Bio 4405- Immunology Lab

Maria Frias, PhD

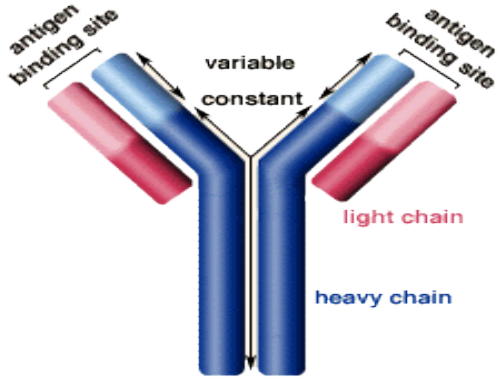
Thursday 1:30-4:30 (Lab)

Objective

- Learn the principles behind an Enzyme linked immunosorbent assay (ELISA)
- Detect an antigen using the ELISA technique

Definitions

- Antibodies (also known as **immunoglobulins** abbreviated **Ig**) are gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.



Definitions- cont.

- Antigens

A substance that when introduced into the body stimulates the production of an antibody

- Immunoassay

A laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in a sample

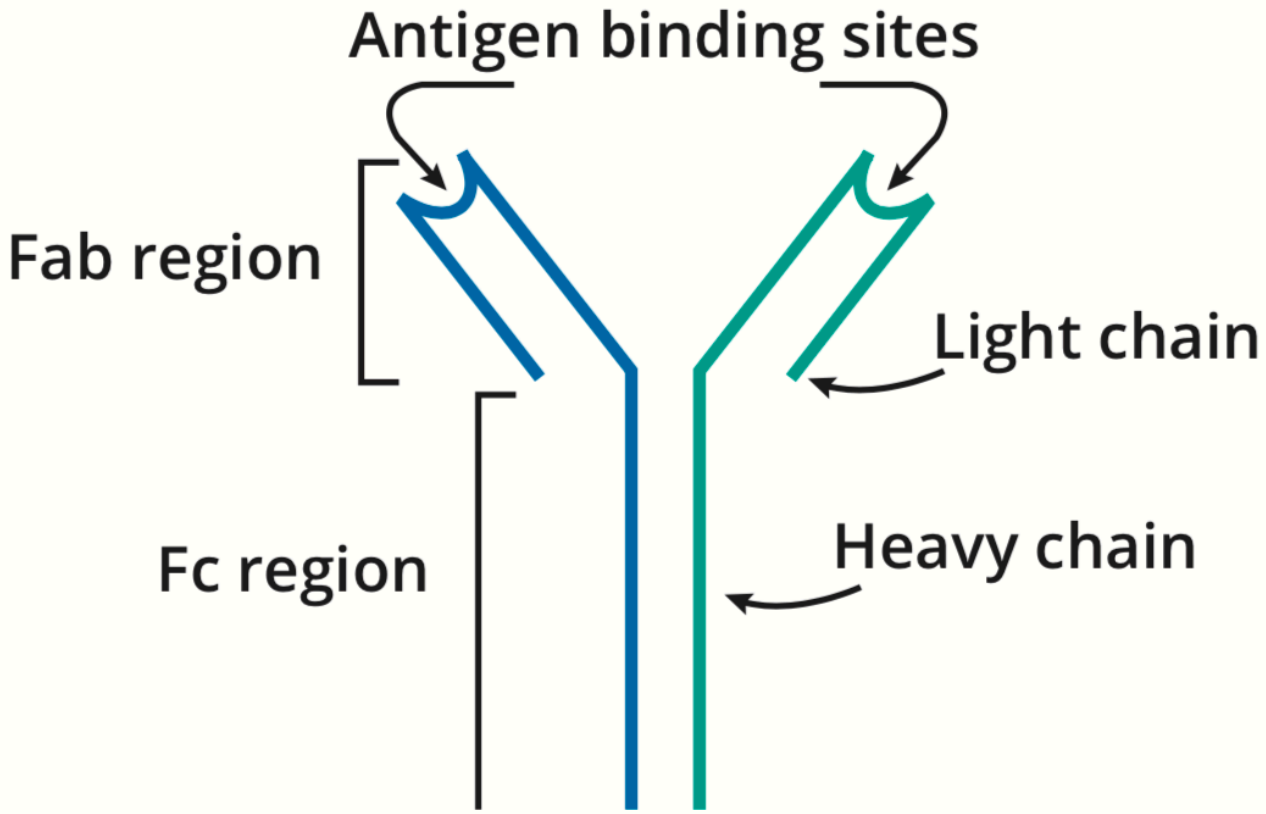


Figure 1:
General Structure of an antibody.

