

Lab : 3/ Second semester (Medical Biology)

Dep: Clinical laboratories / first stage

## **Enzyme Linked Immunosorbent Assay**

### **Introduction of ELISA**

Based on Basic Immunology Response

\* Lock and Key concept .

Antigen (Key) : substance when introduce into the body produce antibodies .

Antibody (Lock) : protein in the body that is used by immune system to identify and neutralize foreign targets (referred to as antigen ).

Key fits into the lock .

### **Enzyme conjugate substrate s:**

The enzyme that convert colorless substrates to a colored product it is bound to the antibody that is part of the antibody – antigen complex , Or bound to a secondary antibody that binds with the antibody – antigen complex .

Four common ELISA tests – based on the binding structure between the antibody and antibody .

1- Direct ELISA . Enzyme conjugated Ab is directly bound the Ag.

2- Indirect ELISA .

3- Sandwich ELISA .

4- Competition ELISA .

### **\* Total Triiodothyronine (tT3 test):**

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassays.

In the method of measurement of T3 serum reference, patient specimen or control is first added to a microplate well. Enzyme-T3 conjugate is added and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well. After the completion of the required incubation period, the antibody-bound T3-enzyme conjugate is separated from the unbound T3-enzyme conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with suitable substrate to produce color.

### **Reagent preparation :**

#### **1- Working Reagent A –T3 – enzyme conjugated solution .**

Dilute the T3 – enzyme conjugate 1:11 with total T3/T4 conjugated buffer in a suitable container. For example, dilute 160 µl of conjugate with 1.6 ml of buffer for 16 wells (a slight excess of solution is made). This reagent should be used within

24 h for maximum performance of the assay . store at 2-8 °c .

## **2- Wash buffer :**

Dilute contents of wash concentrate to 1000ml with D.W in a suitable storage container . store at room temperature 20-27 °c for up to 60 days .

## **3- Working substrate solution :**

Pour the contents of the vial labeled solution A into the vial labeled solution B . next and store at 2- 8 °c use within 60 days .

Note. Do not use the working substrate if it looks blue.

## **Procedure :-**

- 1-Format the microplate s well for each serum reference , control and patient to be assayed in duplicate .
- 2- Pipette (50µl) of the appropriate serum reference , control or specimen into the assigned well.
- 3- Add (100µl) of working reagent A, T3-enzyme conjugate solution to all well .
- 4- Swirl the microplate gently for 20 -30 second to mix and cover .
- 5- Incubate 60 minutes at room tempreature .
- 6- Discard the contents of the microplate by decantation or aspiration . if decanting , blot the plate dry with absorbent paper .
- 7- Add 300µl of wash buffer , decant (tap and blot) or aspirate . repeat two (2) additional times for a total of three (3) washes .

8- Add 100µl of working signal reagent solution to wells .

Do not shake the plate after substrate addition .

9- incubate at room temperature for 15 minutes .

10- Add (50µl)of stop solution to each well and gently mix for 15-20 second .

11- Read the absorbance in each well at 450nm ( using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader .

**Note : the result should be read within 30 minutes of adding the stop solution .**