

PhysioEx Lab Report

Exercise 12: Serological Testing

Activity 3: Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

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Pre-lab Quiz Results

You scored 100% by answering 4 out of 4 questions correctly.

1 For home pregnancy tests

You correctly answered: the antigen is human chorionic gonadotropin.

2 Which of the following is true of the indirect ELISA?

You correctly answered: A positive patient sample contains antibodies specific to the antigen of interest.

3 What does the antigen bind to on the antibody?

You correctly answered: the variable region.

4 Which of the following is true of the direct ELISA?

You correctly answered: The positive patient sample contains the specific antigen.

Experiment Results

Predict Questions

No Predict Questions

Stop & Think Questions

1 What must occur in order for an HIV-infected individual to test positive with the indirect ELISA test?

You correctly answered: seroconversion.

2 What is the key substance found in the developing buffer?

You correctly answered: enzyme linked to the secondary antibody.

Experiment Data

Sample	Optical Density	HIV Test Result
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Positive Control	1.624	+
Negative Control	0.154	-
Patient A	0.054	-
Patient B	0.432	IND
Patient C	1.990	+

Post-lab Quiz Results

You scored 100% by answering 4 out of 4 questions correctly.

1 Antibodies are made of

You correctly answered: **proteins.**

2 How would you describe the binding site of the secondary antibody?

You correctly answered: **the constant region of primary antibody.**

3 A positive result for HIV in the indirect ELISA performed contains

You correctly answered: **antibodies against HIV.**

4 Which patient needs to be retested?

You correctly answered: **patient B.**

Review Sheet Results

1 Describe how the direct and indirect ELISA are different.

Your answer:

The direct and indirect elisa are different because they use a different number of antibodies in the testing. In a direct elisa there is only one antibody used, wich is used directly and the substrate is acted on by the detection enzyme. But in an indirect ELISA, two antibodies are used a primary and secondary antibody which has an enzyme linked to it. The secondary antibody works as a complement to the primary antibody.

2 Discuss why a patient might test indeterminate.

Your answer:

A patient might test indeterminate because they haven't yet been seroconverted. Another reason could be that they have no produced enough antibodies against the antigen to cause the test to show a positive result.

- 3** How would your results have been affected if your negative control had given an indeterminate result?

Your answer:

If the negative control was given an indeterminate result, then the results of the entire experiment would be invalidated. This could happen because the wells were not washed enough and there could have been some type of cross contamination.

- 4** Briefly describe the basic structure of antibodies.

Your answer:

Antibodies are made of two heavy chains and two light chains connected by a hinge region. They are either Y or T shaped and have a constant variable region.