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A Simple Test Tube-Based ELISA Experiment for the High-School Classroom*

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Abstract

Immunology is gaining prominence both in the media as well as on the Advanced Placement (AP) exam in Biology. One of the challenges of teaching modern biological topics such as immunology and biochemistry in the high-school setting is the increased reliance on expensive technology in the research world. To begin to bridge this widening gap, we devised an experiment using a novel macroscale enzyme-linked immunosorbent assay that is suitable for AP-level high-school biology classrooms as well as entry-level collegiate laboratories. This novel method does not require a plate reader for data analysis, but instead relies on more common and inexpensive equipment such as a clinical test tube centrifuge and a simple test tube spectrophotometer. The experimental plan focuses on students measuring antibody concentrations in “unknown” samples and includes the collection and analysis of a standard curve using reagents prepared by the instructor. Students will be introduced to enzyme action, quantitative laboratory technique, antibodies, and the immune system, with the overall goal being to explore and highlight the inherent connections within the fields of biochemistry and immunology.

Keywords

Biotechnology education; clinical biochemistry; enzymes and catalysis; immunology; laboratory exercises

Enzyme-linked immunosorbent assays (ELISA) are biochemical techniques designed to quantitatively detect small amounts of many molecules including proteins, hormones, and antibodies originally developed by Perlmann and coworker in the early 1970s [1]. Although there are some variations in how these are conducted (e.g., direct ELISA vs. sandwich ELISA), ELISA remains a fundamental technique that is extremely common within both clinical [2–5] and investigative laboratories [6,7]. In the clinic, and even at home, ELISA is the basis for a host of molecular diagnostics. At home, these include pregnancy test strips that detect human chorionic gonadotropin and fertility tests strips that detect luteinizing hormone in urine. Within the clinic, ELISA is instrumental in the detection of a host of disease biomarkers. There are hundreds of examples, including prostate-specific antigen (PSA) found in patients with prostate cancer [2,3], anti-nuclear antibody (ANA) in Lupus patients [4], and glycoprotein 120 (GP120) on the surface of the human immunodeficiency

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virus in AIDS patients [5]. As such, the ELISA method is omnipresent in the lives of nearly everyone and therefore serves as an outstanding platform for a laboratory exercise at the high-school level.

In a direct ELISA, the target antigen is typically immobilized to a 96-well microtiter plate via a chemical reaction that results in the covalent attachment of the antigen, most commonly a protein, through free amino groups. Detection is then performed in three simple steps, which include probing the coated well with a primary antibody specific for the target antigen, probing the bound antibody using a secondary antibody specific for the constant region of the primary antibody, and then detection using a number of options. The secondary antibody is typically conjugated to either horseradish peroxidase or alkaline phosphatase. These enzymes are then readily detectable using a colorimetric substrate reaction, thus amplifying the signal and providing the technique with its name: enzyme-linked. The color change is then analyzed in a plate reader equipped to detect changes in absorbance as a measure of either target or primary antibody concentration.

ELISA is performed in a plate to facilitate the analysis of large numbers of samples and primary antibody dilutions. Known standards, typically in triplicate, are needed each time the assay is performed to generate a linear absorbance versus antigen or antibody concentration standard curve which is then used to calculate the concentrations of the unknown samples based on their absorbance values. Completion of an ELISA is therefore dependent on a rudimentary understanding of protein–protein binding and specificity, enzyme action, basic chemistry, and the function of antibodies.

One variation in ELISA can be a constant target antigen coated onto the plate, but with unknown antibodies from patient samples. The ANA test serves as an appropriate example in that the purpose is to detect specific antibody in the patient's blood stream rather than the target of those antibodies [8]. As such, the plate-immobilized antigen remains constant, and the unknown factor becomes the antibody concentration. This arrangement is the basis for the present laboratory exercise.

