Enzyme-linked immunosorbent assay

Enzyme immunoassays exploit an enzymatic reaction for detecting the immune reaction. In 1971, Engvall and Perlmann and van Weemen and Schuurs described independently the use of enzyme-labeled agents. The most common type of enzyme immunoassays in use is *enzyme-linked immunosorbent assays*.

Enzyme-Linked Immuno Sorbent Assay, commonly known as ELISA (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an Ab reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called chromogenic substrate. A number of enzymes have been employed for ELISA, including alkaline phosphatase, horse-radish peroxidase, urease and beta galactosidase.

Table 6.11 : Enzymes used for conjugation of antibodies

Enzyme	*Source*	*Reaction catalyzed*
Peroxidase	Horseradish	H_2O_2 + Oxidisable substrate → Oxidized product + $2H_2O$
Alkaline phosphatase	Calf intestine	$R\text{–}O\text{–}P_i + H_2O \rightarrow R\text{–}OH + P_i$
β-Galactosidase	*E.coli*	β-D-Galactoside + H_2O → Galactose + Alcohol
Urease	Jack bean	$(NH_2)_2CO + 3H_2O \rightarrow CO_2 + 2NH_4OH$

Different variants of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of Ab or Ag. Alternatively, a standard curve based on known concentrations of Ab or Ag is prepared from which the unknown concentrations of a sample can be determined.

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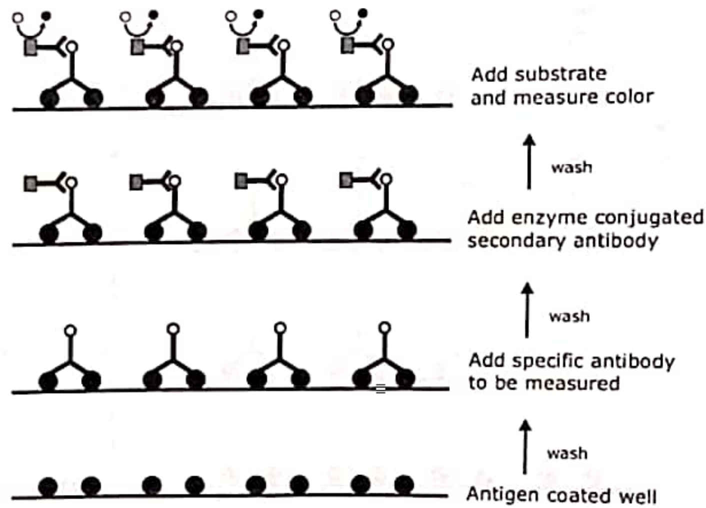
Indirect ELISA

Ab can be detected or quantitatively determined with an indirect ELISA. Serum or some other sample containing primary Ab (Ab1) is added to an Ag-coated micro-titer well and allowed to react with the Ag attached to the well. After any free Ab, is washed away, the presence of Ab bound to the Ag is detected by adding an enzyme-conjugated secondary anti-isotype (Ab2), which binds to the primary Ab. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in less than a few minutes. Indirect ELISA is the method of choice to detect the presence of serum antibodies against HIV.



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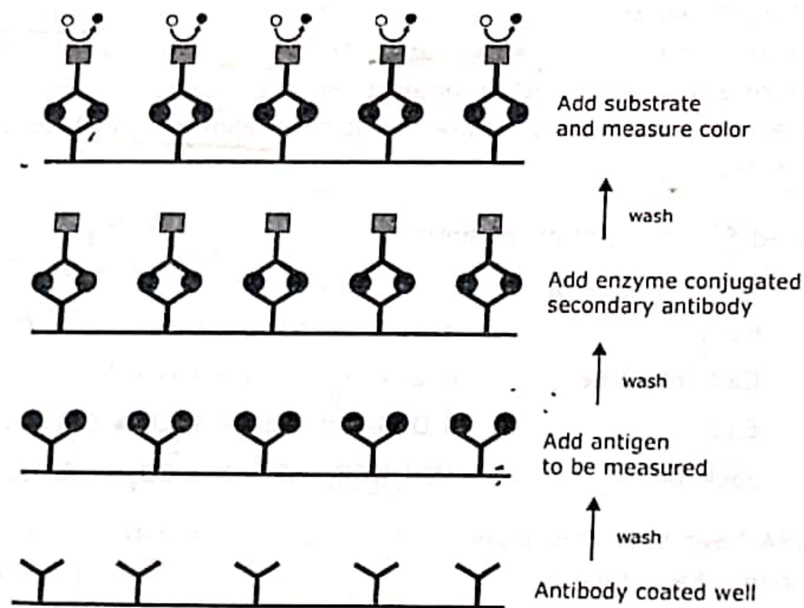
Sandwich ELISA

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked Ab specific for a different epitope on the antigen is added and allowed to react with the bound Ag. Any free second Ab then is washed away and a substrate for the enzyme is added. Finally the amount of colored reaction product that forms is measured.



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