Interpretation of ELISA and Western Blot Assays for HIV Infection Status

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Abstract

Working in pairs, students use an enzyme-linked immunosorbent assay (ELISA) to screen sera for antibodies to human immunodeficiency virus (HIV). Sera that test "positive" in the ELISA are subsequently evaluated by Western blotting to determine whether the patient's infection status is positive, negative, or indeterminate according to criteria set by the Centers for Disease Control and Prevention (CDC). Because surrogate antigens and antibodies are used in this exercise, there is no risk of infection for students or teaching personnel. The students are provided with Western blot strips to which simulated antigens have already been applied, thus eliminating safety concerns associated with gel electrophoresis, such as exposure to acrylamide and the possibility of electrical shock. The banding patterns on the strips are generated using a multi-channel manifold device rather than an electrophoretic cell so that results are highly uniform, reproducible, and simple to interpret.

Activity

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Editor's Note (2008): This Curriculum Resource was published prior to establishment of current criteria of submission, and as such, does not contain all criteria required of current publications. However, the Editorial Committee felt that the activity itself remained worthwhile and relevant, and encourages potential users to contact the authors for clarification as needed. If you do update this activity for use with your students, and are interested in updating the resource for distribution in the library, please contact ASM at MicrobeLibrary@asmusa.org.

INTRODUCTION

Time Required.
Laboratory work - 1 h 15 min

Pedagogical Function.
This activity was designed to demonstrate the usefulness of serological assays in the diagnosis of infectious diseases. The students are given the opportunity to become familiar with the interpretation of assay results and to gain an appreciation for the importance of doing positive and negative controls along with the patient's sample.

Learning Objectives.
At the completion of the exercise, students should:
1. Be familiar with the steps involved in performing the ELISA and Western blot assays.
2. Understand the purpose of each reagent used in the assays, including the antigen, blocking agent, patient serum, enzyme-conjugated secondary antibody, and substrate solution.
3. Be able to interpret assay results, including the circumstances that might lead to "false positive" or "false negative" results.
4. Understand the roles of "screening" versus "confirmatory" assays in diagnosing HIV infection.
5. Be able to suggest alternative diagnostic tests for patients who yield "indeterminate" results in the Western blot assay.

Background.
Before beginning the exercise, students should have a basic understanding of:
1. How the immune system protects the individual from infectious agents, including how antibodies recognize the antigens for which they are specific.
2. The biology of HIV and acquired immunodeficiency syndrome (AIDS).

PROCEDURE

Materials.
ELISA
1. Microcentrifuge tubes containing 3 different simulated patient sera, plus positive and negative control sera, diluted in phosphate-buffered saline
2. An ELISA plate precoated with surrogate HIV antigens and blocking agent
3. A squirt bottle containing ELISA wash buffer
4. Disposable pipets
5. Simulated goat antihuman IgG-horseradish peroxidase conjugate
6. 3,3',5,5'-tetramethyl-benzidine (TMB) substrate

Western blot
1. Three simulated HIV Western blot strips
2. Microcentrifuge tubes containing your positive patient serum from the HIV ELISA, plus positive and negative control sera diluted in tris-buffered saline
3. Incubation tray and forceps
4. A squirt bottle containing Western blot wash buffer
5. Simulated goat antihuman IgG-horseradish peroxidase conjugate
6. 3,3'-diaminobenzidine (DAB) substrate solution

Student Version.
ELISA. The ELISA plate has been coated with surrogate HIV antigens and blocked with irrelevant protein for you by the instructor. Work in pairs and be sure to change pipets between serum samples.

1. Use a pipet to dispense 3 drops of each simulated serum into duplicate wells of the ELISA plate according to the template in Figure 1. Incubate the plate 5 min at room temperature.
2. Shake out the contents of the wells. Flick-wash the test wells 3 times each with ELISA wash buffer.
3. Dispense 3 drops from the tube labeled "goat antihuman IgG-horseradish peroxidase conjugate" into each test well and incubate the plate 5 min.
4. Shake out the contents of the wells. Flick-wash the test wells 3 times each with ELISA wash buffer.
5. Dispense 3 drops of the TMB substrate into each test well. Observe for color development over the next 3-10 min.
6. Record your patients' specimen letters on your answer sheet along with your results (positive or negative).