The most common limitation for high-school classrooms is equipment to carry out modern experimental methods. In the case of ELISA, a plate reader is required, which costs thousands of dollars and is well outside the reach of most high-school budgets. This has led to useful but less satisfying web-based simulations to illustrate how ELISA is done. In this manuscript, we describe a novel macroscale ELISA that relies solely on a clinical-style test tube centrifuge and a basic single-chamber spectrophotometer capable of reading absorbance at 450 nm. Class II major histocompatibility complex (MHCII) is immobilized to resin particles as a substitute for the typical microtiter ELISA plate, which enables all subsequent incubations and data collection to take place in a glass test tube. Standard and “unknown” dilutions of an anti-MHCII monoclonal antibody (mAb) serves as the experimental sample, and detection was accomplished via standard horseradish peroxidase (HRP)-conjugated secondary antibody. We report results from a high-school Advanced Placement (AP) Biology classroom, which show the effectiveness and forgiveness of the protocol in the hands of inexperienced students. This laboratory exercise successfully integrates fundamental laboratory skills such as centrifugation, micropipette technique, and spectrophotometry with the introduction of key concepts in critical AP Biology curriculum areas such as biochemistry of molecular interactions, antibodies, and enzyme action in the context of the immune response.

EXPERIMENTAL PROCEDURES

Antibodies, Cells, and Cell Culture (Preclassroom Experiment Preparatory Work)

Human Raji B cells were cultured in RPMI 1640 media (American Type Culture Collection [ATCC], Manassas, VA) supplemented with 10% fetal bovine serum, penicillin, and streptomycin (Invitrogen, Carlsbad, CA). Mouse HB-55 hybridomas expressing the L243 anti-MHCII monoclonal antibody were cultured in DMEM media (ATCC) supplemented with 10% fetal bovine serum, penicillin, and streptomycin (Invitrogen). The secondary (i.e., detection) antibody used in these experiments was a mouse anti-human IgG₂ horseradish peroxidase-conjugated antibody (Cat. # **05-0520; Invitrogen).

Antigen Isolation and Resin Conjugation (Preclassroom Experiment Preparatory Work)

A total of 10^7 Raji B cells were digested with papain (0.1 mg/mL papain; 10 mM Tris, pH 7.5; 0.5% β -mercaptoethanol; 0.1 mM EDTA) for 1 hour at 37 °C to release surface localized MHCII proteins (see Scheme 1). Protease inhibitor cocktail (Cat. # P8340-1ML; Sigma-Aldrich, St. Louis, MO) was then added to stop the cleavage, the cells were pelleted by centrifugation (5 minutes, 1000 $\times$ g) and the sample (i.e., the supernatant) was collected. The sample was then dialyzed against phosphate-buffered saline (PBS; pH 7.2) overnight. The released MHCII protein was then conjugated to 9 mL of NHS-activated Sepharose 4 affinity resin (Cat. # 17-0906-01; GE Healthcare, Piscataway, NJ) as indicated by the manufacturer. The MHCII-resin was stored at 4 °C in PBS.

Negative control resin lacking MHCII (9 mL total) was created by blocking fresh NHS-activated Sepharose 4 affinity resin with ethanolamine using the conditions recommended by the manufacturer.

Antibody Purification (Preclassroom Experiment Preparatory Work)

HB-55 hybridoma cells expressing the IgG_{2A} mouse antihuman MHCII monoclonal antibody (mAb, clone L243) were cultured continuously for 2 weeks. Spent media was removed daily and replaced with fresh media. The L243 antibody was purified from the spent media using a pre-poured recombinant protein A-conjugated fast-flow agarose chromatography column (HiTrap™ rProtein A FF Column; Cat. # 17-5079-02; GE Healthcare). Briefly, the pH of the spent media was adjusted to pH 7.5 using 0.1 M Tris, pH 7.5 then passed over the rProtein A column pre-equilibrated in 50 mM Tris, pH 7.5. The column was washed with 10 column volumes of 50 mM Tris, pH 7.5 buffer to remove all unbound material. The retained mAb was eluted with 5 column volumes of 50 mM citrate-buffered saline, pH 4.5. The flow through was neutralized with 1 M Tris, pH 7.5 and then dialyzed against PBS overnight at 4 °C. Purified mAb was stored at -20 °C in PBS until use. It should be noted that this mAb is commercially available in purified form (Cat. # 555810, BD Biosciences, San Jose, CA).

Classroom Equipment

Absorbance measurements at 450 nm were taken using a UNICO Visible Spectrophotometer (model S-1200) able to read samples in standard 12 mm $\times$ 75 mm test tubes. Incubations with the resin were performed on an orbital shaker (optional). A standard table-top clinical test tube centrifuge was used for all washing procedures to pellet the resin. A vortex mixer was used to mix reagents (optional). Finally, micropipettes able to transfer 1–10 μ L, 20–200 μ L, and 100–1,000 μ L are needed for accurate reagent transfers.