Definitions- cont

- Analyte

The sample being analyzed and in immunoasssays the analyte is either Antibody or Antigen

ELISA technique

Is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

- The technique is divided into

1- Competitive ELISA

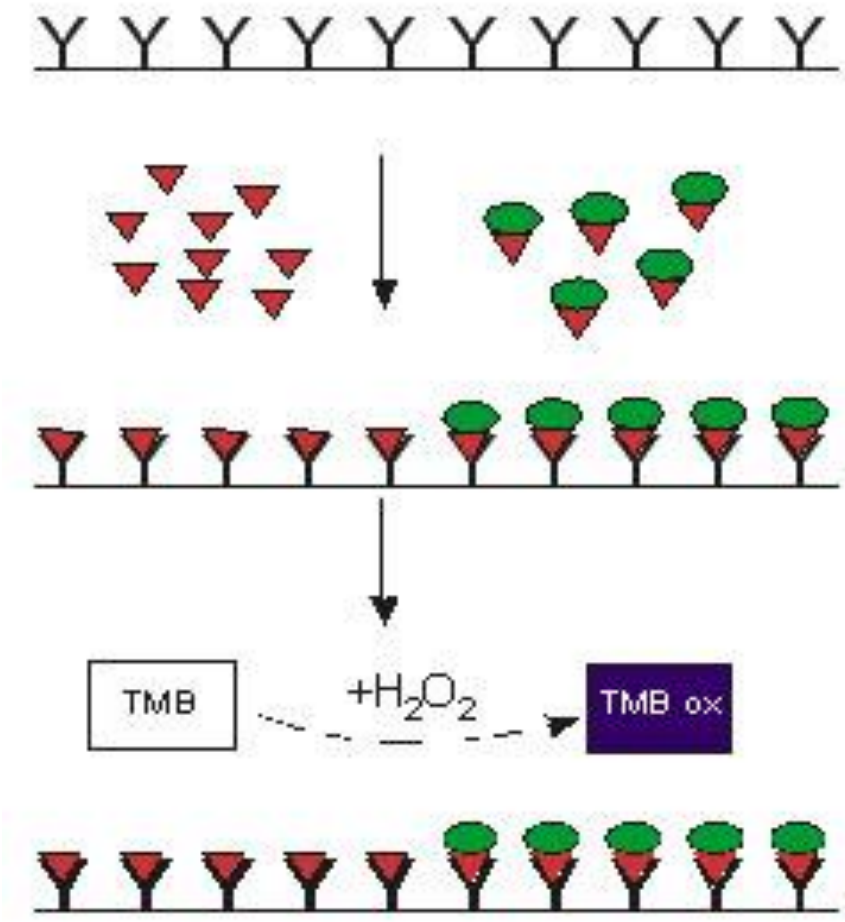
2- Sandwich ELISA (also called direct ELISA)

3- Indirect ELISA

Competitive ELISA

- The labelled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The more antigen in the sample, the less labelled antigen is retained in the well and the weaker the signal).