Figure 1
Answer Sheet

Western blot. Prior to the laboratory, nitrocellulose strips were prepared that simulate HIV antigens separated by polyacrylamide gel electrophoresis according to molecular mass. The strips have been blocked for you by the instructor.

1. Inform the instructor which of your patient sera yielded a positive result in the HIV ELISA. You will be given an HIV Western blot strip that bears the same label as your positive serum sample. In addition, you will receive strips labeled (+) and (-) for incubation with positive and negative control sera, respectively.
2. Using forceps, place the dry strips in separate wells of an incubation tray. Rehydrate each strip with the corresponding serum sample and incubate for 15 min, rocking the incubation tray frequently. For this and all subsequent incubation or washing steps, use enough liquid to completely cover the strips without cross-contaminating adjacent wells.
3. Remove the sera from the strips. Wash each strip 3 times, 2 minutes per wash, rocking the tray constantly.
4. Incubate each strip 5 min with simulated goat antihuman IgG-horseradish peroxidase conjugate, rocking the tray frequently.
5. Remove the conjugate from the strips. Wash each strip 3 times with constant rocking, 2 minutes per wash.
6. Add DAB substrate solution to each strip. Rock the chamber constantly over the next 3-10 min. Stop color development by flushing the strips with water.
7. Using the CDC criteria listed in Table 1 to make your determination, record the results (positive, negative, or indeterminate) obtained for your patient's serum along with the specimen letter.

Table 1

Safety Issues.
Safety concerns are minimal because streptavidin-horseradish peroxidase and biotinylated albumin are used as substitutes for antibodies and HIV antigens, respectively. Exposure of the skin to substrate solutions should be avoided because the chemicals are potentially carcinogenic. Mouth pipetting is prohibited.

All reagents have been chosen for their low toxicity. The students are informed that potentially infectious human serum and HIV antigens are not being used, and so it is not essential to employ universal precautions against bloodborne pathogens as outlined by the CDC (2). However, if this exercise is being performed by allied health or microbiology majors, the instructor may wish to use it as an opportunity to introduce these safety guidelines.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
The answer sheet requires interpretation of the results obtained for patient sera in the HIV ELISA and Western blot assays, and also asks questions that probe the students' understanding of assay methodology and limitations. In addition, students are routinely asked to interpret ELISA and Western blot results during written lecture or laboratory examinations after completion of the exercise to assess their analytical skills.

The instructor may wish to provide background information to facilitate completion of questions asked on the answer sheet. A discussion prior to beginning lab work might include the likelihood of obtaining a false positive ELISA result in plates that have not been properly blocked, or the causes of obtaining false negatives, such as the inadvertent omission of reagents or

the use of expired reagents. In addition to operator error, biological factors that can lead to false positive or false negative results should be discussed. For instance, false positives may occur when human antibodies react with contaminating proteins derived from the cells used to propagate HIV. Such cross-reaction with cellular proteins may occur when women who have been pregnant, or persons who have received transfusions or transplanted tissues, develop antibodies to foreign histocompatibility antigens. The Western blot is a good confirmatory test because it can discriminate between viral antigens and contaminating cellular components on the basis of molecular mass, while the ELISA makes a good screening tool because it is sensitive, relatively inexpensive, and rapid to perform. False negatives may occur if the patient is in the "window" period of infection before detectable antibodies have developed. For these individuals and those who are indeterminate in the Western blot assay, serum or plasma can be examined for the presence of viral antigens. p24 is useful for detecting very recent infections in adults and in neonates. Reverse transcriptase polymerase chain reaction (RT-PCR) detects the presence of the HIV genome during the first 2-4 weeks after infection when patients may be seronegative but infective; it is also useful for detecting HIV infection in neonates. In hospitals affiliated with research facilities, it may be possible to isolate and culture the virus from patient specimens. A decline in the CD4:CD8 T cell ratio may also signal infection in patients who are seronegative.

Problems and Caveats.
Preparation of ELISA plates and Western blot strips is time-consuming and requires advance planning.

