Immune Assay for the Detection of Corona Virus Antibodies

Evaluation of the concentration of antibodies against the envelope protein of the corona virus, in blood samples of subjects, using the ELISA method (serological test)

Developing: The center for development and support for biology schools' labs, Bar Ilan University, Israel

The case

Avi and Daphne, a pair of 16-year-old twins, are eager to commemorate their birthday with their parents in one of the hotels located in Eilat. Despite their parents being diagnosed with COVID-19 approximately a month prior, the twins have not contracted the virus. In light of this, their family doctor advised them to undergo an antibody test to determine their immune response to the virus. The doctor explained that if the blood test detects the presence of COVID-19-specific antibodies, the twins will be immune positive to the virus. Otherwise, they will be advised to either receive a vaccine or undergo a coronavirus test.

In this laboratory, you will have a kit that will allow you to measure the concentration of antibodies present in the blood samples of the twins. Based on the results of the test, you will be able to provide them with guidance on the appropriate course of action.

The ELISA plate

The plate on your desk consists of 96 wells (sockets), organized in a grid of 12 columns and 8 rows (see Figure 1). The plate is made of unique materials - that allow any protein to bind, such as antigens and antibodies, to bind to the surface of the well. The binding of the proteins to the well is non-specific and irreversible

Each well in the plate is designated by its corresponding row and column number, for example: well B5.

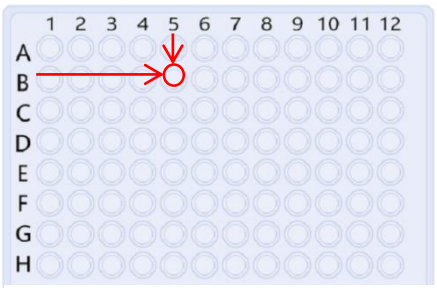

Figure 1: ELISA plate

Tools for two pairs of students working on the same plate:

- 1 ELISA plate
- 1 strip of parafilm paper appropriately sized to cover the entire plate
- 1 container with a waste bag labeled BIOHAZARD
- 1 stand for Eppendorf test tubes
- 12 empty Eppendorf tubes
- 2 glass labeling pens, each of a distinct color
- 2 micropipettes suitable for a volume of 100 µl -1000 µl and suitable tips
- Paper towels

Materials for a pair of students working with the same plate:

- A chemical cup of washing solution, labeled "washing solution."
- A labeled container of crushed ice, labeled "Pair 1" and "Pair 2," containing Eppendorf tubes:
    - A test tube labeled "V" containing a solution that simulates the Corona virus' spike protein (antigen).
    - A test tube labeled "M" containing a solution of blocking protein
    - A test tube labeled "Ab" containing an antibody against the spike protein of the Corona virus (primary antibody)
    - A test tube labeled "Ab2-E" containing an antibody to which an enzyme is bound (secondary antibody)
    - A test tube labeled "S" containing the substrate of the enzyme
    - A test tube labeled " A" containing plasma samples from Avi's blood
    - A test tube labeled "B" containing plasma samples from Daphne's blood
    - A test tube with sodium hydroxide labeled "NaOH"

The experiment procedure

1. Label the border of the wells for each group as shown in Figure 2."

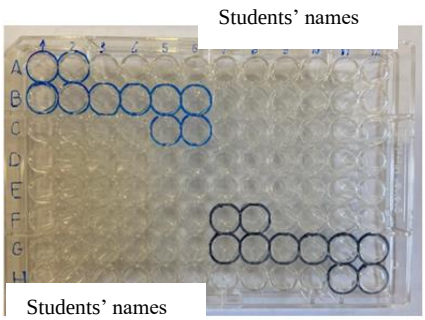

Figure 2: label the wells on the ELISA plate

2. The members of Pair 1 will use one color to label the following wells on the plate: A1-A2, B1-B6, C5-C6. Write down your names on the side of the plate.
3. The members of Pair 2 will use the other color to label the following wells on the plate: F7-F8, G7-G12, H11-H12. Write down your names on the side of the plate.

Step A: Antigen binding (a protein that simulates the protein in the corona virus envelope) to the ELISA plate

Figure 3: Antigen binding to the *ELISA* plate

Pay attention: At this following steps, this protein will be called an antigen.