Stock Solutions and Reagents

One liter of phosphate-buffered saline with tween (PBS-T; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄, 0.1% tween-20), 100 mL 1 M HCl, 100 mL 3,3',5,5'-tetramethylbenzidine peroxidase substrate (TMB; Cat. # T0440, Sigma-Aldrich), phosphate-buffered saline with 5% skim milk (PBS-Milk; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄, 5% w/v skim milk).

Laboratory Supplies (Per Student Group)

Four 15 mL conical tubes (Cat. # S50712, Fisher Scientific, Pittsburgh, PA), four 1.5 mL microcentrifuge tubes (Cat. # 05-406-16, Fisher Scientific), micropipette tips, four glass 12

mm × 75 mm test tubes (Cat. # 14-961-26, Fisher Scientific), eight $\frac{1}{2}$ inch strips of Parafilm (Cat. # 13-374-16, Fisher Scientific), permanent marker, and test tube rack.

Sample Preparation for Student Groups

Six “standard” anti-MHCII mAb dilutions were prepared at 10 ng/mL, 5 ng/mL, 1 ng/mL, 0.5 ng/mL, 0.1 ng/mL, and 0.05 ng/mL, which were used for the creation of a standard curve by the students (see later). In addition, four unknown anti-MHCII mAb dilutions were prepared randomly at concentrations between 10 and 0.05 ng/mL.

Prelaboratory Student Preparation

Before beginning the laboratory experiment, students were given detailed information on the theory of ELISA and how it is used to understand biological phenomena. Of particular importance, the concepts of antibody–antigen specificity and enzyme kinetics were provided through immunology and biochemistry lectures. In addition, students were provided background materials on the variations of ELISA methods, cell-to-cell contact and communication events during an immune response, cytokine production during infection, and modern clinical applications of antibodies. Finally, students were encouraged to follow online “virtual” ELISA experiments as a “dry-run.” An interactive simulation is available through the Howard Hughes Medical Institute-sponsored BioInteractive website at <http://www.hhmi.org/biointeractive/vlabs/>, whereas animations showing the molecular details of ELISA are available at <http://www.sumanasinc.com/webcontent/animations/content/ELISA.html> and http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter33/animation_quiz_1.html.

Macroscale ELISA (Student Experimental Protocol)

This experimental protocol is designed for 12 groups of two students each over a 3-day period. The molecular mechanism of detection is shown in Scheme 2 and the overall student protocol is illustrated in Scheme 3.

Day 1 (about 30 minutes): Each group was given four 1.5 mL microcentrifuge tubes (two with 200 μ L negative control resin; two with 200 μ L MHCII-conjugated resin). In one negative control and one MHCII-resin tubes, the students added 135 μ L of the assigned “standard” mAb dilution (the “standards” were assigned such that all six dilutions were represented twice). In the other negative control and MHCII-resin tubes, the students added 135 μ L of the assigned unknown mAb dilution (the unknowns were assigned such that all four dilutions were represented in triplicate). In all four tubes, 265 μ L of PBS-Milk was added to a final sample volume of 500 μ L and each was sealed with Parafilm. Students inverted the tubes several times to mix the resin with the mAb samples and then the tubes were allowed to mix overnight on an orbital shaker.

Day 2 (about 50 minutes): Each of the four samples in each student group were removed and placed into fresh 15 mL conical tubes. To each sample, 10 mL of PBS-T was added and mixed by inversion. The tubes were centrifuged at 1,000 rpm for 5 minutes to pellet the resin and the supernatant decanted and discarded. This wash procedure was repeated twice more. The final resin pellets were resuspended in 1 mL PBS-Milk with 1 μ L (i.e., 1:1,000 dilution) of the secondary/detection mAb. The tubes were sealed with Parafilm and allowed to mix overnight on an orbital shaker.

Day 3 (about 50 minutes): Each tube was removed from the shaker and diluted with 10 mL of PBS-T. The tubes were centrifuged at 1,000 rpm for 5 minutes to pellet the resin and the supernatant decanted and discarded. This wash procedure was repeated twice more. The final resin pellets were resuspended in 300 μ L of TMB solution, mixed gently, then incubated for 10 minutes at room temperature. Next, 300 μ L of 1 M HCl was added to each tube and centrifuged at 1,000 rpm for 5 minutes to pellet the resin. The supernatants were transferred to a clean 12 mm $\times$ 75 mm test tube and the absorbance of the samples at 450 nm was measured and recorded.