ELISA plates work best if they are incubated with surrogate antigen overnight at 4^{o}C and then blocked in the morning before their use in an afternoon lab period. Ten wells (see Fig. 1) in each Falcon Probind assay plate are coated with 100 µl/well of biotin-labeled bovine serum albumin diluted to 20 mg/ml in phosphate-buffered saline (PBS), pH 7.4. Plates are washed 3 times with 200 µl/well of PBS containing 0.05% Tween 20 (PBST, ELISA wash buffer). To prevent nonspecific binding, unsaturated binding sites in the wells are blocked by a 1-h incubation at room temperature in 200 µl/well of PBS containing 1% unlabeled bovine serum albumin. After removing the blocking agent and washing the wells 3 times with PBST, the plates can be shaken dry and stored at 4^{o}C in resealable plastic bags until used.

For each pair of students, 5 simulated sera should be prepared—2 positive and 3 negative samples. The positive control and positive patient serum are made by diluting streptavidin-horseradish peroxidase to 1 µg/ml in PBS. Negative control and negative patient sera consist of PBS alone. The sera are dispensed at 250 µl/vial in labeled microcentrifuge tubes. The simulated goat antihuman IgG-horseradish peroxidase also consists of PBS alone and can be provided in larger bottles for several students to share. TMB substrate solution is supplied as a ready-to-use solution. All reagents are dispensed into the ELISA wells using disposable plastic transfer pipets. Typical results obtained for the HIV ELISA and their interpretation are illustrated in Figure 2.

Figure 2

Simulated Western blot strips are prepared by placing a sheet of nitrocellulose wetted with tris-buffered saline (TBS), pH 7.5, into a Mini-Protean II Multiscreen Apparatus, and then loading specific lanes with 600 µl of biotinylated BSA diluted to 5 µg/ml in TBS. For instance, to generate the banding pattern of the positive control illustrated in Fig. 3, lanes 1, 2, 7, 9, 10, 12, 15, 17, and 19 of the Multiscreen Apparatus were loaded with biotinylated BSA. Additional banding patterns we typically use also are illustrated in Fig. 3; they may be altered by the instructor so long as the new banding patterns follow the criteria listed in Table 1. After a 1-h incubation at room temperature, the surrogate antigen is removed by vacuum aspiration, the nitrocellulose is removed from the apparatus and rinsed with TBS, and the membrane is blocked by a 1-h incubation in TBS containing 3% unlabeled BSA. After rinsing in TBS, the nitrocellulose is cut into uniform horizontal strips perpendicular to the antigen lanes and dried at room temperature. The strips are labeled (see the letter codes in the appendix) and then stored between pieces of cardboard in resealable plastic bags at -20^{o}C for up to 2 months. Negative control strips are cut to the same size as the surrogate HIV strips from sheets of nitrocellulose that have been incubated in blocking agent.

Figure 3

For the Western blot, each pair of students will require 3 simulated serum samples and 3 corresponding nitrocellulose strips representing the positive control, negative control, and the patient serum that yielded a positive result in the ELISA. It is important to remember that a serum yielding a positive ELISA result can subsequently be positive, negative, or indeterminate by Western blot analysis (1). Positive and indeterminate sera are prepared by diluting streptavidin-horseradish peroxidase to 1 µg/ml in TBS containing 0.5% unlabeled BSA; since the sera are the same, the outcome of the Western blot is determined by the pattern of antigen bands the instructor chooses to apply to the nitrocellulose. Negative serum consists of TBS alone. The strips are placed in the wells of mini-incubation trays for incubation and washing steps. After incubation in simulated human serum, the strips are washed in TBS containing 0.05% Tween-20 (TTBS, Western blot wash buffer). The wash buffer is replaced by simulated goat antihuman IgG-horseradish peroxide which consists of TBS alone. After further washes, the strips are incubated in an metal-enhanced DAB substrate solution, which is supplied as a two-component system that can be mixed just prior to the laboratory period.