4. Use an appropriate micropipette to transfer 100 µl of antigen from the Eppendorf test tube labeled "V" (Coronavirus spike protein), to each of the wells you have labeled.
5. Cover the ELISA plate with a strip of Parafilm paper.
6. Place the plate into the incubator that is set to 37°C. Write down the time: ____
7. Wait for 5 minutes.

Washing procedure (a-h):

Cleanse the plate by washing it with the "Washing Solution" (WS) to eliminate any antigen (or antibodies) residues that did not bind to the well. Work according to the following instructions:

While waiting, prepare your workspace by placing four layers of paper towels on the table.
After a duration of five minutes from the time you wrote down in section 7, proceed to carry out the washing procedure:

a) Take out the plate from the incubator and remove the parafilm paper from the plate.
b) Flip the plate upside down over the waste container and pour the liquid into it.
c) To remove any remaining liquid, hold the plate in one hand with the wells facing downwards and tap the bottom of the plate 5 times on top of the paper towels.
d) On your desk you will find a vessel labeled "washing solution".
e) Use an appropriate micropipette to transfer 370 µl of the WS to each labeled well.
f) Wait for 5 seconds.
g) Remove the WS from the wells into the waste container as described in step b.
h) Repeat the washing process one more time using the WS.
i) If necessary, replace the paper towels with new ones.

Step B: Linkage of the blocking protein to the Elisa wells

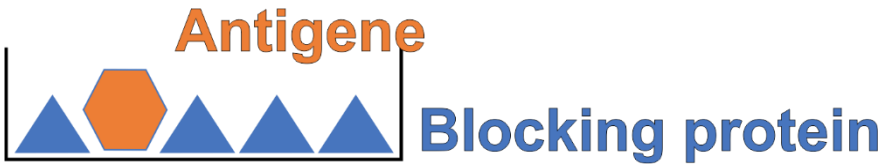

Figure 4: The blocking protein

Pay attention: The blocking protein binds to areas of the well where the antigen is not bound, preventing subsequent protein molecules from binding in those locations during the following steps.

8. Use an appropriate micropipette to transfer 100 µl of blocking protein from an Eppendorf test tube labeled "M" to each of the wells you labeled.
9. Cover the ELISA plate with a strip of Parafilm paper.
10. Place the plate in the incubator and leave it there until the procedure of multipole dilutions is complete.

Prepare antibody multipole dilutions according to the information in table 1

Pay attention: The antibody is against the Corona antigen. The antibody used at this step and in the following steps will be referred to as the 'Primary antibody'."

11. Labeled 6 empty Eppendorf test tubes with the numbers 1-6.
12. Use an appropriate micropipette to transfer 150 µl of washing solution to each of the test tubes you have labeled."
13. Use an appropriate micropipette to transfer 150 µl of antibody solution from the tube labeled 'Ab' to the tube labeled '1', following the guidance provided in Figure 4.
14. Gently mix the liquid in the tube labeled '1' by pipetting up and down several times using the micropipette. Then, place the tube in a container with ice."
15. Exchange the tip.

16. Use an appropriate micropipette to transfer 150 µl of the antibody solution from the Eppendorf test tube labeled '1' to the tube labeled '2'."
17. Gently mix the liquid in the tube labeled '2' by pipetting up and down several times using the micropipette.
18. Repeat steps 13-16 for test tubes 3-6, using the same procedure to withdraw 150 µl of the solution from one tube to the next, as shown in Figure 5.