Data Analysis

All analyses were performed using Microsoft Excel. Briefly, the values obtained for the negative control samples were subtracted from the MHCII-resin samples to correct for assay background. Then, student “standard” absorbance data were collated, averaged, and fit by linear regression on a plot of $\log_{10}$ of the mAb concentration and the absorbance at 450 nm. Using the extrapolated linear equation, the students then calculated the unknown mAb sample concentrations using the measured absorbance data collated from the entire classroom. Finally, the values obtained were compared with the actual mAb concentrations.

Laboratory Safety and Hazards

This laboratory exercise contains very little biohazardous steps for the students. The only chemical hazard is the 1 M HCl at the end of the protocol to stop the HRP reaction, although it is highly recommended that all students wear laboratory goggles throughout the wet lab portion of the protocol. The only physical hazards are the micropipette tips, as they are relatively sharp, and the glass test tubes. All solutions and used tubes can be safely discarded in a standard sink and trash, respectively.

RESULTS AND DISCUSSION

Resin Preparation and mAb Purification

Raji B cells were grown to reach a concentration of 10^7 cells/dish. The cells were collected, digested as described, and the extract collected. Although this yielded easily detectable MHCII ectodomain antigen from the cell surface (Fig. 1), this preparatory step can be modified through using any of a number of commercially available soluble antigens (e.g., glutathione S-transferase; GST; Cat. # G6511; Sigma-Aldrich).

Next, the antigen was conjugated to NHS-activated affinity resin to create covalent bonds between the released molecules and the resin (Scheme 1). Negative control resin was created by conjugating resin with ethanolamine to block all the reactive NHS groups. Both resins were washed with PBS and stored at 4 °C (do not freeze) until use.

The MHCII mAb was produced in hybridoma cell culture over the period of 1 week. mAb molecules were purified using recombinant Protein A resin as described. We obtained ~3 mg of purified mAb using this method (data not shown). We chose MHCII because of the availability of anti-MHCII mAbs in the Cobb laboratory at Case Western Reserve

University, however, if GST was used in place of MHCII as the target antigen, anti-GST mAbs are readily available (Cat. # G7781; Sigma-Aldrich).

Laboratory Testing of the Protocol

The macroscale ELISA was first tested within a research laboratory setting to assess the reliability and feasibility of the protocol for inexperienced high-school students. Using only the equipment listed in the Experimental Procedures section, we performed the assay using five known anti-MHCII mAb amounts ranging from 100 ng to 1 pg. The final supernatants following HCl addition were diluted 1:5 and then measured for absorbance at 450 nm using a classroom test tube spectrophotometer. We found a high degree of linearity in our results (Fig. 1), leading to a highly reproducible standard curve from which we could easily calculate unknown mAb sample concentrations.

Classroom Implementation

To test this laboratory exercise under the intended conditions, the experiment was performed by a total of 64 high-school students within the context of three separate course sections of AP Biology at Rocky River High School (Rocky River, OH). Within each class, the students were broken into 12 groups with two students per group. As described in the Experimental Procedures section, each student group was given four 1.5 mL microfuge tubes containing pre-prepared MHCII-resin or negative control resin, a standard mAb dilution and an unknown mAb dilution (see Scheme 3). The standard dilutions were assigned a letter designation (SA through SF) and divided among the groups so that each dilution was represented at least twice, although it was stressed to the students that in a “real” research setting, all standard dilutions would be carried out by the individual investigator rather than pooling data from others. Likewise, the unknown dilutions were assigned a letter designation (UA through UD) and divided such that each dilution was represented at least three times. Actual dilutions and concentrations were noted by the instructor for later reference.

Each group of students went through the protocol, ultimately generating four absorbance values per group (two negative controls, one standard, and one unknown). The students subtracted the negative control values from the experimental values and then pooled their two data points, one background-corrected standard and one background-corrected unknown, with the other student groups. The data obtained from the six standards were plotted with the mAb concentration and fit to a linear regression algorithm (Fig. 2a). The equation of the line was calculated from the fit and a 95% confidence interval is shown. Using this equation, the students calculated the mAb concentration of their unknown samples using their experimental absorbance data. These values were pooled and compared with the actual concentration of mAb (Fig. 2b). It should be noted that a few data point were omitted because the resin was accidentally discarded rather than the supernatant during one of the wash steps. However, the students as a whole generated highly accurate results that closely match the actual mAb concentrations.

