change. The patient's serum was added to some of the wells instead of the anti-ENA antibodies.

The results were as below:

Discussion

The standard curve created from the ELISA shown in Figure 6 is very linear and has a high correlation – this shows a high degree of control and will give an accurate positive or negative result when the patient's serum results are compared. The error bars are slightly large and do overlap, showing there is no statistical difference between lower concentrations of Anti-ENA – there is,

however a statistical difference between the higher concentrations of Anti-ENA (1500U/L and 3000U/L).