SUPPLEMENTARY MATERIALS

Possible Modifications.
Because this exercise was designed to be completed within 2 h including discussion time, lengthy antigen adsorption and blocking steps have been performed by the instructor prior to class. If time constraints are less strict, students should be allowed to coat their own ELISA plates with surrogate antigen and to block unsaturated binding sites with irrelevant protein. Three drops of antigen solution should be applied to each well and the plate incubated at room temperature for 1 h. The plates should be washed 3 times with ELISA wash buffer. Six drops/well of blocking agent should then be applied and incubation allowed to proceed for 1 h. After washing the wells an additional 3 times with wash buffer, the plate is ready for the application of the simulated serum samples. The instructor may also wish to have the students block their own nitrocellulose strips containing surrogate HIV antigens for Western blot analysis. Blocking is accomplished by a 1-h incubation in blocking agent at room temperature.

Commercial HIV ELISA and Western blot kits compare a single dilution (typically 1:100) of the patient's serum to three

controls—negative, weak positive, and positive. If the instructor wishes to enhance the authenticity of this exercise, a weak positive control can be included to enable the construction of a standard curve from which to calculate antibody concentrations in the patient specimens. ELISA readers that accommodate microwell strips can be purchased for around $2000; these are quite suitable for use in the student laboratory if quantitative measurements of ELISA absorbance values are desired.

General Information on the HIV ELISA and Western Blot Techniques.
The ELISA is used as a screening tool to identify individuals potentially infected by HIV, the causative agent of AIDS. Partially purified, inactivated HIV antigens are precoated onto the ELISA plate, and remaining sites on the plastic support are saturated (blocked) with an irrelevant protein such as bovine serum albumin to prevent subsequent reagents from binding nonspecifically. Patient serum is added to the wells; if HIV-specific antibodies are present in the serum, they will bind to the HIV antigens on the plate. Antibodies not specific for HIV are washed away during subsequent rinsing. An antihuman IgG secondary antibody conjugated to the enzyme horseradish peroxidase is then added to the wells; it binds to any human IgG that previously bound to HIV. After further washing, a colorless substrate solution is added to the wells, and the plate is inspected for a colored product that forms upon cleavage of the substrate by the peroxidase enzyme. The results can be measured qualitatively by visual inspection or quantitatively using a spectrophotometer. In this laboratory exercise, biotinylated albumin and streptavidin-horseradish peroxidase are used in place of HIV antigens and patient serum, respectively. Streptavidin is a tetrameric protein that binds with high affinity to biotin, a small B vitamin. The interaction is rapid and nearly irreversible, and thus it provides a useful alternative to a genuine antigen-antibody interaction for use in the teaching laboratory.

In an HIV Western blot, HIV-infected cells are lysed and electrophoresed into a polyacrylamide gel. As the proteins migrate, they separate based upon mass and charge, with smaller proteins migrating through the gel faster than larger proteins. The separated proteins are then transferred to a nitrocellulose membrane by passing a uniform electric current through the gel so that the proteins retain the same pattern on the membrane as on the gel. All sites on the membrane which do not contain blotted antigens from the gel can then be blocked with irrelevant protein to prevent nonspecific binding by subsequent reagents. After blocking, the membrane is incubated first with patient serum, then with goat antihuman IgG-horseradish peroxidase conjugate, and lastly with substrate solution. Upon reaction with the conjugate enzyme, the substrate precipitates, resulting in a visible band wherever the patient's antibody bound to an antigen on the membrane. As in the ELISA exercise, HIV antigens and human sera are replaced by biotinylated albumin and streptavidin-horseradish peroxidase, respectively, in the simulated HIV Western blot. The biotinylated albumin is applied to the nitrocellulose in a pattern that closely mimics the pattern that results when HIV antigens are separated by electrophoresis through a continuous 10% polyacrylamide gel.

References.
1. Centers for Disease Control and Prevention. Interpretation and use of the western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR 1989; **38** (suppl no. 7)**:**1-7.
2. Centers for Disease Control and Prevention. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987; **36**(suppl no. 2)**:**1-18.

Appendices.
Appendix 1. Letter Codes for Simulated Patient Sera
Appendix 2. Suggested Serum Groupings

Recipes for Media.
PBS, pH 7.4

NaCl	8.0 g
KH_2PO_4	0.2 g
Na_2HPO_4	1.15 g
KCl	0.2 g

Q.s. to 1 L with distilled water.

