Practical immunity **Ass. Lec. Heba shehab**

Lecture:

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) the most widely used of a group of tests known as enzyme immunoassay (EIA) there are two basic methods The direct ELISA detects Ag and the indirect ELISA detects Ab Amicrotiter plat with numerous shallow wells is used in both procedure.

Sandwich ELISA (Direct ELISA)

A "sandwich" ELISA, is used to detect sample antigen. The steps are :

1- In the first step the Ab specific to Ag to be detected is adsorbed to the surface of the wells of the microtiter plate .

2- A patient sample containing unidentified Ag is then added to each well , if the Ag specifically with the Ab adsorbent to the well , the Ag (drug in urine test , for example). will be retained there when the well is washed free of unbound Ag

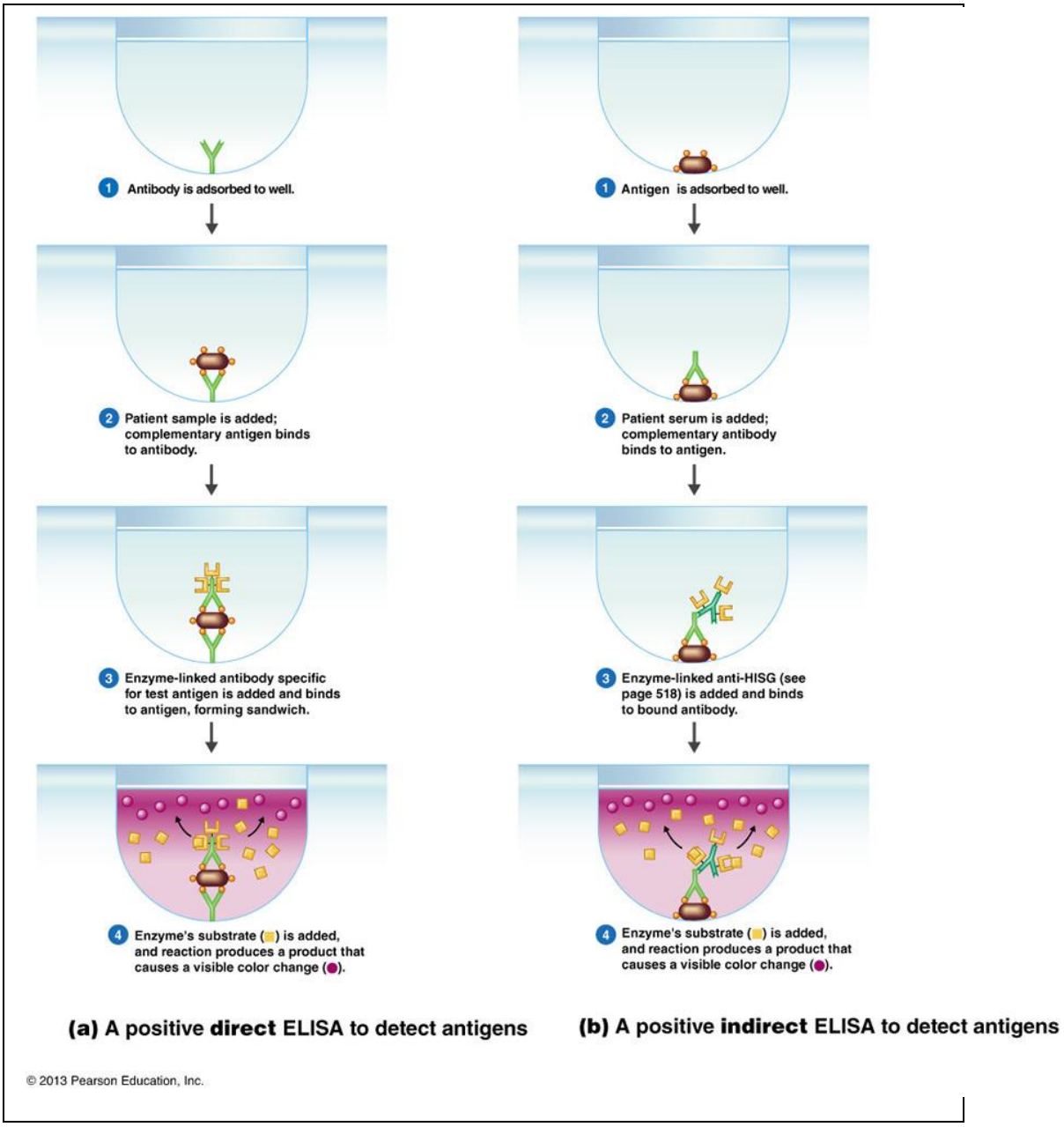
3-A specific antibody is added , and binds to antigen (hence the 'sandwich': the Ag is stuck between two antibodies) , the secondary Ab added is linked to an enzyme such as

alkaline phosphatase , unbound enzyme –linked Ab is washed from the well .

4-The plate is washed to remove the unbound antibody-enzyme conjugates.

5-the enzymes substrate is added to it enzymatic activity is indicated by a color change that can be visually detected the test will be positive if the Ag has reacted with adsorbed Ab in the first step.

If the Ag was not specific for the Ab adsorbed to the wall of the well , the test will be negative because the unbound Ag will have been washed



A common use of the direct ELISA test to detect the presence of drug in urine the availability of monoclonal Ab has been essential to the widespread use of the type of test.

Indirect ELISA

1. Micro-well plates are incubated with antigens , washed up and blocked with BSA bovine serum albumin (BSA) as a blocking agent to prevent non-specific binding of antigens and antibodies to the microtiter well.

2. Samples with antibodies are added , incubate and washed.

3.Enzyme linked secondary antibody are added , incubate and washed.

4. A substrate is added , and enzymes on the antibody elicit a chromogenic or fluorescent signal.

The addition of an enzyme substrate - chromogen reagent causes color to develop. This color is directly proportional to the amount of bound sample antibody.

The more antibody present in the sample , the stronger the color development in the test wells. This format of indirect ELISA is suitable for determining total antibody level in samples (Newcastle disease virus , **B. *abortus*** , etc.).