Directions for Workshop HIV/AIDS Lab Exercise

Introduction
This lab is a simulation of an ELISA test for detecting the presence of antigen from a sample of body fluid. The antigen is not actually related to the HIV virus, but safety procedures will be practiced as though it were both contagious and dangerous. The antigen used is a harmless protein chosen because it binds strongly to a second protein. The second protein, representing the antibody, will have been linked to the enzyme peroxidase. The protein used as the antibody binds very strongly to the antigen.

Each workshop participant will be given a solution representing his/her body fluid. Participants will then exchange body fluid with three other individuals. The ELISA will be used to test for the simulated disease agent in the mixed body fluids. Finally, the lab will trace back through the transmission routes to determine the identity of the original carrier.

Experimental Materials
- tube rack
- Washing solution (1X PBS /0.1% Tween)
- microtiter (assay) plates
- Antibody solution (streptavidin-peroxidase solution)
- Negative Control and “body fluid” solution (sodium carbonate buffer solution, pH 9.6)
- TMB - (3,3’,5,5’-tetramethylbenzidine) Colorizing solution
- Positive Control and “body fluid” solution (biotinylated albumin solution)
- transfer pipets

Protocol
1. Find one other participant in the workshop and exchange fluids by transferring the contents of one tube into the other using a transfer pipet. Using the same pipet, split the contents back into the two original tubes. Record the name of the person you first made contact with. (You can use the same transfer pipet throughout all 3 rounds of sharing.)
2. At the instructor’s signal find a different participant to exchange with, as you did in #1 above. Record the name of your second contact.
3. At the instructor’s signal exchange with one more participant and record the name of your third contact.

Note: be sure to choose participants from among all workshop participants, and not those just in your immediate area.

4. When all three contacts have been completed, an assistant will perform an ELISA test on your tube of fluid.
5. During this time you will learn about recent advances in the genetics of HIV/AIDS.
6. The workshop assistant will do one positive and one negative control for you to view. To do this, they will add 4 drops (100 µL) of a positive test solution into 3 wells and 4 drops (100 µL) of a negative test solution into a different set of three wells. The wells will be marked to indicate positive and negative controls. The 4 drops will fill one third to one half of the well.
7. The assistant will then add 4 drops (100 µL) from each sharing tube fluid into each of three wells. As you observe this, you should record which wells contain your fluid to avoid confusing your wells with another participant’s.
8. You will then see the assistant rinse the plate with washing solution, dumping off the excess, repeating this technique a total of three times.
9. Once all data is recorded, the assistant will shake off the fluid into a nearby sink or designated container, making sure that the fluid has emptied from each well. Then the plate will be turned upside down onto the paper towel to remove any excess liquid or bubbles.
10. The assistant will remove excess solution and bubbles by again tapping the plate face down onto a paper towel. You will either observe or help with this procedure.
11. You will then be instructed to add 4 drops (100 µL) of the antibody solution to each well.
12. Incubate for 5 minutes on the lab table and then shake off the fluid.
13. Rinse the plate with washing solution, dumping off the excess. Repeat a total of three times (a repeat of steps 10 and 11).
14. Add 4 drops (100 µL) of the colorizing (TMB peroxidase substrate) solution to each well.
15. Make observations after 5 minutes.
16. The assistant will make an announcement indicating the infected participants. The positive participants and a list of their contacts should also be recorded on a white board.
Discussion Topics for Workshop Participants

1. From the data on the blackboard, how many people could be the source of the “virus”? The Instructor should be able to tell you who “Patient 0” really was.
2. Discuss what is meant by a false positive test.
3. Explain what is meant by a false negative test. Does this mean a person shouldn’t take precautions in future sexual contacts?
4. What was the significance of mixing the body fluids with other participants?
5. After doing this exercise, would you agree or disagree with the following statement: “When you have sex with someone, you are also having sex with everyone they have previously had sex with.” Explain your answer.
6. Was it possible to know who was infected before the interaction where fluids were exchanged? In what ways is this situation similar to HIV infection in the real world?
7. What steps can you take to protect yourself from sexually transmitted diseases?