ELISA Protocol for ZIKA VIRUS

1. Gather your materials:
   - 12 well ELISA strip
   - + control
   - Negative control
   - Patient serum (2 samples)
   - Wash
   - Blocker
   - Disposable pipettes
   - Paper towels

   **ELISA Test Key**

<table>
<thead>
<tr>
<th>Positive Control</th>
<th>Negative Control</th>
<th>Patient’s Serum</th>
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<td>+</td>
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2. Select one patient serum tube, and using one disposable micropipette, place one drop of the patient’s serum into wells 7-9. When you are done, put pipette aside.

3. Choose another patient tube and using a NEW disposable micropipette, place one drop of that patient’s serum from the tube into wells 10-12. When you are done, put pipette aside.

   You have now added **antibodies (Y shaped protein)** from the patient blood plasma. If the patient has formed **antibodies to the antigen**, the antibodies will attach to the antigen when it is placed in the well.

4. Place a small stack of paper towels down onto the desk. Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down onto the paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

5. Find the **large tube labeled "Wash"**. Using one NEW pipette, place one drop of the Wash solution into wells 7-12. Do NOT throw this pipette away, you will use it again for the wash steps.

6. Carefully take the ELISA well strip into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

   You have now washed away any **antibodies** that were not attached to the wall of the wells.
7. Find the tube labeled "Blocker" and using a NEW disposable pipette place one drop of the blocker protein solution into wells 1-12 in.

8. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

   You have now blocked all the sites that the antibodies were not attached to in the well.

9. Find the large tube labeled "Wash". Using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1-12. Do NOT throw this pipette away, you will use it again for the wash solution.

10. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

   You have now washed away any blocker proteins that were not attached to the wall of the wells (purple dot protein).

11. Find the tube labeled "Positive Control" and using a NEW micropipette place one drop of the positive control solution into wells 1-3.

   You have now added a solution that is known to contain antibodies for Zika – we know this sample will react, which is why it is called a positive control. No antibodies have been placed in wells 4-6 – we know these samples will not react, which is why it is called a negative control.

12. Find the tube labeled "Negative Control" and using a NEW micropipette place one drop of the negative control solution into wells 4-6.

13. Find the tube labeled "Zika". This tube contains a sample of virus antigen. Using a NEW disposable micropipette, place one drop of the antigen into wells 1-12. DO NOT use this pipette again.

14. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times, and then place back on the desk.

   You have now placed Zika antigen (red circle protein) into the wells where they can interact with the specific antibodies.
15. Find the large tube labeled "Wash". Using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1-12. Do NOT throw this pipette away, you will use it again for the wash solution.

16. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

You have now washed away any antibodies that had not attached to antigens in the wells.

17. Find the tube labeled "Fluorescent Tag" and using a NEW disposable micro pipette place one drop of the tagging solution into wells 1-12.

18. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

You have placed antibodies with fluorescent molecules (star shape molecule) attached into the wells. These fluorescent molecules will attach to any antibodies present in the well. Antibodies will only be found in a well if they were placed there (as in the positive control), OR if they were in the patients’ blood.

19. Find the large tube labeled "Wash". Using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1-12.

20. Carefully take the ELISA tray into the palm of your hand and quickly turn it upside down onto the stack of paper towels to empty the wells. Tap the tray several times then place it back on the desk right side up.

You have washed fluorescent molecules that have NOT attached to an antibodies in the wells.

21. Obtain an Ultraviolet light and shine it over the ELISA plate. Use the ELISA Test Key to record which of the patients tested positive for Zika.