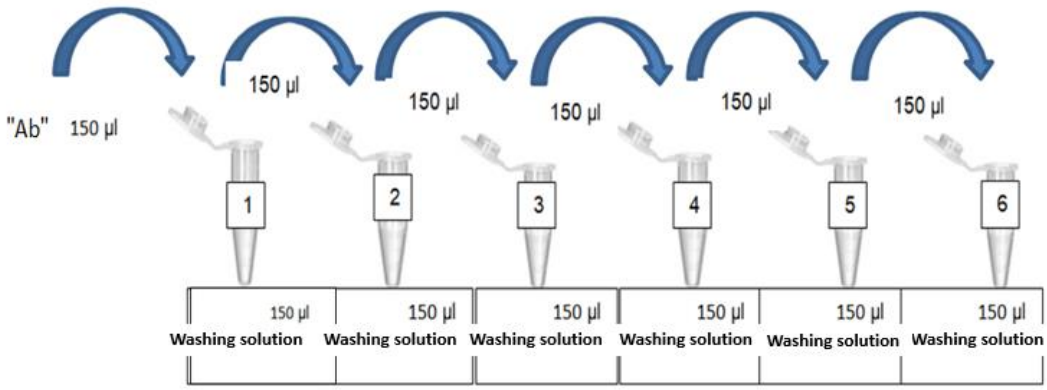

Figure 5: Double dilutions of antibody against the antigen

Table 1: Calculation of final concentrations of primary antibody

No. of tube	Volume of washing (µl) solution	Volume of primary antibody(µl)	Original concentration of primary antibody (µg/ml)	Final concentration of primary antibody (µg/ml)
1	150	150	1000	500
2	150	150		
3	150	150		
4	150	150		
5	150	150		
6	150	150		

19. Washing the plate: After the solutions have been prepared, take out the plate from the incubator and wash the plate using the washing procedure described earlier. This step is necessary to remove any blocking protein molecules that have not bound to the wells."

Step C: Adding the specific primary antibody

At this stage you will add known concentrations solutions of the primary antibody to the labeled wells, as well as solutions 'A' and 'B' containing plasma samples from Avi and Daphne, respectively."

Do not add the antibody to the cells labeled with an X.

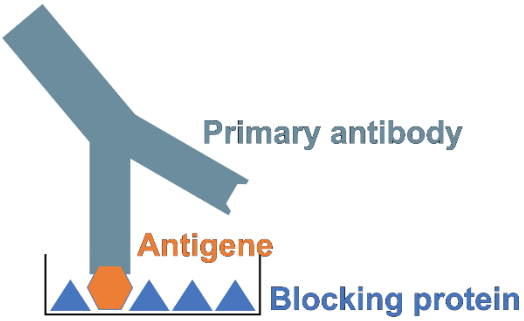

*Figure 6:*The specific primary antibody

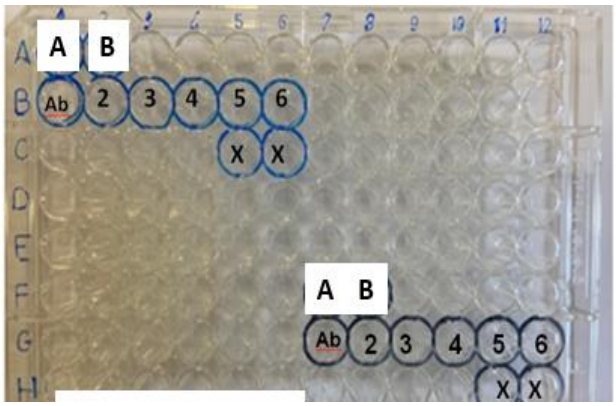

*Figure 7:*Wells with specific primary antibody

<u>Working instructions for pair 1</u>

Pay attention A/b are the samples (not Ab- antibody)

20. Use an appropriate micropipette to transfer 100 µl of antibody solution from the Ab-(or1) labeled test tube to the Ab-labeled well in row B.
21. Use an appropriate micropipette to transfer 100 µl of diluted antibody solution from the test tubes labeled 1-6, to the wells labeled 1-6 in row B respectively.
22. Use an appropriate micropipette to transfer 100 µl from the test tube labeled " A" to the well that is labeled "A" in row A.
23. Use an appropriate micropipette to transfer 100 µl of the solution from the test tube labeled "B" to the well labeled "B" in row B.
24. Continue to section 30- incubation.

Working instructions for pair 2

Pay attention A/b are the samples (not Ab- antibody)

25. Use an appropriate micropipette to transfer 100 µl of antibody solution from the Ab-labeled test tube to the Ab-labeled bar in row G.
26. Use an appropriate micropipette to transfer 100 µl of diluted antibody solution from the test tubes labeled 6-1, to each of the wells 1-6 in row G respectively.
27. Use an appropriate micropipette to transfer 100 µl from the test tube labeled " A" to the well that is labeled " A" in row F. Replace tip.
28. Withdraw 100 µl from the test tube labeled "B" to the well that is labeled "B" in row F.
29. Cover the plate with parafilm paper.
30. Place the plate into the incubator that is set to 37°C. Write down the time: ____
31. Wait for 5 minutes.
32. Washing the plate: Prepare the workspace for washing the ELISA plate according to the instructions in washing procedure:
    The purpose of this washing is to remove the molecules of the primary antibody that have not bound to the antigen.

