Chapter 5 Protein Isolation and Analysis

Lab 5a The Specificity of Antibody Proteins

Objective: Students produce a model of allergen antibody-antigen testing.

Timing:

One 50-minute lab period for preparing samples and materials

One 50-minute lab period for conducting the lab

One 25-minute lab post-lab for results and discussion

Student Groups:

- Student pairs

Materials: (Current materials lists are available at http://www.sargentwelch.com/biotech.)

LB agar Petri plates 60×15 mm, sterile, 2/3 full
Transfer pipets, 3 mL
Rocky's blood serum antibody solution
Permanent lab marker pens
Prepared antigen solutions
Pipets, 1 mL and Pipet pump, blue, or P-1000 and tips

Antibody Lab Preparation

This activity is a simulation of an Ouchterlony test using inorganic compounds to represent antibodies and antigens. When certain compounds bond with each other, a precipitation occurs. The compounds used here are calcium nitrate, sodium chloride, potassium chloride, water, and sodium carbonate. When calcium nitrate and sodium carbonate react, they produce a white precipitate (calcium carbonate), which is visible at a zone of interaction in the agar. The other compounds do not produce a precipitate.

Provide students with "serum" and 5 "antigens" to test. The compounds used are—

Serum (central well) = Rocky's serum, containing IgE (calcium nitrate)

Antigen choices =

- Flea extract (sodium chloride)
- Itchless® flea powder (potassium chloride)
- Puppystew® dog food (sodium carbonate)
- Cleantooth® dog biscuits (water)
- Fluffy® dog shampoo (sodium carbonate)

Recipes for Allergen and IgE Antibody Solutions

For the class, prepare 500 mL of each of the following antibody and antigen solutions. Label all solutions, and store at room temperature.

Rocky's serum IgE (antibody solution) = 0.5 *M* calcium nitrate

flea extract = 1 *M* sodium chloride

Itchless® flea powder = 1 *M* potassium chloride

Puppystew® dog food = 1 *M* sodium carbonate

Cleantooth® dog biscuits = distilled water

Fluffy® dog shampoo = 0.75 *M* sodium carbonate

Safety Issues and Tips:

The simulated "antibody" and "antigen" samples are inorganic chemicals. Gloves and goggles should be worn when handling chemicals.

Text Support:

Text Sections 2.3 and 5.1 have extensive discussions and several good graphics of proteins and, specifically, antibody structure and function.

Anticipatory Set:

Project an image of a pregnancy test kit, an HIV test kit, and a hepatitis test kit. Ask, "What do these have in common?" The answer is that they all use specific antibody proteins to recognize other specific proteins (called the antigens) in a solution.

Antibodies are just one category of protein molecules, but along with enzymes, they are often the target product of a biotechnology company.

Instruction:

Proteins are long, twisted chains of amino acids that are bound by peptide bonds. Since there are 20 different amino acids found in proteins, and they can be arranged in any amount or order, the possible variety of proteins is endless.

Protein function depends on the final three-dimensional shape of a protein. Protein structure is discussed in depth in the text. Proteins are usually classified into nine groups based on their functions.

Use the text's Table 2.1 Proteins Grouped by Function chart, to review the nine groups of proteins and their functions.

The two groups of proteins that are garnering the most interest in biotechnology because of their importance in research and as products are antibodies and enzymes. Enzymes are studied in the next lesson.

Read through the background of the lab activity with your students. Define the terms, "antibody" and "antigen." Antibodies are proteins developed by the immune systems that recognize specific molecules called antigens. Antigens are foreign proteins or molecules that are the target of antibody binding. Use the text Figures 5.8 and 5.9 to discuss the complexity of antibody molecules and how they recognize antigens. Use lab Figure 5.1 to show how antibodies recognize and clump antigens.

Use lab Figure 5.2 to discuss how an Ouchterlony test can be used to measure the amount of antibody-antigen recognition.

Have students complete procedure steps 1 through 8, produce a data table, and turn in the resulting plate.

Things to Stress with Students:

- Although the Ouchterlony test is the older type of test for checking antibody-antigen reaction, it will clearly show when an antibody recognizes and binds with its specific antigen. The antibody-antigen complex precipitates out of solution and is seen in the agar as a white band.

- Students should label the underside of the bottom of their Petri plates before trying to add any solution.
- The antibody solution is a simulation of antibodies from dog blood. In a real sample, there will be hundreds or even thousands of different kinds of antibodies in the blood serum. In this activity, we are looking for five specific antibodies for five specific antigens being tested. If precipitation between an antibody and antigen occurs, it means that the dog has antibodies to the specific antigens tested, possibly in high enough concentration to give an allergic response.
- Students should fill each well with sample to the top, but not overflowing onto the surface of the agar. This forces the antibody or antigen to diffuse through the agar equally and in all directions. Molecules diffuse based on their size. Some samples may diffuse more quickly than others. It may take 30-60 minutes for the antibody and the antigens to diffuse enough to meet each other. Leaving samples overnight ensures plenty of diffusion and precipitation.
- In step 7, students are converting qualitative data into quantitative data, so that they can average the results.

Tips, Tricks, and Hints:

- To increase student interest, do not tell students that they are using inorganic chemicals instead of real antibodies and antigens until after the reaction. Play up the dog allergy story line since most students love dogs. After the testing is complete, the simulation may be revealed. The "dog food" and "dog shampoo" will cause precipitant bands.
- Make up the solutions at least 1 day in advance to give them enough time to go completely into solution.
- If a photo-imaging system is available, photographing plate data is a good way to document the results.

Other Teaching Tools:

Assign, from the text Chapter 5, *Biotech Live* Activity 4.1, "Gathering Information on the Structure and Function of Proteins."

Assign, from the text Section 5.1, *Biotech Online*, "Antibody-Producing Companies."

A set of Microsoft® PowerPoint® lecture notes are available at http://biotech.emcp.net/immunology-lecture. In the Microsoft® PowerPoint® presentation, there are useful visuals that can help students to understand immunology topics, as well as antibody and antigen reactions.

There is an Ouchterlony simulator and a document explaining Ouchterlony analysis in greater detail that can be found online at http://biotech.emcp.net/immunology.

Checking for Understanding:

Assign the Data Analysis/Conclusion section, found at the end of the activity.

Assign students the *Thinking Like a Biotechnician* questions at the end of Lab 5a.

1. How likely is it that the results of one Ouchterlony test will give results that lead to the understanding of an organism's allergic response? Explain.

   Answer: It is not very likely that the results of one Ouchterlony test will give results that lead to the understanding of an organism's allergic response since we only test a small number of antigens as possible allergens.

2. Why is the speed of agglutination or precipitation not a valuable piece of data in this experiment?

   Answer: The speed of agglutination has to do with how soon the antibody and antigen come in contact, which depends on how quickly they diffuse through the agar. The diffusion of a molecule through the agar depends on its size, not its attraction to an antibody or antigen.

3. Setting up an Ouchterlony test may be time consuming. Why not just mix two solutions together to see if they clump? Suggest an advantage to having the molecules diffuse through and precipitate in the agar.