Sandwich Dot ELISA test for antigen

Principle:

Dot-ELISA (Enzyme Linked Immunosorbent Assay) is an extensively used immunological tool in research as well as analytical/diagnostic laboratories. In sandwich Dot-ELISA, the antigen is sandwiched directly between two antibodies which react with two different epitopes on the same antigen. Here one of the antibodies is immobilized onto a solid support and the second antibody is linked to an enzyme. Antigen in the test sample first reacts with the immobilized antibody and then with the second enzyme-linked antibody. The amount of enzyme linked antibody bound is assayed by incubating the strip with an appropriate chromogenic substrate, which is converted to a coloured, insoluble product. The latter precipitates onto the strip in the area of enzyme activity, hence the name **Dot-ELISA.** The enzyme activity is indicated by intensity of the spot, which is directly proportional to the antigen concentration.

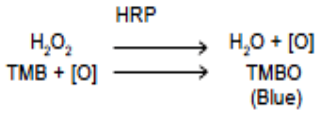
ELISA strips are supplied having three well defined zones:

• **Negative control zone** that is blocked with an inert protein.

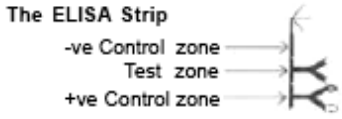
• **Test zone** having an antibody immobilized on it and then blocked with an inert protein.

• **Positive control zone** having the antibody immobilized on it, blocked with inert protein and has a specific antigen bound to the immobilized antibody.

These strips will be used to detect the antigen in the test serum samples supplied, by using a secondary antibody conjugated to Horse radish perxoidase (HRP). HRP is then detected using hydrogen peroxide as a substrate and Tetramethylbenzidine (TMB) as a chromogen. HRP acts on hydrogen peroxide to release oxygen, which oxidizes the TMB to TMB oxide. The TMB oxide is deposited wherever enzyme is present and appears as a blue spot.



If the test sample does not contain the antigen specific to the antibody, there will be no enzyme reaction and no spot develops.



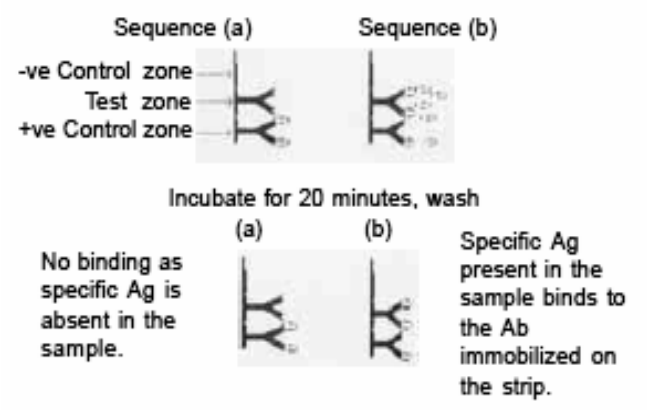
The Reaction Sequence

Negative Control Zone: In this zone immobilized antibody is not present and hence, there is no reaction when the reagents are added.

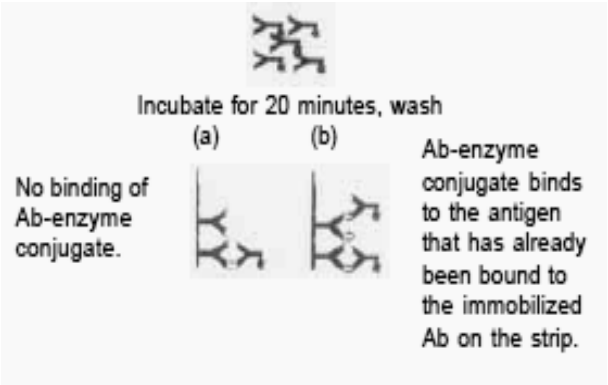
Positive control zone: In this zone antigen is bound to immobilized antibody. The antigen binds to antibody enzyme conjugate in step 2 and develops spot in step 3.

Note: When sample does not contain specific antigen, reaction follows sequence (a) and when sample contains specific antigen, it follows sequence (b). Reaction sequences for negative and positive control remains same in sequences (a) and (b).

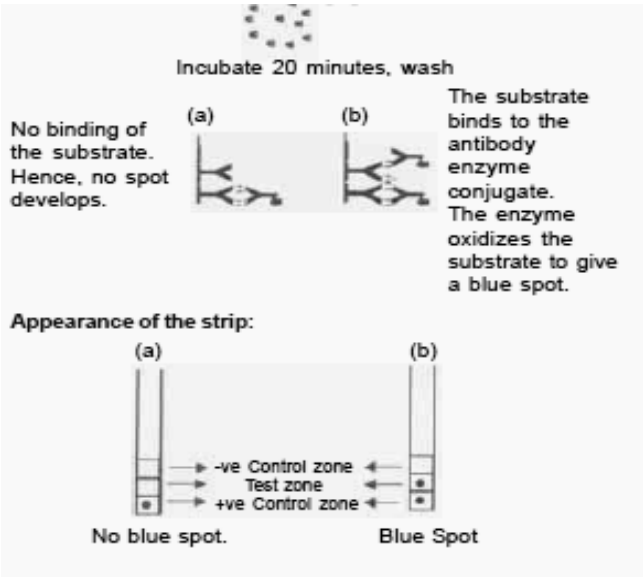
Step-1: Dot ELISA strip + 1X Assay Buffer +Serum Samples.



Step-2: Add enzyme-antibody conjugate (antibody-HRP).



Step-3: Add substrate (TMB/H2O2)



Procedure:

1. In a test tube/vial, take 1 ml of 1X assay buffer and 50 µl of serum sample. Mix thoroughly. Insert a Dot-ELISA strip.

2. Allow the reaction to occur at room temperature for 20 minutes.

3. Wash the strip three times by dipping it in 1 ml of 1X assay buffer for about 5 minutes each. Replace the buffer each time.

4. Take 1 ml of 1X assay buffer in a fresh tube or vial, add 10 µl antibody-HRP conjugate to it. Mix thoroughly. Dip the strip; allow the reaction to take place for 20 minutes.

5. Wash the strip as in step # 3, three times.

6. In a fresh tube/vial, take 0.1 ml of 10X TMB/H2O2 and 0.9 ml of distilled water, mix thoroughly. Dip the strip in this substrate solution.

7. Observe the strip after 10 - 20 minutes for appearance of a blue/grey spot.

8. Rinse the strip with distilled water.

Observation:

Record your observations as follows:

Test serum sample:

Zone	Spot
Negative zone	
Test zone	
Positive zone	

Note: Denote +ve : on appearance of a blue spot, and -ve : on absence of a blue spot

Interpretation:

• Spot in positive control zone and no spot in the negative control zone indicates proper performance of test.

• Spot in test zone indicates presence of specific antigen in the sample.

Note: Intensity of the spot will vary depending upon the test sample used.

• No spot in the test zone indicates the absence of specific antigen in the sample.