TBS, pH 7.5

Tris base	4.84 g
NaCl	58.48 g

Adjust pH to 7.5 with HCl.
Q.s. to 2 L with distilled water.

Reagents and Equipment.
Table 2 lists suppliers and costs for materials used in this laboratory. The cost per group is estimated at $8.20 for disposable supplies; it was derived by prorating each price by the shelf life and/or minimum sufficient quantity of the reagent required for 50 groups of students per laboratory session. This figure does not include the cost of reusable items such as the Multiscreen Apparatus, forceps, and incubation trays.

Table 2

Related Activities.
The laboratory presented here was inspired by the AIDS laboratory activity written by William J. Grimes, Linda Chambers,

and Martha Narro, contributors to The Biology Project, an online educational resource at the University of Arizona. In the original activity, students learn about the transmission of HIV by simulating the exchange of bodily fluids. This is accomplished by transferring aliquots of solutions, some of which contain simulated HIV antigens, between test tubes. Infected individuals are identified by an ELISA that assays for the presence of HIV antigens (rather than antibodies) in the fluids. Each group is then asked to work through the routes of transmission to determine the identity of the original carrier(s). The activity, as well as the entire Immunology Module offered through The Biology Project, can be accessed at: http://www.biology.arizona.edu/immunology/immunology.html

Acknowledgments.
We thank N. R. Chamberlain for suggesting the use of the Mini-Protean II Multiscreen Apparatus to prepare simulated Western blot strips.

Curriculum Resources

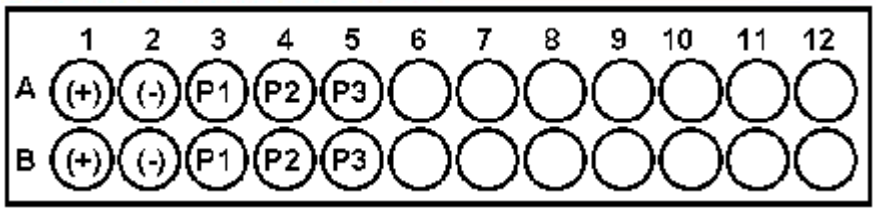

FIG 1. Template for loading the ELISA plate with positive (+), negative (-), and patient sera (P1-P3) in duplicate wells.

Curriculum Resources

Answer Sheet

A. HIV ELISA.
Record the letter assigned to each of your patient sera. Indicate whether each serum was positive (+) or negative (-) in the ELISA. Correct assessment of each patient serum is worth 1 point.

Serum	Reaction
+ Control	.
- Control	.
Patient:	.
Patient:	.
Patient:	.

B. HIV Western blot.
Record the letter assigned to the patient serum that you tested in the Western blot assay. Indicate whether each serum was POSITIVE, NEGATIVE, or INDETERMINATE in the Western blot according to the criteria listed in Table 1. Correct assessment of the patient serum is worth 3 points.

Serum	Reaction
+ Control	.
- Control	.
Patient:	.

C. Questions.
1. Why is it necessary to "block" the ELISA plate and nitrocellulose strip with "irrelevant" protein before application of subsequent reagents? (1 pt)

2. Why is it necessary to perform a confirmatory Western blot on serum that tests positive in the HIV ELISA? (1 pt)

3. What circumstances can lead to obtaining a false positive or false negative result in the ELISA and/or Western blot? (1 pt)

4. What are some alternative diagnostic tests that can be performed on patients who yield "indeterminate" results in the Western blot assay? (1 pt)

Curriculum Resources

Table 1. CDC Criteria for Scoring a Western Blot for Reactivity to HIV (1)

Positive result	*At least two* of the following HIV bands are recognized: **p24, gp41,** & **gp120/160**. The presence of both gp160 and 120 counts as one band. Note: Any number of *additional* bands may be present.
Negative result	*No bands whatsoever* are recognized.
Indeterminate	One or more bands of any size are recognized, but the criteria for a "positive" result are not met.