Competitive Enzyme Immunoassay



Solid phase coated with antibody

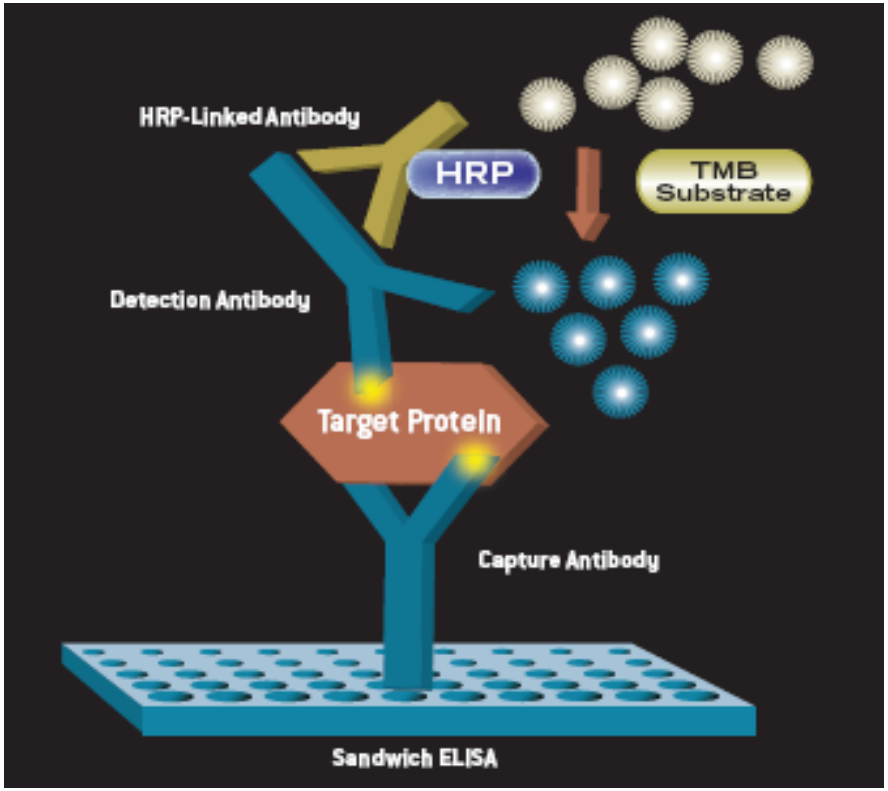
Add free and labelled antigen

Free and labelled antigen are captured

Color formation by oxidation of substrate into a colored compound

Sandwich ELISA

- The ELISA plate is coated with Antibody to detect specific antigen



Indirect ELISA

1. The protein antigen to be tested for is added to each well of ELISA plate, where it is given time to adhere to the plastic through charge interactions

2. A solution of non-reacting protein is added to block any plastic surface in the well that remains uncoated by the protein antigen

Indirect ELISA-Cont

3. Then the serum is added, which contains a mixture of the serum antibodies, of unknown concentration, some of which may bind specifically to the test antigen that is coating the well.

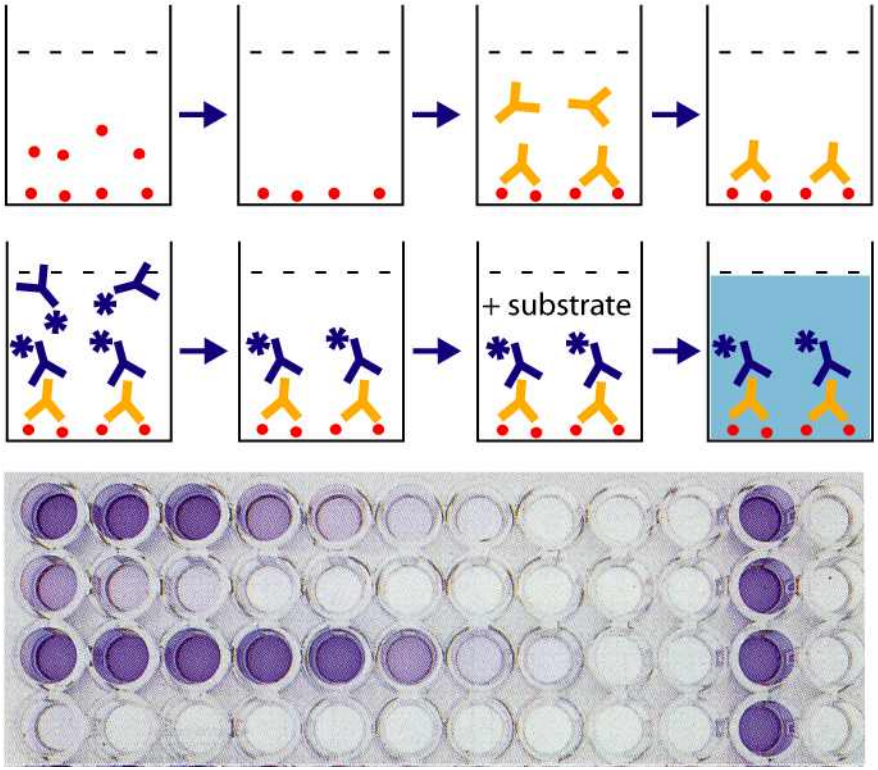
4. Afterwards, a secondary antibody is added, which will bind to the antibody bound to the test antigen in the well. This secondary antibody often has an enzyme attached to it

Indirect ELISA-Cont

5. A substrate for this enzyme is then added. Often, this substrate changes colour upon reaction with the enzyme. The colour change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen.

