PhysioEx Lab Report

Exercise 12: Serological Testing

Activity 4: Western Blotting Technique

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Date: 1 April 2025

Session ID: session-c91d152a-71c5-328d-889b-acbd43119663

Pre-lab Quiz Results

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

1 Which of the following tests is not correctly matched to what it tests for?

You correctly answered: These are all correctly matched.

2 Gel electrophoresis is used to separate proteins on the basis of

You correctly answered: size and charge.

3 Western blotting can be used to test for

You correctly answered: HIV and Lyme disease.

4 In this activity you will use Western blotting to test for the presence of

You correctly answered: antibodies (like the indirect ELISA).

Experiment Results

Predict Questions

No Predict Questions

Stop & Think Questions

1 What is present on the nitrocellulose strips?

You correctly answered: HIV antigens separated by electrophoresis.

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

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

Experiment Data

Sample	gp160	gp120	p55	p31	p24	HIV Test Result
Patient A	no	no	no	no	no	-

Patient B	no	no	yes	no	yes	IND
Patient C	yes	yes	yes	no	yes	+
Positive Control	yes	yes	yes	yes	yes	+
Negative Control	no	no	no	no	no	-

Post-lab Quiz Results

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

1 The patient samples being tested contain

You correctly answered: serum.

2 The primary antibodies in the Western blot

You correctly answered: come from the patient sample.

3 A key difference between the ELISA and the Western blot technique is that the

You correctly answered: Western blot looks at separated antigens.

4 The antibodies in a positive patient sample bind to the

You correctly answered: constant region of the enzyme-linked antibody and the antigen bound to the nitrocellulose strip.

Review Sheet Results

1 Describe why the HIV Western blot is a more specific test than the indirect ELISA for HIV.

Your answer:

Indirect Elisa uses wells that correspond with a mixture of different antigens. The western blot tests a specific protein that corresponds to a specific antigen.

2 Explain the procedure for a patient with an indeterminate HIV Western blot result.

Your answer:

This could be due to possible errors during the experiment.

3 Briefly describe how the nitrocellulose strips were prepared before reacting them with the patient samples.

Your answer:

An electric current separates proteins. the proteins are transferred to the nitrocellulose membrane to make nitrocellulose strips.

4 Describe the importance of the washing steps in the procedure.

Your answer:

It removes other nonspecific binding antibodies. There could be a false positive if not washed properly.