Molecular Biology Name _____

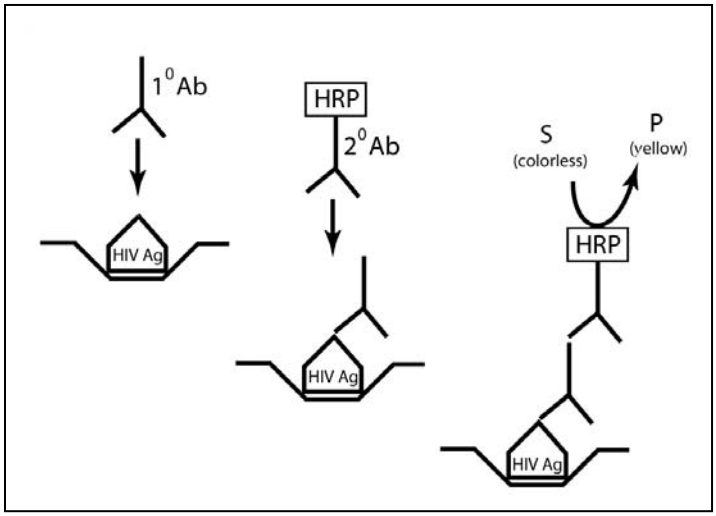
Lab #8 ELISA

Human Immunodeficiency Virus (HIV) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). HIV primarily infects and kills T helper lymphocytes, which are an essential component of the immune system. In the initial stages of the disease, the immune system mounts a strong response against HIV that includes both antibody-mediated and cell-mediated immunity. However, in the absence of antiviral drugs the virus almost always defeats the immune system, and the number of T lymphocytes slowly declines. As the T-cell count of an HIV-positive (HIV+) person drops from 1200/ml blood (normal) to <200/ml and the symptoms of immune suppression begin to manifest themselves, the clinical status of the individual changes from being HIV+ to having AIDS. A combination of opportunistic infections, cancers, wasting and neuropathies in AIDS patients results in serious illness and eventual death.

The HIV virus is relatively labile (fragile) outside the body and is easily destroyed by environmental conditions such as heat, desiccation and pH changes. Transmission of HIV occurs through the exchange of bodily fluids that contain live virus; blood, semen, vaginal secretions and breast milk.

The treatment of AIDS includes antiviral drugs (reverse-transcriptase inhibitors and protease inhibitors) to inhibit viral replication and conventional drugs to treat opportunistic infections such as PCP pneumonia, shingles, thrush and Taxoplasmosis.

The Enzyme-Linked ImmunoSorbant Assay (ELISA) is designed to detect small amounts of antigen (Ag) or antibody (Ab) in a complex liquid sample. The ELISA is the primary test for HIV. When an individual is infected with HIV their immune system will produce antibodies that specifically recognize and bind to HIV antigens (proteins). The ELISA can test for the presence of HIV-specific antibodies in a person's blood. If HIV-specific antibodies are present in a sample of blood then the person must be HIV positive (HIV+).



The experiment we will perform today is a simulation of an HIV-AIDS test. Refer to the figure to the right throughout the following discussion. The ELISA is typically performed in a microtiter plate. These transparent plastic plates contain multiple wells, into which liquid samples are placed.

First, antigen is added to the wells. The antigen binds non-specifically to the plastic surface. In the case of an HIV test, the antigens are purified viral proteins derived from the HIV virus. Viral antigens that do not bind to the plastic well are washed away in a subsequent step.

Second, donor serum (the liquid portion of the blood) from the person being tested is added to the wells. If antibodies (IgG) specific for HIV viral antigens exist in the donor's serum then these antibodies will bind tightly to the viral antigens adsorbed to the plastic well.

Antibodies that do not bind to antigens are washed away in a subsequent step. In this experiment, the antibodies that bind HIV antigens are termed the primary antibodies (1^0 Ab)

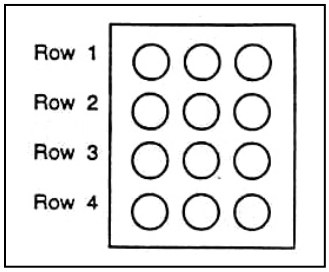
Third, a rabbit antibody that specifically recognizes and binds to human IgG is added to the wells. The rabbit antibody binds to human IgG and remains in the well even after the subsequent wash step. The rabbit antibody is conjugated (linked) to an enzyme called horseradish peroxidase. This enzyme is capable of converting a colorless substrate (S) into a yellow product (P). In this experiment, the enzyme-conjugated rabbit antibody is termed the secondary antibody (2^0 Ab).