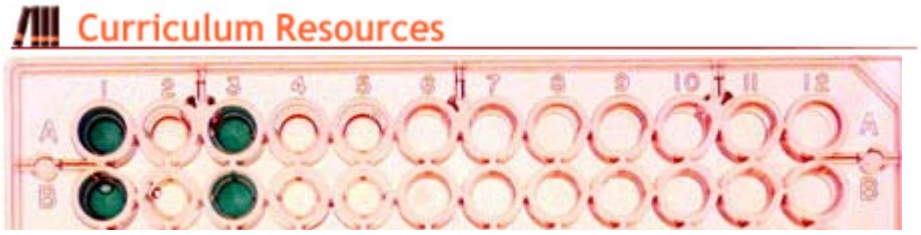

FIG 2. Typical results obtained in the HIV ELISA after 10 min of substrate incubation. Wells were loaded with sera as outlined in Fig. 1. The positive control and patient serum P1 yielded positive results.

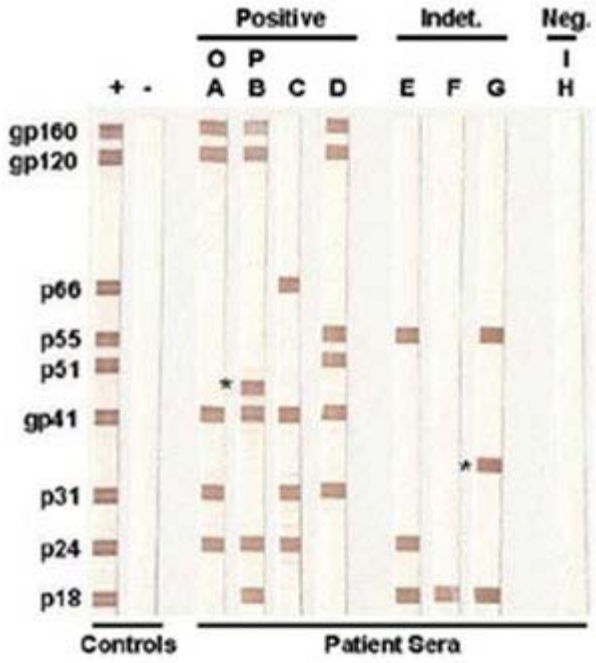

FIG 3. Banding patterns obtained in the HIV Western blot assay. Molecular weights (expressed in kD) simulating those found in HIV are labeled to the left of the positive control (+). Strips are labeled with letter codes of patient sera yielding positive, indeterminate (Indet.), or negative (Neg.) Western blot results. The letter codes correspond to those listed in the appendices. Bands denoted with asterisks (*) represent spurious cross-reactivities, such as those that occur with cellular contaminants in the HIV antigen preparation.

Curriculum Resources

Appendix 1 - Letter Codes for Simulated Patient Sera

Positive ELISA, positive Western blot	A, B, C, D, O, P
Positive ELISA, indeterminate Western blot	E, F, G
Positive ELISA, negative Western blot	H, I
Negative ELISA, Western blot not performed	J, K, L, M, N, Q, R, S, T, U, V, W, X, Y, Z

Curriculum Resources

Appendix 2. Suggested Serum Groupings

Note: Each pair of students receives 3 simulated patient sera for the ELISA, in addition to a positive control and negative control. The single patient serum that yields a positive result is listed under the "Western blot" column heading, along with the reaction obtained for that serum in the Western blot assay: positive (+), negative (-), or indeterminate (I).

Group numbers	ELISA - patient samples	Western blot - patient samples
1	Z, N, C	C (+)
2	Q, X, E	E (I)
3	U, V, F	F (I)
4	T, L, G	G (I)
5	R, M, A	A (+)
6	K, W, B	B (+)
7	J, S, I	I (-)
8	T, Y, P	P (+)
9	X, U, H	H (-)
10	S, N, D	D (+)
11	L, J, O	O (+)
12	W, V, H	H (-)
13	K, Z, O	O (+)
14	Q, R, F	F (I)
15	M, U, P	P (+)
16	X, Z, D	D (+)
17	W, L, E	E (I)
18	Q, N, A	A (+)
19	S, T, B	B (+)
20	J, K, I	I (-)
21	V, Y, G	G (I)
22	R, N, C	C (+)
23	W, U, I	I (-)
24	S, L, C	C (+)
25	M, X, P	P (+)
26	Z, K, F	F (I)
27	Y, T, A	A (+)