6. The higher the concentration of the primary antibody that was present in the serum, the stronger the colour change. Often a spectrometer is used to give quantitative values for colour strength

Indirect ELISA



Procedure

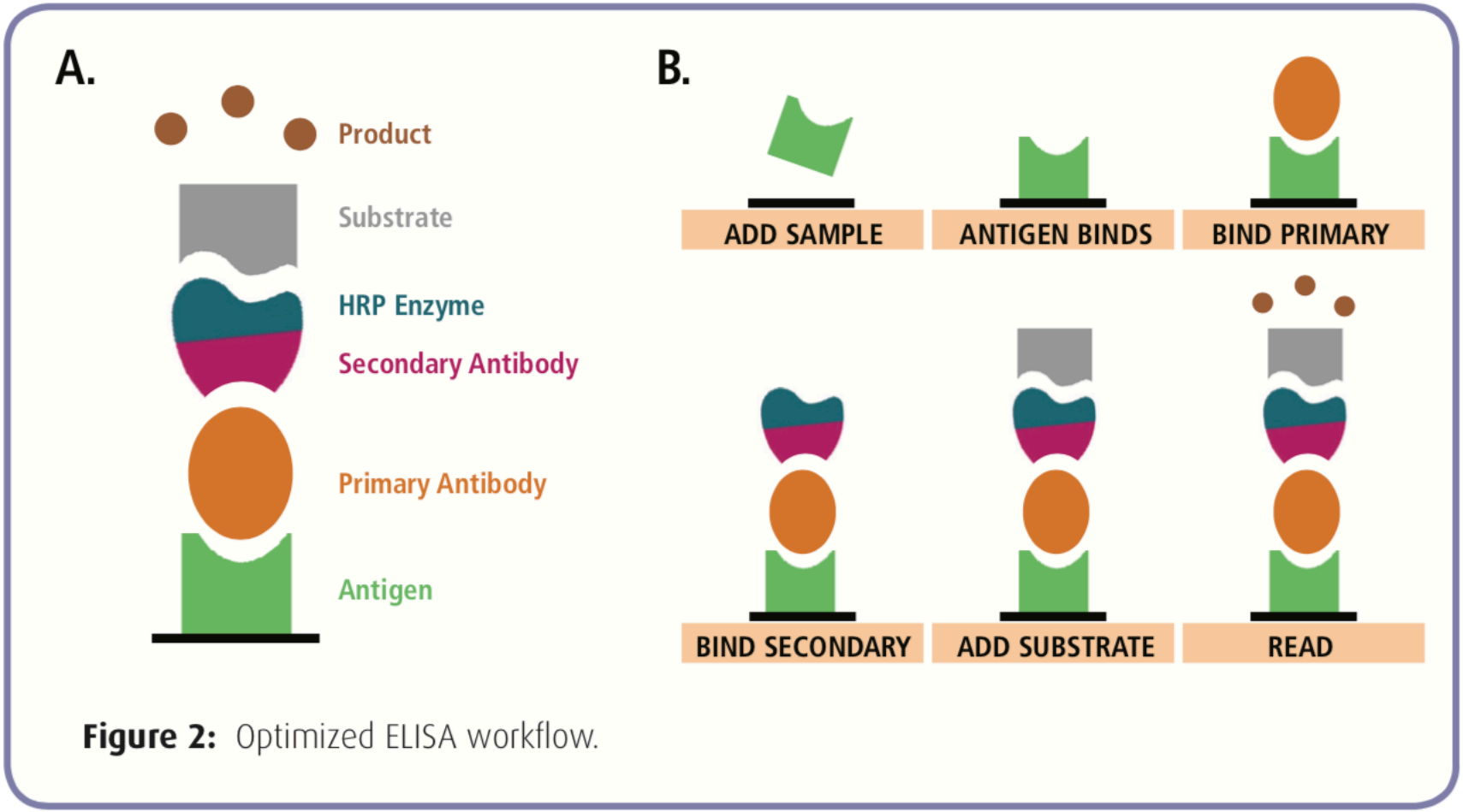


Figure 2: Optimized ELISA workflow.

Procedure

LABORATORY NOTEBOOKS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

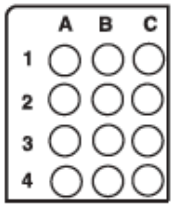
- Record (draw) your observations, or photograph the results.