Reaching the Learning Objectives

As a portion of this laboratory exercise, the students were expected to understand the concept and procedure of an ELISA, how to develop a standard curve using Microsoft Excel, how to determine unknowns from a standard curve, develop new laboratory skills (e.g., using a micropipette), and to develop an appreciation for the practical uses of ELISA in today’s society. Each student was required to complete a worksheet on the experiment (Table I) along with a laboratory report that included their raw data, calculated values for their unknown samples, and the standard curve graph using the pooled classroom data. We found that most students understood the basic molecular concepts centered around an

antibody binding to its antigen and the use of a color-changing enzymatic reaction as a detection method. However, though these concepts were generally understood, some of the students did have difficulty with the underlying chemistry, especially the role of HCl, and this difficulty depended on their exposure to high-school chemistry. Nonetheless, many of the students were able to draw connections between the laboratory exercise and the use of this technology in the clinic, especially in the HIV example. Initially, the students seemed to not fully appreciate the importance of controls and careful washes during the procedure, but by the end of the experiment, they could see firsthand the need for the control resin as the background and the potential for false-positive and false-negative results. Overall, it appeared that the students gained a strong appreciation for the concepts and their application to “real world” research and clinical testing, while earning a greater understanding of controls and other fundamental experimental design issues.

Common Student Pitfalls

The primary mistake to be watchful for is accidental discarding of the resin pellet during the several wash steps. Accuracy of this assay also depends on the students decanting the supernatant with care, so as to not remove resin each time the resin is washed or incubated. Finally, it is important to make certain the students are aware of the difference between microliter and milliliter volumes, as this can lead to highly inaccurate results and a waste of reagents.

CONCLUSIONS

This laboratory experiment was designed to measure the amount of specific antibody in a sample using ELISA. This is analogous to a number of real-world clinical tests where doctors need to know if a patient has antibodies against a particular disease marker (e.g., the ANA test for Lupus patients). The experiment was also designed to bypass the typical equipment limitations by using a macroscale resin-based ELISA rather than a microtiter plate-based assay. As such, this experiment is proposed for advanced high-school level biology students or entry level college students and both authors (brian.cobb@case.edu and brokaw.ann@rrcs.org) are happy to advise in the implementation of the exercise.

The experiment requires ~4 hours to perform, spread over 4 days to allow for overnight incubations, data analysis, and discussion. As a result of the background lectures and materials together with the laboratory experience, students were able to make natural interdisciplinary connections, such as the ability of the immune system to recognize “self” and “nonself” based on cell surface molecules to identify foreign cells or molecules and the concept of antigen recognition by immunoglobulins/antibodies and the subsequent immune response.

Although this exercise was carried out with MHCII from cultured B cells and laboratory-expressed mAb to reduce costs, this protocol is easily modified to strictly use commercially available antigen and primary mAb (see Experimental Procedures section for specifics). The protocol for conjugation of the antigen to the resin would remain the same, as would all subsequent steps. Some test run titrations of the experiment, however, may be necessary to ensure that the dilutions of the mAb “sample” fall within the linear range of detection.

The mission of the John H. Wallace High School Teachers Program administered by The American Association of Immunologists, the program under which this exercise was developed, is to promote science education through the creation of new laboratory exercises to illustrate key concepts in biology. This approach and the observed effect on reaching the learning objectives by the participating students is supported by two studies focused on the evaluation of teaching methods in the modern age of near-ubiquitous computer access in the

classroom. Although the use of simulation technology and activities appears to be an effective learning tool, its effectiveness has been shown to be limited to its use as part of a comprehensive curriculum, and does not replace practice-based didactic experiences [8,9], especially when the emulation activity does not include the full pedagogical scope of the laboratory exercise. To this end, we have demonstrated that a macroscaled ELISA is easily performed using very basic equipment that is common to most high-school biology/chemistry classrooms. The students gained experience with modern laboratory techniques, witnessed enzymatic events via colorimetric substrates, obtained hands-on understanding of antibody–antigen interactions, and learned valuable data analysis skills that are highly relevant to the clinical and investigative biomedical world. As such, this exercise provides a useful tool with which to illustrate traditional biochemical topics with real-world methodology to draw students' attention and excitement to an area of study (i.e., science and technology) that is continuing to expand in importance to our daily lives and national economy.