28	V, Q, D	D (+)
29	J, W, B	B (+)
30	Q, N, G	G (I)
31	X, Z, E	E (I)
32	U, M, O	O (+)
33	T, L, H	H (-)
34	S, R, E	E (I)
35	K, Y, B	B (+)
36	V, T, P	P (+)
37	W, R, A	A (+)
38	K, S, D	D (+)
39	Z, Y, I	I (-)
40	J, Q, G	G (I)
41	L, U, F	F (I)
42	X, N, O	O (+)
43	M, J, H	H (-)
44	U, T, C	C (+)
45	S, Z, P	P (+)
46	Z, N, C	C (+)
47	Q, X, E	E (I)
48	U, V, F	F (I)
49	T, L, G	G (I)
50	R, M, A	A (+)
51	K, W, B	B (+)
52	J, S, I	I (-)
53	T, Y, P	P (+)
54	X, U, H	H (-)
55	S, N, D	D (+)
56	L, J, O	O (+)
57	W, V, H	H (-)
58	K, Z, O	O (+)
59	Q, R, F	F (I)
60	M, U, P	P (+)
61	X, Z, D	D (+)
62	W, L, E	E (I)

63	Q, N, A	A (+)
64	S, T, B	B (+)
65	J, K, I	I (-)
66	V, Y, G	G (I)
67	R, N, C	C (+)
68	W, U, I	I (-)
69	S, L, C	C (+)
70	M, X, P	P (+)
71	Z, K, F	F (I)
72	Y, T, A	A (+)
73	V, Q, D	D (+)
74	J, W, B	B (+)
75	Q, N, G	G (I)
76	X, Z, E	E (I)
77	U, M, O	O (+)
78	T, L, H	H (-)
79	S, R, E	E (I)
80	K, Y, B	B (+)
81	V, T, P	P (+)
82	W, R, A	A (+)
83	K, S, D	D (+)
84	Z, Y, I	I (-)
85	J, Q, G	G (I)
86	L, U, F	F (I)
87	X, N, O	O (+)
88	M, J, H	H (-)
89	U, T, C	C (+)
90	S, Z, P	P (+)

Curriculum Resources

Table 2. Reagents and Equipment.

Suppliers and costs (based on 2000 figures) for materials used in the laboratory exercise.

Item	Supplier	Catalog #	Quantity	Useful Life	Price (in 2000)
Microcentrifuge tubes	Fisher	05-408-10	1 case of 500	1 session	$35.45
Falcon ELISA plates	Fisher	08-772-3C	1 case of 60	1 session	$105.80
Pasteur pipets	Fisher	13-711-7	1 case of 500	1 session	$32.80
NitroPure supported nitrocellulose	Fisher	WP4HYB0010	15cm x 3m roll	4 sessions	$108.35
NaCl	Fisher	BP358-1	1 kg	10 years	$22.70
Tris base	Fisher	BP152-1	500 g	10 years	$39.02
Bovine serum albumin	Sigma	A7906	10 g	1 session	$25.40
Biotinylated albumin (aliquot and store at -20^{o}C)	Sigma	A8549	10 mg	2 sessions	$39.10
KCl	Sigma	P3911	500 g	10 years	$24.40
KH_2PO_4	Sigma	P0662	500 g	10 years	$32.80
$Na_2HPO_4(7H_2O)$	Sigma	S9390	100 g	10 years	$14.70
Streptavidin-HRP (aliquot and store at -20^{o}C)	Sigma	S5512	250 mg	2 sessions	$49.80
Tween 20	Sigma	P1379	500 ml	10 years	$17.30
TMB liquid substrate	Sigma	T8665	100 ml	1 session	$27.90
Metal-enhanced DAB substrate	Pierce	34065ZZ	250 ml	1 session	$95.00
Dissecting forceps	Carolina Biological Supplies	BA-62-3990	50 @ $0.75 per piece	Indefinite	$37.50
Mini-incubation trays	BioRad	170-3902	3 cases of 20 @ $66.00/case	Indefinite	$198.00
Mini-Protean II Multiscreen Apparatus	BioRad	170-4017	1	Indefinite	$495.00