Finally the enzyme's substrate is added to the well and the reaction is given time to occur. If the donor serum does indeed contain HIV-specific antibodies then the wells will turn yellow. If the donor serum lacks HIV-specific antibodies then the well will remain relatively colorless.

It is essential that the ELISA have both a positive and a negative control. The positive control is a sample that is guaranteed to produce a positive result (yellow color). Our positive control tube, labeled (+), contains "HIV"-specific antibodies. The negative control is a sample that is guaranteed to give negative results (colorless). Our negative control tube, labeled (-), contains bovine serum albumin, a cow plasma protein. The controls tell us that the experimental system is working, that is to say that positive and negative results are at least possible under our experimental conditions. The controls also provide a basis for comparison with experimental results.

The ELISA is a sensitive and relatively accurate test. Occasionally however, the test provides false information. A "false positive" is a test result that indicates a person is HIV positive when in fact they are not. A "false negative" is a test result that indicates a person is HIV negative when in fact they are infected with HIV. The frequency of false positives and false negatives is less than 1/1000, which means that more than 99.9% of the time the test is accurate.

Procedure



1. Label the microtiter plate with your initials and number the rows 1-4.
2. To all wells add 100 microliters (μl) of "HIV" viral antigens.
3. Incubate at room temperature for 5 minutes
4. Using a micropipettor, remove the unbound viral antigen from each well.
5. Wash each well twice with phosphate buffered saline (PBS).
    - Use a Pasteur pipet to add fresh PBS to each well. Wells should be ¾ full. Cross-contamination will occur if you allow wells to overflow.
    - Remove the PBS with a Pasteur pipet and put it in the beaker labeled "waste"
    - Repeat again

6. Using a fresh tip for each new reagent, add 100 μl of each reagent to the wells as outlined below:
    - Add the positive control, marked (+), to each of the 3 wells in row 1.
    - Add the negative control, marked (-), to each of the 3 wells in row 2.
    - Add donor serum #1 (DS1) to each of the 3 wells in row 3.
    - Add donor serum #2 (DS2) to each of the 3 wells in row 4.

7. Incubate at 37^0C for 15 minutes.

8. Using a new tip for each row, remove the reagents from each well with a micropipettor.

9. Wash each well twice with PBS

10. Using a new tip, add 100 μl of secondary antibody (2^0 Ab) to each well.

11. Incubate at 37^0C for 15 minutes. *At the start of this incubation ask the instructor to prepare the substrate, which must be made fresh*.

12. Using a new tip for each row, remove the reagents from each well with a micropipettor.

13. Wash each well twice with PBS

14. Using a new tip, add 100 μl of substrate to each well.

15. Incubate at 37^0C for 10 minutes.

16. Analyze your results

Questions

1. Produce a drawing of your results.

2. Which, if any, of the donors tested positive for HIV? Provide a rationale for your answer.

3. With regards to an ELISA test for HIV, what is a false positive? What is a false negative?

4. The acronym ELISA stands for what?

5. Can the ELISA detect only antibodies in a sample, or can it also detect antigens? Explain.

6. What is the role of horse radish peroxidase in the ELISA.

7. What is the function of positive and negative controls?

8. What other diagnostic test (besides the ELISA) is used to test for HIV infection?

9. A pregnancy test is an ELISA that assays for the presence of human chorionic gonadotropin (hCG) in the urine of the female subject. HCG is a protein hormone produced only by the placenta and thus is an excellent indicator of pregnancy. Design an ELISA that would function as a pregnancy test. Assume you have access to any antibodies you wish. Draw a picture of your design and describe how it would work.

10. An ELISA is used to screen hybridomas for the production of monoclonal antibodies. Let's consider the following hypothetical situation. You wish to make monoclonal antibodies specific for the gp120 protein of HIV. You begin by repeatedly injecting purified gp120 protein into a mouse. Next you remove the spleen and isolate B lymphocytes. Next you fuse these B lymphocytes with $HGPRT^-$ myeloma cells and select for hybridomas in HAT media. You isolate many different hybridomas. How can you use the ELISA assay to identify those few hybridomas that make antibodies specific for gp120?

11. What specific cells does HIV infect?

12. What types of drugs are used to inhibit viral replication in HIV+ individuals?