# **LAB 9 PROTOCOL: IMMUNOASSAYS (ELISA) AND BLOOD TYPING**

## **ACTIVITY 1: EXPERIMENTAL PROCEDURE FOR THE DETECTION OF HIV ANTIBODIES BY ELISA**

1. Place the microtiter plate vertically to show 4 rows and 3 columns. Mark the plate with your initials and **label** the 4 rows from top to bottom as follows:

**(-)** (negative control)



**(+)** (positive control)

**P1** (donor serum 1)

**P2** (donor serum 2)

1. **Label** the 8 plastic transfer pipettes as follows:

**(-)** (negative control)

**(+)** (positive control)

**P1** (donor serum 1)

**P2** (donor serum2)

**2oAB** (secondary antibody)

**Ag** (HIV antigen)

**ABTS** (ABTS substrate)

**Wash** (1x PBST Wash Buffer).

**These 8 pipets will be used to add and remove liquid from the wells.**

1. Using the “Ag” transfer pipet or a micropipette, **ADD** 3 drops of 50 ul of Antigen (Ag) to all 12 wells.
2. **INCUBATE** the plate at room temperature for 5 minutes.
3. Using the “Ag” pipet **REMOVE** all of the liquid from the wells. Extract all liquid using the HIV pipette. The waste may go in the sink.
4. **Wash each well using the following procedure:**

Use the “WASH” pipette **WASH** each well by adding wash buffer to the wells in all rows. Add the WASH buffer until each well is almost full. *Do not allow the liquid to spill over into adjacent wells.*

1. ***REMOVE*** *all of the wash buffer using the transfer pipet designated for each row.* Dispose of the liquid in the sink.
2. **REPEAT** STEP 6 and 7 to wash the well once more.
3. Using the “-“ transfer pipet or a micropipette, **ADD** 3 drops or 50 uL of the negative control to all three wells in the top row.
4. As in step 9, **ADD** the “+”, “P1”, and “P2” samples to all three wells in the appropriate rows, taking care to use the correct pipets or changing tips between each sample.
5. **INCUBATE** the plate at room temperature for 5 minutes.
6. Using the correct transfer pipet for each row, **REMOVE** all of the primary antibody from each well.
7. **WASH** each well twice with fresh wash buffer. Between washes **REMOVE** all of the wash buffer using the transfer pipet designated for each row.
8. Using the “2 AB” labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 uL of the sencondary antibody to each well.
9. **INCUBATE** the plate at room temperature for 5 minutes.
10. Using the labeled transfer pipet for each row, **REMOVE** all of the secondary antibody from each well.
11. **WASH** each well twice with fresh wash buffer. Between washes **REMOVE** all of the wash buffer using the transfer pipet designated for each row.
12. Using the “ABTS” labeled transfer pipet or micropipette**, ADD** 3 drops or 50 uL of ABTS substrate to all wells.
13. **INCUBATE** the plate at room temperature for 5 minutes.
14. Immediately **ANALYZE** the plate for color changes in the substrate. If the color is not fully developed it can be left for a longer of time.

**\*\*\*STUDENTS WHO DID NOT SIGN THE CONSENT FORM MAY DUE THE BELOW LAB PROCEDURE AS AN ALTERNATIVE**

## **ACTIVITY 2: BLOOD TYPING PROCEDURE**

1. LABEL EACH BLOOD TYPING SLIDE:

SLIDE #1: Mr. Smith

SLIDE #2: Mr. Jones

SLIDE #3: Mr. Green

SLIDE #4: Mr. Brown

1. PLACE TWO DROPS OF Mr. Smith’s blood in each of the A, B, and Rh wells of SLIDE #1.
2. PLACE TWO DROPS OF Mr. Jones’s blood in each of the A, B, and Rh wells of SLIDE #2.
3. PLACE TWO DROPS OF Mr. Green’s blood in each of the A, B, and Rh wells of SLIDE #3.
4. PLACE TWO DROPS OF Mr. Brown’s blood in each of the A, B, and Rh wells of SLIDE #4.
5. PLACE TWO DROPS OF the simulated anti-A serum in each A well on the four slides.
6. PLACE TWO DROPS OF the simulated anti- B serum in each B well on the four slides.
7. PLACE TWO DROPS OF the simulated anti-Rh serum in each Rh well on the four slides.
8. OBTAIN THREE TOOTHPICKS PER BLOOD TYPING SLIDE. Stir each well with a separate clean toothpick for 30 seconds. To avoid splattering the simulated blood, DO NOT PRESS TOO HARD ON THE TYPING TRAY.
9. OBSERVE EACH SLIDE. Record observations in your table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **NAME** | **Anti-A Serum** | **Anti-B serum** | **Anti-Rh Serum** | **Blood Type** |
| **Mr. Smith** |  |  |  |  |
| **Mr. Jones** |  |  |  |  |
| **Mr. Green** |  |  |  |  |
| **Mr. Brown** |  |  |  |  |

**AGGLUTINATION = CLUMPING**

**HINT:** If agglutination occurs only in the suspension to which the anti-A serum was added, the blood type is A.

POPS PROJECT:

Record your own blood type