After the Experiment:

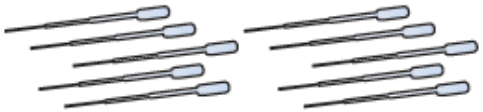
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Procedure - visual

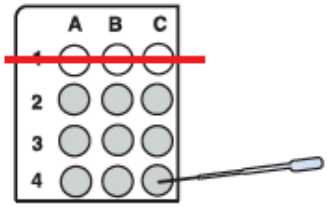
1. LABEL the microtiter plate.



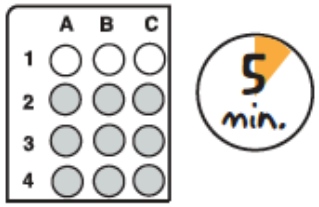
2. LABEL the transfer pipets.



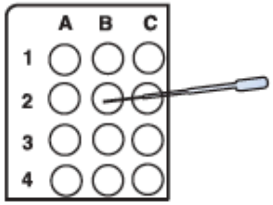
3. ADD 3 drops Ag to rows 2, 3, and 4.



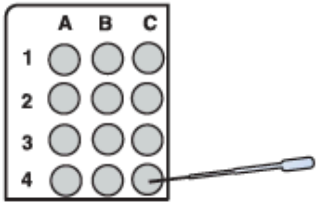
4. INCUBATE microtiter plate at room temp.



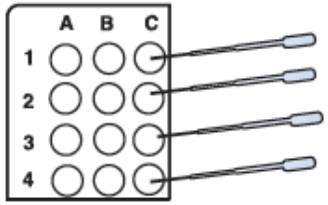
5. REMOVE liquid from wells.



6. WASH each well with 1X PBST buffer.



7. REMOVE all PBST using pipet for each row.



8. REPEAT Steps 6 and 7.

Procedure – step by step

Wear gloves and safety goggles

1. **LABEL** the wells of the microtiter plate as shown.

2. If not using an adjustable volume micropipette, **LABEL** the transfer pipets as outlined in the box below. These 10 pipets will be used to add and remove liquid from the wells.

(PBS)	Phosphate Buffered Saline	(S2)	Substrate 2
(Ag)	Antigen	(Row1)	Used to remove samples from row 1
(1°AB)	Primary antibody	(Row2)	Used to remove samples from row 2
(2°AB)	Secondary antibody	(Row3)	Used to remove samples from row 3
(S1)	Substrate 1	(Row4)	Used to remove samples from row 4

3. Using the "Ag" transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of Antigen (Ag) to all of the wells in rows 2, 3, and 4. Do not add antigen to the wells in row 1.

4. **INCUBATE** the plate at room temperature for 5 minutes.

5. Using the "Ag" pipet **REMOVE** all of the liquid from the wells.

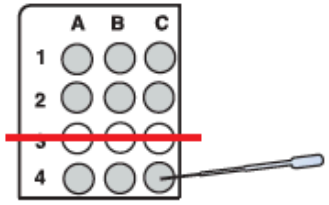
6. Using the "PBS" transfer pipet **WASH** each well by adding 1X PBST buffer until the wells are almost full (~200 µL). Do not allow the buffer to spill over into adjacent wells.

7. **REMOVE** all of the 1X PBST using the transfer pipet designated for each row.

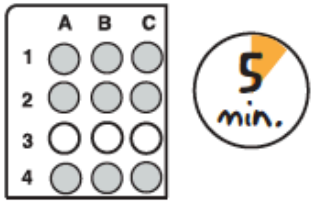
8. **REPEAT** steps 6 and 7 to wash the wells once more.

Procedure – visual

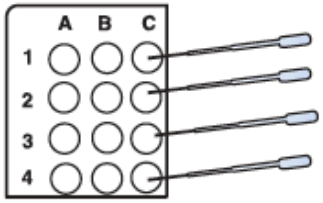
9. ADD 3 drops 1°AB to rows 1, 2, and 4.



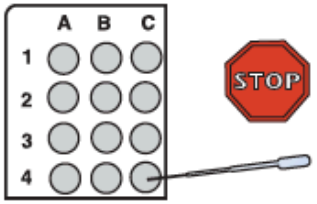
10. INCUBATE microtiter plate at room temp.



