



## UBL201-L Introductory Biology III - Immunology

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### Detection and determination of the concentration of an antibody by an indirect ELISA

#### Principle:

ELISA (enzyme linked immunosorbent assay) is a laboratory technique used to detect the presence of antigens (Ag) or antibodies (Ab) in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate. The substrate is converted to a detectable signal by the enzyme. The amount of signal is directly proportional to the amount of antibody bound to the antigen. The antibody concentration can be determined by comparing the signal obtained from the sample to the standard curve. The standard curve is generated by plotting the known concentrations of the antibody against the signal obtained from each standard. The signal is usually measured by absorbance at 450 nm. The ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag). In principle, there are two possible approaches to the implementation of an ELISA test: Direct ELISA and Indirect ELISA.

#### Direct ELISA:

Direct ELISA involves directly detecting antigen in the test samples by antibodies (primary/capture antibodies). These antibodies are directed against the antigen being sought and are bound to the microtitre plate. The antigen binds the antibodies and can be detected by a secondary antibody-enzyme conjugate.

#### Indirect ELISA:

Indirect ELISA involves detecting the antibody specific for the antigen, which in turn, is bound to the plate. The antibody being detected binds the antigen and can be detected in a second step by a secondary antibody-enzyme conjugate. The capture antibody needs to be different from the antibody used for detection. Often a monoclonal antibody is used to capture and a polyclonal antiserum is used for detection. The indirect ELISA is useful for screening specific antibodies.

#### Detection principle using Hydrogen peroxidase (HRP)-TMB/ $H_2O_2$

The amount of primary antibody bound will be detected using a secondary antibody conjugated to the enzyme, Horse radish peroxidase (HRP). Upon incubation with the substrate i.e., hydrogen peroxide ( $H_2O_2$ ), HRP acts on  $H_2O_2$  to release nascent oxygen, which acts on the chromogen, Tetramethylbenzidine (TMB) resulting in the formation of a blue coloured product. The reaction is stopped using 1M sulphuric acid ( $H_2SO_4$ ), addition of which leads to formation of yellow color which is read at 450 nm in spectrophotometer (ELISA reader).

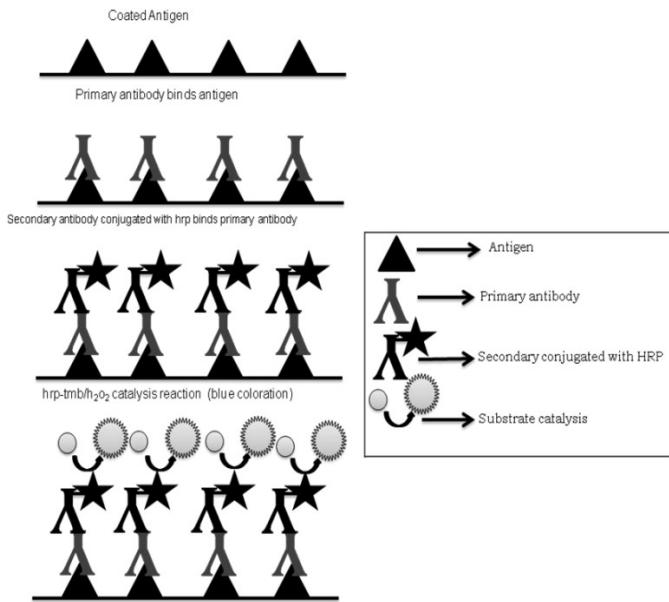


Figure 1: Indirect ELISA

## Results and discussion

The following are the readings obtained from the spectrophotometer. The readings are in the form of absorbance at 450 nm.

17	Measurement Wavelength	450 nm
18	Bandwidth	9 nm
19	Number of Flashes	25
20	Settle Time	0 ms
21	Part of Plate	A1-H3
22	Start Time	01-09-2023 15:57:35
23		+
24	Temperature:	27.1 °C
25	↔	1    2    3
26	A	3.3812    3.567    0.1076
27	B	2.0353    1.9127    0.0796
28	C	1.1093    1.0787    0.3134
29	D	0.5273    0.5354    0.3377
30	E	0.2637    0.2073    0.1463
31	F	0.1483    0.1319    0.1352
32	G	0.1143    0.0906    0.1035
33	H	0.1021    0.0878    0.0981
34		
35		
36		
37		
38	End Time:	01-09-2023 15:58:01

Figure 2: spectrophotometer readings



Figure 3: Sample after adding substrate



Figure 4: Sample after adding stop solution

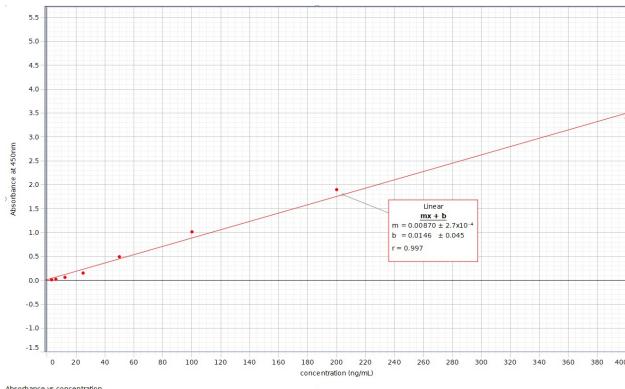


Figure 5: antibody concentration plot

### Antibody concentration

The antibody concentration is calculated using the following formula:

$$\text{Concentration of antibody (in mg/mL)} = \frac{\text{Concentration of antibody from graph (ng/mL)}}{10^6} \times \text{Dilution Factor} \quad (1)$$

From the plot we get the concentration of the test samples as follows:

Test sample 1 = **0.0582 mg/mL**

Test sample 2 = **0.0256 mg/mL**

Test sample 3 = **0.0116 mg/mL**

Hence, the concentration of the antibody in the test samples is 0.0582 mg/mL, 0.0256 mg/mL and 0.0116 mg/mL respectively. We could see that the absorbance from the blank was extremely lower confirming that our negative control worked well. The absorbance values of the test samples were higher than the blank confirming that the test samples were also working well. The plot was so used as there was linear relationship between the concentration of the antibody and the absorbance values. Using the equation of the line  $y = mx + b$ , we can determine the concentration of the unknown sample.

### Interpretation:

We can see a decrease in the concentration of the antibody as we go from test sample 1 to test sample 3. This suggests that the test sample 1 is at a higher risk of being exposed to the antigen than the other two test samples. This could be due to the fact that the test sample 1 was exposed to the antigen for a longer duration than the other two test samples.

### Precautions:

- Wear appropriate personal protective equipments, including lab coats, gloves, and safety goggles, to protect against chemical exposure.
- Use sterile techniques and clean glassware to prevent contamination of samples and reagents.
- Calibrate and standardize the spectrophotometer using appropriate standards and controls to ensure accurate measurements.
- Avoid pipetting errors and ensure that the pipettes are calibrated.
- Keep everything properly thawed as the samples and reactions are temperature sensitive.
- Perform duplicates and triplicates to reduce the error and enhance reproducibility of results.
- Careful disposal of biohazardous waste.