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REFERENCES

- Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 1971;8:871–874. [PubMed: 5135623]
- Benson MC, Whang IS, Pantuck A, Ring K, Kaplan SA, Olsson CA, Cooner WH. Prostate specific antigen density: A means of distinguishing benign prostatic hypertrophy and prostate cancer. *J. Urol* 1992;147:815–816. [PubMed: 1371554]
- Catalona WJ, Hudson MA, Scardino PT, Richie JP, Ahmann FR, Flanigan RC, Dekernion JB, Ratliff TL, Kavoussi LR, Dalkin BL. Selection of optimal prostate specific antigen cut-offs for early detection of prostate cancer: Receiver operating characteristic curves. *J. Urol* 1994;152:2037–2042. [PubMed: 7525995]
- Jaskowski TD, Schroder C, Martins TB, Mouritsen L, Hill HR. Comparison of three commercially available enzyme immunoassays for the screening of autoantibodies to extractable nuclear antigens. *J. Clin. Lab Anal* 1995;9:166–172. [PubMed: 7602423]
- Moore JP, Jarrett RF. Sensitive ELISA for the gp120 and gp160 surface glycoproteins of HIV-1. *AIDS Res. Hum. Retroviruses* 1988;4:369–379. [PubMed: 2848557]
- Pecora ND, Gehring AJ, Canaday DH, Boom WH, Harding CV. Mycobacterium tuberculosis LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. *J. Immunol* 2006;177:422–429. [PubMed: 16785538]
- Wang Q, McLoughlin RM, Cobb BA, Charrel-Dennis M, Zaleski KJ, Golenbock D, Tzianabos AO, Kasper DL. A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *J. Exp. Med* 2006;203:2853–2863. [PubMed: 17178920]
- Korfiatis K, Papatheodorou E, Stamou GP, Paraskevopoulos S. An investigation of the effectiveness of computer simulation programs as tutorial tools for teaching population ecology at university. *Int. J. Sci. Educ* 1999;21:1269–1280.
- McGaghie WC, Siddall VJ, Mazmanian PE, Myers J. Lessons for continuing medical education from simulation research in undergraduate and graduate medical education: Effectiveness of continuing medical education: American College of Chest Physicians Evidence-Based Educational Guidelines. *Chest* 2009;135:62S–68S. [PubMed: 19265078]

FIG. 1. Research laboratory standard detection curve

Negative control resin and MHCII-Resin were probed using the macro-ELISA protocol outlined with five mAb concentrations. The final supernatants were diluted 1:5 and detected using a test tube spectrophotometer. The raw data were plotted for each resin and fit by linear regression analysis. In addition, the MHCII-resin data were scaled by a factor of 5 to reflect actual absorbance values before dilution. These data show the feasibility of the experiment for a classroom setting.

FIG. 2. Student data from the classroom implementation

(a) Standard curve data and linear regression fit using student data with dotted lines representing the 95% confidence interval. (b) Student mAb concentration values for the unknown samples compared with the calculated values based on the actual known concentration of each unknown sample. These data not only demonstrate the feasibility of this experiment, but also show that the procedure is adequately robust to ensure reasonably accurate results within the high-school classroom setting.

SCHEME 1.

Isolation of the target MHCII antigen and conjugation of the resin.

SCHEME 2.
Mechanism of macro-ELISA detection.

SCHEME 3.

Student protocol schematic with expected color changes.

TABLE I

Student worksheet questions following the completion of the laboratory exercise

-
1. Why is the control resin necessary?
 2. Identify the following reagent by name:
(a) primary antibody, (b) secondary antibody, (c) enzyme,
and (d) substrate
 3. Why is each standard and each unknown tested multiple
times in all classes?
 4. What is the purpose of washing the resin before adding
each reagent?
 5. What is the purpose of adding the 1 M HCl at the end of
Day 3? Explain.
 6. Explain what is meant by a false-positive result. Name one
error that would result in a false-positive.
 7. Explain what is meant by a false-negative result. Name one
error that would result in a false-negative.
 8. Using your ELISA knowledge, the textbook, and the
internet if needed, explain how this particular type of
assay is used to diagnose HIV, the virus associated with
AIDS.
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