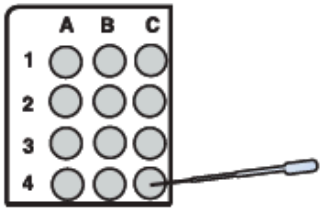
11. REMOVE all 1°AB using pipet for each row.



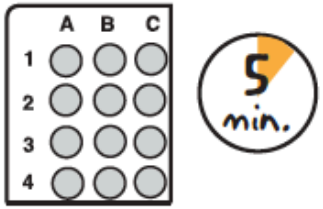
12. WASH each well with 1X PBST buffer. Remove. Repeat.



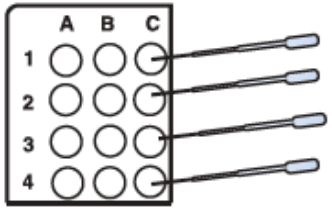
13. ADD 3 drops 2°AB to each well.



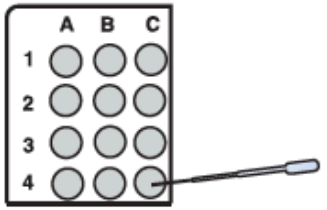
14. INCUBATE microtiter plate at room temp.



15. REMOVE all 2°AB using pipet for each row.



16. WASH each well with 1X PBST buffer. Remove. Repeat.



Procedure – step by step


Wear gloves and safety goggles

9. Using the "1°AB" transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of the primary antibody to the wells in rows 1, 2, and 4. Do not add primary antibody to the wells in row 3.

10. **INCUBATE** the plate at room temperature for 5 minutes.

11. Using the labeled transfer pipet for each row, **REMOVE** all of the primary antibody from each well.

12. **WASH** each well twice with fresh 1X PBST buffer. Between washes **REMOVE** all of the 1X PBST using the transfer piper designated for each row.

OPTIONAL STOPPING POINT: For overnight storage, **ADD** 200 µL of PBS to each well. Carefully cover the samples and place the plate in the refrigerator. The experiment should be resumed during the next lab period. Remove the 1X PBST and continue with Step 13.

13. Using the "2°AB" labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of the secondary antibody to each well.

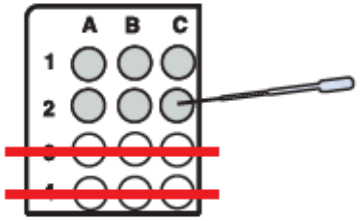
14. **INCUBATE** the plate at room temperature for 5 minutes.

15. Using the labeled transfer pipet for each row, **REMOVE** all of the secondary antibody from each well.

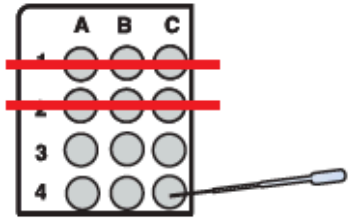
16. **WASH** each well twice with fresh 1X PBST buffer. Between washes **REMOVE** all of the 1X PBST using the transfer piper designated for each row.

Procedure – visual + step by step

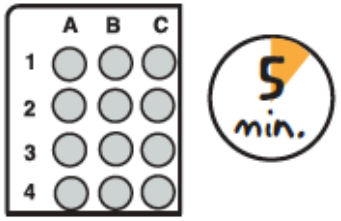
17. ADD 3 drops S1 to rows 1 and 2.



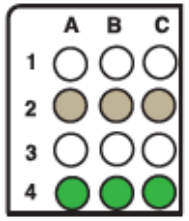
18. ADD 3 drops S2 to rows 3 and 4.



19. INCUBATE microtiter plate at room temp.



20. ANALYZE plate for color changes.



17. Using the "S1" labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of Substrate 1 to rows 1 and 2.

18. Using the "S2" labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of Substrate 2 to rows 3 and 4.

19. **INCUBATE** the plate at room temperature for 5 minutes.

20. Immediately **ANALYZE** the plate for color changes in the substrates. If the color is not fully developed it can be left for a longer period of time.

Results and analysis

- Record results and conclusions
- Interpret results
- Think about these questions

Study Questions

1. What is the ELISA? Describe the purpose of each component used in an ELISA.
2. What is the effect of not including the antigen or the primary antibody in the ELISA reaction?
3. Why is it important to wash all the wells between the additions of the various components?
4. Can nucleic acids be detected by the ELISA format?