Step D: Adding the secondary antibody

At this stage, you will add the secondary antibody, which has an enzyme bound to it, to the wells. This antibody will bind to the primary antibody.

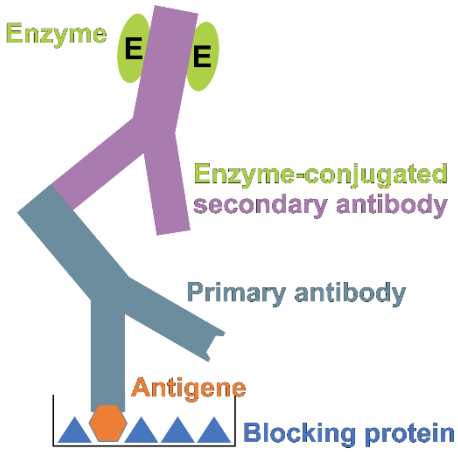

*Figure 8:*The secondary antibody

33. Use an appropriate micropipette to transfer 100 µl of the secondary antibody from the Eppendorf test tube labeled "Ab2-E" to all the labeled wells.
34. Cover the plate with parafilm paper.
35. Place the plate into the incubator that is set to 37°C. Write down the time: ____
36. Wait for 5 minutes for the incubation.
37. Washing the plate: Prepare the workspace for washing the ELISA plate according to the instructions in washing procedure:
    This process is designed to remove the molecules of the secondary antibody that have not bound to the primary antibody.

Step E: Adding substrate to the enzymes that is bound to the secondary antibody

Pay attention: The intensity of yellow color is directly proportional to the concentration of primary antibody in the subject's sample. Higher concentration of primary antibody results in more enzyme molecules in the well, leading to a higher intensity of yellow color

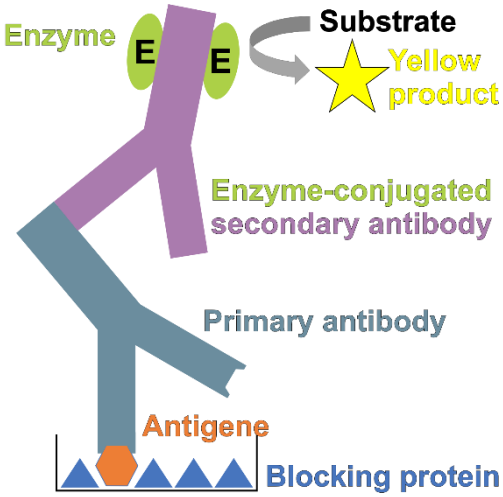

Figure 9: The enzyme substrate reaction

38. Use an appropriate micropipette to transfer 200 µl of medium from the tube labeled "S" to all the labeled wells.
39. Cover the plate with parafilm paper.
40. Place the plate into the incubator that is set to 37°C. Write down the time: ____
41. Wait for 5 minutes for the incubation.
42. after five minutes take the plate out from the incubator and look at results.
    The color of the solution should change gradually in wells B1-6.
43. stop the enzymatic process by adding 100 µl of Sodium hydroxide from an Eppendorf test labeled NaOH to each well

Pay attention: If the gradation in color intensity has not yet appeared in rows B and G, return the plate to the incubator and wait another 5 minutes. (If the color does not appear within 20 minutes, leave the palette for overnight incubation.

Table 2: Summary of the entire process

Action performed in the step	Step number
Addition of antigen to the wells	
Incubation for 5 minutes	
Washing the wells	
Addition of blocking protein to the wells	
Incubation until done with primary antibody solutions	
preparation of primary antibody solutions	
Washing the wells	
Addition of primary antibody to the wells	
Incubation for 5 minutes	
Washing the wells	
Addition of secondary antibody to the wells	
Incubation for 5 minutes	
Washing the wells	
Addition of substrate to the wells	
Incubation until yellow color appears	

Table 3: Summary of ingredients added to the wells

Substrate	**Secondary antibody**	**Primary antibody**	**Blocking protein**	**Antigen**	**Wells**
X	X	X	X	X	A1/F1
X	X	X	X	X	A2/F2
X	X	X	X	X	B1-B6/ G1-G6
X	X		X	X	C5-C6/ H5-H6

Table 4 : Preparation of washing solution

Ingredients	Quantity (g)
NaCl	8
KH_2PO_4	0.2
$Na_2HPO_4 \cdot 12H_20$	2.89
Tween-20	0.5
Distilled water	1 liter